



IMMY

K112422

MAR 28 2012

510(k) Summary CrAg Lateral Flow Assay

This 510(k) summary is submitted in accordance with 21 CFR §807.92

Owner: Immuno-Mycologics, Inc.
2700 Technology Place
Norman, OK 73071
Tel: 405-360-4669
Fax: 405-364-1058
Contact: Dr. Sean K. Bauman, President & CEO
Sean-Bauman@immy.com

Prepared: March 26, 2012

Trade Name: CrAg Lateral Flow Assay

Common Name: Cryptococcal Antigen Lateral Flow Immunoassay

Regulation: 866.3165

Predicate Device: Immuno-Mycologics' CrAg Lateral Flow Assay (K102286)

Intended Use: The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) in serum and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription-use laboratory assay, which can aid in the diagnosis of Cryptococcosis.

Device Description:

Explanation:

Detection of cryptococcal antigen in serum and CSF has been used for over forty years to aid in the diagnosis of cryptococcosis with very high sensitivity and specificity (9,14,15). Current guidelines for the management of cryptococcal disease partially base treatment recommendations on cryptococcal antigen presence and more specifically on cryptococcal antigen titers (16).

Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) (5,6,12,13). Individuals with impaired cell-mediated

immune (CMI) function due to acquired immunodeficiency syndrome (AIDS) (19), lymphoproliferative disorders (18), steroid therapy (8), and organ transplantation (7) are at increased risk of cryptococcosis. AIDS accounts for 80-90% of cryptococcal infections (11). The incidence of cryptococcosis in AIDS patients in the United States is estimated to be 5-10% (11), while the incidence of cryptococcosis in other parts of the world, such as Africa, is as high as 30% (3). Cryptococcosis is the fourth most common opportunistic, life-threatening infection among AIDS patients (10).

Description:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in serum and CSF. For the qualitative procedure, specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For the semi-quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the qualitative procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold-conjugated, anti-cryptococcal monoclonal antibodies and gold-conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it binds to the gold-conjugated, anti-cryptococcal antibodies. The gold-labeled antibody-antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti-cryptococcal monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold-labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold-conjugated goat IgG antibody to move to the Control Line (C) which is immobilized bovine anti-goat IgG antibody. The immobilized anti-goat antibody will bind to the gold-conjugated goat IgG Control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line (Figure 1). If the control line fails to develop a line, then the test is not valid.

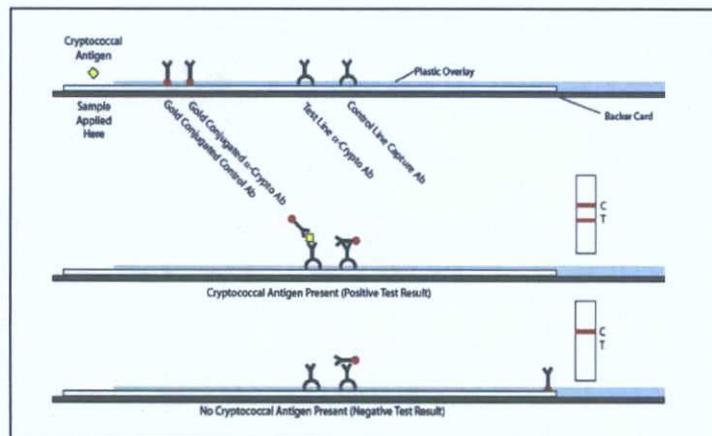


Figure 1. CrAg Lateral Flow Assay Schematic

Technological Characteristics Summary

A comparison between the CrAg LFA and the CrAg LFA (K102286 - Serum only) is presented in Table 1.

Table 1. Comparison with Predicate Device

SIMILARITIES		
Feature	CrAg LFA (New Device)	CrAg LFA (Serum Only) (K102286)
Intended Use		
Intended Use	Immunochromatographic test system for the qualitative or semi-quantitative detection of the capsular polysaccharide antigens of <i>Cryptococcus</i> species complex (<i>Cryptococcus neoformans</i> and <i>Cryptococcus gattii</i>) in serum	Immunochromatographic test system for the qualitative or semi-quantitative detection of the capsular polysaccharide antigens of <i>Cryptococcus</i> species complex (<i>Cryptococcus neoformans</i> and <i>Cryptococcus gattii</i>) in serum
Indication For Use	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis
Device Description		
Technology	Lateral Flow Assay	Lateral Flow Assay
Sample Matrix	Serum	Serum
Instruments	None	None
Assay Components	Specimen diluent, lateral flow strips, built-in control, gold conjugated antibodies	Positive control, negative control, latex cards, latex conjugated antibodies
Specimen Pre-Treatment	Dilution	Dilution
Detection Antibody	Anti-cryptococcal monoclonal antibody	Anti-cryptococcal monoclonal antibody
Storage Requirements	20-25°C	20-25°C
DIFFERENCES		
Feature	Cryptococcal Antigen Lateral Flow Assay	Latex- <i>Cryptococcus</i> Antigen Detection System
Intended Use		
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum and CSF
Indication For Use	No differences	No differences

Performance Summary

A. Precision Studies (Repeatability & Reproducibility)

Serum repeatability and reproducibility results can be found in the predicate device 510(k) (K102286)

Repeatability and reproducibility with CSF specimens were determined by spiking a mock CSF that was negative by the IMMY Latex-*Cryptococcus* Antigen Detection System with cryptococcal antigen at four concentrations: Negative, high negative (C₅), low positive (near C₉₅), and medium positive. The samples were analyzed on the CrAg Lateral Flow Assay in triplicate on five different days, at three different sites with a total of five different operators, on one lot, according to EP5-A2. One site was internal (Site 1) and the remaining two were a US reference laboratory (Site 2) and a US hospital laboratory (Site 3). For repeatability, percent positive and percent negative detected were calculated for each site (Table 2). For reproducibility, overall percent positive and percent negative detected were calculated by combining the data from all three sites (last two rows of Table 2).

Table 2. Repeatability at 3 Different Sites

Sample	CSF							
	1		2		3		4	
	Med. Pos	Low Pos	High Neg	Neg				
Neg/Pos	-	+	-	+	-	+	-	+
Site 1	0	30	0	30	27	3	30	0
Percent %	0	100	0	100	90	10	100	0
Site 2	0	30	0	30	30	0	30	0
Percent %	0	100	0	100	100	0	100	0
Site 3	0	15	0	15	15	0	15	0
Percent %	0	100	0	100	100	0	100	0
Total No.	0	75	0	75	72	3	75	0
Percent %	0	100	0	100	96	4	100	0

B. Analytical Sensitivity (lower limits of the assay/analytical cut-off)

Serum analytical sensitivity can be found in the predicate device 510(k) (K102286)

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running 24 replicates of varying concentrations of cryptococcal antigen diluted in mock CSF on one lot of kits, according to EP12-A2. The analytical cut-off was defined as the concentration where 50% of the results were positive and 50% of the results were negative. The analytical cut-off is 1.25ng/ml.

C. Analytical Specificity (cross-reactivity)

Serum analytical specificity can be found in the predicate device 510(k) (K102286)

Due to specimen availability, the following CSF conditions were not tested in the CrAg Lateral Flow Assay: *S. pneumonia*, *Enterovirus*, *Enterobacteriaceae*, *Streptococcus* spp., *Staphylococcus* spp., *diphtheroid*, *H. influenzae* type B, *N. meningitidis*, *Enterococcus* spp., *Epstein Barr*, *Herpes simplex virus* Type 1 and 2, *Listeria monocytogenes*, *Trichosporon beigelii*, and samples with syneresis fluid condensation.

This assay was not evaluated for potential interference related to specimen pretreatment with 2-mercaptoethanol or with specimens including the following substances or conditions: bloody CSF, cloudy CSF, white blood cells, xanthochromic CSF, bilirubin, protein, systemic lupus erythmatosus (SLE), sarcoidosis, or *N. meningitides*.

D. Linearity

N/A

E. High Dose Hook Effect

High dose hook effect concentrations with specimens were determined by spiking negative serum that was negative by the IMMY Latex-*Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at various concentrations between 20 and 500ug/ml. Each concentration was tested in triplicate at IMMY on one lot of CrAg Lateral Flow Assay, according to the package insert. It was determined that serum specimens with a cryptococcal antigen concentration higher than 200ug/ml can produce a high dose hook effect and therefore may produce a false negative result.

F. Method Comparisons

Predicate Device Method Comparison

Not Applicable

Other Method Comparison – Culture/India Ink (Gold Standards)

Serum method comparison to gold standards can be found in the predicate device 510(k) (K102286)

The CrAg Lateral Flow Assay was compared to the gold standard for the diagnosis of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay in CSF. These studies contained a mix of both prospective and retrospective specimens. A summary of the data collected is included in Tables 3 and 4 below:

Table 3. CSF 2x2 Contingency Table: Culture/India Ink

		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	65	0
	Negative	0	99

Table 4. CSF Statistical Analysis: Culture/India Ink

	Calculated	95% CI
Sensitivity	100%	94.4-100.0%
Specificity	100%	96.3-100%

Conclusion

The information submitted in this premarket notification is complete and supports a substantial equivalence decision.



Immuno-Mycologics, Inc.
c/o Sean K. Bauman, Ph.D.
President and CEO
2700 Technology PL
Norman, OK 73071

MAR 28 2012

Re: K112422

Trade/Device Name: CrAg Lateral Flow Assay (CrAg LFA)
Regulation Number: 21 CFR § 866.3165
Regulation Name: Cryptococcal antigen lateral flow assay
Regulatory Class: II
Product Code: GMD
Dated: March 26, 2012
Received: March 27, 2012

Dear Dr. Bauman:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

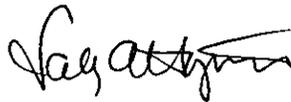
Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice

requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to <http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm> for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address <http://www.fda.gov/cdrh/industry/support/index.html>.

Sincerely yours,



Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure



IMMY

Indications for Use Statement

510(k) Number (if known): K112422

Device Name: CrAg Lateral Flow Assay

Indications for Use:

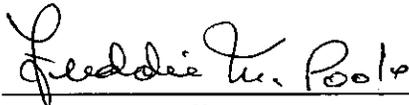
The Cryptococcal Antigen Lateral Flow Assay (CrAg LFA) is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis.

Prescription Use X AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)



Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k): K112422

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center - WO66-G609
10903 New Hampshire Ave.
Silver Spring, MD 20993-0002

April 3, 2012

Immuno-Mycologics, Inc.
c/o Sean K Bauman
2700 Technology Place
Norman, OK 73071 US

Document No: k112422
Re: k112422
Received: August 23, 2011

Categorization Notification

Regulations codified at 42 CFR 493.17 et. seq., implementing the Clinical Laboratory Improvement Amendments of 1988, require the Secretary to provide for the categorization of specific clinical laboratory test systems by the level of complexity. Based upon these regulations, the following commercially marketed test system or assay for the analyte is categorized below:

Test System/Analyte (s) : (SEE ATTACHMENT)

This complexity categorization is effective as of the date of this notification and will be reported on FDA's home page <http://www.fda.gov/cdrh/clia>. This categorization information may be provided to the user of the commercially marketed test system or assay as specified for the analyte indicated. It will also be announced in a Federal Register Notice, which will provide opportunity for comment on the decision. FDA reserves the right to reevaluate and recategorize this test based upon the comments received in response to the Federal Register Notice.

If you change the test system name or your company's name or if a distributor's name replaces your name, you must request another categorization by sending in the revised labeling along with a letter to FDA referencing the document number above.

If you have any questions regarding this complexity categorization, please contact Freddie M. Poole at 301-796-5467.

Sincerely yours,

Alberto Gutierrez, Ph.D.
Director
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

ATTACHMENT

Document Number : k112422

Test System : Immuno-Mycologics, Inc Cryptococcal lateral Flow Assay (Test Strip)

Analyte : Cryptococcus Antibodies

Complexity : MODERATE

CLIA Routing Slip

Document No : k112422

Re : k112422

Division: DMD

Branch: BACB

Applicant: Immuno-Mycologies, Inc.

Contact Name: Sean K Bauman

Contact Address: 2700 Technology Place, Norman, OK 73071 US

Contact Phone(s): (405) 360-4669

Contact Fax(es): (405) 364-1058

Contact Email:

Trade Name: Crag lateral flow assay (lfa)

DMC Date Received: August 23, 2011

Division Date Received: August 25, 2011

Categorization Information

CLIA Reviewer: Michael White [MWW]

Date Review Completed: March 30, 2012

Date Branch Concurred: March 30, 2012

Date Coordinator Concurred: APR 03 2012 

Effective Date:

Test Systems/Analytes/Grading

(See Attachment)

Document Number : k112422

Test System: Immuno-Mycologics, Inc Cryptococcal lateral Flow Assay (Test Strip)

Analyte : Cryptococcus Antibodies

Complexity : MODERATE [11]

Knowledge [2]; Training and Experience [2]; Reagents Preparation [1];

Operational Steps [2]; Quality Control [1];

Troubleshooting and Maintenance [1]; Interpretation and Judgment [2]

Rationale : IMM-015

CLIA Routing Slip

Document No : k112422

Re : k112422

Division: DMD
Branch: BACB

Applicant: Immuno-Mycologics, Inc.
Contact Name: Sean K Bauman
Contact Address: 2700 Technology Place, Norman, OK 73071 US
Contact Phone(s): (405) 360-4669
Contact Fax(es): (405) 364-1058
Contact Email:
Trade Name: Crag lateral flow assay (Ifa)

DMC Date Received: August 23, 2011
Division Date Received: August 25, 2011

Categorization Information

CLIA Reviewer: Michael White [MWW]
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(See Attachment)

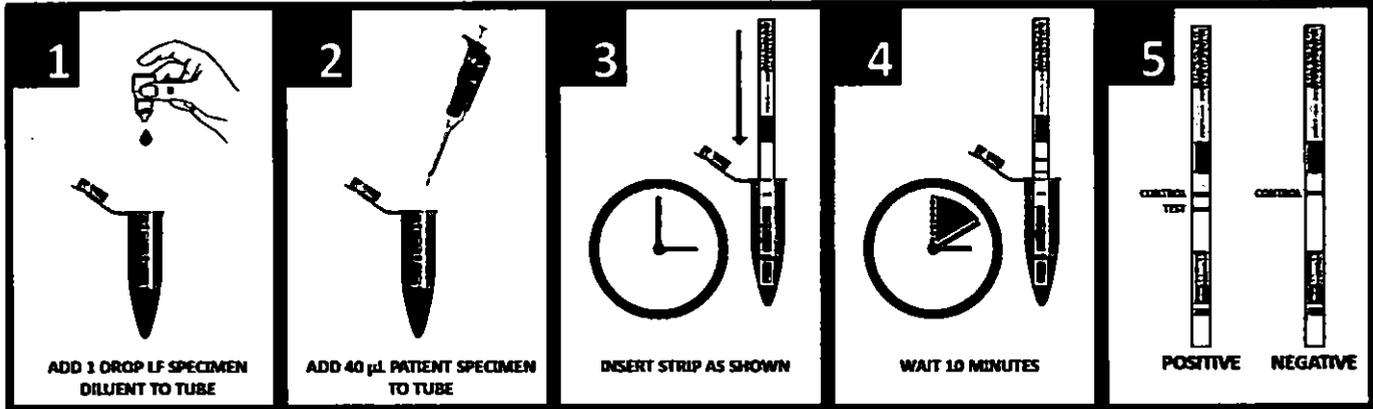


CrAg Lateral Flow Assay

For the Detection of Cryptococcal Antigen - REF CR2003



QUALITATIVE – BASIC PROCEDURE



INTENDED USE

The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of the capsular polysaccharide antigens of *Cryptococcus neoformans* and *Cryptococcus gattii* in serum and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription-use laboratory assay which can aid in the diagnosis of cryptococcosis.

SUMMARY and EXPLANATION of the Test

Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) (4). Individuals with impaired cell-mediated immunity are at greatest risk of infection (8). Cryptococcosis is one of the most common opportunistic infections in AIDS patients (6). Detection of cryptococcal antigen (CrAg) in serum and CSF has been extensively utilized with very high sensitivity and specificity (1-3).

BIOLOGICAL PRINCIPLES

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay. Specimens and specimen diluent are added into an appropriate reservoir, such as a test tube, and the lateral flow device is placed into the reservoir. The test uses specimen wicking to capture gold-conjugated, anti-CrAg monoclonal antibodies and gold-conjugated control antibodies deposited on the test membrane. If CrAg is present in the specimen, then it binds to the gold-conjugated, anti-CrAg antibodies. The gold-labeled antibody-antigen complex continues to wick up the membrane where it will interact with the test line, which has immobilized anti-CrAg monoclonal antibodies. The gold-labeled antibody-antigen complex forms a sandwich at the test line causing a visible line to form. With proper flow and reagent reactivity, the wicking of any specimen, positive or negative, will cause the gold-conjugated control antibody to move to the control line. Immobilized antibodies at the control line will bind to the gold-conjugated control antibody and form a visible control line. Positive test results create two lines (test and control). Negative test results form only one line (control). If a control line fails to develop then the test is not valid.

WARNINGS and PRECAUTIONS

For In Vitro Diagnostic Use only.

REAGENT PRECAUTIONS

1. Specific standardization is necessary to produce our high-quality reagents and materials. The user assumes full responsibility for any modification to the procedures published herein.

2. When handling patient specimens, adequate measures should be taken to prevent exposure to etiologic agents potentially present in the specimens.
3. Always wear gloves when handling reagents in this kit as some reagents are preserved with 0.095% (w/w) sodium azide. Sodium azide should never be flushed down the drain as this chemical may react with lead or copper plumbing to form potentially explosive metal azides. Excess reagents should be discarded in an appropriate waste receptacle.

REAGENTS

1. LF Specimen Diluent (2.5 mL, REF GLF025): Glycine-buffered saline containing blocking agents and a preservative
2. CrAg LF Test Strips (50 strips in desiccant vial, REF LFCR50)
3. CrAg Positive Control (1 mL, REF CB1020): Glycine-buffered saline spiked with cryptococcal antigen (strain 184A – clinical isolate from Tulane University (Infection & Immunity, June 1983, p. 1052-1059))
4. Package insert

MATERIALS NOT PROVIDED

1. Pipettor (40-µL and 80-µL)
2. Timer
3. Disposable micro-centrifuge tubes, test tubes, or a micro-titer plate

REAGENT PREPARATIONS

The entire kit should be at room temperature (22-25 °C) before and during use.

REAGENT STABILITY AND STORAGE

All reagents included in this kit should be stored at room temperature (22-25°C) until the expiration dates listed on the reagent labels.

Unused test strips should be stored in the LF test strip vial with the desiccant cap firmly attached.

SPECIMEN COLLECTION & PREPARATION

For optimal results, sterile non-hemolyzed serum should be used. Collect CSF specimens aseptically following accepted procedures. If a delay is encountered in specimen processing, storage at 2-8°C for up to 72 hours is permissible. Specimens may be stored for longer periods at <-20°C, provided they are not repeatedly thawed and refrozen. Specimens in transit should be maintained at 2-8°C or <-20°C.

PROCEDURE

REFER TO REAGENTS SECTION FOR A LIST OF MATERIALS PROVIDED.

Qualitative Procedure

1. Add 1 drop of LF Specimen Diluent (REF GLF025) to an appropriate reservoir (disposable micro-centrifuge tube, test tubes, or micro-titer plate, etc.).
2. Add 40 µL of specimen to the container and mix.
3. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip (REF LFCR50) into the specimen.
4. Wait 10 minutes.
5. Read and record the results (See READING THE TEST).

Semi-Quantitative Titration Procedure

1. Prepare dilutions starting with an initial dilution of 1:5, followed by 1:2 serial dilutions to 1:2560.
2. Place 10 micro-centrifuge or test tubes in an appropriate rack and label them 1-10 (1:5 through 1:2560). Additional dilutions may be necessary if the specimen is positive at 1:2560.
3. Add 4 drops of LF Specimen Diluent (REF GLF025) to tube #1.
4. Add 2 drops of LF Specimen Diluent to each of the tubes labeled 2-10.
5. Add 40 µL of specimen to tube #1 and mix well.
6. Transfer 80 µL of specimen from tube #1 to tube #2 and mix well. Continue this dilution procedure through tube #10. Discard 80 µL from tube 10 for a final tube volume of 80 µL.
7. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip into the specimen in each of the 10 tubes.
8. Wait 10 minutes.
9. Read and record the results (See READING THE TEST).

READING THE TEST

Read the reactions. The presence of two lines (test and control), regardless of the intensity of the test line, indicates a positive result.

For the semi-quantitative titration procedure, the patient's titer should be reported as the highest dilution that yields a positive result.

A single control line indicates a negative result. If the control line does not appear, the results are invalid and the test should be repeated.

REPRODUCIBILITY AND PRECISION

The CrAg Lateral Flow Assay was evaluated for reproducibility and precision by spiking serum and mock CSF with cryptococcal antigen to produce a panel consisting of a negative sample, a high-negative (C_s) sample, a low-positive sample and a moderate-positive sample. This panel was tested twice per day at three sites with a total of five operators over a five-day period in order to determine both the inter-lab and the intra-lab reproducibility and precision of the assay. The results of this study are shown in the tables below.

SERUM PANEL	Site 1 % Pos (n/N)	Site 2 % Pos (n/N)	Site 3 % Pos (n/N)	Overall % Pos (n/N)
Negative	0% (0/30)	0% (0/30)	0% (0/15)	0% (0/75)
High Negative	7% (2/30)	0% (0/30)	0% (0/15)	3% (2/75)
Low Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)
Moderate Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)

CSF PANEL	Site 1 % Pos (n/N)	Site 2 % Pos (n/N)	Site 3 % Pos (n/N)	Overall % Pos (n/N)
Negative	0% (0/30)	0% (0/30)	0% (0/15)	0% (0/75)
High Negative	10% (3/30)	0% (0/30)	0% (0/15)	3% (2/75)
Low Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)
Moderate Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)

BIBLIOGRAPHY

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 WEB: www.immy.com

CE
EC REP
 MDSS
 Schödiggraben 41
 30175 Hannover, Germany

International Symbol Usage

	Storage 20-25 C		Lot Number
	Manufactured by		Reference Number In Vitro Diagnostics Sufficient for "R" Tests
	Expiration Date		
	Conforms to European Union Requirements		Protect from Humidity



10903 New Hampshire Avenue
Silver Spring, MD 20993

Immuno-Mycologics, Inc.
c/o Sean K. Bauman, Ph.D.
President and CEO
2700 Technology PL
Norman, OK 73071

MAR 28 2012

Re: K112422

Trade/Device Name: CrAg Lateral Flow Assay (CrAg LFA)
Regulation Number: 21 CFR § 866.3165
Regulation Name: Cryptococcal antigen lateral flow assay
Regulatory Class: II
Product Code: GMD
Dated: March 26, 2012
Received: March 27, 2012

Dear Dr. Bauman:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice

requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to <http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm> for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address <http://www.fda.gov/cdrh/industry/support/index.html>.

Sincerely yours,



Sally A. Hojvat, M.Sc., Ph.D.

Director

Division of Microbiology Devices

Office of *In Vitro* Diagnostic Device

Evaluation and Safety

Center for Devices and Radiological Health

Enclosure



IMMY

Indications for Use Statement

510(k) Number (if known): K112422

Device Name: CrAg Lateral Flow Assay

Indications for Use:

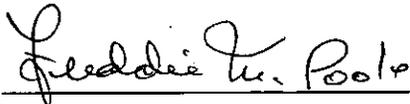
The Cryptococcal Antigen Lateral Flow Assay (CrAg LFA) is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis.

Prescription Use X AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)



Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k): K112422

www.immy.com | 2700 Technology PI Norman, OK 73071 USA | 405.360.4669



U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

March 27, 2012

IMMUNO-MYCOLOGICS, INC.
2700 TECHNOLOGY PLACE
NORMAN, OKLAHOMA 73071
ATTN: SEAN K. BAUMAN

510k Number: K112422

Product: CRAG LATERAL FLOW ASSAY (LFA)

The additional information you have submitted has been received.

We will notify you when the processing of this submission has been completed or if any additional information is required. Please remember that all correspondence concerning your submission **MUST** be sent to the Document Mail Center at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089402.htm>. On August 12, 2005 CDRH issued the Guidance for Industry and FDA Staff: Format for Traditional and Abbreviated 510(k)s. This guidance can be found at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm084365.htm>. Please refer to this guidance for assistance on how to format an original submission for a Traditional or Abbreviated 510(k).

The Safe Medical Devices Act of 1990, signed on November 28, states that you may not place this device into commercial distribution until you receive a letter from FDA allowing you to do so. As in the past, we intend to complete our review as quickly as possible. Generally we do so in 90 days. However, the complexity of a submission or a requirement for additional information may occasionally cause the review to extend beyond 90 days. Thus, if you have not received a written decision or been contacted within 90 days of our receipt date you may want to check with FDA to determine the status of your submission.

Please ensure that whether you submit a 510(k) Summary as per 21 CFR 807.92, or a 510(k) Statement as per 21 CFR 807.93, it meets the content and format regulatory requirements.

If you have procedural questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301)796-7100 or at their toll-free number (800)638-2041, or contact the 510k staff at (301)796-5640.

Sincerely,

510(k) Staff

Mcdonald, Lisa *

From: Microsoft Outlook
To: 'sean-bauman@immy.com'
Sent: Tuesday, March 27, 2012 12:21 PM
Subject: Relayed: K112422 AI Letter

Delivery to these recipients or distribution lists is complete, but delivery notification was not sent by the destination:

'sean-bauman@immy.com'

Subject: K112422 AI Letter

Sent by Microsoft Exchange Server 2007

Nichols, Karl *

From: Microsoft Exchange
To: 'sean-bauman@immy.com'
Sent: Wednesday, August 24, 2011 11:43 AM
Subject: Relayed: K112422- Acknowledgement Letter

Delivery to these recipients or distribution lists is complete, but delivery notification was not sent by the destination:

'sean-bauman@immy.com'

Subject: K112422- Acknowledgement Letter

Sent by Microsoft Exchange Server 2007



U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

August 24, 2011

IMMUNO-MYCOLOGICS, INC.
2700 TECHNOLOGY PLACE
NORMAN, OKLAHOMA 73071
ATTN: SEAN K. BAUMAN

510k Number: K112422
Received: 8/23/2011
Product: CRAG LATERAL FLOW ASSAY (LFA)

The Center for Devices and Radiological Health (CDRH), Office of Device Evaluation (ODE), has received the Premarket Notification you submitted in accordance with Section 510(k) of the Federal Food, Drug, and Cosmetic Act (Act) for the above referenced product. We have assigned your submission a unique 510(k) number that is cited above. Please refer prominently to this 510(k) number in any future correspondence that relates to this submission. We will notify you when the processing of your premarket notification has been completed or if any additional information is required. **YOU MAY NOT PLACE THIS DEVICE INTO COMMERCIAL DISTRIBUTION UNTIL YOU RECEIVE A LETTER FROM FDA ALLOWING YOU TO DO SO.**

On May 21, 2004, FDA issued a Guidance for Industry and FDA Staff entitled, "FDA and Industry Actions on Premarket Notification (510(k)) Submissions: Effect on FDA Review Clock and Performance Assessment". The purpose of this document is to assist agency staff and the device industry in understanding how various FDA and industry actions that may be taken on 510(k)s should affect the review clock for purposes of meeting the Medical Device User Fee and Modernization Act. Please review this document at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089735.htm>.

In future premarket submissions, we encourage you to provide an electronic copy of your submission. By doing so, you will save FDA resources and may help reviewers navigate through longer documents more easily. Under CDRH's eCopy Program, you may replace one paper copy of any premarket submission (e.g., 510(k), IDE, PMA, HDE) with an electronic copy. For more information about the program, including the formatting requirements, please see <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/ucm134508.htm>.

We remind you that Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) amended the PHS Act by adding new section 402(j) (42 U.S.C. § 282(j)), which expanded the current database known as ClinicalTrials.gov to include mandatory registration and reporting of results for applicable clinical trials of human drugs (including biological products) and devices. Section 402(j) requires that a certification form (<http://www.fda.gov/opacom/morechoices/fdaforms/FDA-3674.pdf>) accompany 510(k)/HDE/PMA submissions. The agency has issued a draft guidance titled: "Certifications To Accompany Drug, Biological Product, and Device Applications/Submissions: Compliance with Section 402(j) of The Public Health Service Act, Added By Title VIII of The Food and Drug Administration

Amendments Act of 2007" (http://www.fda.gov/oc/initiatives/fdaaa/guidance_certifications.html). According to the draft guidance, 510(k) submissions that do not contain clinical data do not need the certification form.

The Clinical Laboratory Improvement Amendments of 1988 (CLIA) requires the categorization of commercially marketed test systems by level of complexity. If your device is a test system that requires categorization you will be notified of your complexity as an enclosure with any clearance letter.

Please remember that all correspondence concerning your submission MUST be sent to the Document Mail Center (DMC) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review". Please refer to this guidance for information on current fax and e-mail practices at <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089402.htm>.

Please ensure that whether you submit a 510(k) Summary as per 21 CFR 807.92, or a 510(k) Statement as per 21 CFR 807.93, it meets the content and format regulatory requirements.

You should be familiar with the regulatory requirements for medical device available at Device Advice <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm>". If you have other procedural questions, or want information on how to check on the status of your submission, please contact DSMICA at (301)796-7100 or its toll-free number (800)638-2041, or at their Internet address <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm> or the 510k staff at (301)796-5640 .

Sincerely,

510(k) Staff



10903 New Hampshire Avenue
Silver Spring, MD 20993

Immuno-Mycologics, Inc.
c/o Sean K. Bauman, Ph.D.
President and CEO
2700 Technology Place.
Norman, OK 73071

Re: K112422

Trade Name: CrAg Lateral Flow Assay
Dated: August 22, 2011
Received: August 23, 2010

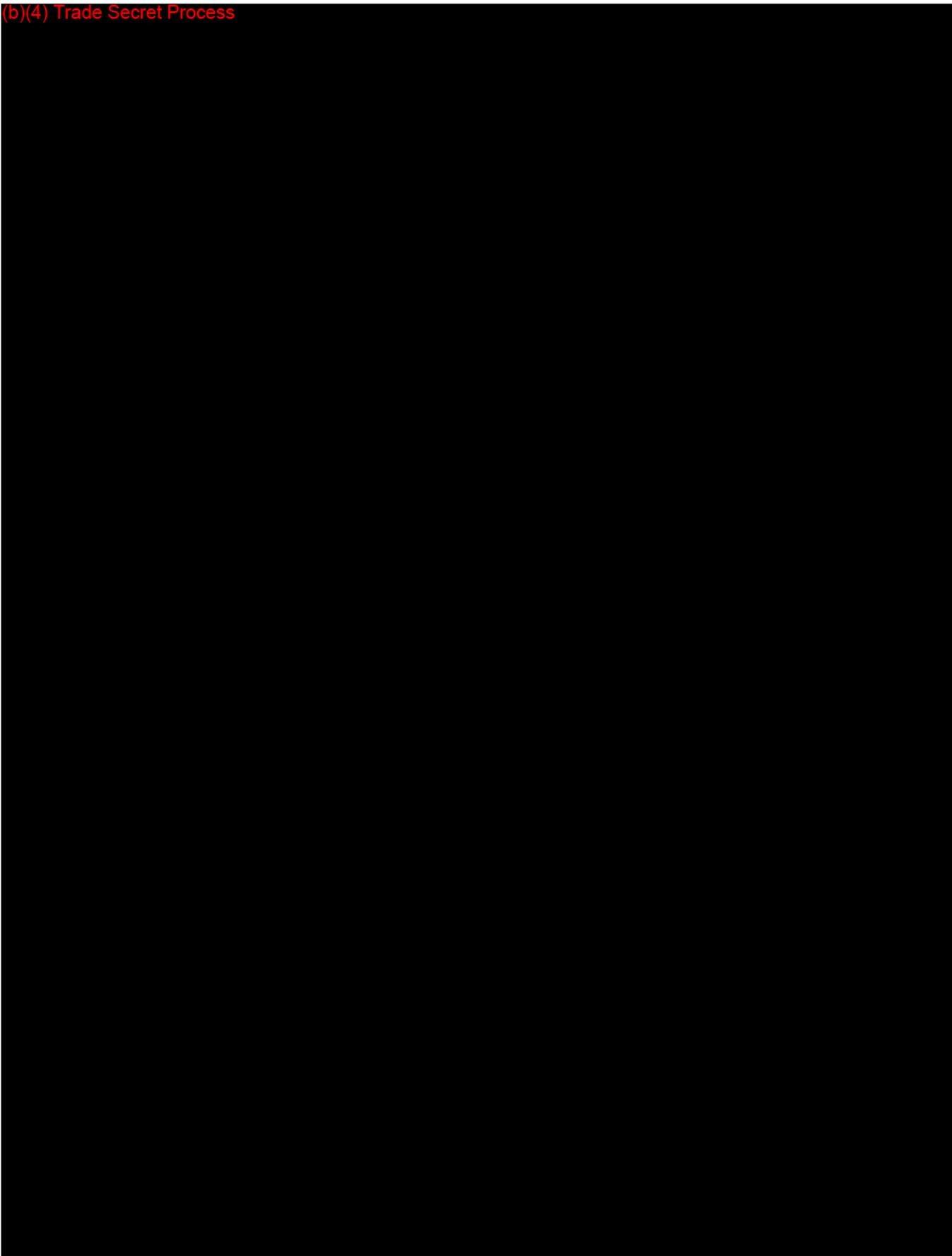
Dear Dr. Bauman:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above. [REDACTED]

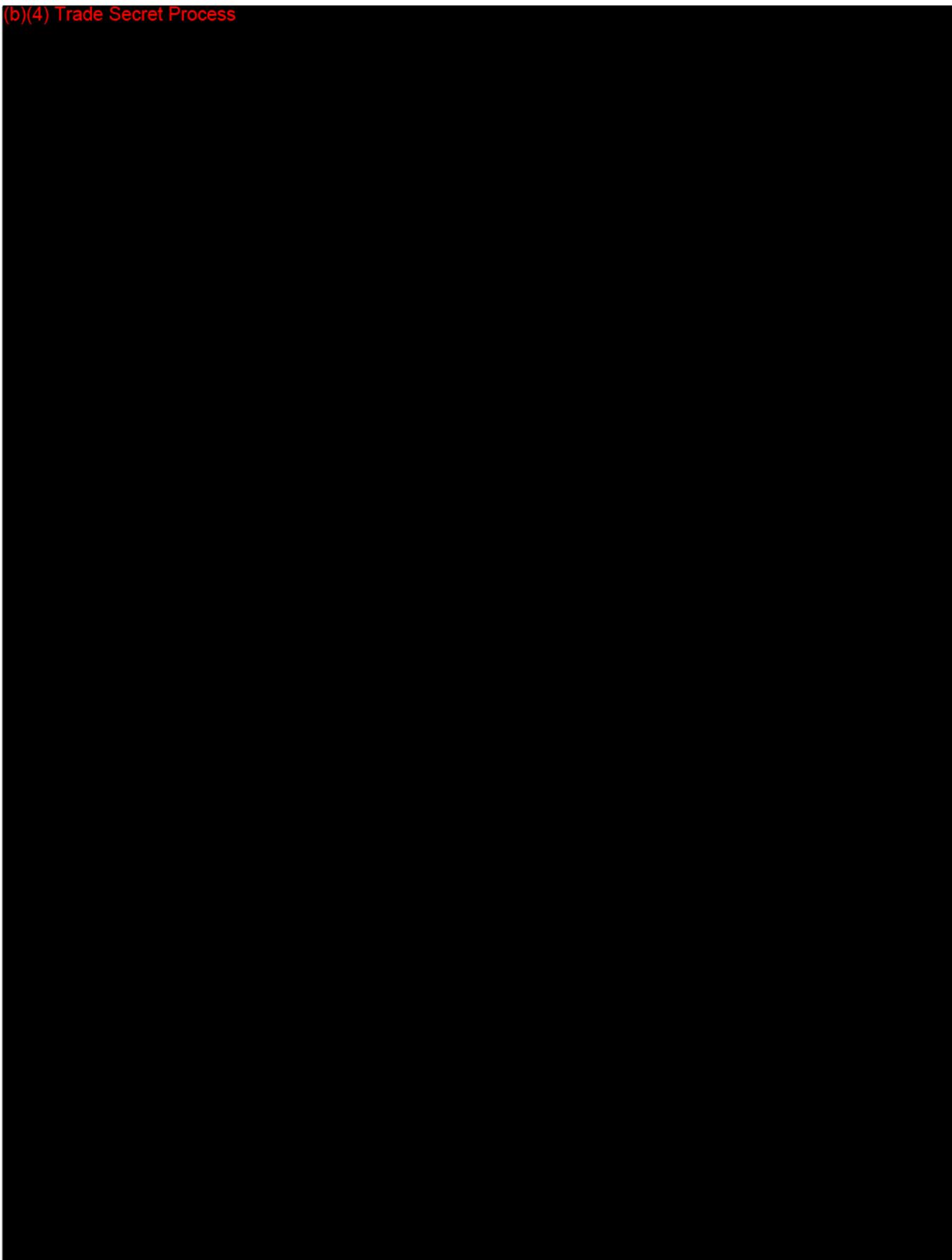
(b)
()
()

(b)(4) Trade Secret Process

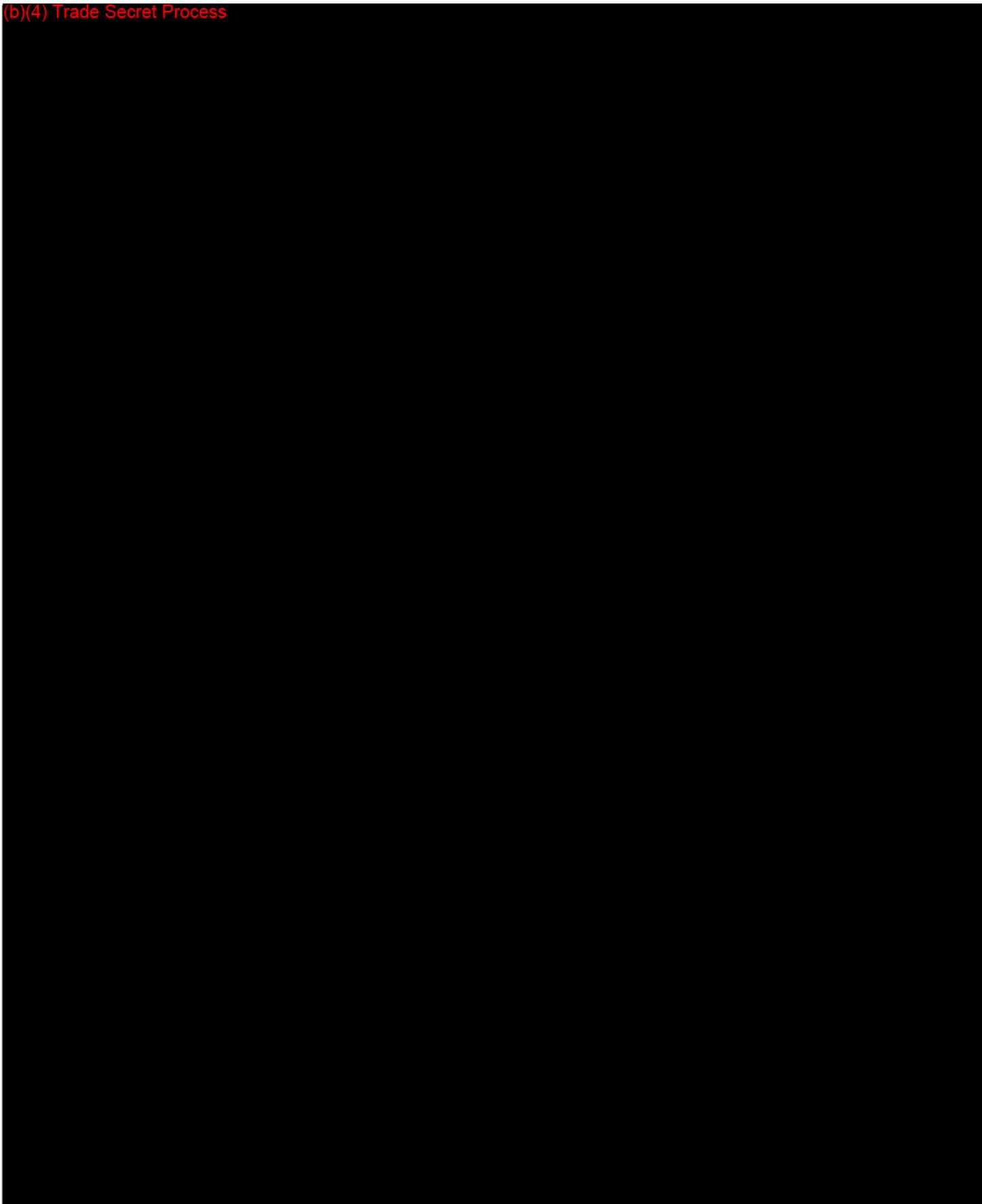
(b)(4) Trade Secret Process



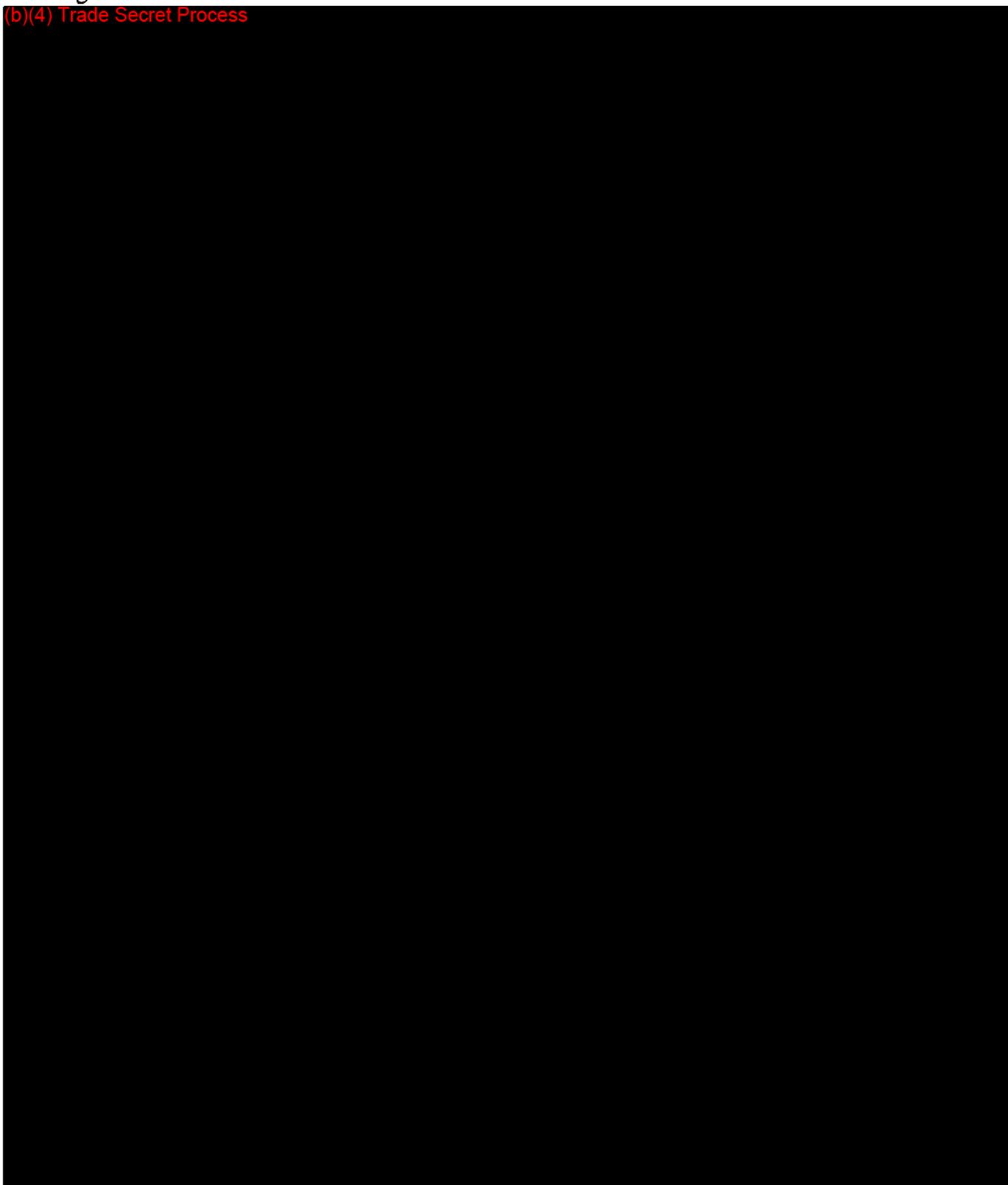
(b)(4) Trade Secret Process



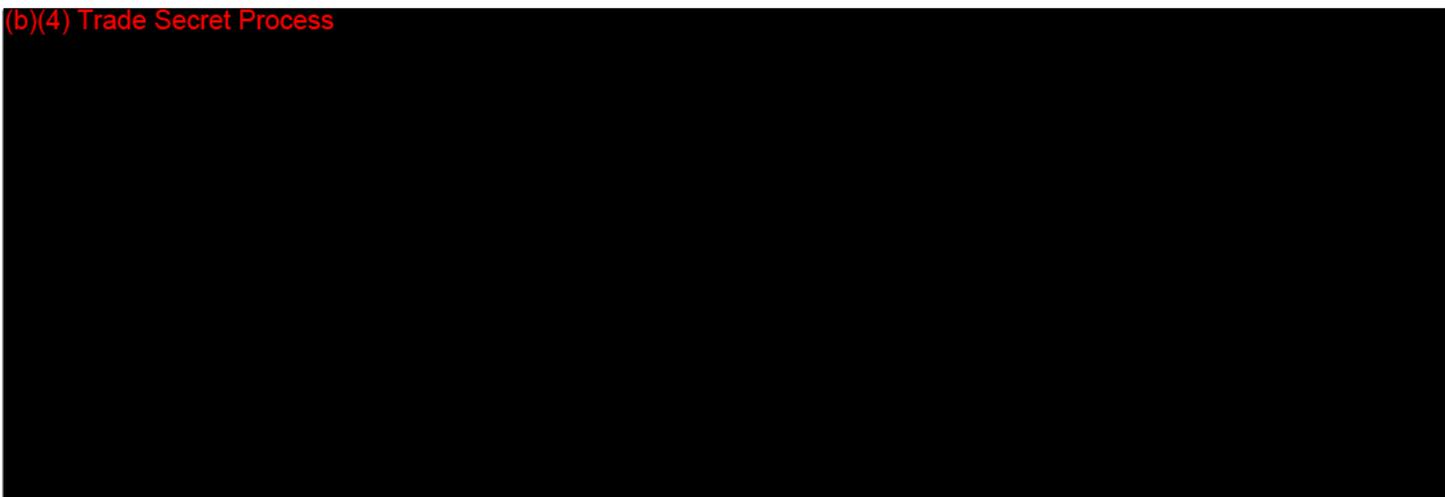
(b)(4) Trade Secret Process



(b)(4) Trade Secret Process



(b)(4) Trade Secret Process



Sincerely yours,



Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

FAX HEADER 1:
FAX HEADER 2:

TRANSMITTED/STORED : NOV. 14. 2011 9:05AM
FILE MODE OPTION

ADDRESS

RESULT

PAGE

6 MEMORY TX

4053641058

OK

6/6

REASON FOR ERROR
E-1) HANG UP OR LINE FAIL
E-3) NO ANSWER

E-2) BUSY
E-4) NO FACSIMILE CONNECTION



DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration

10903 New Hampshire Avenue
Silver Spring, MD 20993

Immuno-Mycologics, Inc.
c/o Sean K. Bauman, Ph.D.
President and CEO
2700 Technology Place.
Norman, OK 73071

Re: K112422

Trade Name: CrAg Lateral Flow Assay
Dated: August 22, 2011
Received: August 23, 2010

Dear Dr. Bauman:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above. (b)(4)

Trade Secret Process

(b)(4) Trade Secret Process

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CrAg Lateral Flow Assay 510(k)

KLI

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510(k) Screening Checklist

CrAg Lateral Flow ImmunoAssay (LFA) 510(k)

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Section 20: Performance Testing – Clinical	20-1		



DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Certification of Compliance, under 42 U.S.C. § 282(j)(5)(B), with Requirements of ClinicalTrials.gov Data Bank (42 U.S.C. § 282(j))

(For submission with an application/submission, including amendments, supplements, and resubmissions, under §§ 505, 515, 520(m), or 510(k) of the Federal Food, Drug, and Cosmetic Act or § 351 of the Public Health Service Act.)

SPONSOR / APPLICANT / SUBMITTER INFORMATION

1. NAME OF SPONSOR/APPLICANT/SUBMITTER Sean K. Bauman, PhD, President and CEO	2. DATE OF THE APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES Aug 24, 2011
3. ADDRESS (Number, Street, State, and ZIP Code) 2700 Technology Place Norman, OK 73071	4. TELEPHONE AND FAX NUMBERS (Include Area Code) (Tel.) 405-360-4669 (Fax) 405-364-1058

PRODUCT INFORMATION

5. FOR DRUGS/BIOLOGICS: Include Any/All Available Established, Proprietary and/or Chemical/Biochemical/Blood/Cellular/Gene Therapy Product Name(s)
FOR DEVICES: Include Any/All Common or Usual Name(s), Classification, Trade or Proprietary or Model Name(s) and/or Model Number(s)
(Attach extra pages as necessary)

Cryptococcal Antigen Lateral Flow Assay

CrAg Lateral Flow Assay (CrAg LFA)

APPLICATION / SUBMISSION INFORMATION

6. TYPE OF APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES

IND NDA ANDA BLA PMA HDE 510(k) PDP Other

7. INCLUDE IND/NDA/ANDA/BLA/PMA/HDE/510(k)/PDP/OTHER NUMBER (If number previously assigned)

8. SERIAL NUMBER ASSIGNED TO APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES

CERTIFICATION STATEMENT / INFORMATION

9. CHECK ONLY ONE OF THE FOLLOWING BOXES (See instructions for additional information and explanation)

A. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, do not apply because the application/submission which this certification accompanies does not reference any clinical trial.

B. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, do not apply to any clinical trial referenced in the application/submission which this certification accompanies.

C. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, apply to one or more of the clinical trials referenced in the application/submission which this certification accompanies and that those requirements have been met.

10. IF YOU CHECKED BOX C, IN NUMBER 9, PROVIDE THE NATIONAL CLINICAL TRIAL (NCT) NUMBER(S) FOR ANY "APPLICABLE CLINICAL TRIAL(S)," UNDER 42 U.S.C. § 282(j)(1)(A)(i), SECTION 402(j)(1)(A)(i) OF THE PUBLIC HEALTH SERVICE ACT, REFERENCED IN THE APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES (Attach extra pages as necessary)

NCT Number(s):

The undersigned declares, to the best of her/his knowledge, that this is an accurate, true, and complete submission of information. I understand that the failure to submit the certification required by 42 U.S.C. § 282(j)(5)(B), section 402(j)(5)(B) of the Public Health Service Act, and the knowing submission of a false certification under such section are prohibited acts under 21 U.S.C. § 331, section 301 of the Federal Food, Drug, and Cosmetic Act.

Warning: A willfully and knowingly false statement is a criminal offense, U.S. Code, title 18, section 1001.

11. SIGNATURE OF SPONSOR/APPLICANT/SUBMITTER OR AN AUTHORIZED REPRESENTATIVE (Sign) 	12. NAME AND TITLE OF THE PERSON WHO SIGNED IN NO. 11 (Name) Sean K. Bauman, Ph.D. (Title) President and CEO
13. ADDRESS (Number, Street, State, and ZIP Code) (of person identified in Nos. 11 and 12) 2700 Technology Place Norman, OK 73071	14. TELEPHONE AND FAX NUMBERS (Include Area Code) (Tel.) 405-360-4669 (Fax) 405-364-1058
15. DATE OF CERTIFICATION Aug 22, 2011	

Form Approved: OMB No. 0910-511. See Instructions for OMB Statement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES FOOD AND DRUG ADMINISTRATION MEDICAL DEVICE USER FEE COVER SHEET		PAYMENT IDENTIFICATION NUMBER: (b)(4) Trade Secret Write the Payment Identification number on your check.
A completed cover sheet must accompany each original application or supplement subject to fees. If payment is sent by U.S. mail or courier, please include a copy of this completed form with payment. Payment and mailing instructions can be found at: http://www.fda.gov/oc/mdufma/cover sheet.html		
1. COMPANY NAME AND ADDRESS (Include name, street address, city state, country, and post office code) IMMUNO MYCOLOGICS INC 2700 TECHNOLOGY PLACE NORMAN OK 73071 US 1.1 EMPLOYER IDENTIFICATION NUMBER (EIN) (b)(4)	2. CONTACT NAME Sean Bauman 2.1 E-MAIL ADDRESS sean-bauman@immy.com 2.2 TELEPHONE NUMBER (include Area code) 405-360-4669 2.3 FACSIMILE (FAX) NUMBER (Include Area code)	
3. TYPE OF PREMARKET APPLICATION (Select one of the following in each column; if you are unsure, please refer to the application descriptions at the following web site: http://www.fda.gov/oc/mdufma) <u>Select an application type:</u> <input checked="" type="checkbox"/> Premarket notification(510(k)); except for third party <input type="checkbox"/> 513(g) Request for Information <input type="checkbox"/> Biologics License Application (BLA) <input type="checkbox"/> Premarket Approval Application (PMA) <input type="checkbox"/> Modular PMA <input type="checkbox"/> Product Development Protocol (PDP) <input type="checkbox"/> Premarket Report (PMR) <input type="checkbox"/> Annual Fee for Periodic Reporting (APR) <input type="checkbox"/> 30-Day Notice		
3.1 Select a center <input checked="" type="checkbox"/> CDRH <input type="checkbox"/> CBER 3.2 Select one of the types below <input checked="" type="checkbox"/> Original Application <u>Supplement Types:</u> <input type="checkbox"/> Efficacy (BLA) <input type="checkbox"/> Panel Track (PMA, PMR, PDP) <input type="checkbox"/> Real-Time (PMA, PMR, PDP) <input type="checkbox"/> 180-day (PMA, PMR, PDP)		
4. ARE YOU A SMALL BUSINESS? (See the instructions for more information on determining this status) <input type="checkbox"/> YES, I meet the small business criteria and have submitted the required qualifying documents to FDA <input checked="" type="checkbox"/> NO, I am not a small business 4.1 If Yes, please enter your Small Business Decision Number:		
5. FDA WILL NOT ACCEPT YOUR SUBMISSION IF YOUR COMPANY HAS NOT PAID AN ESTABLISHMENT REGISTRATION FEE THAT IS DUE TO FDA. HAS YOUR COMPANY PAID ALL ESTABLISHMENT REGISTRATION FEES THAT ARE DUE TO FDA? <input checked="" type="checkbox"/> YES (All of our establishments have registered and paid the fee, or this is our first device, and we will register and pay the fee within 30 days of FDA's approval/clearance of this device.) <input type="checkbox"/> NO (If "NO," FDA will not accept your submission until you have paid all fees due to FDA. This submission will not be processed; see http://www.fda.gov/cdrh/mdufma for additional information)		
6. IS THIS PREMARKET APPLICATION COVERED BY ANY OF THE FOLLOWING USER FEE EXCEPTIONS? IF SO, CHECK THE APPLICABLE EXCEPTION. <input type="checkbox"/> This application is the first PMA submitted by a qualified small business, including any affiliates <input type="checkbox"/> This biologics application is submitted under section 351 of the Public Health Service Act for a product licensed for further manufacturing use only <input type="checkbox"/> The sole purpose of the application is to support conditions of use for a pediatric population <input type="checkbox"/> The application is submitted by a state or federal government entity for a device that is not to be distributed commercially		
7. IS THIS A SUPPLEMENT TO A PREMARKET APPLICATION FOR WHICH FEES WERE WAIVED DUE TO SOLE USE IN A PEDIATRIC POPULATION THAT NOW PROPOSES CONDITION OF USE FOR ANY ADULT POPULATION? (If so, the application is subject to the fee that applies for an original premarket approval application (PMA). <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO		
PAPERWORK REDUCTION ACT STATEMENT Public reporting burden for this collection of information is estimated to average 18 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to the address below. Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, 1350 Piccard Drive, 4th Floor Rockville, MD 20850 [Please do NOT return this form to the above address, except as it pertains to comments on the burden estimate.]		
8. USER FEE PAYMENT AMOUNT SUBMITTED FOR THIS PREMARKET APPLICATION (b)(4)		22-Aug-2011

Form FDA 3501 (01/2007)

["Close Window"](#) [Print Cover sheet](#)

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CDRH PREMARKET REVIEW SUBMISSION COVER SHEET

Date of Submission 08/22/2011	User Fee Payment ID Number (b)(4) Trade	FDA Submission Document Number (if known)
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SECTION A TYPE OF SUBMISSION

PMA <input type="checkbox"/> Original Submission <input type="checkbox"/> Premarket Report <input type="checkbox"/> Modular Submission <input type="checkbox"/> Amendment <input type="checkbox"/> Report <input type="checkbox"/> Report Amendment <input type="checkbox"/> Licensing Agreement	PMA & HDE Supplement <input type="checkbox"/> Regular (180 day) <input type="checkbox"/> Special <input type="checkbox"/> Panel Track (PMA Only) <input type="checkbox"/> 30-day Supplement <input type="checkbox"/> 30-day Notice <input type="checkbox"/> 135-day Supplement <input type="checkbox"/> Real-time Review <input type="checkbox"/> Amendment to PMA & HDE Supplement <input type="checkbox"/> Other	PDP <input type="checkbox"/> Original PDP <input type="checkbox"/> Notice of Completion <input type="checkbox"/> Amendment to PDP	510(k) <input checked="" type="checkbox"/> Original Submission: <input checked="" type="checkbox"/> Traditional <input type="checkbox"/> Special <input type="checkbox"/> Abbreviated (Complete section I, Page 5) <input type="checkbox"/> Additional Information <input type="checkbox"/> Third Party	Meeting <input type="checkbox"/> Pre-510(K) Meeting <input type="checkbox"/> Pre-IDE Meeting <input type="checkbox"/> Pre-PMA Meeting <input type="checkbox"/> Pre-PDP Meeting <input type="checkbox"/> Day 100 Meeting <input type="checkbox"/> Agreement Meeting <input type="checkbox"/> Determination Meeting <input type="checkbox"/> Other (specify):
IDE <input type="checkbox"/> Original Submission <input type="checkbox"/> Amendment <input type="checkbox"/> Supplement	Humanitarian Device Exemption (HDE) <input type="checkbox"/> Original Submission <input type="checkbox"/> Amendment <input type="checkbox"/> Supplement <input type="checkbox"/> Report <input type="checkbox"/> Report Amendment	Class II Exemption Petition <input type="checkbox"/> Original Submission <input type="checkbox"/> Additional Information	Evaluation of Automatic Class III Designation (De Novo) <input type="checkbox"/> Original Submission <input type="checkbox"/> Additional Information	Other Submission <input type="checkbox"/> 513(g) <input type="checkbox"/> Other (describe submission):

Have you used or cited Standards in your submission? Yes No (If Yes, please complete Section I, Page 5)

SECTION B SUBMITTER, APPLICANT OR SPONSOR

Company / Institution Name Immuno-Mycologics, Inc.	Establishment Registration Number (if known) 1627497		
Division Name (if applicable)	Phone Number (including area code) 405-360-4669		
Street Address 2700 Technology Place	FAX Number (including area code) 405-364-1058		
City Norman	State / Province OK	ZIP/Postal Code 73071	Country USA
Contact Name Dr. Sean K. Bauman, Ph.D			
Contact Title President and CEO		Contact E-mail Address Sean-Bauman@immy.com	

SECTION C APPLICATION CORRESPONDENT (e.g., consultant, if different from above)

Company / Institution Name			
Division Name (if applicable)	Phone Number (including area code)		
Street Address	FAX Number (including area code)		
City	State / Province	ZIP Code	Country
Contact Name			
Contact Title		Contact E-mail Address	

SECTION D1 REASON FOR APPLICATION - PMA, PDP, OR HDE

<input type="checkbox"/> New Device <input type="checkbox"/> Withdrawal <input type="checkbox"/> Additional or Expanded Indications <input type="checkbox"/> Request for Extension <input type="checkbox"/> Post-approval Study Protocol <input type="checkbox"/> Request for Applicant Hold <input type="checkbox"/> Request for Removal of Applicant Hold <input type="checkbox"/> Request to Remove or Add Manufacturing Site	<input type="checkbox"/> Change in design, component, or specification: <input type="checkbox"/> Software / Hardware <input type="checkbox"/> Color Additive <input type="checkbox"/> Material <input type="checkbox"/> Specifications <input type="checkbox"/> Other (<i>specify below</i>)	<input type="checkbox"/> Location change: <input type="checkbox"/> Manufacturer <input type="checkbox"/> Sterilizer <input type="checkbox"/> Packager
<input type="checkbox"/> Process change: <input type="checkbox"/> Manufacturing <input type="checkbox"/> Packaging <input type="checkbox"/> Sterilization <input type="checkbox"/> Other (<i>specify below</i>)	<input type="checkbox"/> Labeling change: <input type="checkbox"/> Indications <input type="checkbox"/> Instructions <input type="checkbox"/> Performance Characteristics <input type="checkbox"/> Shelf Life <input type="checkbox"/> Trade Name <input type="checkbox"/> Other (<i>specify below</i>)	<input type="checkbox"/> Report Submission: <input type="checkbox"/> Annual or Periodic <input type="checkbox"/> Post-approval Study <input type="checkbox"/> Adverse Reaction <input type="checkbox"/> Device Defect <input type="checkbox"/> Amendment
<input type="checkbox"/> Response to FDA correspondence:		<input type="checkbox"/> Change in Ownership <input type="checkbox"/> Change in Correspondent <input type="checkbox"/> Change of Applicant Address

Other Reason (*specify*):

SECTION D2 REASON FOR APPLICATION - IDE

<input type="checkbox"/> New Device <input type="checkbox"/> New Indication <input type="checkbox"/> Addition of Institution <input type="checkbox"/> Expansion / Extension of Study <input type="checkbox"/> IRB Certification <input type="checkbox"/> Termination of Study <input type="checkbox"/> Withdrawal of Application <input type="checkbox"/> Unanticipated Adverse Effect <input type="checkbox"/> Notification of Emergency Use <input type="checkbox"/> Compassionate Use Request <input type="checkbox"/> Treatment IDE <input type="checkbox"/> Continued Access	<input type="checkbox"/> Change in: <input type="checkbox"/> Correspondent / Applicant <input type="checkbox"/> Design / Device <input type="checkbox"/> Informed Consent <input type="checkbox"/> Manufacturer <input type="checkbox"/> Manufacturing Process <input type="checkbox"/> Protocol - Feasibility <input type="checkbox"/> Protocol - Other <input type="checkbox"/> Sponsor	<input type="checkbox"/> Response to FDA Letter Concerning: <input type="checkbox"/> Conditional Approval <input type="checkbox"/> Deemed Approved <input type="checkbox"/> Deficient Final Report <input type="checkbox"/> Deficient Progress Report <input type="checkbox"/> Deficient Investigator Report <input type="checkbox"/> Disapproval <input type="checkbox"/> Request Extension of Time to Respond to FDA <input type="checkbox"/> Request Meeting <input type="checkbox"/> Request Hearing
<input type="checkbox"/> Report submission: <input type="checkbox"/> Current Investigator <input type="checkbox"/> Annual Progress Report <input type="checkbox"/> Site Waiver Report <input type="checkbox"/> Final		

Other Reason (*specify*):

SECTION D3 REASON FOR SUBMISSION - 510(k)

<input type="checkbox"/> New Device	<input checked="" type="checkbox"/> Additional or Expanded Indications	<input type="checkbox"/> Change in Technology
-------------------------------------	--	---

Other Reason (*specify*):

SECTION E ADDITIONAL INFORMATION ON 510(K) SUBMISSIONS

Product codes of devices to which substantial equivalence is claimed								Summary of, or statement concerning, safety and effectiveness information <input checked="" type="checkbox"/> 510 (k) summary attached <input type="checkbox"/> 510 (k) statement
1	GMD	2		3		4		
5		6		7		8		

Information on devices to which substantial equivalence is claimed (if known)

	510(k) Number	Trade or Proprietary or Model Name	Manufacturer
1	K102286	1 CrAg LFA	1 Immuno-Mycologics, Inc.
2		2	2
3		3	3
4		4	4
5		5	5
6		6	6

SECTION F PRODUCT INFORMATION - APPLICATION TO ALL APPLICATIONS

Common or usual name or classification name
 Cryptococcal Antigen Lateral Flow Assay

	Trade or Proprietary or Model Name for This Device	Model Number
1	CrAg Lateral Flow Assay (LFA)	1 CR2003
2		2
3		3
4		4
5		5

FDA document numbers of all prior related submissions (regardless of outcome)

1	2	3	4	5	6
7	8	9	10	11	12

Data Included in Submission
 Laboratory Testing Animal Trials Human Trials

SECTION G PRODUCT CLASSIFICATION - APPLICATION TO ALL APPLICATIONS

Product Code GMD	C.F.R. Section (if applicable) 866.3165	Device Class <input type="checkbox"/> Class I <input checked="" type="checkbox"/> Class II <input type="checkbox"/> Class III <input type="checkbox"/> Unclassified
Classification Panel Microbiology		

Indications (from labeling)
 The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) in serum, (b)(4) Trade cerebral spinal fluid (CSF).
 The CrAg Lateral Flow Assay is a prescription-use laboratory assay, which can aid in the diagnosis of Cryptococcosis.

Note: Submission of this information does not affect the need to submit a 2891 or 2891a Device Establishment Registration form.

FDA Document Number (if known)

SECTION H MANUFACTURING / PACKAGING / STERILIZATION SITES RELATING TO A SUBMISSION

<input type="checkbox"/> Original <input type="checkbox"/> Add <input type="checkbox"/> Delete	Facility Establishment Identifier (FEI) Number	<input type="checkbox"/> Manufacturer <input type="checkbox"/> Contract Manufacturer	<input type="checkbox"/> Contract Sterilizer <input type="checkbox"/> Repackager / Relabeler
Company / Institution Name		Establishment Registration Number	
Division Name (if applicable)		Phone Number (including area code)	
Street Address		FAX Number (including area code)	
City		State / Province	ZIP Code Country
Contact Name	Contact Title	Contact E-mail Address	

<input type="checkbox"/> Original <input type="checkbox"/> Add <input type="checkbox"/> Delete	Facility Establishment Identifier (FEI) Number	<input type="checkbox"/> Manufacturer <input type="checkbox"/> Contract Manufacturer	<input type="checkbox"/> Contract Sterilizer <input type="checkbox"/> Repackager / Relabeler
Company / Institution Name		Establishment Registration Number	
Division Name (if applicable)		Phone Number (including area code)	
Street Address		FAX Number (including area code)	
City		State / Province	ZIP Code Country
Contact Name	Contact Title	Contact E-mail Address	

<input type="checkbox"/> Original <input type="checkbox"/> Add <input type="checkbox"/> Delete	Facility Establishment Identifier (FEI) Number	<input type="checkbox"/> Manufacturer <input type="checkbox"/> Contract Manufacturer	<input type="checkbox"/> Contract Sterilizer <input type="checkbox"/> Repackager / Relabeler
Company / Institution Name		Establishment Registration Number	
Division Name (if applicable)		Phone Number (including area code)	
Street Address		FAX Number (including area code)	
City		State / Province	ZIP Code Country
Contact Name	Contact Title	Contact E-mail Address	

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SECTION I

UTILIZATION OF STANDARDS

Note: Complete this section if your application or submission cites standards or includes a "Declaration of Conformity to a Recognized Standard" statement.

	Standards No.	Standards Organization	Standards Title	Version	Date
1	EP5-A2	CLSI	Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline	2nd Edition	07/15/2011
2	EP12-A2	CLSI	User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline	2nd Edition	07/15/2011
3	EP17-A	CLSI	Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline	1st Edition	07/15/2011
4					
5					
6					
7					

Please include any additional standards to be cited on a separate page.

Public reporting burden for this collection of information is estimated to average 0.5 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

Department of Health and Human Services
 Food and Drug Administration
 Office of the Chief Information Officer (HFA-710)
 5600 Fishers Lane
 Rockville, Maryland 20857

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

K112422



U.S. Food and Drug Administration
Center for Devices and Radiological Health (CDRH)
Document Mail Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, Maryland 20993-0002

~~Received~~

AUG 23 2011

K11

FDA CDRH DMC

Submission Date: August 22, 2011

Re: Cryptococcal Antigen Lateral Flow Assay (CrAg Lateral Flow Assay)

Please find enclosed our traditional 510(k) submission for the Cryptococcal Antigen Lateral Flow Assay (CrAg Lateral Flow Assay), which expands the intended use of K102286 to include **(b)(4) Trade Secret** cerebral spinal fluid (CSF).

Submission Type	Traditional 510(k) for a new device
Submitter	Immuno-Mycologics, Inc (Registration # 1627497; Owner Operator # 9916020)
Contact Person	Sean K. Bauman, Ph.D., President/CEO 2700 Technology Place Norman, OK 73071 Phone: (800) 654-3639 Fax: (405) 364-1058 Sean-Bauman@immy.com
Continued Confidentiality	Yes
Common Name	Cryptococcal Antigen Lateral Flow Assay
Trade Name	CrAg Lateral Flow Assay (CrAg LFA)
Class	II
Classification Regulation	866.3165
Review Panel	Microbiology
Predicate Device	CrAg Lateral Flow Assay (CrAg LFA) (K102286)
Special Controls	No applicable mandatory performance standards or special controls exist for this device.

Kind regards,

Sean K. Bauman, Ph.D.
President/CEO

Enclosures: CrAg Lateral Flow Assay 510(k) paper copy and electronic copy (CD)

www.immy.com



Toll Free: 800-654-3639 Fax:405-364-1058 2700 Technology Pl Norman, OK 73071 U.S.A.

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Indications for Use Statement

510(k) Number (if known): _____

Device Name: CrAg Lateral Flow Assay

Indications for Use:

The Cryptococcal Antigen Lateral Flow Assay (CrAg LFA) is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum, (b)(4) Trade Secret, and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis.

Prescription Use _____ AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k):

510(k) Summary CrAg Lateral Flow Assay

This 510(k) summary is submitted in accordance with 21 CFR §807.92

Owner: Immuno-Mycologies, Inc.
2700 Technology Place
Norman, OK 73071
Tel: 405-360-4669
Fax: 405-364-1058
Contact: Dr. Sean K. Bauman, President & CEO
Sean-Bauman@immy.com

Prepared: August 15, 2011

Trade Name: CrAg Lateral Flow Assay

Common Name: Cryptococcal Antigen Lateral Flow Immunoassay

Regulation: 866.3165

Predicate Device: Immuno-Mycologies' CrAg Lateral Flow Assay (K102286)

Intended Use: The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) in serum, (b)(4) Trade Secret, and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription-use laboratory assay, which can aid in the diagnosis of Cryptococcosis.

Device Description:

Explanation:

Detection of cryptococcal antigen in serum and CSF has been used for over forty years to aid in the diagnosis of cryptococcosis with very high sensitivity and specificity (9,14,15). Current guidelines for the management of cryptococcal disease partially base treatment recommendations on cryptococcal antigen presence and more specifically on cryptococcal antigen titers (16).

Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) (5,6,12,13). Individuals with impaired cell-mediated immune (CMI) function due to acquired immunodeficiency syndrome (AIDS) (19),

lymphoproliferative disorders (18), steroid therapy (8), and organ transplantation (7) are at increased risk of cryptococcosis. AIDS accounts for 80-90% of cryptococcal infections (11). The incidence of cryptococcosis in AIDS patients in the United States is estimated to be 5-10% (11), while the incidence of cryptococcosis in other parts of the world, such as Africa, is as high as 30% (3). Cryptococcosis is the fourth most common opportunistic, life-threatening infection among AIDS patients (10).

Description:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in serum, (b)(4) Trade, and CSF. For the qualitative procedure, specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For the semi-quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the qualitative procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold-conjugated, anti-cryptococcal monoclonal antibodies and gold-conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it binds to the gold-conjugated, anti-cryptococcal antibodies. The gold-labeled antibody-antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti-cryptococcal monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold-labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold-conjugated control goat IgG antibody to move to the Control Line (C) which is immobilized bovine anti-goat IgG antibody. The immobilized anti-goat antibody will bind to the gold-conjugated goat IgG Control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line (Figure 1). If the control line fails to develop a line, then the test is not valid.

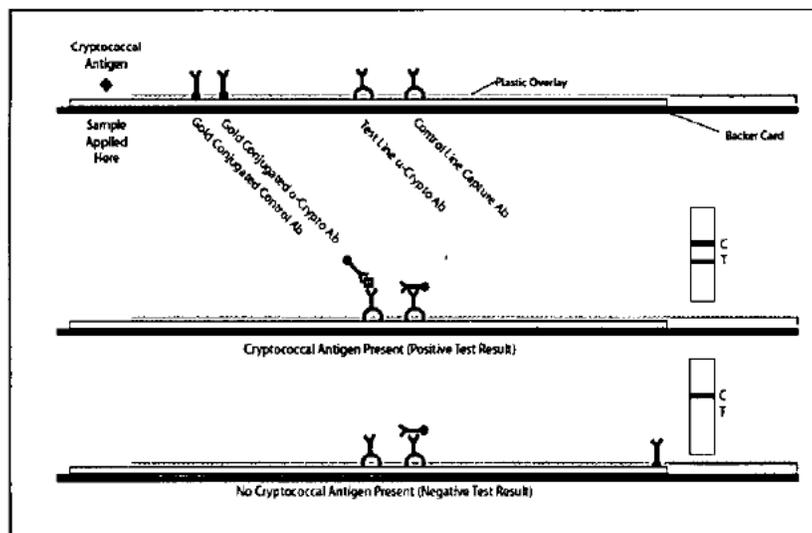


Figure 1. CrAg Lateral Flow Assay Schematic

Technological Characteristics Summary

A comparison between the CrAg LFA and the CrAg LFA (K102286 - Serum only) is presented in Table 1.

Table 1. Comparison with Predicate Device

SIMILARITIES		
Feature	CrAg LFA (New Device)	CrAg LFA (Serum Only) (K102286)
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum
Indication For Use	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis
Device Description		
Technology	Lateral Flow Assay	Lateral Flow Assay
Sample Matrix	Serum	Serum
Instruments	None	None
Assay Components	Specimen diluent, lateral flow strips, built-in control, gold conjugated antibodies	Positive control, negative control, latex cards, latex conjugated antibodies
Specimen Pre-Treatment	Dilution	Dilution
Detection Antibody	Anti-cryptococcal monoclonal antibody	Anti-cryptococcal monoclonal antibody
Storage Requirements	20-25°C	20-25°C
DIFFERENCES		
Feature	Cryptococcal Antigen Lateral Flow Assay	Latex- <i>Cryptococcus</i> Antigen Detection System
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum, (b)(4), and CSF	
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum, (b)(4), and CSF
Indication For Use	No differences	No differences

Performance Summary

A. Precision Studies (Repeatability & Reproducibility)

Repeatability and reproducibility with serum specimens were determined by spiking a serum specimen pool that was negative by the IMMY Latex-*Cryptococcus* Antigen Detection System with cryptococcal antigen at four concentrations: Negative, high negative (C₅), low positive (near C₉₅), and medium positive. The samples were analyzed on the CrAg Lateral Flow Assay in triplicate on five different days, at three different sites with a total of five different operators, on one lot, according to EP5-A2. One site was internal (Site 1) and the remaining two were a US reference laboratory (Site 2) and a US hospital laboratory (Site 3).

For repeatability, percent positive and percent negative detected were calculated for each site (Table 2). For reproducibility, overall percent positive and percent negative detected were calculated by combining the data from all three sites (last two rows of Table 2).

Table 2. Repeatability at 3 Different Sites

Sample	Serum							
	1		2		3		4	
	Med. Pos		Low Pos		High Neg		Neg	
Neg/Pos	-	+	-	+	-	+	-	+
Site 1	0	30	0	30	28	2	30	0
Percent %	0	100	0	100	93	7	100	0
Site 2	0	30	0	30	30	0	30	0
Percent %	0	100	0	100	100	0	100	0
Site 3	0	15	0	15	15	0	15	0
Percent %	0	100	0	100	100	0	100	0
Total No.	0	75	0	75	73	2	75	0
Percent %	0	100	0	100	97	3	100	0

B. Analytical Sensitivity (lower limits of the assay/analytical cut-off)

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running varying concentrations of cryptococcal antigen diluted in Lateral Flow (LF) Specimen Diluent, according to EP12-A2. The concentration where 50% of the results were positive and 50% of the results were negative determined our analytical cut-off. The analytical cut-off is 1.25ng/ml.

C. Analytical Specificity (cross-reactivity)

Analytical specificity for the CrAg Lateral Flow Assay was determined by running potentially cross-reacting medical conditions unrelated to cryptococcosis. A total of 118 serum specimens were tested in triplicate. Percent positive was determined for each condition (Table 3).

Table 3. Analytical Specificity

Pathology	# of Samples	% Positive
Penicilliosis	5	0 % (0/5)
Sporothrichosis	6	0 % (0/6)
HAMA	5	0 % (0/5)
Syphilis	10	0 % (0/10)
Rubella	5	0 % (0/5)
Mycoplasmosis	10	0 % (0/10)
Toxoplasmosis	7	0 % (0/7)
CMV	10	0 % (0/10)
Blastomycosis	10	0 % (0/10)
Coccidiomycosis	10	0 % (0/10)
Histoplasmosis	10	0 % (0/10)
Candidiasis	10	0 % (0/10)
Aspergillus GM+	10	10 % (1/10)

Rheumatoid Factor*	10	0 % (0/10)
--------------------	----	------------

* Rheumatoid factor concentrations tested ranged from 112IU/ml to 6479IU/ml.

Additionally, cross-reactivity was assessed by testing crude culture filtrate antigens at a range of concentrations using the CrAg Lateral Flow Assay. At high concentrations (>0.1 mg/ml), antigens from *Paracoccidioides brasiliensis* exhibited some cross-reactivity. Antigens from *Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus* did not exhibit cross-reactivity.

Interference testing was also performed on five icteric, five hemolyzed, and five lipemic serum specimens. Each specimen was spiked with cryptococcal antigen at three times the C95 concentration. All specimens were then tested at IMMY, on one lot of CrAg Lateral Flow assay in triplicate: spiked and unspiked. Percent positivity was determined for each condition. All of the unspiked specimens had negative results on the CrAg Lateral Flow Assay. All spiked specimens were positive, thus, these types of serum specimens do not interfere with the CrAg Lateral Flow Assay. However, it is possible that hemolyzed samples could lead to false negatives due to the high background color on the strip.

The effect of pronase on the CrAg LFA was determined by pronase-treating 5 Cryptococcal EIA positive specimens and 5 Cryptococcal EIA negative specimens. The samples were analyzed both untreated and pronase-treated. All treated, positives samples remained positive and all treated, negative samples remained negative. Therefore, pronase does not affect the CrAg LFA.

Due to specimen availability, the following conditions were not tested in the CrAg Lateral Flow Assay: *Candida dubliniensis*, *Candida tropicalis*, *Candida parapsidosis*, *Candida krusei*, *Candida glabrata*, *Cladosporium trichoides*, *Neisseria meningitidis*, *Salmonella typhi*, *Pneumocystis carinii*, *Trichosporon beigeli*, *Zygomycetes*, (b)(4) Trade Secret Process

ANA+, HAV, HCV, *Staph*, and *Strep*.

D. Linearity

N/A

E. High Dose Hook Effect

High dose hook effect concentrations with serum specimens were determined by spiking negative serum that was negative by the IMMY Latex-*Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at various concentrations between 20 and 500ug/ml. Each concentration was tested in triplicate at IMMY on one lot of CrAg Lateral Flow Assay, according to the package insert. It was determined that serum specimens with a cryptococcal antigen concentration higher than 200ug/ml can produce a high dose hook effect and therefore may produce a false negative result.

F. Method Comparisons

Predicate Device Method Comparison

Not Applicable

Other Method Comparison – Culture/India Ink (Gold Standards)

The CrAg Lateral Flow Assay was compared to the gold standard for the diagnosis of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay in (b)(4) Trade Secret Process, and CSF. These studies contained a mix of both prospective and retrospective specimens. A summary of the data collected is included in Tables 4-9 below:

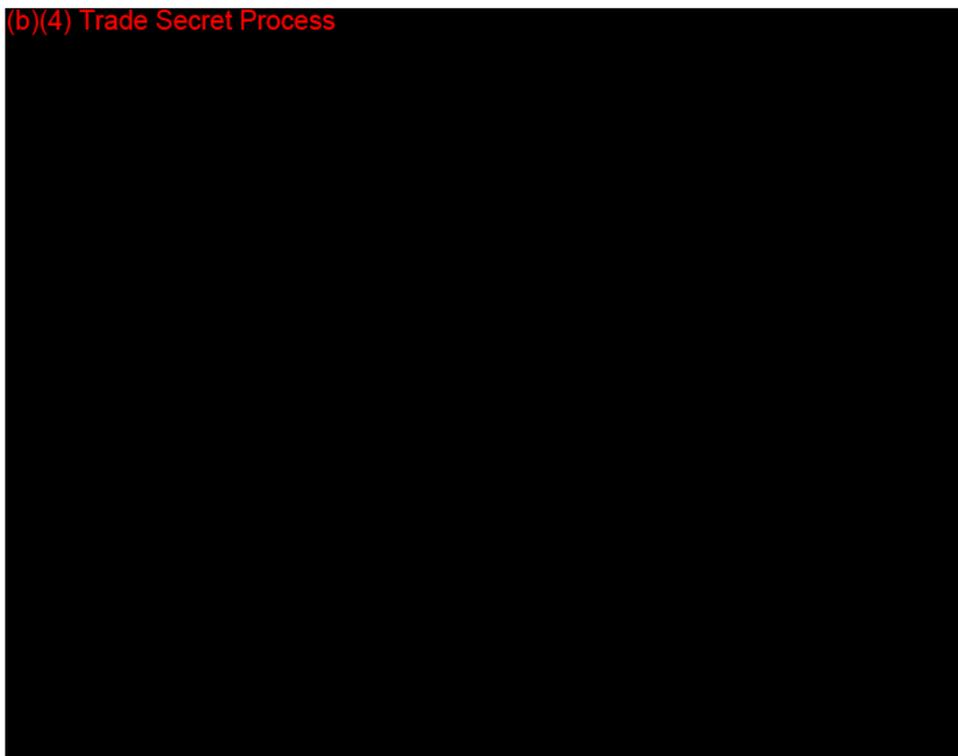


Table 8. CSF 2x2 Contingency Table: Culture/India Ink

		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	65	0
	Negative	0	99

Table 9. CSF Statistical Analysis: Culture/India Ink

	Calculated	95% CI
Sensitivity	100%	94.4-100.0%
Specificity	100%	96.3-100%

Conclusion

The information submitted in this premarket notification is complete and supports a substantial equivalence decision.

Premarket Notification Truthful And Accurate Statement

[As Required by 21 CFR 807.87(k)]

I certify that, in my capacity as President and CEO of Immuno-Mycologics, Inc., I believe to the best of my knowledge, that all data and information submitted in the premarket notification are truthful and accurate and that no material fact has been omitted.


(Signature)

Sean K. Bauman
(Typed Name)

8/22/2011
(Date)

*(Premarket Notification [510(k)] Number)

*For a new submission, leave the 510(k) number blank.

Must be signed by a responsible person of the firm required to submit the premarket notification [e.g., not a consultant for the 510(k) submitter].

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Section 07 Class III Summary and Certification

N/A

CERTIFICATION: FINANCIAL INTERESTS AND ARRANGEMENTS OF CLINICAL INVESTIGATORS

TO BE COMPLETED BY APPLICANT

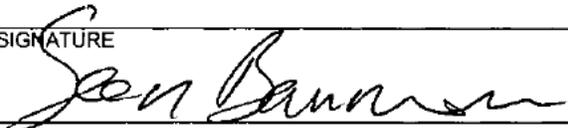
With respect to all covered clinical studies (or specific clinical studies listed below (if appropriate)) submitted in support of this application, I certify to one of the statements below as appropriate. I understand that this certification is made in compliance with 21 CFR part 54 and that for the purposes of this statement, a clinical investigator includes the spouse and each dependent child of the investigator as defined in 21 CFR 54.2(d).

Please mark the applicable checkbox.

- (1) As the sponsor of the submitted studies, I certify that I have not entered into any financial arrangement with the listed clinical investigators (enter names of clinical investigators below or attach list of names to this form) whereby the value of compensation to the investigator could be affected by the outcome of the study as defined in 21 CFR 54.2(a). I also certify that each listed clinical investigator required to disclose to the sponsor whether the investigator had a proprietary interest in this product or a significant equity in the sponsor as defined in 21 CFR 54.2(b) did not disclose any such interests. I further certify that no listed investigator was the recipient of significant payments of other sorts as defined in 21 CFR 54.2(f).

Clinical Investigators	(b) (6)	(b) (6)
	(b) (6)	
	(b) (6)	

- (2) As the applicant who is submitting a study or studies sponsored by a firm or party other than the applicant, I certify that based on information obtained from the sponsor or from participating clinical investigators, the listed clinical investigators (attach list of names to this form) did not participate in any financial arrangement with the sponsor of a covered study whereby the value of compensation to the investigator for conducting the study could be affected by the outcome of the study (as defined in 21 CFR 54.2(a)); had no proprietary interest in this product or significant equity interest in the sponsor of the covered study (as defined in 21 CFR 54.2(b)); and was not the recipient of significant payments of other sorts (as defined in 21 CFR 54.2(f)).
- (3) As the applicant who is submitting a study or studies sponsored by a firm or party other than the applicant, I certify that I have acted with due diligence to obtain from the listed clinical investigators (attach list of names) or from the sponsor the information required under 54.4 and it was not possible to do so. The reason why this information could not be obtained is attached.

NAME Sean K. Bauman, Ph.D.	TITLE President and CEO
FIRM/ORGANIZATION Immuno-Mycologics, Inc. (IMMY)	
SIGNATURE 	DATE (mm/dd/yyyy) 08/22/2011

Paperwork Reduction Act Statement

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. Public reporting burden for this collection of information is estimated to average 1 hour per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the necessary data, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information to the address to the right:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
1350 Piccard Drive, 420A
Rockville, MD 20850

Section 09 Declarations of Conformity and Summary Reports

N/A

Executive Summary

Trade Name: CrAg Lateral Flow Assay

Common Name: Cryptococcal Antigen Lateral Flow Assay

Regulation: 866.3165

Predicate Device: CrAg Lateral Flow Assay (K102286)

Intended Use: The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) in serum, (b)(4) Trade S t, and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription-use laboratory assay, which can aid in the diagnosis of Cryptococcosis.

Device Description:

Explanation:

Detection of cryptococcal antigen in serum and CSF has been used for over forty years to aid in the diagnosis of cryptococcosis with very high sensitivity and specificity (6,11,12). Current guidelines for the management of cryptococcal disease partially base treatment recommendations on cryptococcal antigen presence and more specifically on cryptococcal antigen titers (13).

Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) (2,3,9,10). Individuals with impaired cell-mediated immune (CMI) function due to acquired immunodeficiency syndrome (AIDS) (15), lymphoproliferative disorders (14), steroid therapy (5), and organ transplantation (4) are at increased risk of Cryptococcosis. AIDS accounts for 80-90% of Cryptococcal infections (8). The incidence of cryptococcosis in AIDS patients in the United States is estimated to be 5-10% (8) while the incidence of Cryptococcosis in other parts of the world, such as Africa, is as high as 30% (1). Cryptococcosis is the fourth most common opportunistic, life-threatening infection among AIDS patients (7).

Description:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in serum, (b)(4) Trade S t, and CSF. For qualitative procedure,

specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For semi-quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the screening procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold-conjugated, anti-cryptococcal antigen antibodies and gold-conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it picks up and binds to the gold-conjugated, anti-cryptococcal antigen antibodies. The gold-labeled antibody-antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti-cryptococcal antigen monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold-labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold-conjugated control goat IgG antibody to move to the Control Line (C) which is immobilized bovine anti-goat IgG antibody. The immobilized anti-goat antibody will bind to the gold-conjugated goat IgG Control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line (Figure 1). If the control line fails to develop a line, then the test is not valid.

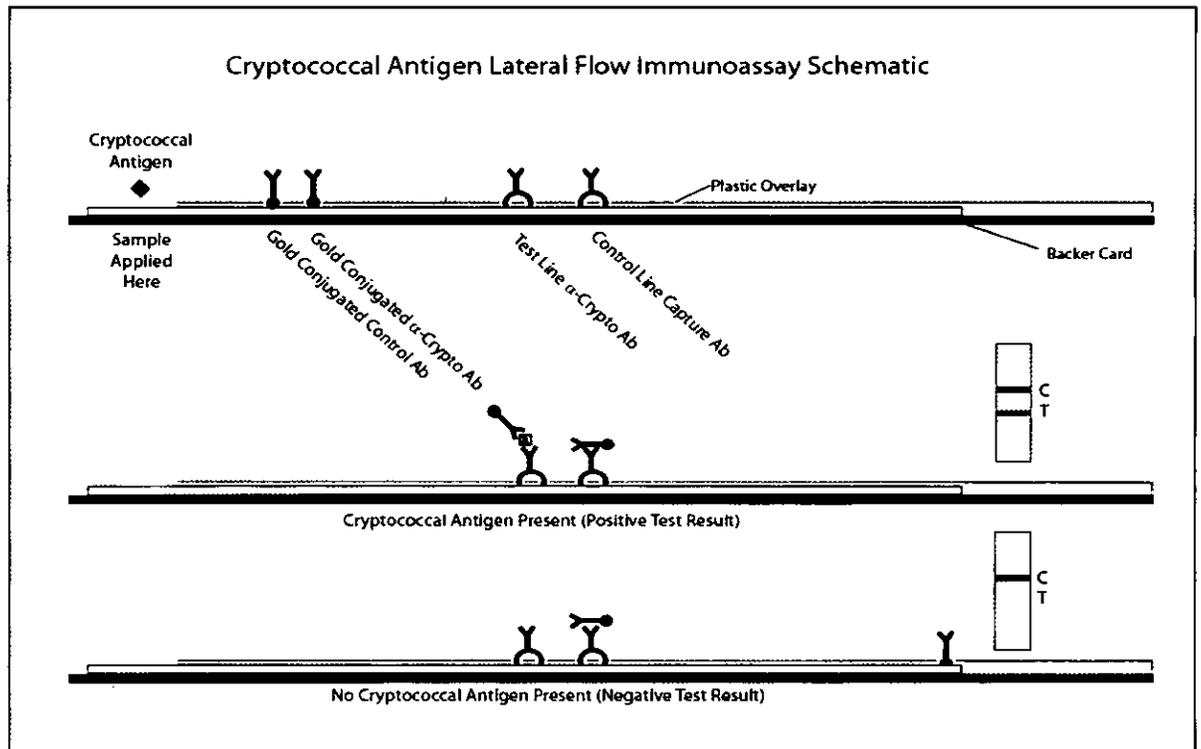


Figure 1. Cryptococcal Antigen Lateral Flow Immunoassay Schematic

Technological Characteristics Summary

A comparison between the Cryptococcal Antigen Lateral Flow Immunoassay and the Latex-*Cryptococcus* Antigen Detection System is presented in Table 1.

Table 1. Comparison between Cryptococcal Antigen LFI and *Cryptococcus* Antigen Detection System

SIMILARITIES		
Feature	CrAg LFA	CrAg LFA (Serum Only) (K102286)
Intended Use		
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum
Indication For Use	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis
Device Description		
Technology	Lateral Flow Assay	Lateral Flow Assay
Sample Matrix	Serum	Serum
Instruments	None	None
Assay Components	Specimen diluent, lateral flow strips, built-in control, gold conjugated antibodies	Positive control, negative control, (b)(4) conjugated antibodies
Specimen Pre-Treatment	Dilution	Dilution
Detection Antibody	Anti-cryptococcal monoclonal antibody	Anti-cryptococcal monoclonal antibody
Storage Requirements	20-25°C	20-25°C
DIFFERENCES		
Feature	Cryptococcal Antigen Lateral Flow Assay	Latex- <i>Cryptococcus</i> Antigen Detection System
Intended Use		
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum, (b)(4), and CSF
Indication For Use	No differences	No differences

This comparison supports substantial equivalence in many ways. The only difference between the two devices is the sample matrix.

Performance Summary

A. Precision Studies (Repeatability & Reproducibility)

Repeatability and reproducibility with serum specimens were determined by spiking a serum specimen pool that was negative by the IMMY Latex-*Cryptococcus* Antigen Detection System with cryptococcal antigen at four concentrations: Negative, high negative (C₅), low positive (near C₉₅), and medium positive. The samples were analyzed on the CrAg Lateral Flow Assay in triplicate on five different days, at three different sites with a total of five different operators, on one lot, according to EP5-A2. One site was internal (Site 1) and the remaining two were a US reference laboratory (Site 2) and a US hospital laboratory (Site 3). For repeatability, percent positive and percent negative detected were calculated for each site (Table 2). For reproducibility, overall percent positive and percent negative detected were calculated by combining the data from all three sites (last two rows of Table 2).

Table 2. Repeatability at 3 Different Sites

Sample	Serum							
	1		2		3		4	
	Med. Pos	Low Pos	High Neg	Neg				
Neg/Pos	-	+	-	+	-	+	-	+
Site 1	0	30	0	30	28	2	30	0
Percent %	0	100	0	100	93	7	100	0
Site 2	0	30	0	30	30	0	30	0
Percent %	0	100	0	100	100	0	100	0
Site 3	0	15	0	15	15	0	15	0
Percent %	0	100	0	100	100	0	100	0
Total No.	0	75	0	75	73	2	75	0
Percent %	0	100	0	100	97	3	100	0

As expected, the high negative (C₅) gave a positive result nearly 5% of the time - 3% in serum. All other samples performed 100% as expected across all sites, all runs, and all operators.

B. Analytical Sensitivity (lower limits of the assay/analytical cut-off)

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running varying concentrations of cryptococcal antigen diluted in Lateral Flow (LF) Specimen Diluent, according to EP12-A2. The concentration where 50% of the results were positive and 50% of the results were negative determined our analytical cut-off. The analytical cut-off is 1.25ng/ml (Figure 2 and Table 3).

(b)(4) Trade Secret Process

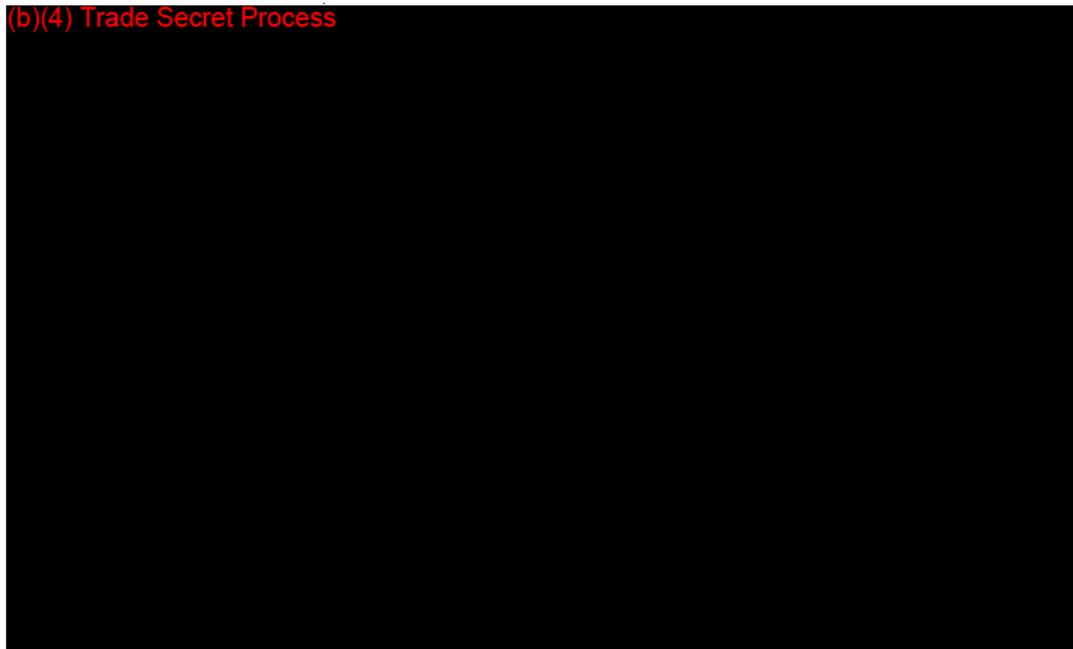


Table 3. Analytical Cut-off Analysis

Sample Concentration (ng/ml)	No. Positive	No. Tested	% Positive
(b)(4) Trade Secret Process			

C. Analytical Specificity/Interference

Analytical Specificity

Analytical specificity for the CrAg Lateral Flow Assay was determined by running potentially cross-reacting medical conditions unrelated to cryptococcosis. A total of 118 serum specimens were tested in triplicate. Percent positive was determined for each condition (Table 4).

Table 4. Analytical Specificity

Pathology	# of Samples	% Positive
Penicilliosis	5	0% (0/5)
Sporothrichosis	6	0% (0/6)
HAMA	5	0% (0/5)

Syphilis	10	0 % (0/10)
Rubella	5	0 % (0/5)
Mycoplasmosis	10	0 % (0/10)
Toxoplasmosis	7	0 % (0/7)
CMV	10	0 % (0/10)
Blastomycosis	10	0 % (0/10)
Coccidiomycosis	10	0 % (0/10)
Histoplasmosis	10	0 % (0/10)
Candidiasis	10	0 % (0/10)
Aspergillus GM+	10	10 % (1/10)
Rheumatoid Factor*	10	0 % (0/10)

* Rheumatoid factor concentrations tested ranged from 112IU/ml to 6479IU/ml.

Additionally, cross-reactivity was assessed by testing crude culture filtrate antigens at a range of concentrations using the CrAg Lateral Flow Assay. At high concentrations (>0.1 mg/ml), antigens from *Paracoccidioides brasiliensis* exhibited some cross-reactivity. Antigens from *Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus* did not exhibit cross-reactivity.

Interference

In addition to the cross-reactivity study, interference testing was also performed on five icteric, five hemolyzed, and five lipemic serum specimens. Each specimen was spiked with cryptococcal antigen at three times the C95 concentration. All specimens were then tested at IMMY, on one lot of CrAg Lateral Flow assay in triplicate: spiked and unspiked. Percent positivity was determined for each condition. All of the unspiked specimens had negative results on the CrAg Lateral Flow Assay. All spiked specimens were positive, thus, these types of serum specimens do not interfere with the CrAg Lateral Flow Assay. However, it is possible that hemolyzed samples could lead to false negatives due to the high background color on the strip. As such, a statement is included in the Limitations of the Procedure section of the package insert:

"Hemolyzed serum samples could lead to false negative results due to the high background color on the strip."

The effect of pronase on the CrAg LFA was determined by pronase-treating 5 Cryptococcal EIA positive specimens and 5 Cryptococcal EIA negative specimens. The samples were analyzed both untreated and pronase-treated. All treated, positives samples remained positive and all treated, negative samples remained negative. Therefore, pronase does not affect the CrAg LFA.

Due to specimen availability, the following conditions were not tested in the CrAg Lateral Flow Assay: *Candida dubliniensis*, *Candida tropicalis*, *Candida parapsidosis*, *Candida krusei*, *Candida glabrata*, *Cladosporium trichoides*, *Neisseria meningitidis*, *Salmonella typhi*, *Pneumocystis carinii*, *Trichosporon beigeli*, *Zygomycetes*, (b)(4) Trade Secret Process

ANA+, HAV, HCV, *Staph*, and *Strep*. Therefore, the package insert for

the CrAg Lateral Flow Assay will include a limitations statement regarding these organisms and substances.

D. Linearity

N/A

E. High Dose Hook Effect

High dose hook effect concentrations with serum specimens were determined by spiking negative serum that were negative by the IMMY Latex-*Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at various concentrations between 20 and 500ug/ml. Each concentration was tested in triplicate at IMMY on one lot of CrAg Lateral Flow Assay, according to the package insert. It was determined that serum specimens with a cryptococcal antigen concentration higher than 200ug/ml can produce a high dose hook effect and therefore may produce a false negative result.

F. Method Comparison

Predicate Device Method Comparison

Not Applicable

Other Method Comparison – Culture/India Ink (Gold Standards)

The CrAg Lateral Flow Assay was compared to the gold standard diagnosis of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay in (b)(4) [redacted] CSF. These studies contained a mix of both prospective and retrospective specimens. A summary of the data collected is included in Tables 5-10 below: Trade Secret

(b)(4) Trade Secret Process



(b)(4) Trade Secret Process



Table 9. CSF 2x2 Contingency Table: Culture/India Ink

		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	65	0
	Negative	0	99

Table 10. CSF Statistical Analysis: Culture/India Ink

	Calculated	95% CI
Sensitivity	100%	94.4-100.0%
Specificity	100%	96.3-100%

Summary and Conclusion

The information submitted in this premarket notification is complete and supports a substantial equivalence decision.

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1. **Clumeck, N., J. Sonnet, H. Taelman, F. Mascart-Lemone, B. M. De, P. Vandepierre, J. Dasnoy, L. Marcelis, M. Lamy, C. Jonas, and .** 1984. Acquired immunodeficiency syndrome in African patients. *N. Engl. J. Med.* **310**:492-497.
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11. **Patterson, T. F. and V. T. Andriole.** 1989. Current concepts in cryptococcosis. *Eur. J. Clin. Microbiol. Infect. Dis.* **8**:457-465.
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15. **Zuger, A., E. Louie, R. S. Holzman, M. S. Simberkoff, and J. J. Rahal.** 1986. Cryptococcal disease in patients with the acquired immunodeficiency syndrome. Diagnostic features and outcome of treatment. *Ann. Intern. Med.* **104**:234-240.

510(k) Device Description CrAg Lateral Flow Assay

Description and Performance Specifications:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in serum, (b)(4) Trade, and cerebral spinal fluid (CSF). For the qualitative procedure, specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For the semi-quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the qualitative procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold-conjugated, anti-cryptococcal monoclonal antibodies and gold-conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it binds to the gold-conjugated, anti-cryptococcal antibodies. The gold-labeled antibody-antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti-cryptococcal monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold-labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold-conjugated control goat IgG antibody to move to the Control Line (C) which is immobilized bovine anti-goat IgG antibody. The immobilized anti-goat antibody will bind to the gold-conjugated goat IgG Control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line (Figure 1). If the control line fails to develop a line, then the test is not valid.

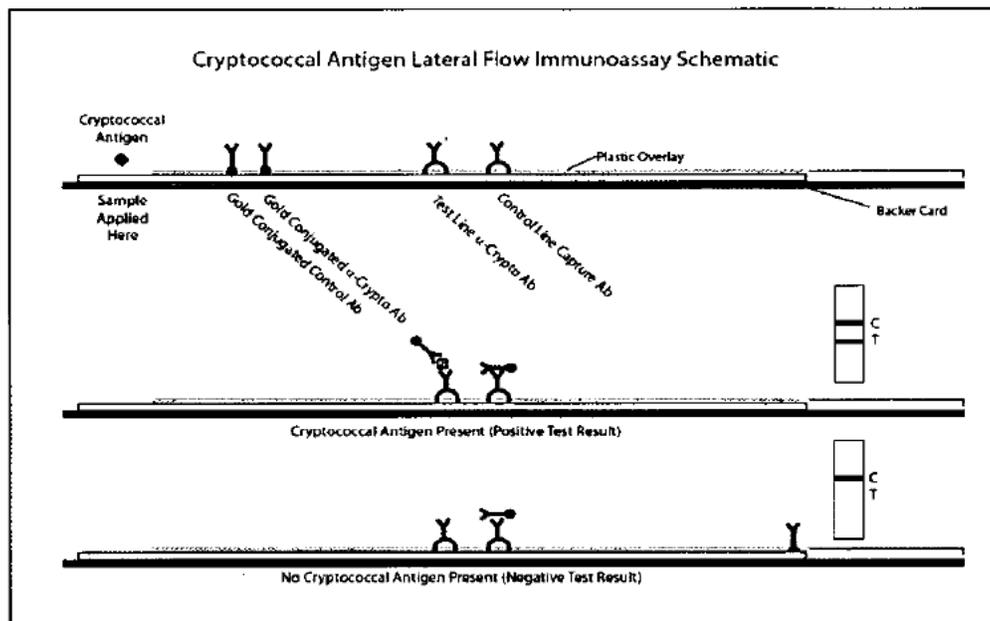


Figure 1. CrAg Lateral Flow Assay Schematic

The kit components and specifications are described in Table 1.

Table 1. CrAg Lateral Flow Assay Components

Kit Component	Component		Specification
	Reference Number	Volume/Kit	
LF Specimen Diluent	GLF070	2.5 ml	Creates a negative result on the CrAg LF Test Strips
CrAg LF Test Strips	LFCR01	50 strips	1 line with negative control and 2 lines with <i>Cryptococcus</i> -positive specimens

510(k) Substantial Equivalence Determination Decision Summary CrAg Lateral Flow Assay

A. 510(k) Number

B. Purpose for Submission

To expand the Intended Use Statement to include (b)(4) Trade Secret, and cerebral spinal fluid (CSF) specimens.

C. Measurand

Capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*)

D. Type of Test

Qualitative and semi-quantitative dipstick sandwich lateral flow immunochromatographic assay

E. Applicant

Immuno-Mycologies, Inc.
2700 Technology Place
Norman, OK 73071
Tel. 405.360.4669
Fax. 405.364.1058

F. Proprietary and Established Names:

CrAg Lateral Flow Assay

G. Regulatory Information

a. Regulation Section:

866.3165

b. Classification

Class II

c. Product Code

GMD

d. Panel

Microbiology

H. Intended Use

a. Intended Use

The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum, (b)(4) Trade, and cerebral spinal fluid (CSF).

b. Indication for Use

The CrAg Lateral Flow Assay is a prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis.

I. Device Description

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay which detects cryptococcal antigen in serum, (b)(4) Trade, and cerebral spinal fluid (CSF). The assay consists of CrAg Lateral Flow test strips which have a gold-conjugated antibody and a gold-conjugated, anti-cryptococcal antibody deposited onto a sample membrane and anti-Crypto antibody and control-line capture antibody striped onto a membrane. Also in the kit is a specimen diluent.

J. Substantial Equivalence Information

a. Predicate Device Name

Immuno-Mycologics' CrAg Lateral Flow Assay (serum only)

b. Predicate 510(k) Number

K102286

c. Comparison with predicate

Table 1: Comparison Between New Device and Predicate Device

SIMILARITIES		
Feature	CrAg LFA (New Device)	CrAg LFA (Serum Only) (K102286)
Intended Use	Intended Use	
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum
Indication For Use	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis
Device Description	Device Description	
Technology	Lateral Flow Assay	Lateral Flow Assay
Sample Matrix	Serum	Serum
Instruments	None	None

Assay Components	Specimen diluent, lateral flow strips, built-in control, gold conjugated antibodies	Positive control, negative control, latex cards, latex conjugated antibodies
Specimen Pre-Treatment	Dilution	Dilution
Detection Antibody	Anti-cryptococcal monoclonal antibody	Anti-cryptococcal monoclonal antibody
Storage Requirements	20-25°C	20-25°C
DIFFERENCES		
Feature	Cryptococcal Antigen Lateral Flow Assay	Latex-Cryptococcus Antigen Detection System
Intended Use		
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum, urine, plasma, and CSF
Indication For Use	No differences	No differences

K. Standard/Guidance Document Referenced (if applicable)

Not Applicable

Predicate: Not Applicable

L. Test Principle

Immunochromatographic Assay

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in serum, (b)(4) Trade, and CSF. For the qualitative procedure, specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For the semi-quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the qualitative procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold-conjugated, anti-cryptococcal monoclonal antibodies and gold-conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it binds to the gold-conjugated, anti-cryptococcal antibodies. The gold-labeled antibody-antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti-cryptococcal monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold-labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold-conjugated control goat IgG antibody to move to the

Control Line (C) which is immobilized bovine anti-goat IgG antibody. The immobilized anti-goat antibody will bind to the gold-conjugated goat IgG Control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line. If the control line fails to develop a line, then the test is not valid.

Predicate: Same

M. Performance Characteristics

a. Analytical Performance

i. Precision

Repeatability and reproducibility with serum specimens were determined by spiking a serum specimen pool that was negative by the IMMY Latex-*Cryptococcus* Antigen Detection System with cryptococcal antigen at four concentrations: Negative, high negative (C₅), low positive (near C₉₅), and medium positive. The samples were analyzed on the CrAg Lateral Flow Assay in triplicate on five different days, at three different sites with a total of five different operators, on one lot, according to EP5-A2. One site was internal (Site 1) and the remaining two were a US reference laboratory (Site 2) and a US hospital laboratory (Site 3). For repeatability, percent positive and percent negative detected were calculated for each site (Table 2). For reproducibility, overall percent positive and percent negative detected were calculated by combining the data from all three sites (last two rows of Table 2).

Table 2. Repeatability at 3 Different Sites

Sample	Serum							
	1		2		3		4	
	Med. Pos	Low Pos	High Neg	Neg				
Neg/Pos	-	+	-	+	-	+	-	+
Site 1	0	30	0	30	28	2	30	0
Percent %	0	100	0	100	93	7	100	0
Site 2	0	30	0	30	30	0	30	0
Percent %	0	100	0	100	100	0	100	0
Site 3	0	15	0	15	15	0	15	0
Percent %	0	100	0	100	100	0	100	0
Total No.	0	75	0	75	73	2	75	0
Percent %	0	100	0	100	97	3	100	0

As expected, the high negative tested positive nearly 5% of the time - 3% of the time in serum. All other samples performed 100% as expected across all sites, all runs, and all operators.

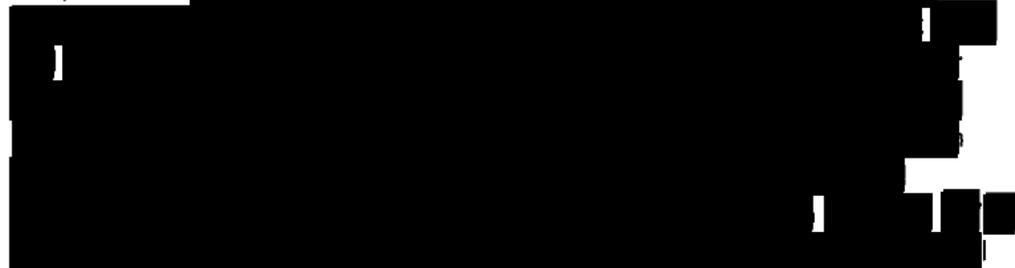
ii. Linearity

N/A

Predicate: N/A

iii. Traceability/Stability/Expected Values (controls, calibrators, or methods)

The CrAg Lateral Flow Assay has a two-year expiration date when stored at room temperature. (b)(4) Trade Secret Process



indicate that the CrAg Lateral Flow Assay is stable at room temperature for two years.

Predicate: Same

iv. Detection Limit/Analytical Cut-off

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running varying concentrations of cryptococcal antigen diluted in LF Specimen Diluent. Test results determined that the analytical cut-off is 1.25ng/ml (Table 3).

Table 3. Analytical Cut-Off

Sample Concentration (ng/ml)	No. Positive	No. Tested	% Positive
0.50	0	24	0%
0.75	0	24	0%
1.00	4	24	17%
1.25	12	24	50%
1.50	21	24	88%
1.75	24	24	100%
2.00	24	24	100%
2.50	24	24	100%
3.00	24	24	100%
3.50	24	24	100%
4.00	24	24	100%

v. Analytical Specificity/Interference

Analytical Specificity

Analytical Specificity for the CrAg Lateral Flow Assay was determined by running potentially interfering medical conditions unrelated to cryptococcosis.

Table 4. Analytical Specificity

Pathology	# of Samples	% Positive
Penicilliosis	5	0 % (0/5)
Sporothrichosis	6	0 % (0/6)
HAMA	5	0 % (0/5)
Syphilis	10	0 % (0/10)
Rubella	5	0 % (0/5)
Mycoplasmosis	10	0 % (0/10)
Toxoplasmosis	7	0 % (0/7)
CMV	10	0 % (0/10)
Blastomycosis	10	0 % (0/10)
Coccidiomycosis	10	0 % (0/10)
Histoplasmosis	10	0 % (0/10)
Candidiasis	10	0 % (0/10)
Aspergillus GM+	10	10 % (1/10)
Rheumatoid Factor*	10	0 % (0/10)

* Rheumatoid factor concentrations tested ranged from 112IU/ml to 6479IU/mls.

Additionally, cross-reactivity was assessed by testing crude culture filtrate antigens at a range of concentrations using the CrAg Lateral Flow Assay. At high concentrations (>0.1 mg/ml), antigens from *Paracoccidioides brasiliensis* exhibited some cross-reactivity. Antigens from *Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus* did not exhibit cross-reactivity.

In addition to the cross-reactivity study, interference testing was also performed on five icteric, five hemolyzed, and five lipemic serum specimens. Each specimen was spiked with cryptococcal antigen at three times the C95 concentration. All specimens were then tested at IMMY, on one lot of CrAg Lateral Flow assay in triplicate: spiked and unspiked. Percent positivity was determined for each condition. All of the unspiked specimens had negative results on the CrAg Lateral Flow Assay. All spiked specimens were positive, thus, these types of serum specimens do not interfere with the CrAg Lateral Flow Assay. However, it is possible that hemolyzed samples could lead to false negatives due to the high background color on the strip. As such, a statement is included in the Limitations of the Procedure section of the package insert:

“Hemolyzed serum samples could lead to false negative results due to the high background color on the strip.”

The effect of pronase on the CrAg LFA was determined by pronase-treating 5 Meridian EIA positive specimens and 5 Meridian EIA negative specimens. The samples were analyzed both untreated and pronase-treated. All treated, positives samples remained positive and all treated, negative samples remained negative. Therefore, pronase does not affect the CrAg LFA.

Due to specimen availability, the following conditions were not tested in the CrAg Lateral Flow Assay: *Candida dubliniensis*, *Candida tropicalis*, *Candida parapsidosis*, *Candida krusei*, *Candida glabrata*, *Cladosporium trichoides*, *Neisseria meningitidis*, *Salmonella typhi*, *Pneumocystis carinii*, *Trichosporon beigeli*, *Zygomycetes*, (b)(4) Trade Secret Process

ANA+, HAV, HCV, *Staph*, and *Strep*.

vi. High Dose Hook Effect

High dose hook effect concentrations with serum specimens were determined by spiking negative serum that were negative by the IMMY Latex-*Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at various concentrations between 20 and 500ug/ml. Each concentration was tested in triplicate at IMMY on one lot of CrAg Lateral Flow Assay, according to the package insert. It was determined that serum specimens with a cryptococcal antigen concentration higher than 200ug/ml can produce a high dose hook effect and therefore may produce a false negative result.

b. Comparison Studies

Predicate Device Method Comparison

Not Applicable

Other Method Comparison – Culture/India Ink (Gold Standards)

The CrAg Lateral Flow Assay was compared to the gold standard diagnosis of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay in (b)(4) and CSF. These studies contained a mix of both prospective and retrospective specimens. A summary of the data collected is included in Tables 5-10 below:

(b)(4) Trade Secret Process

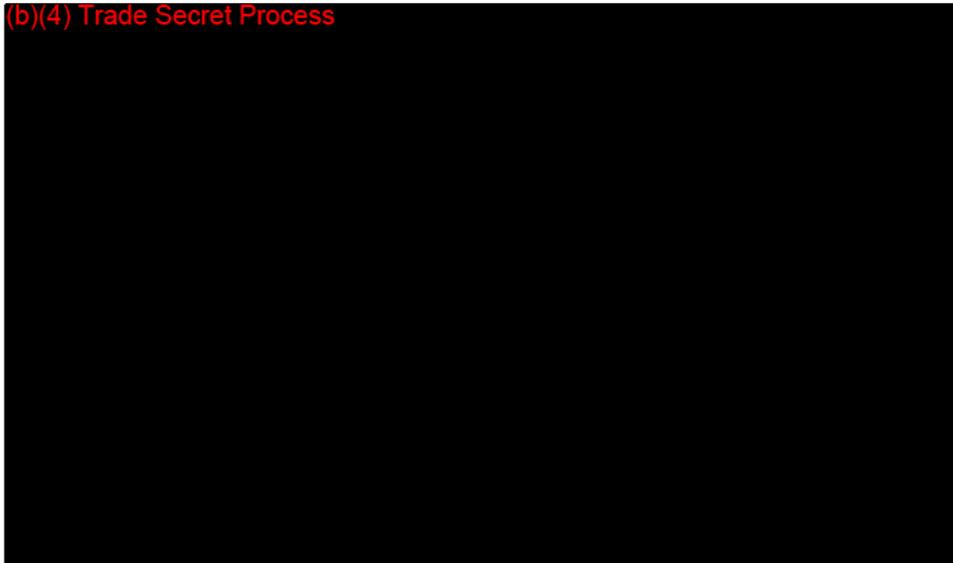


Table 9. CSF 2x2 Contingency Table: Culture/India Ink

		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	65	0
	Negative	0	99

Table 10. CSF Statistical Analysis: Culture/India Ink

	Calculated	95% CI
Sensitivity	100%	94.4-100.0%
Specificity	100%	96.3-100%

Predicate Device:

Table 11. Serum 2x2 Contingency Table: Culture/India Ink

		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	91	0
	Negative	0	123

Table 12. Serum Statistical Analysis: Culture/India Ink

	Calculated	95% CI
Sensitivity	100%	96.0%-100%
Specificity	100%	97.0%-100%

c. Clinical Studies
 Not Applicable

d. Clinical Cut-Off
 Not Applicable

e. Expected Values/Reference Range
Not Applicable

N. Proposed Labeling

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusions

The submitted information in the premarket notification is complete and supports a substantial equivalence decision.

Section 13 Proposed Labeling

I. Kit Label:

CrAg Lateral Flow Assay

For the Detection of Cryptococcal Antigen

IVD 20C-25C

REF CR2003 50 Tests

LOT SAMPLE 2011-06

Kit Contents		
REF	Description	Qty
LFCR01	CrAg Lateral Flow Test Strips	50 strips
GLF070	LF Specimen Diluent	2.5 ml
PKGINS	Package Insert	N/A

Immuno-Mycologics, Inc. - 2700 Technology PI - Norman OK 73071 USA - (800)654-3639

1

ADD 1 DROP LF SPECIMEN
DILUENT TO TUBE

2

ADD 40 µL PATIENT SPECIMEN
TO TUBE

3

INSERT STRIP AS SHOWN

4

WAIT 10 MINUTES

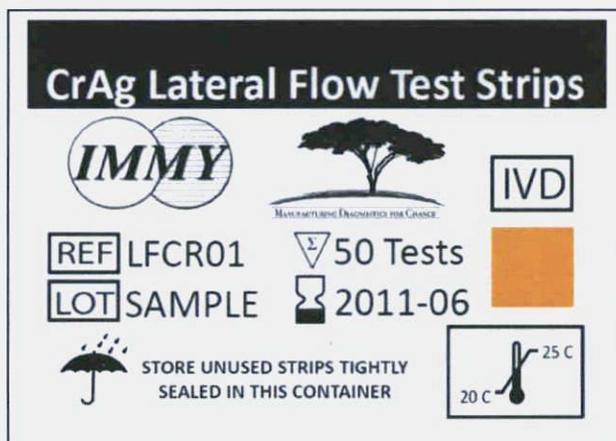
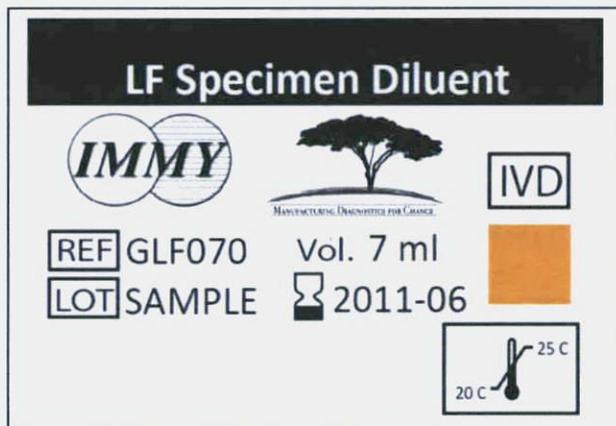
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CONTROL TEST CONTROL

POSITIVE NEGATIVE

Section 13 Proposed Labeling

II. Kits Components' Labels



III. Package Insert (see next 2 pages)

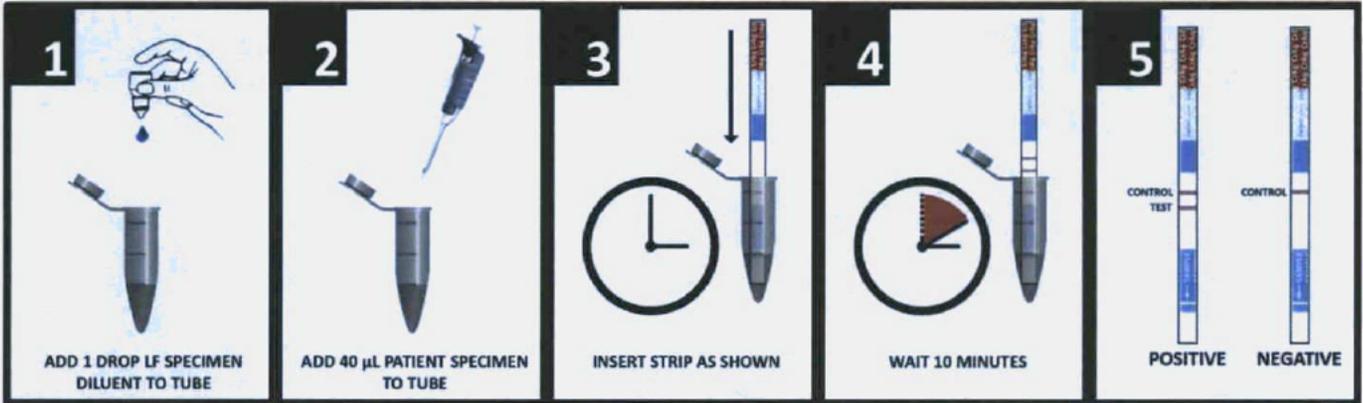


CrAg Lateral Flow Assay

For the Detection of Cryptococcal Antigen - REF CR2003



QUALITATIVE – BASIC PROCEDURE



INTENDED USE

The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of the capsular polysaccharide antigens of *Cryptococcus neoformans* and *Cryptococcus gattii* complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum, (b)(4) and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription-use laboratory assay which can aid in the diagnosis of cryptococcosis.

SUMMARY and EXPLANATION of the Test

Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) (4). Individuals with impaired cell-mediated immunity are at greatest risk of infection (6). Cryptococcosis is one of the most common opportunistic infections in AIDS patients (5). Detection of cryptococcal antigen (CrAg) in serum and CSF has been extensively utilized with very high sensitivity and specificity (1-3).

BIOLOGICAL PRINCIPLES

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay. Specimens and specimen diluent are added into an appropriate reservoir, such as a test tube, and the lateral flow device is placed into the reservoir. The test uses specimen wicking to capture gold-conjugated, anti-CrAg monoclonal antibodies and gold-conjugated control antibodies deposited on the test membrane. If CrAg is present in the specimen, then it binds to the gold-conjugated, anti-CrAg antibodies. The gold-labeled antibody-antigen complex continues to wick up the membrane where it will interact with the test line, which has immobilized anti-CrAg monoclonal antibodies. The gold-labeled antibody-antigen complex forms a sandwich at the test line causing a visible line to form. With proper flow and reagent reactivity, the wicking of any specimen, positive or negative, will cause the gold-conjugated control antibody to move to the control line. Immobilized antibodies at the control line will bind to the gold-conjugated control antibody and form a visible control line. Positive test results create two lines (test and control). Negative test results form only one line (control). If a control line fails to develop then the test is not valid.

WARNINGS and PRECAUTIONS

For In Vitro Diagnostic Use only.

REAGENT PRECAUTIONS

1. Specific standardization is necessary to produce our high-quality reagents and materials. The user assumes full responsibility for any modification to the procedures published herein.

2. When handling patient specimens, adequate measures should be taken to prevent exposure to etiologic agents potentially present in the specimens.
3. Always wear gloves when handling reagents in this kit as some reagents are preserved with 0.095% (w/w) sodium azide. Sodium azide should never be flushed down the drain as this chemical may react with lead or copper plumbing to form potentially explosive metal azides. Excess reagents should be discarded in an appropriate waste receptacle.

REAGENTS

1. LF Specimen Diluent (2.5 mL, REF GLF025): Glycine-buffered saline containing blocking agents and a preservative
2. CrAg LF Test Strips (50 strips in desiccant vial, REF LFCR50)
3. CrAg Positive Control (1 mL, REF CB1020): Glycine-buffered saline spiked with cryptococcal antigen (strain 184A – clinical isolate from Tulane University (Infection & Immunity, June 1983, p. 1052-1059))
4. Package insert

MATERIALS NOT PROVIDED

1. Pipettor (40-µL and 80-µL)
2. Timer
3. Disposable micro-centrifuge tubes, test tubes, or a micro-titer plate

REAGENT PREPARATIONS

The entire kit should be at room temperature (22-25 °C) before and during use.

REAGENT STABILITY AND STORAGE

All reagents included in this kit should be stored at room temperature (22-25°C) until the expiration dates listed on the reagent labels.

Unused test strips should be stored in the LF test strip vial with the desiccant cap firmly attached.

SPECIMEN COLLECTION & PREPARATION

For optimal results, sterile non-hemolyzed serum should be used. If a delay is encountered in specimen processing, storage at 2-8°C for up to 72 hours is permissible. Specimens may be stored for longer periods at <-20°C, provided they are not repeatedly thawed and refrozen. Specimens in transit should be maintained at 2-8°C or <-20°C.

PROCEDURE

REFER TO REAGENTS SECTION FOR A LIST OF MATERIALS PROVIDED.

Qualitative Procedure

1. Add 1 drop of LF Specimen Diluent (REF GLF025) to an appropriate reservoir (disposable micro-centrifuge tube, test tubes, or micro-titer plate, etc.).
2. Add 40 µL of specimen to the container and mix.
3. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip (REF LFCR50) into the specimen.
4. Wait 10 minutes.
5. Read and record the results (See READING THE TEST).

Semi-Quantitative Titration Procedure

1. Prepare dilutions starting with an initial dilution of 1:5, followed by 1:2 serial dilutions to 1:2560.
2. Place 10 micro-centrifuge or test tubes in an appropriate rack and label them 1-10 (1:5 through 1:2560). Additional dilutions may be necessary if the specimen is positive at 1:2560.
3. Add 4 drops of LF Specimen Diluent (REF GLF025) to tube #1.
4. Add 2 drops of LF Specimen Diluent to each of the tubes labeled 2-10.
5. Add 40 µL of specimen to tube #1 and mix well.
6. Transfer 80 µL of specimen from tube #1 to tube #2 and mix well. Continue this dilution procedure through tube #10. Discard 80 µL from tube 10 for a final tube volume of 80 µL.
7. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip into the specimen in each of the 10 tubes.
8. Wait 10 minutes.
9. Read and record the results (See READING THE TEST).

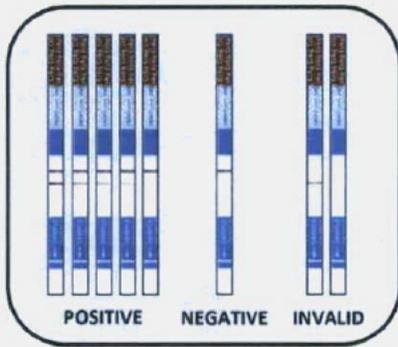
READING THE TEST

Read the reactions. The presence of two lines (test and control), regardless of the intensity of the test line, indicates a positive result.

For the semi-quantitative titration procedure, the patient's titer should be reported as the highest dilution that yields a positive result.

A single control line indicates a negative result. If the control line does not appear, the results are invalid and the test should be repeated.

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QUALITY CONTROL

A positive control (CrAg Positive Control REF CB0020) can be evaluated by adding 1 drop of LF Specimen Diluent (REF GLF025) followed by 1 drop of CrAg Positive Control to a tube. A negative control can be evaluated by adding 2 drops of LF Specimen Diluent (REF GLF025) to a tube. Insert a test strip into the tubes and read after 10 minutes. Two (2) lines (test and control) indicate a positive result and one line (control) indicates a negative result.

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION OF RESULTS

The control line must be present for a valid test. The presence of two lines (a control line and a line in the test zone) indicates a positive result.

Negative results do not rule out the diagnosis of disease. The specimen may be drawn before detectable antigen is present.

The magnitude of the measured result, above the cutoff, is not indicative of the total amount of antigen present.

LIMITATIONS OF THE PROCEDURE

- The assay performance characteristics have not been established for matrices other than serum, [REDACTED], and CSF.
- Depending on the disease and organism prevalence, testing should not be performed as a screening procedure for the general population. The predictive value of a positive or negative serologic result depends on the pretest likelihood of cryptococcal disease being present. Testing should only be done when clinical evidence suggests the diagnosis of cryptococcal disease.
- Testing hemolyzed serum samples could lead to false negatives due to the high background color on the strip.
- This assay was not evaluated for potential interference related to specimen pretreatment with 2-mercaptoethanol or with specimens including the following substances: (b)(4) Trade Secret Process, (b)(4) Trade Secret Process.

CROSS-REACTIVITY ANALYSIS

The CrAg Lateral Flow Assay was evaluated for cross-reactivity against a panel of patients' specimens across a variety of different pathologies. The results of this testing are shown in the table below.

Pathology	# of Samples	% Positive
Penicilliosis	5	0% (0/5)
Sporothrichosis	6	0% (0/6)
HAMA	5	0% (0/5)
Syphilis	10	0% (0/10)
Rubella	5	0% (0/5)
Mycoplasmosis	10	0% (0/10)
Toxoplasmosis	7	0% (0/7)
CMV	10	0% (0/10)
Blastomycosis	10	0% (0/10)
Coccidioidomycosis	10	0% (0/10)

Histoplasmosis	10	0% (0/10)
Candidiasis	10	0% (0/10)
Aspergillus GM+	10	10% (1/10)
Rheumatoid Factor	10	0% (0/10)

Additionally, cross-reactivity was assessed by testing crude culture filtrate antigens at a range of concentrations using the CrAg Lateral Flow Assay. At high concentrations (>0.1 mg/mL) antigens from *Paracoccidioides brasiliensis* exhibited some cross-reactivity.

Antigens from the following organisms were tested and exhibited no cross-reactivity:

- Aspergillus terreus*
- Aspergillus niger*
- Aspergillus fumigatus*
- Aspergillus flavus*

This assay was not evaluated for cross-reactivity against the following organisms or pathologies:

- Candida dubliniensis*
- Candida tropicalis*
- Candida parapsidosis*
- Candida krusei*
- Candida glabrata*
- Cladosporium trichoides*
- Neisseria meningitidis*
- Salmonella typhi*
- Mycobacterium tuberculosis*
- Pneumocystis carinii*
- Trichosporon beigelii*
- Zygomycetes*
- Antinuclear antibody +
- Hepatitis A Virus
- Hepatitis C Virus
- Staphylococcus aureus*
- Streptococcus pneumoniae*

HIGH DOSE HOOK EFFECT (PROZONING)

Although rare, extremely high concentrations (>0.140 mg/mL) of cryptococcal antigen can result in weak test lines and, in extreme instances, yield negative test results. If prozoning is suspected in weakly positive or negative test results, the semi-quantitative titration procedure should be followed to rule out false negative results.

EXPECTED VALUES

The frequency of cryptococcosis is dependent on several factors including: patient population, type of institution, and epidemiology. In this study, 100% of true positives as determined by culture and/or India Ink were detected.

SPECIFIC PERFORMANCE CHARACTERISTICS

The CrAg Lateral Flow Assay was compared to the gold standard diagnoses of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay. These studies contained a mix of both prospective and retrospective specimens. A summary table of the data collected is included below.

Serum	CrAg LFA Assay	Culture/India Ink	
		Positive	Negative
	Positive	91	0
	Negative	0	123

Serum	Calculated	95% CI
Sensitivity	100%	96.0% - 100%
Specificity	100%	97.0% - 100%

(b)(4) Trade Secret Process

(b)(4) Trade Secret Process

CSF	CrAg LFA Assay	Culture/India Ink	
		Positive	Negative
	Positive	65	0
	Negative	0	99

CSF	Calculated	95% CI
Sensitivity	100%	96.0% - 100%
Specificity	100%	97.0% - 100%

EIA METHOD COMPARISON

The CrAg Lateral Flow Assay was evaluated using 197 serum specimens that were submitted to a US reference laboratory for cryptococcal antigen testing. These specimens were tested using the CrAg Lateral Flow Assay and a commercially available cryptococcal antigen EIA. The results of these comparisons are shown in the tables below.

Serum	CrAg LFA Assay	CrAg EIA	
		Positive	Negative
	Positive	96	7
	Negative	0	94

Serum	Calculated	95% CI
% Positive Agreement	100% (96/96)	96% - 100%
% Negative Agreement	93% (94/101)	86% - 97%

IMMY LATEX AGGLUTINATION METHOD COMPARISON

The CrAg Lateral Flow Assay was evaluated using 197 serum specimens that were submitted to a US reference laboratory for cryptococcal antigen testing. These specimens were tested using the CrAg Lateral Flow Assay and the IMMY Cryptococcal Antigen Latex Agglutination Assay. This comparison yielded an overall percent agreement of 99%.

SEMI-QUANTITATIVE METHOD COMPARISON

In addition, 62 of these specimens were tested using the semi-quantitative titration procedure in both the CrAg Lateral Flow Assay and the IMMY Latex Cryptococcal Antigen Detection System (REF CR1003). Linear regression analysis of the data yielded an R² value of 0.905.

LIMIT OF DETECTION

In order to establish the limit of detection, a C₅ - C₉₅ experiment was conducted by diluting purified cryptococcal antigen in LF Specimen Diluent (REF GLF025) and testing 24 replicates per concentration using the CrAg Lateral Flow Assay. The results of this testing are shown in the following table:

Concentration	# Positive	% Positive
0.50 ng/mL	0	0% (0/24)
0.75 ng/mL	0	0% (0/24)
1.00 ng/mL	4	17% (4/24)
1.25 ng/mL	12	50% (12/24)
1.50 ng/mL	21	88% (21/24)
1.75 ng/mL	24	100% (24/24)
2.00 ng/mL	24	100% (24/24)
2.50 ng/mL	24	100% (24/24)
3.00 ng/mL	24	100% (24/24)

C ₅ - C ₉₅ Interval	1.0 - 1.5 ng/mL
---	-----------------

REPRODUCIBILITY AND PRECISION

The CrAg Lateral Flow Assay was evaluated for reproducibility and precision by spiking serum with cryptococcal antigen to produce a panel consisting of a negative sample, a high-negative (C₅) sample, a low-positive sample and a moderate-positive sample. This panel was tested twice per day at three sites with a total of five operators over a five-day period in

order to determine both the inter-lab and the intra-lab reproducibility and precision of the assay. The results of this study are shown in the table below.

PANEL	Site 1 % Pos	Site 2 % Pos	Site 3 % Pos	Overall % Pos
Negative	0% (0/30)	0% (0/30)	0% (0/15)	0% (0/75)
High Negative	7% (2/30)	0% (0/30)	0% (0/15)	3% (2/75)
Low Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)
Moderate Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)

BIBLIOGRAPHY

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3. Kozel, T. R. 1995. Trends Microbiol. 3:295-299.
4. Lin, X. and J. Heltman. 2006. T. Annu. Rev. Microbiol. 60:69-105.
5. Park, B. J., K. A. Wannemuehler, B. J. Marston, N. Govender, P. G. Pappas, and T. M. Chiller. 2009. AIDS 23:525-530.
6. Zhou, Q. and W. J. Murphy. 2006. Immunol. Res. 35:191-208.

 Immuno-Mycologics, Inc.
2700 Technology Place
Norman OK 73071 U.S.A.
(405) 360-4669 / (800) 654-3639
Fax: (405) 364-1058
E-mail: info@immy.com
WEB: www.immy.com



EC REP

MDSS
Schiffgraben 41
30175 Hannover, Germany

International Symbol Usage

	Storage 20-25 C		Lot Number
	Manufactured by		Reference Number
	Expiration Date		In Vitro Diagnostics
	Conforms to European Union Requirements		Sufficient for "IIR" Tests
	Protect from Humidity		

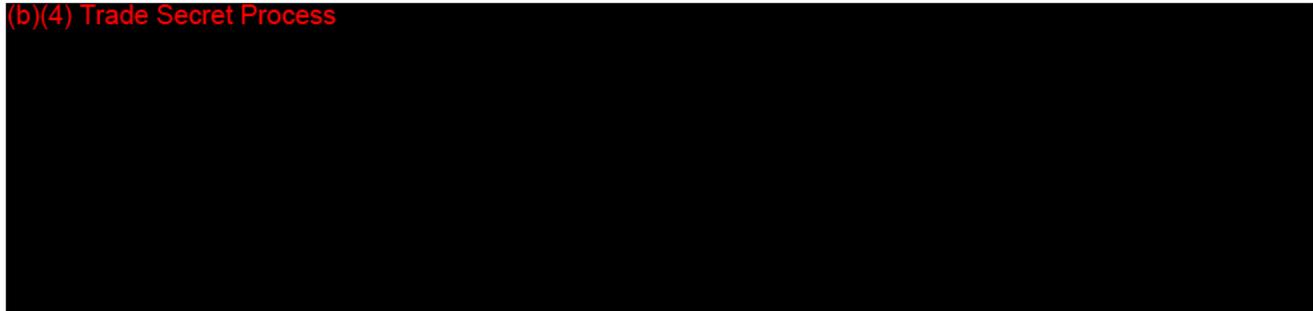
Rev. 08-16-2011

Sterilization and Shelf Life

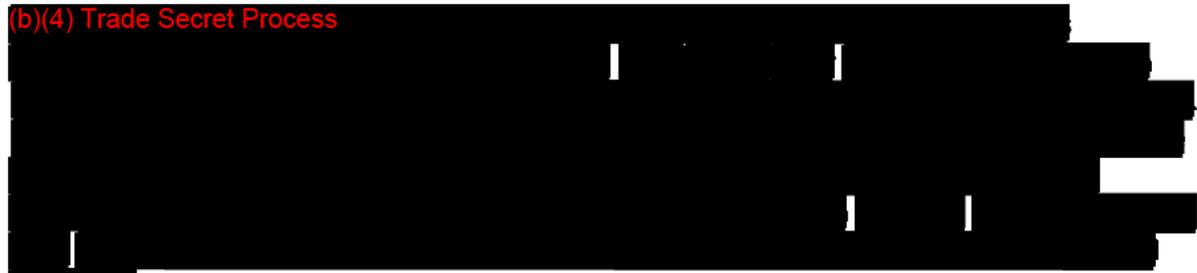
Sterilization: N/A

Stability: 2 years

(b)(4) Trade Secret Process



(b)(4) Trade Secret Process



indicate that the CrAg Lateral Flow Test Kit is stable at room temperature for two years.

(b)(4) Trade Secret Process



Results:

Testing	Pre-Defined Acceptance Criteria	Pass/Fail?
Transportation	Samples and Controls run as expected after shipping domestically and internationally	Pass
Container Closure System	Containers remain closed for 1 year with no deterioration or leaking	Pass
In-Use Stability	N/A	
Shelf Life at 3 Months	Samples and Controls run as expected	Pass
Shelf Life at 6 Months	Samples and Controls run as expected	Pass
Shelf Life at 12 Months	Samples and Controls run as expected	Pass
Shelf Life at 24 Months	Samples and Controls run as expected	Pass

Conclusions: All data support a 24-month shelf life for the CrAg Lateral Flow Test Kit.

Section 15 Biocompatibility

N/A

Section 16 Software

N/A

Section 17 Electromagnetic Compatibility and Electrical Safety

N/A

Performance Testing - Bench CrAg Lateral Flow Assay

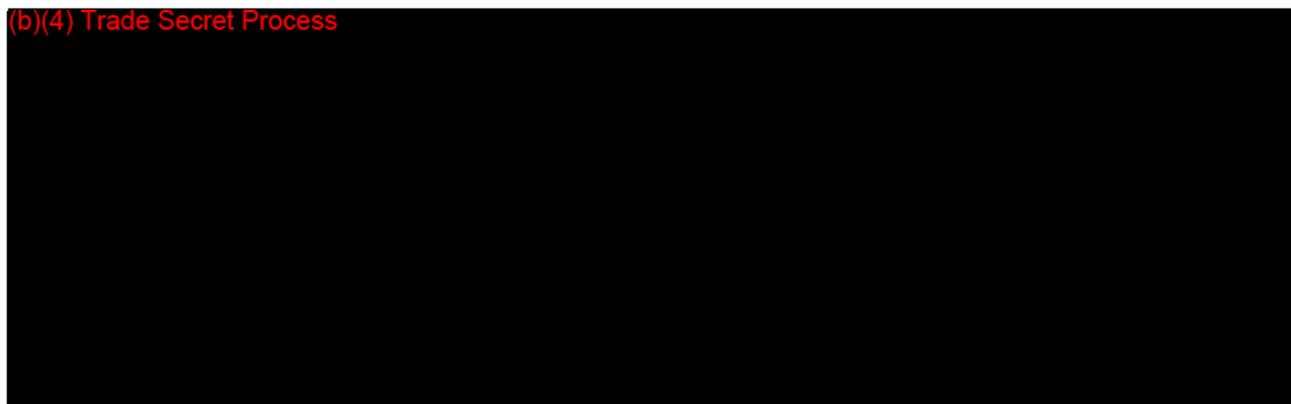
A. Precision Studies (Repeatability & Reproducibility)

(b)(4) Trade Secret Process



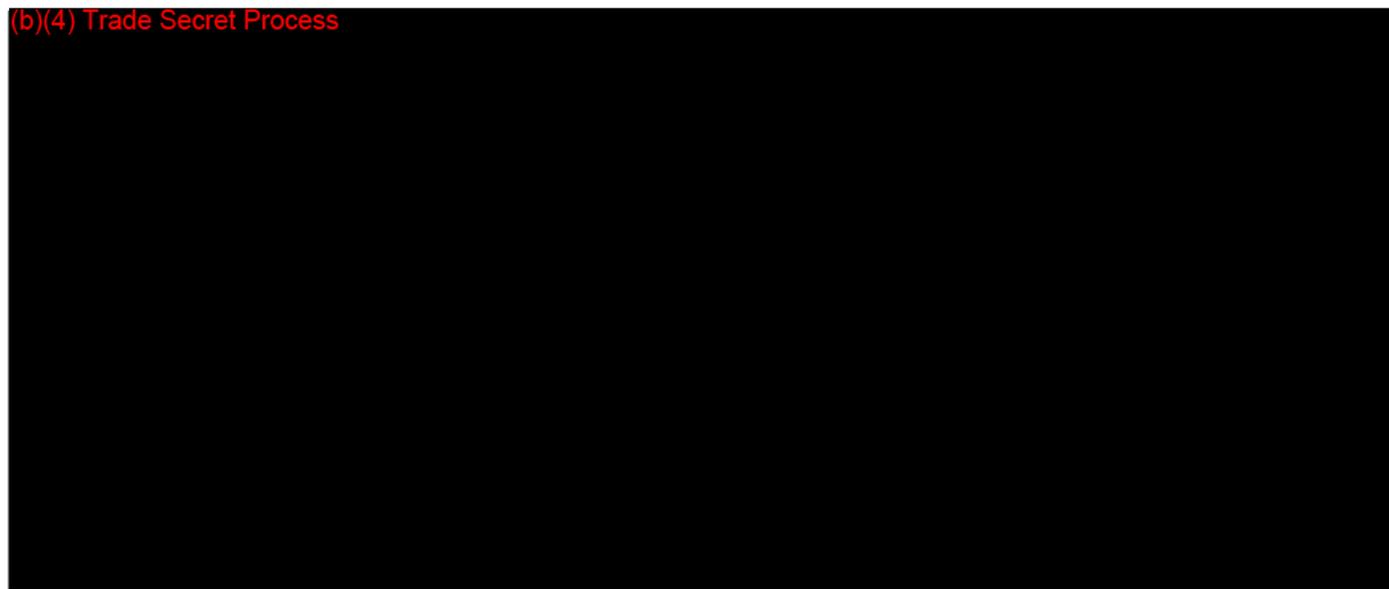
1. Precision Test Methods:

(b)(4) Trade Secret Process

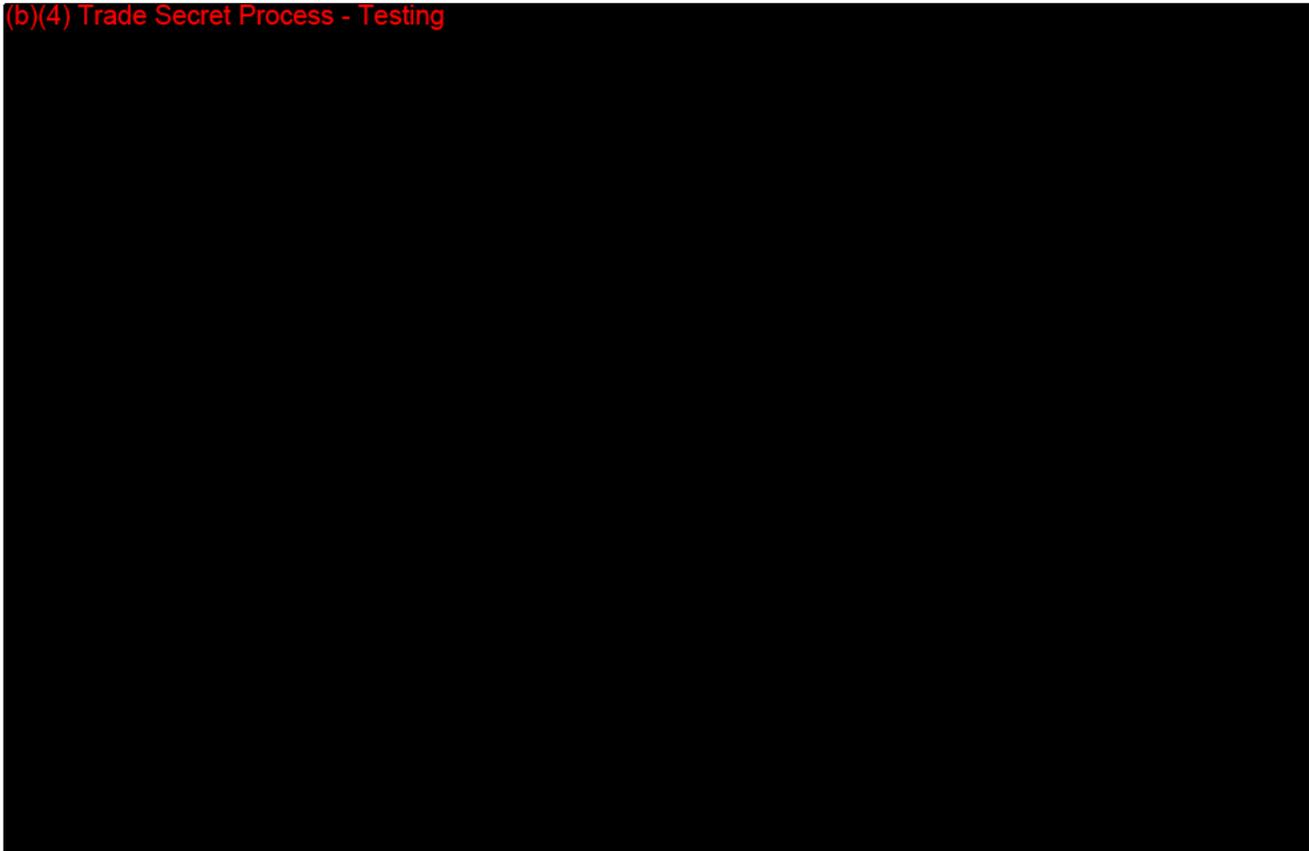


2. Precision Results:

(b)(4) Trade Secret Process



(b)(4) Trade Secret Process - Testing



3. Precision Analysis:

For repeatability, percent positives detected and percent negatives detected were calculated for each site (Table 2). For reproducibility, overall percent positives detected were calculated by combining the data from all three sites (Table 3).

Table 2. Repeatability

Sample	Serum							
	Med. Pos		Low Pos		High Neg		Neg	
	-	+	-	+	-	+	-	+
Site 1	0	30	0	30	28	2	30	0
Percent %	0	100	0	100	93	7	100	0
Site 2	0	30	0	30	30	0	30	0
Percent %	0	100	0	100	100	0	100	0
Site 3	0	15	0	15	15	0	15	0
Percent %	0	100	0	100	100	0	100	0

Table 3. Reproducibility

Sample	Serum							
	Med. Pos		Low Pos		High Neg		Neg	
	-	+	-	+	-	+	-	+
Total No.	0	75	0	75	73	2	75	0

Percent %	0	100	0	100	97	3	100	0
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4. Precision Conclusions:

Percent negatives on negative specimens are greater than 90%, percent positives on low positive samples are greater than 90%, and percent positives on medium positive specimen are greater than 95%. Therefore, the assay is precise with serum samples across operators, days, and sites.

B. Analytical Sensitivity (Lower Limits of the Assay/Analytical Cut-Off)

Test Objective: To determine the lower limits of the assay
 Test Articles Used: Cryptococcal antigen spiked into LF Specimen Diluent
 Test Methods: CLSI EP12-A2
 Study Endpoint: C₅₀ (Concentration where sample runs positive 50% of the time)
 Acceptance Criteria: N/A

1. Analytical Sensitivity Methods:

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running 24 replicates of varying concentrations of cryptococcal antigen diluted in Lateral Flow Specimen Diluent, on one lot of kits. The analytical cut-off was defined as the concentration where 50% of the results were positive and 50% of the results were negative (Table 4 and Figure 1).

2. Analytical Sensitivity Results/Analysis:

Table 4. Analytical Cut-Off

Sample Concentration (ng/ml)	No. Positive	No. Tested	% Positive
0.50	0	24	0%
0.75	0	24	0%
1.00	4	24	17%
1.25	12	24	50%
1.50	21	24	88%
1.75	24	24	100%
2.00	24	24	100%
2.50	24	24	100%
3.00	24	24	100%
3.50	24	24	100%
4.00	24	24	100%

(b)(4) Trade Secret Process - Testing

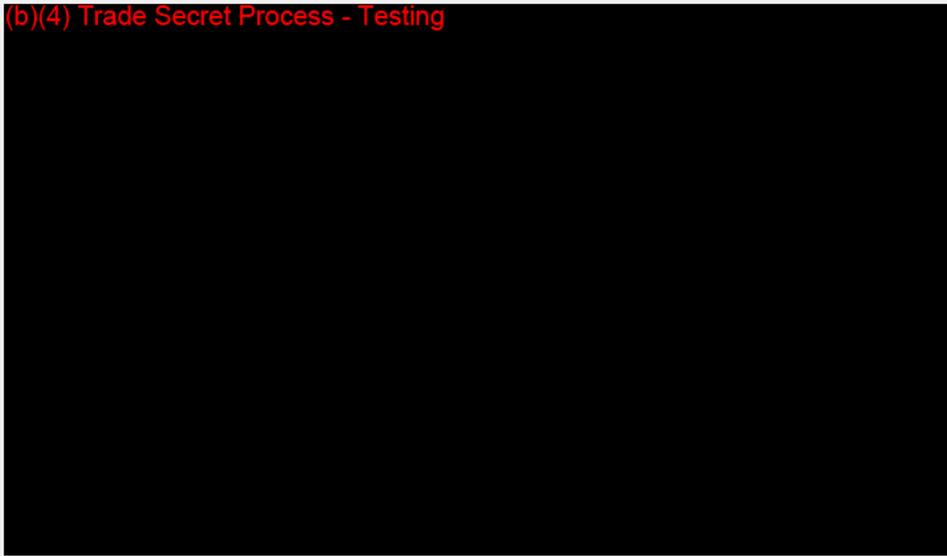


Figure 1. Analytical Cut-off

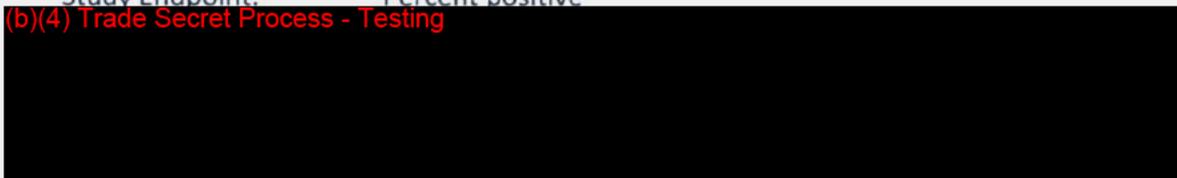
3. Analytical Sensitivity Conclusions:

Analysis shows that the analytical sensitivity (analytical cut-off) is 1.25 ng/ml.

C. Analytical Specificity/Interference

Test Objective:	To determine assay cross-reactivity
Test Articles Used:	Specimens representative of potentially cross-reacting medical conditions unrelated to cryptococcosis; specimens containing potentially interfering substances.
Test Methods:	See below
Study Endpoint:	Percent positive

(b)(4) Trade Secret Process - Testing



1. Analytical Specificity

a. Analytical Specificity Methods:

Analytical specificity for the CrAg Lateral Flow Assay was determined by running potentially cross-reacting medical conditions unrelated to cryptococcosis. The following specimens were run in triplicate on one lot of the CrAg Lateral Flow Assay. A total of 118 serum specimen and 15 fungal culture filtrates were tested. (b)(4) Trade Secret Process - Testing

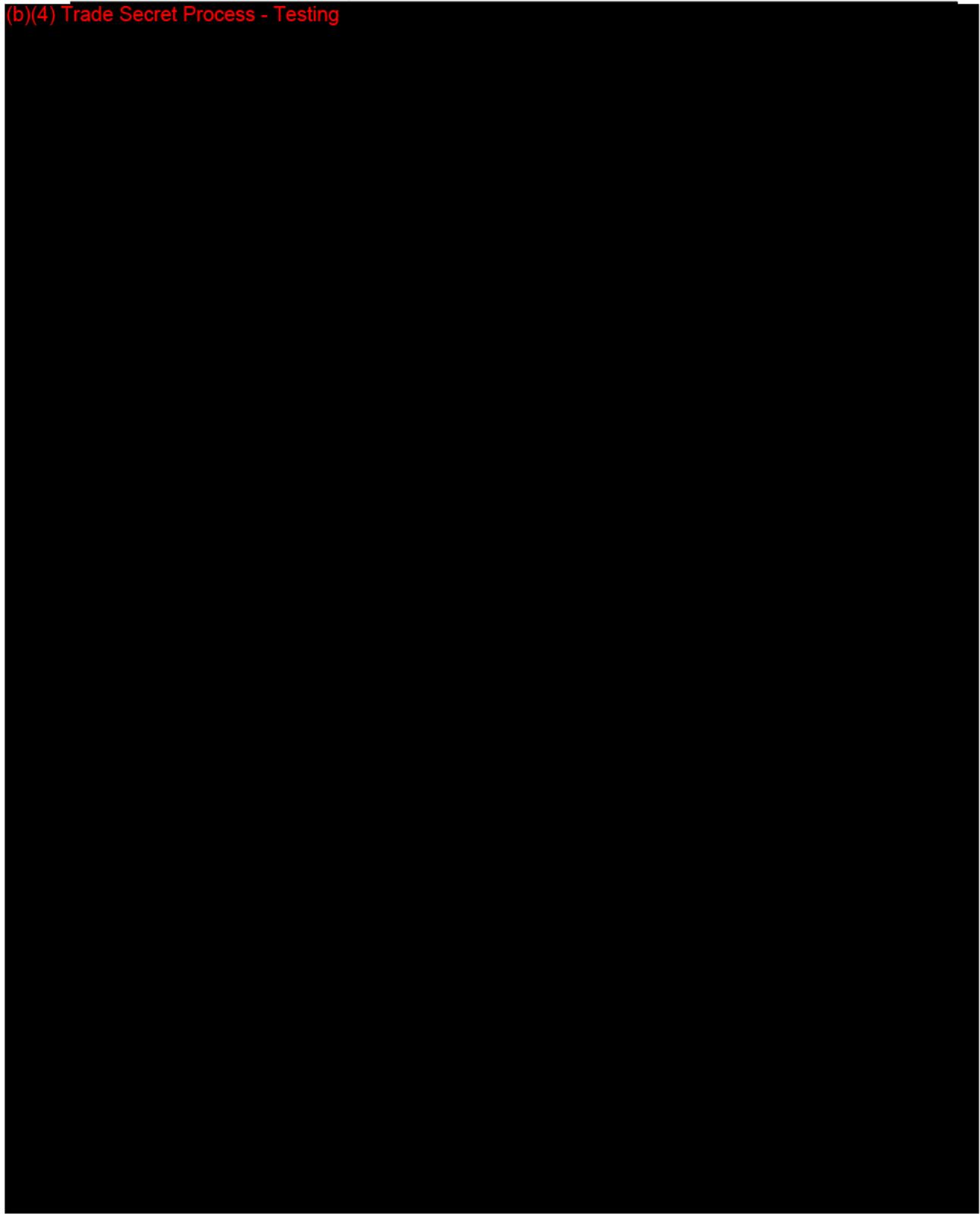


Percent positive was determined for each condition (Table 5).

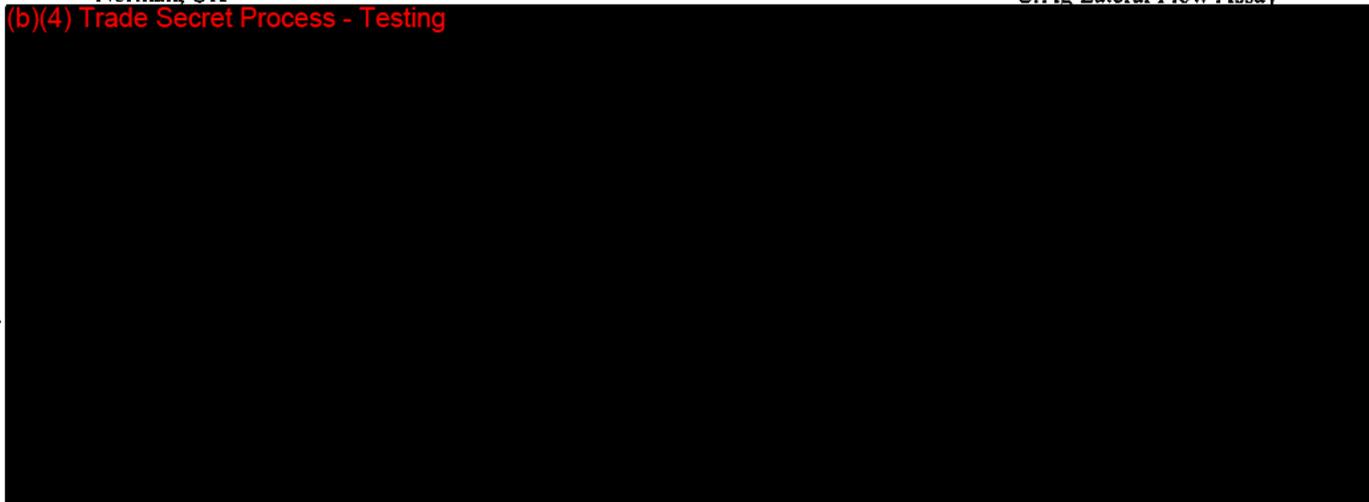
b. Analytical Specificity Results/Analysis:

Table 5. Analytical Specificity Analysis

(b)(4) Trade Secret Process - Testing

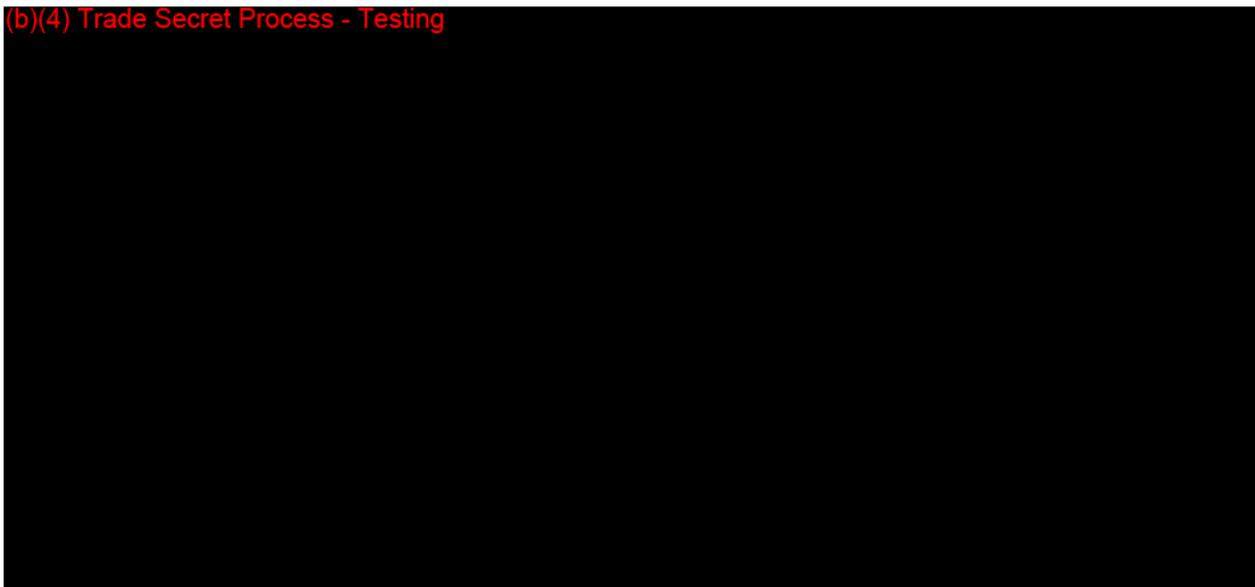


(b)(4) Trade Secret Process - Testing



c. Analytical Specificity Conclusions:

(b)(4) Trade Secret Process - Testing



1. Interference

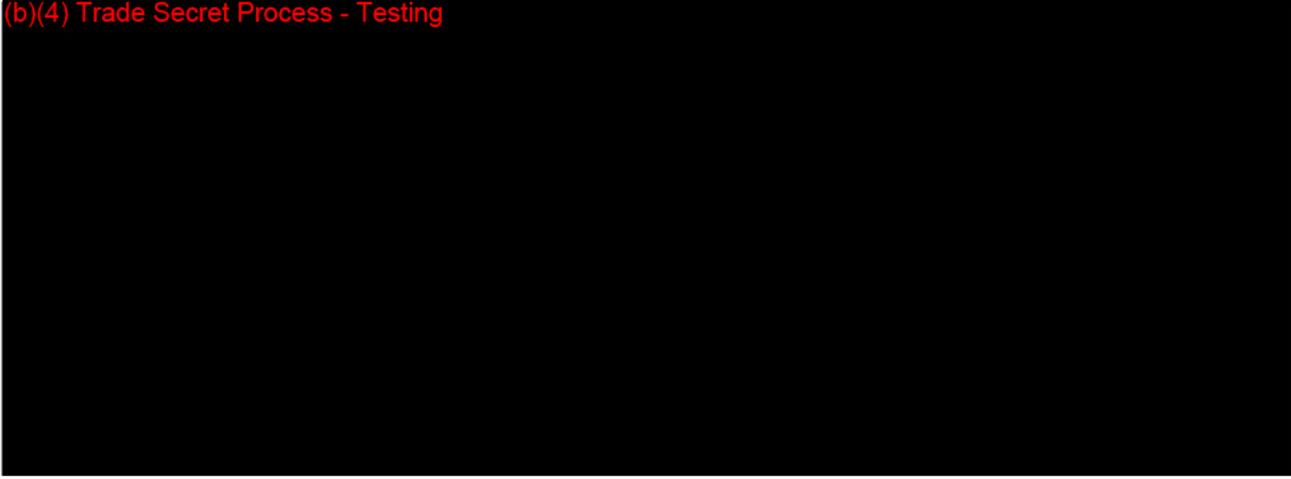
a. Interference Methods:

In addition to the cross-reactivity study, interference testing was also performed on five icteric, five hemolyzed, and five lipemic serum specimens. Each specimen was spiked with cryptococcal antigen at three times the C_{95} concentration. All specimens were then tested at IMMY, on one lot of CrAg Lateral Flow assay in triplicate: spiked and unspiked. The effect of pronase on the CrAg LFA was determined by pronase-treating 5 Meridian EIA positive specimens and 5 Meridian EIA negative specimens. The samples were analyzed both untreated and pronase-treated. Percent positivity was determined for each condition (Table 6).

b. Interference Results/Analysis:

Table 6. Interference Analysis

(b)(4) Trade Secret Process - Testing



c. Interference Conclusions:

All of the unspiked iceteric, hemolyzed, and lipemic specimens had negative results on the CrAg Lateral Flow Assay. All spiked specimens were positive, thus, these types of serum specimens do not interfere with the CrAg Lateral Flow Assay. However, it is possible that hemolyzed samples could lead to false negatives due to the high background color on the strip. As such, a statement is included in the Limitations of the Procedure section of the package insert:

“Hemolyzed serum samples could lead to false negative results due to the high background color on the strip.”

Pronase does not affect the CrAg LFA results.

D. High Dose Hook Effect

Test Objective:	To determine if specimens with high concentrations of cryptococcal antigen will produce a negative result on the CrAg Lateral Flow Assay
Test Articles Used:	Human serum specimens spiked with cryptococcal antigen at various concentrations, CrAg Lateral Flow Assay
Test Methods:	See Below
Study Endpoint:	The concentration of cryptococcal antigen well above the analytical sensitivity that fails to produce a positive result in the CrAg Lateral Flow Assay.

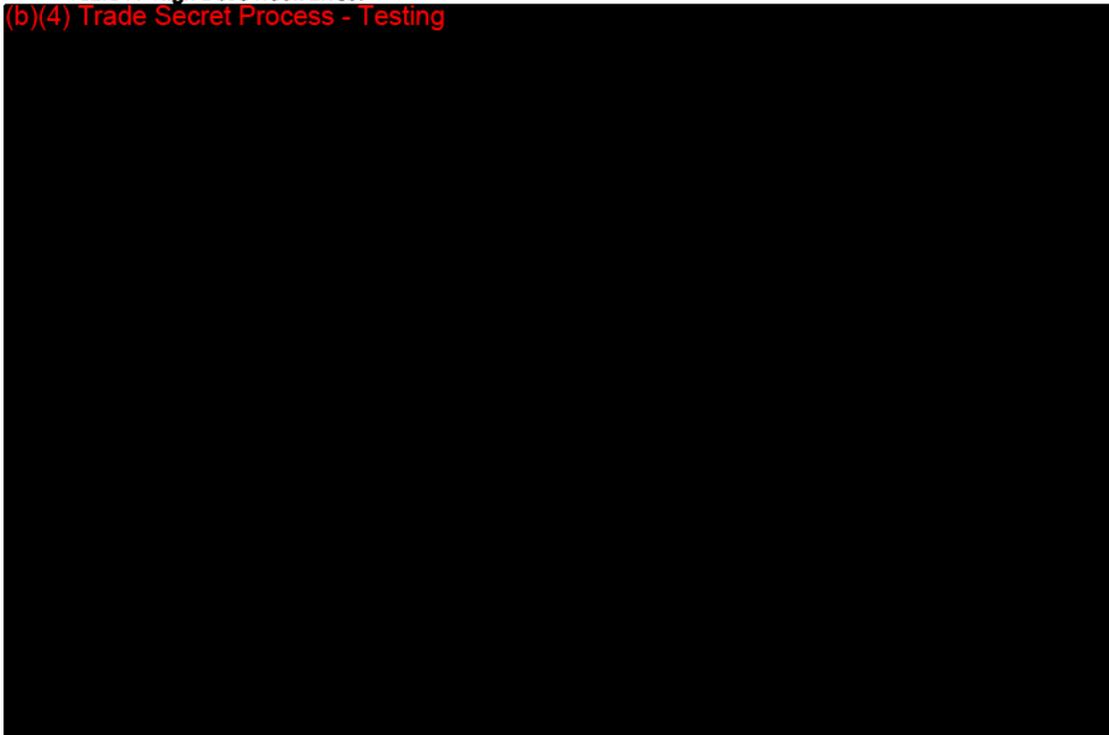
1. High Dose Hook Effect Methods

High dose hook effect concentrations with serum specimens were determined by spiking a serum specimen pool that was negative by the IMMY Latex-*Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at various concentrations between 20 and 500ug/ml. (b)(4) Trade Secret Process - Testing

2. High Dose Hook Effect Results/Analysis

Table 7. High Dose Hook Effect

(b)(4) Trade Secret Process - Testing

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3. High Dose Hook Effect Conclusions

(b)(4) Trade Secret Process - Testing

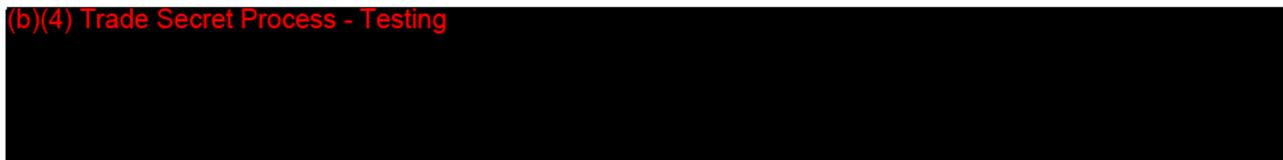
any serum specimen containing more than 200ug/ml of cryptococcal antigen may produce a false negative result in the CrAg Lateral Flow Assay. As such, a statement will be included in the package insert as a limitation of the procedure.

E. Freeze/Thaw Studies

Test Objective:	To establish equality between fresh and frozen samples
Test Articles Used:	Cryptococcal antigen-negative serum samples spiked with cryptococcal antigen
Test Methods:	See below
Acceptance Criteria:	100% Correlation between fresh and frozen samles

1. Freeze/Thaw Study Methods:

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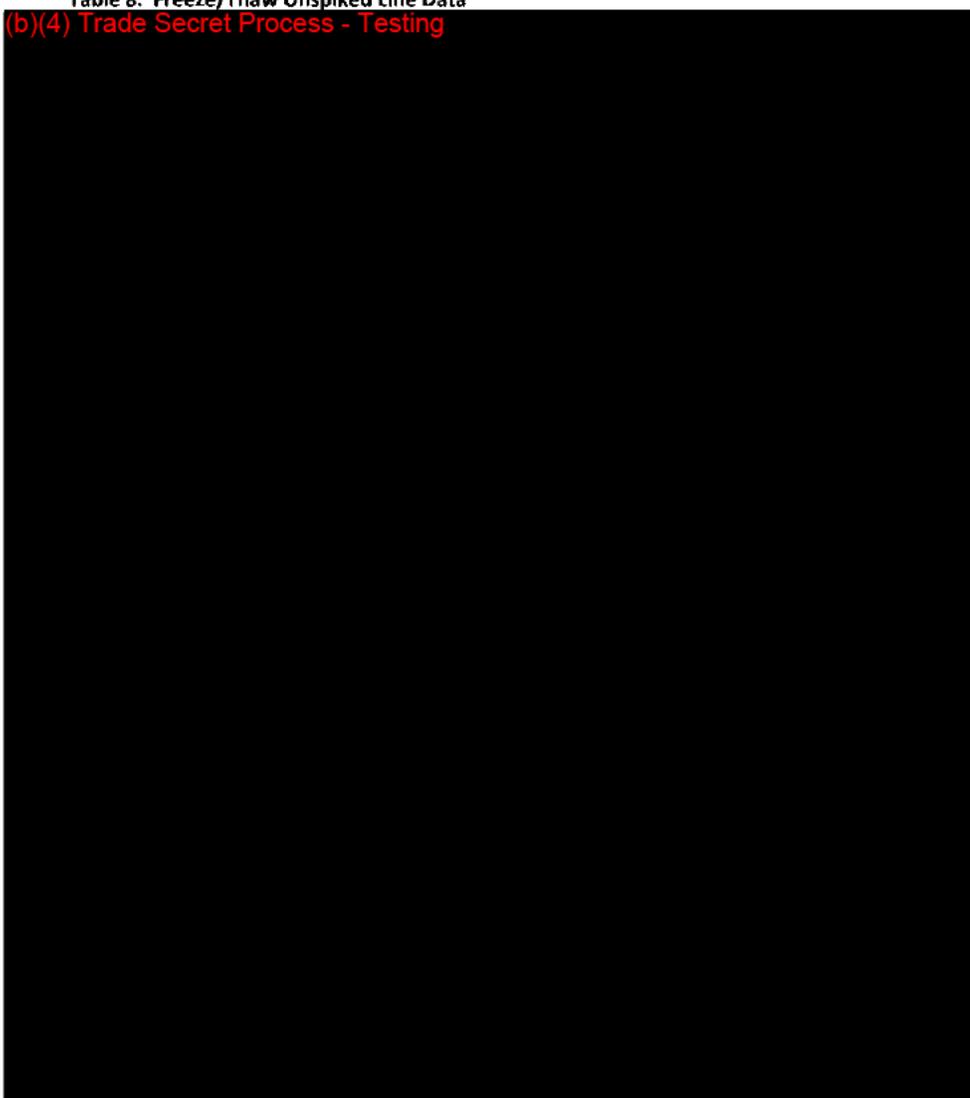
2. Freeze/Thaw Study Results

(b)(4) Trade Secret Process - Testing



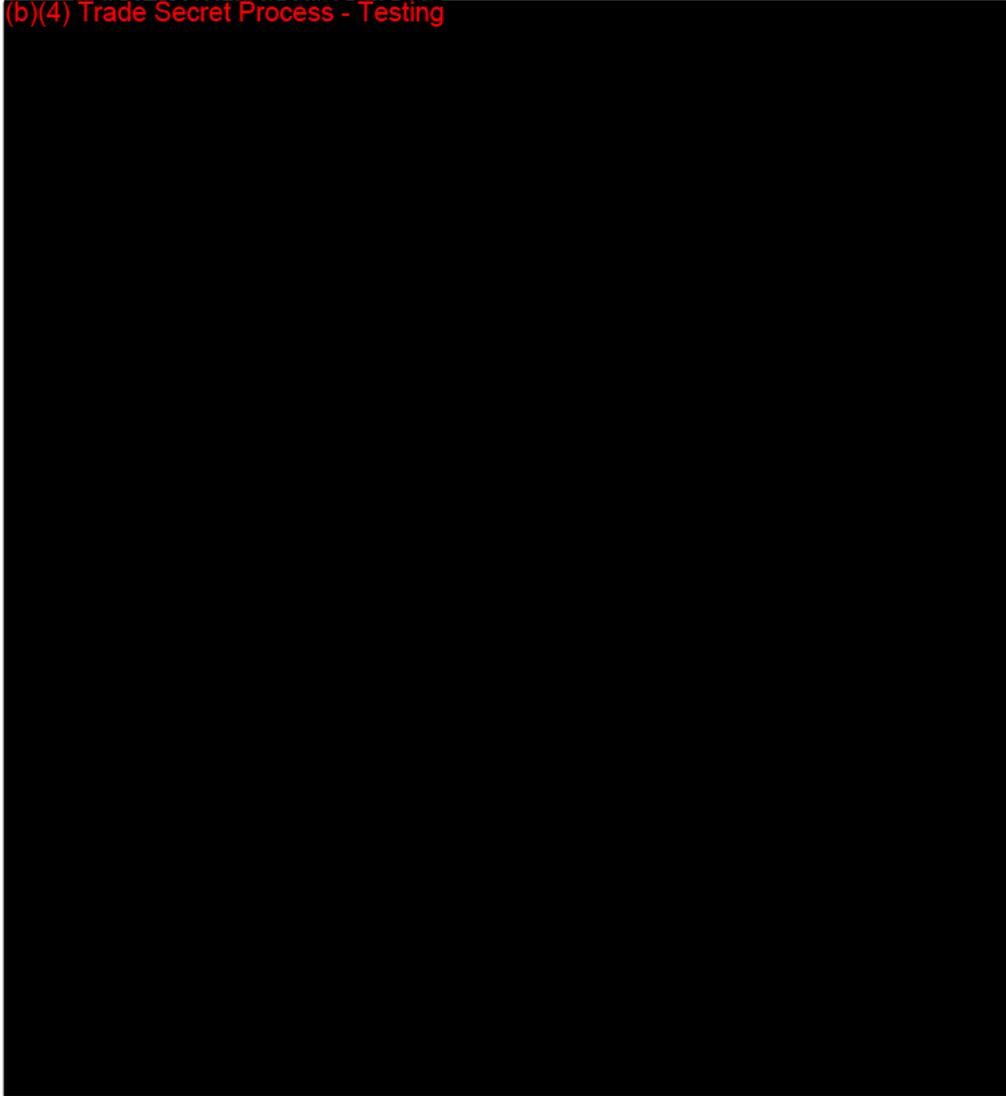
Table 8. Freeze/Thaw Unspiked Line Data

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Table 9. Freeze/Thaw Spiked Line Data
(b)(4) Trade Secret Process - Testing



3. Freeze/Thaw Study Conclusion:

There is 100% agreement (95-100%, 95% CI) between frozen and fresh specimens. Therefore, frozen specimens are equivalent to fresh specimens.

F. Method Comparisons – Culture/India Ink

Test Objective:	To establish the new device's sensitivity and specificity
Test Articles Used:	Culture-confirmed human (b)(4) Trade Secret, and CSF specimens tested collected retrospectively and prospectively.
Test Methods:	EP12-A2

Study Endpoint: Sensitivity and Specificity
Acceptance Criteria: Sensitivity values greater than 90% and specificity values greater than 90%.

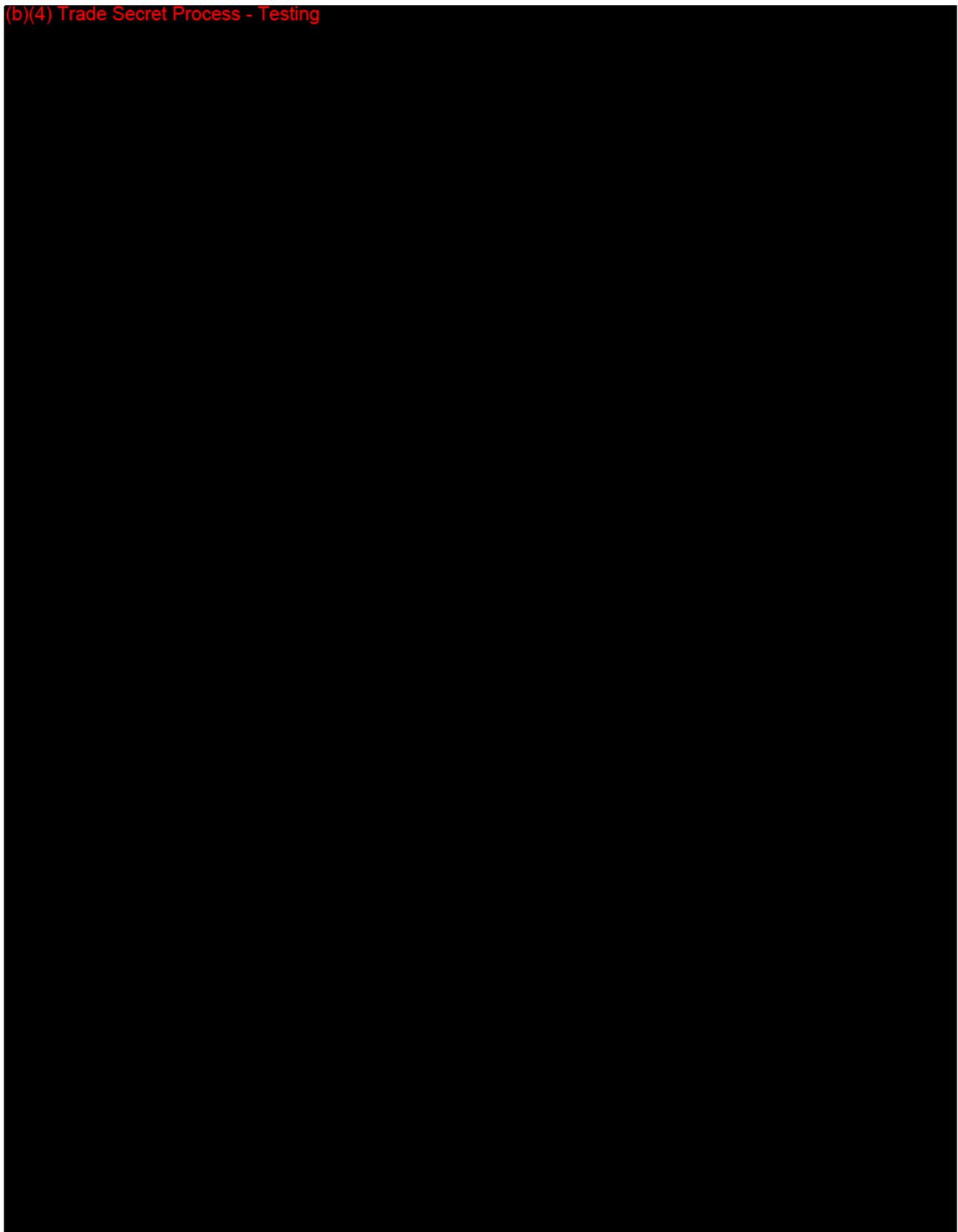
1. Comparison Methods:

(b)(4) Trade Secret CSF specimens were collected from patients suspected of active cryptococcosis. Disease was confirmed through CSF culture or India Ink, the current gold standard for diagnosis. Specimens were then either immediately tested in the LFA (prospective) or frozen and tested on the LFA (retrospective). (b)(4) Trade Secret Process - Testing

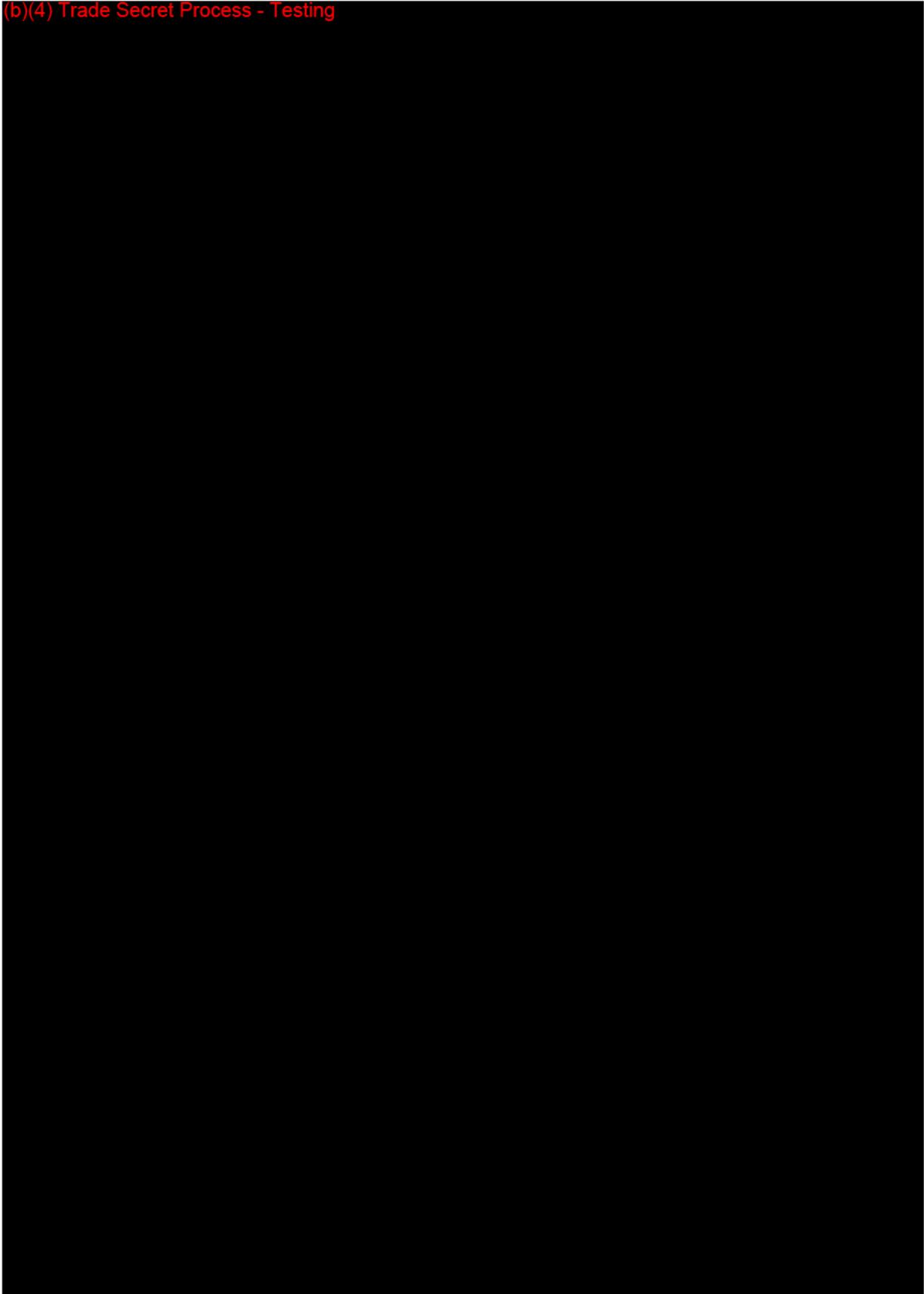
2. Comparison Results:

(b)(4) Trade Secret Process - Testing

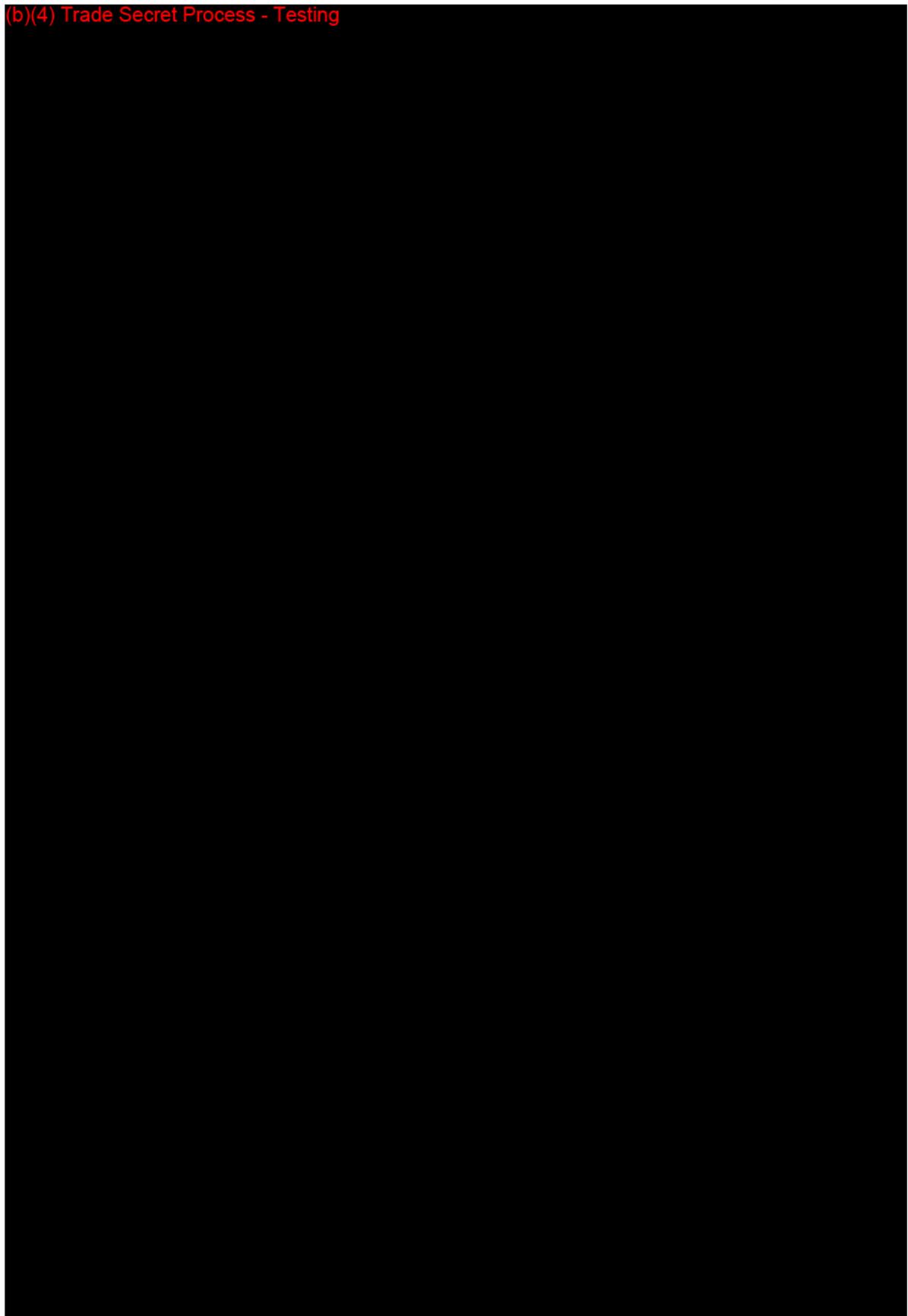
(b)(4) Trade Secret Process - Testing



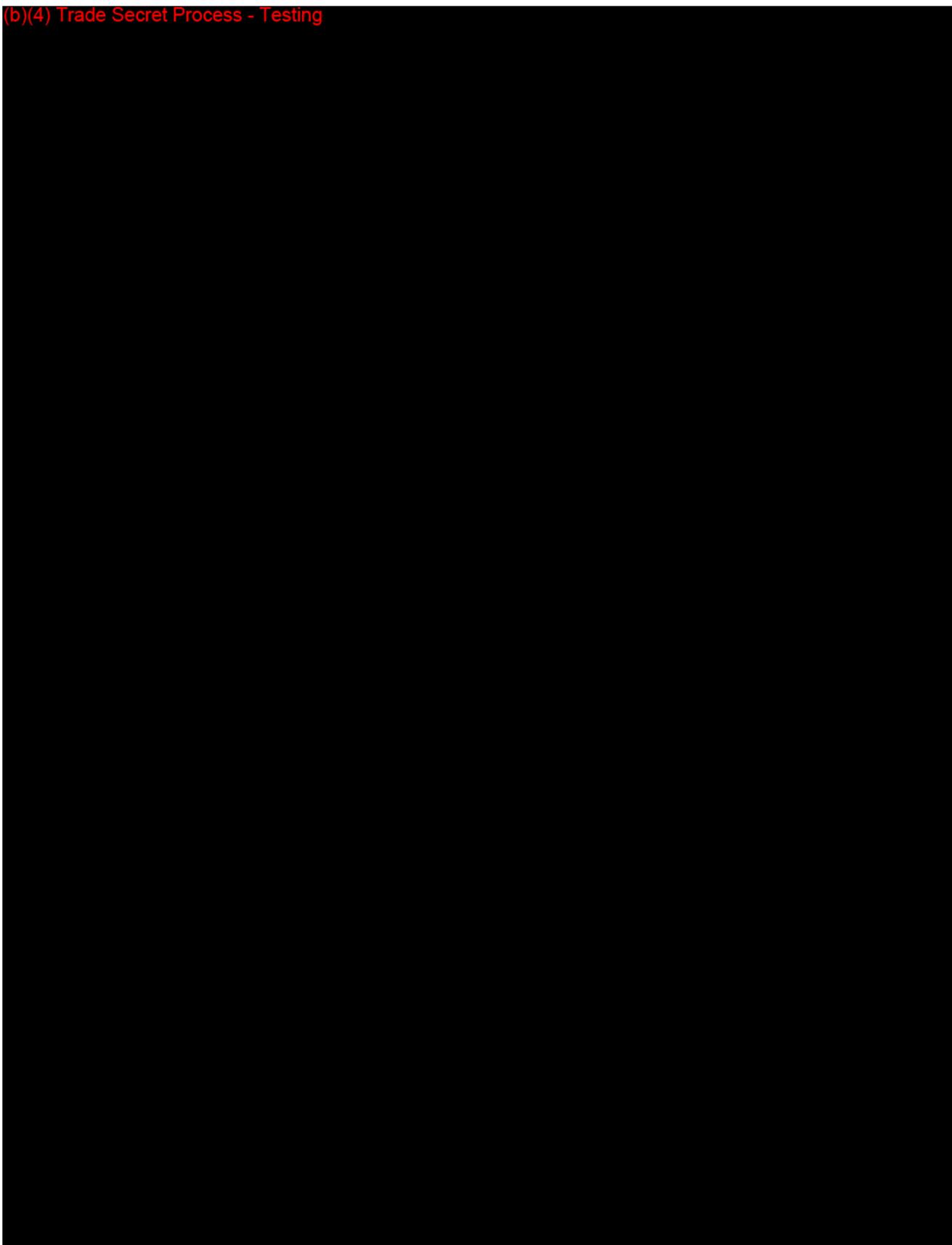
(b)(4) Trade Secret Process - Testing



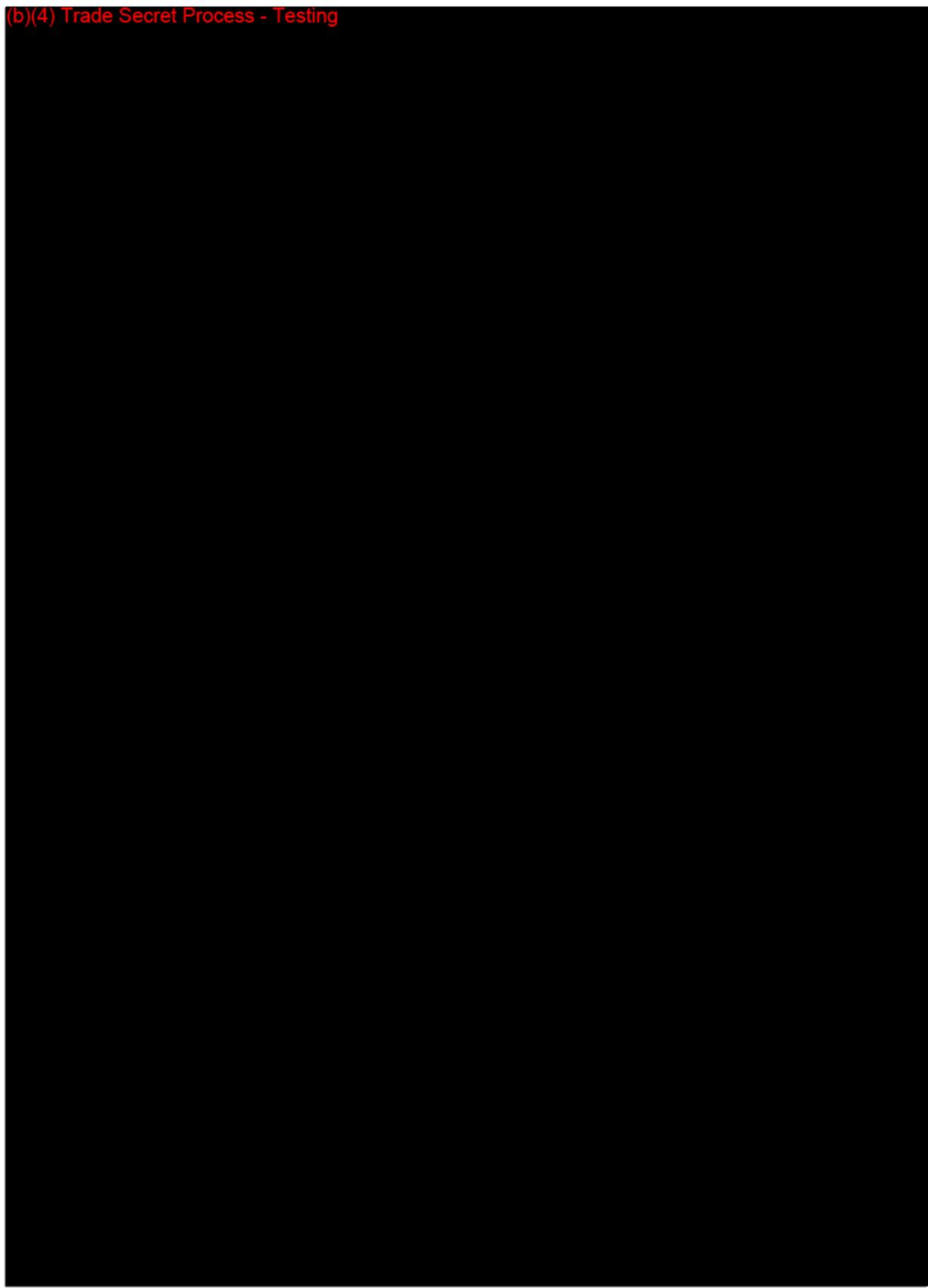
(b)(4) Trade Secret Process - Testing



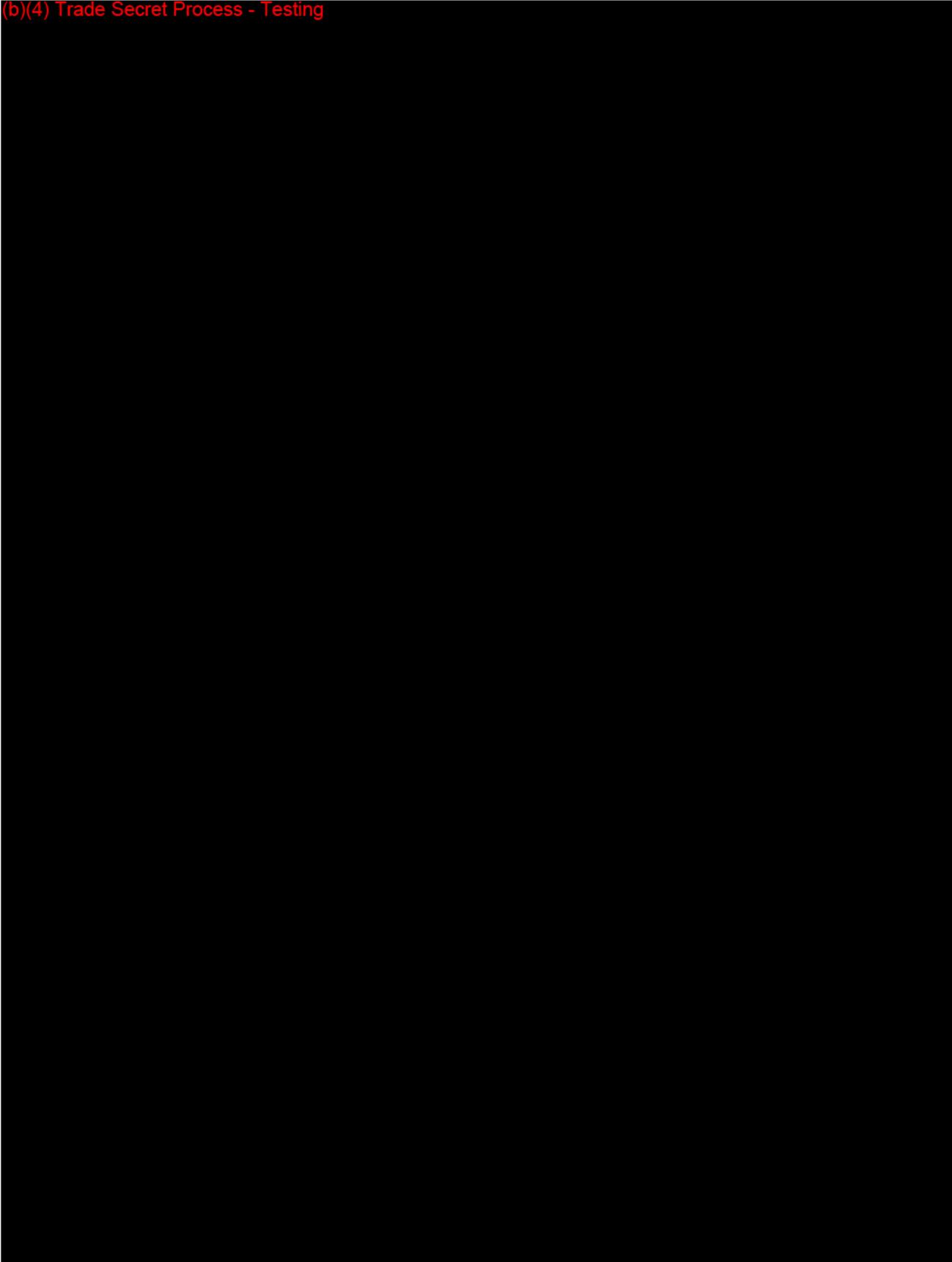
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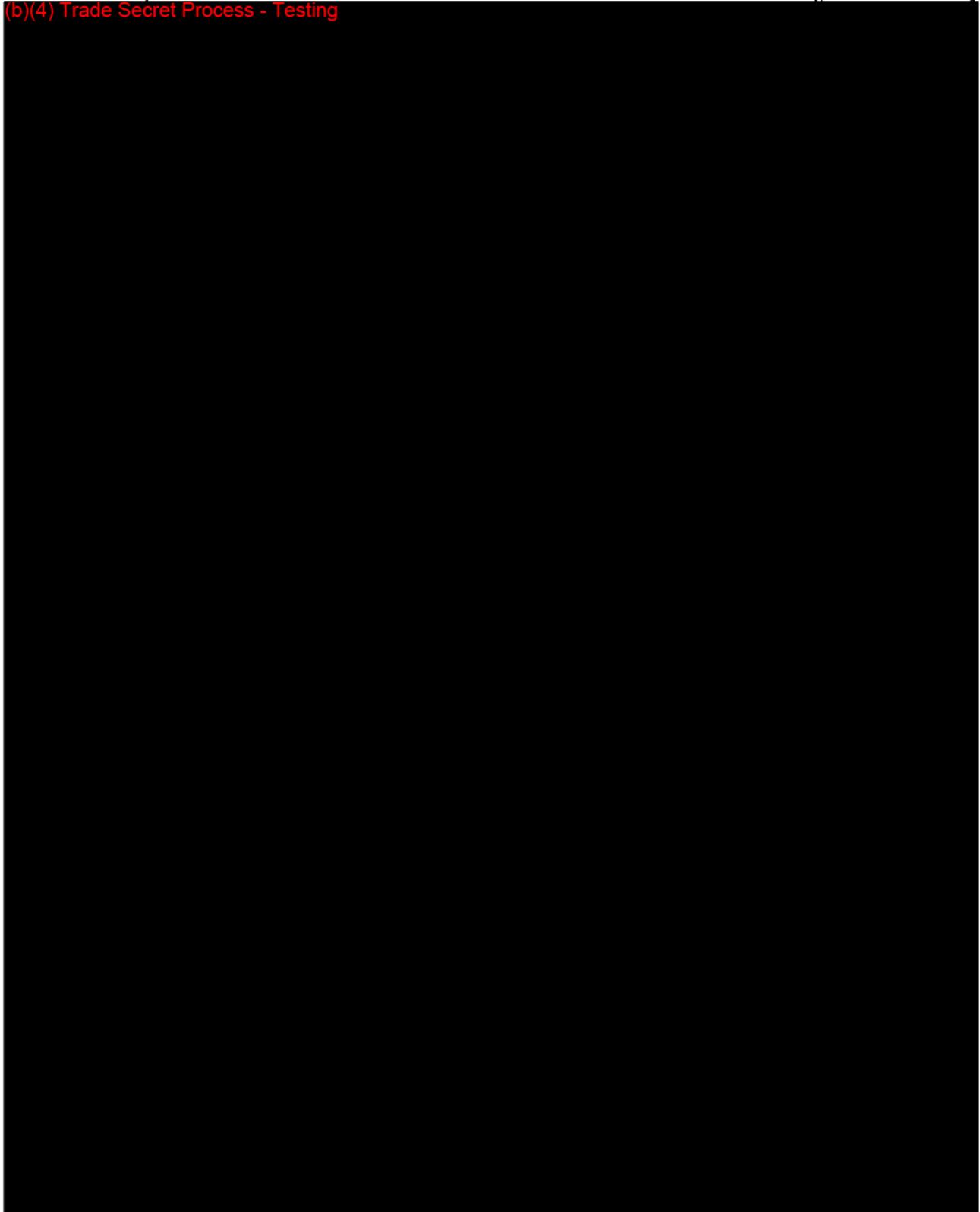
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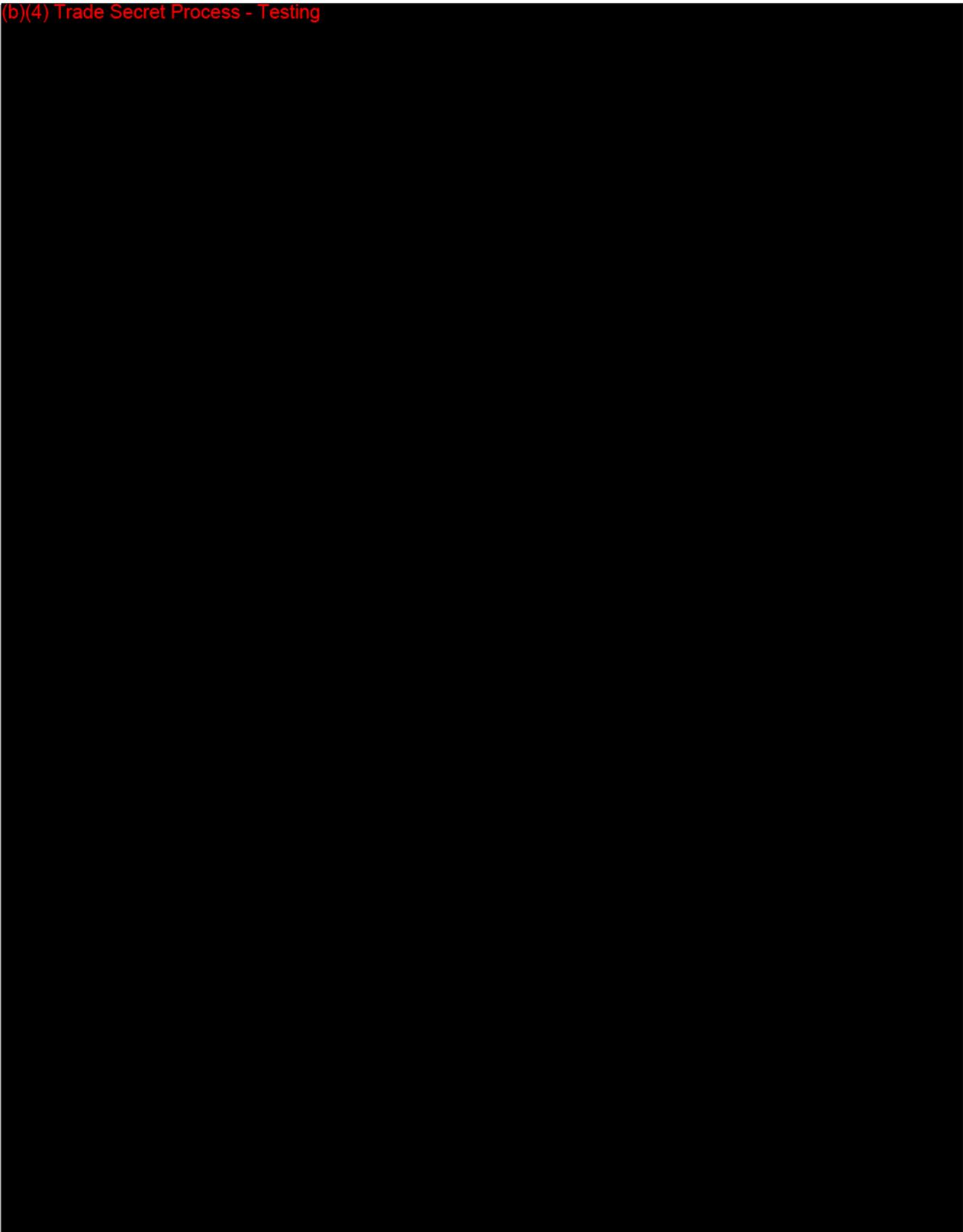
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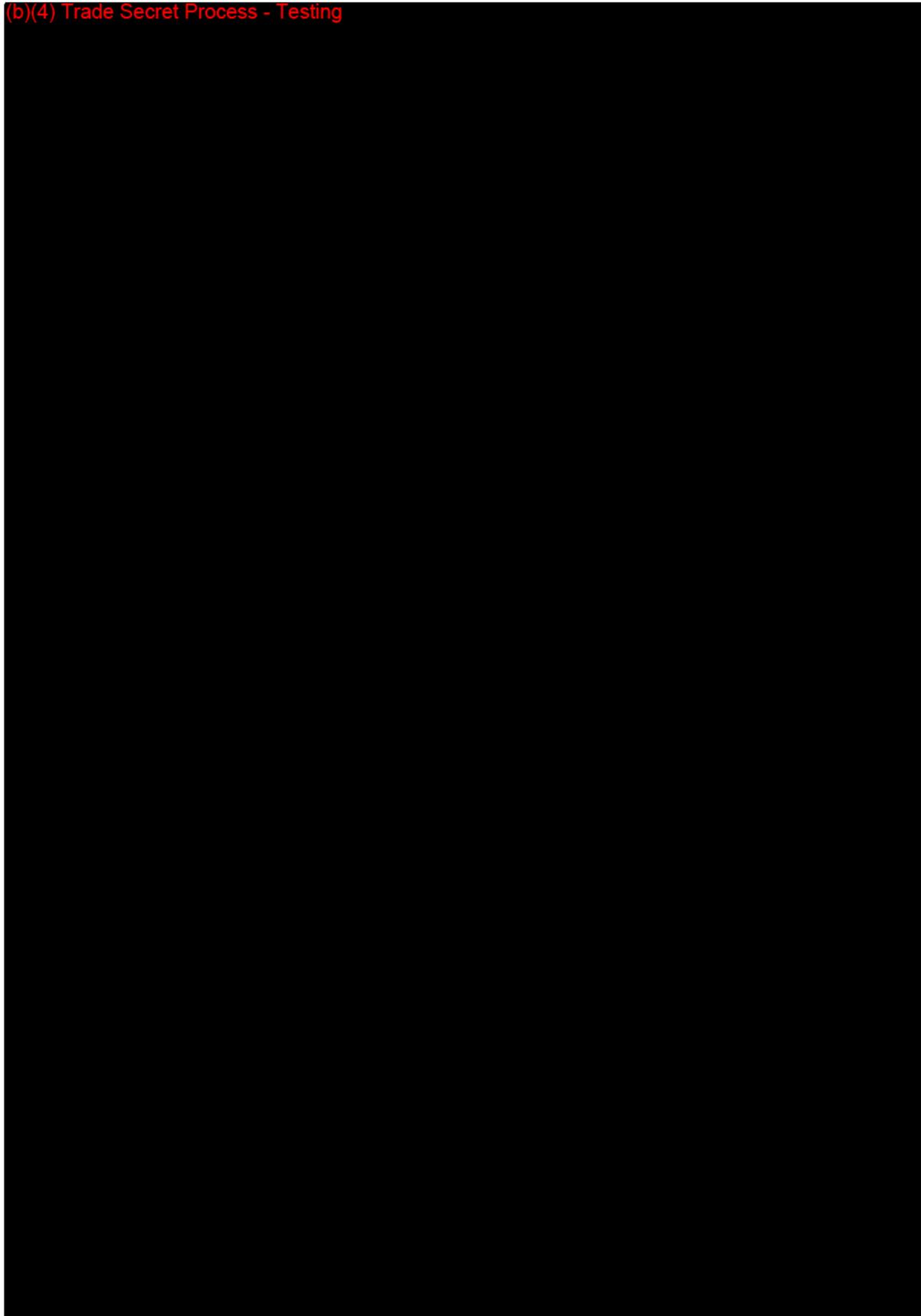
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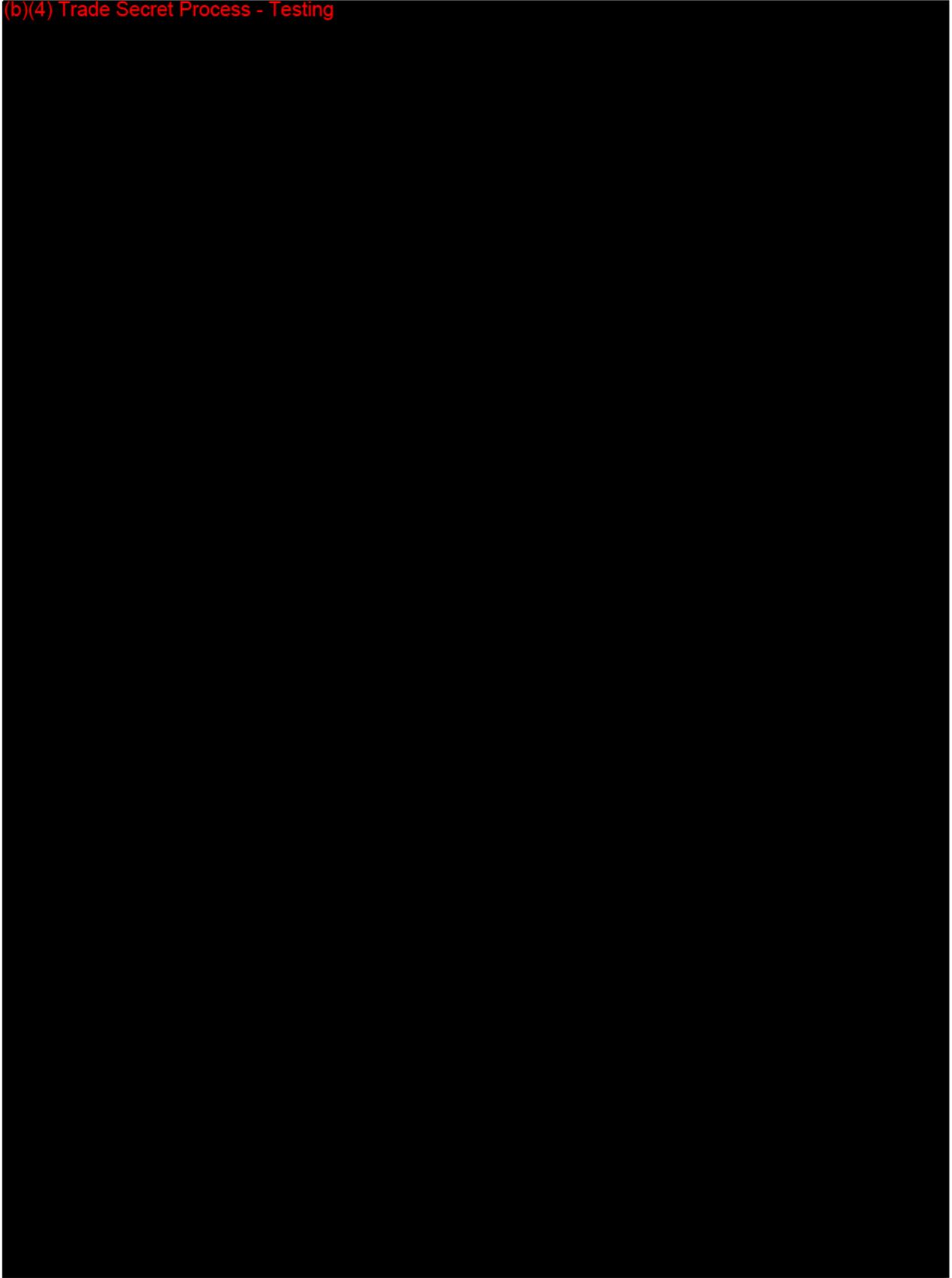
(b)(4) Trade Secret Process - Testing



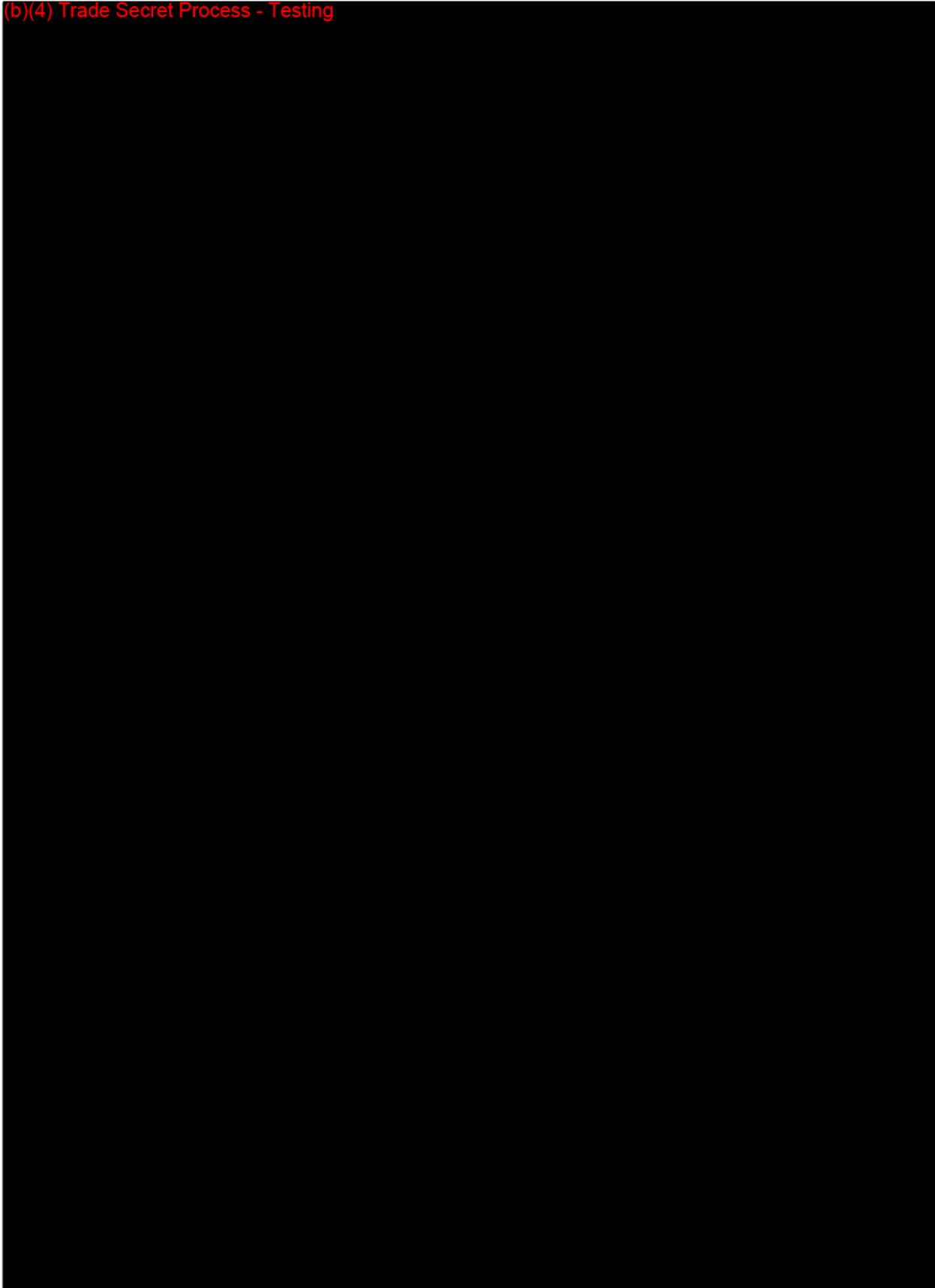
(b)(4) Trade Secret Process - Testing



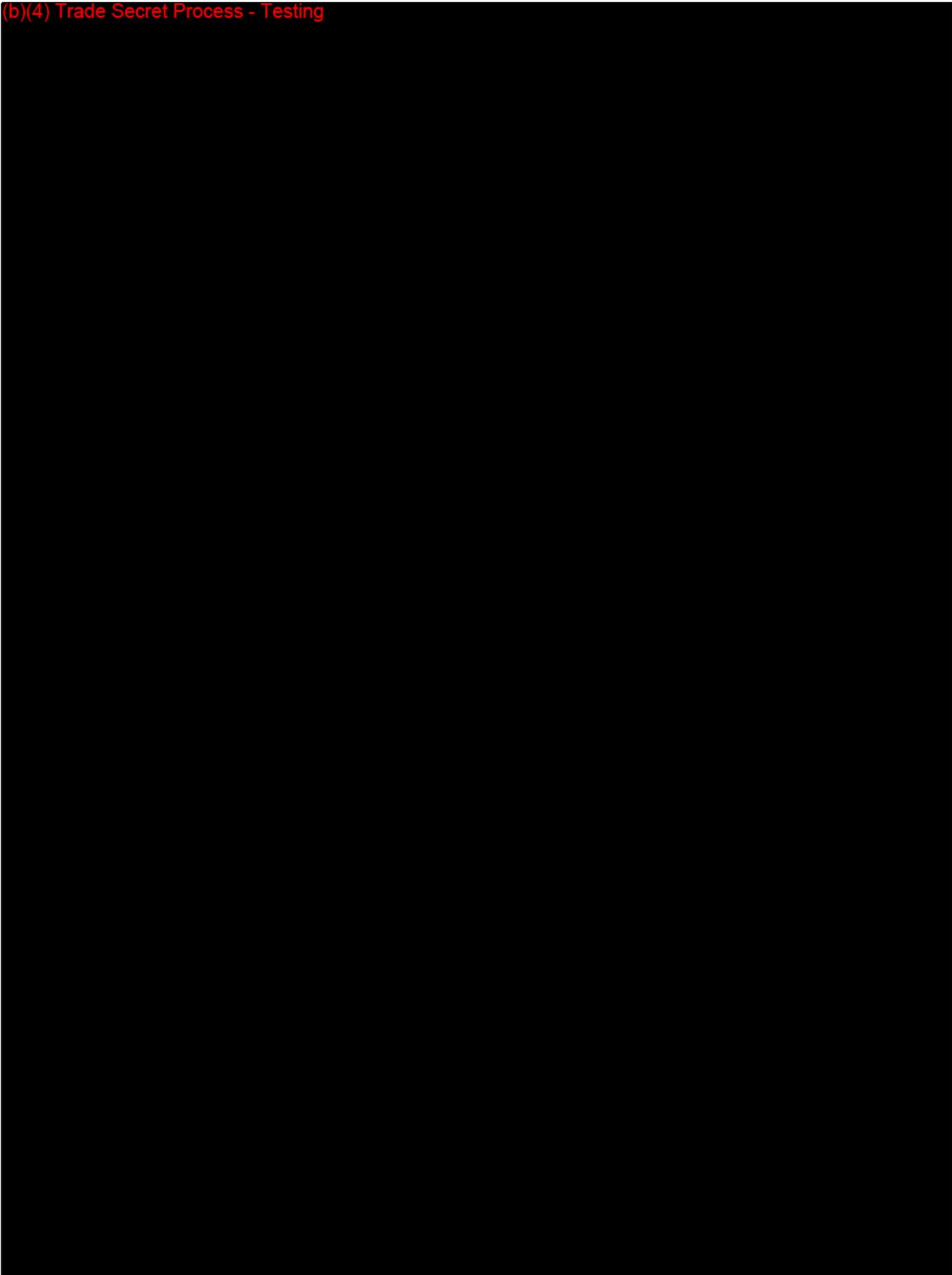
(b)(4) Trade Secret Process - Testing



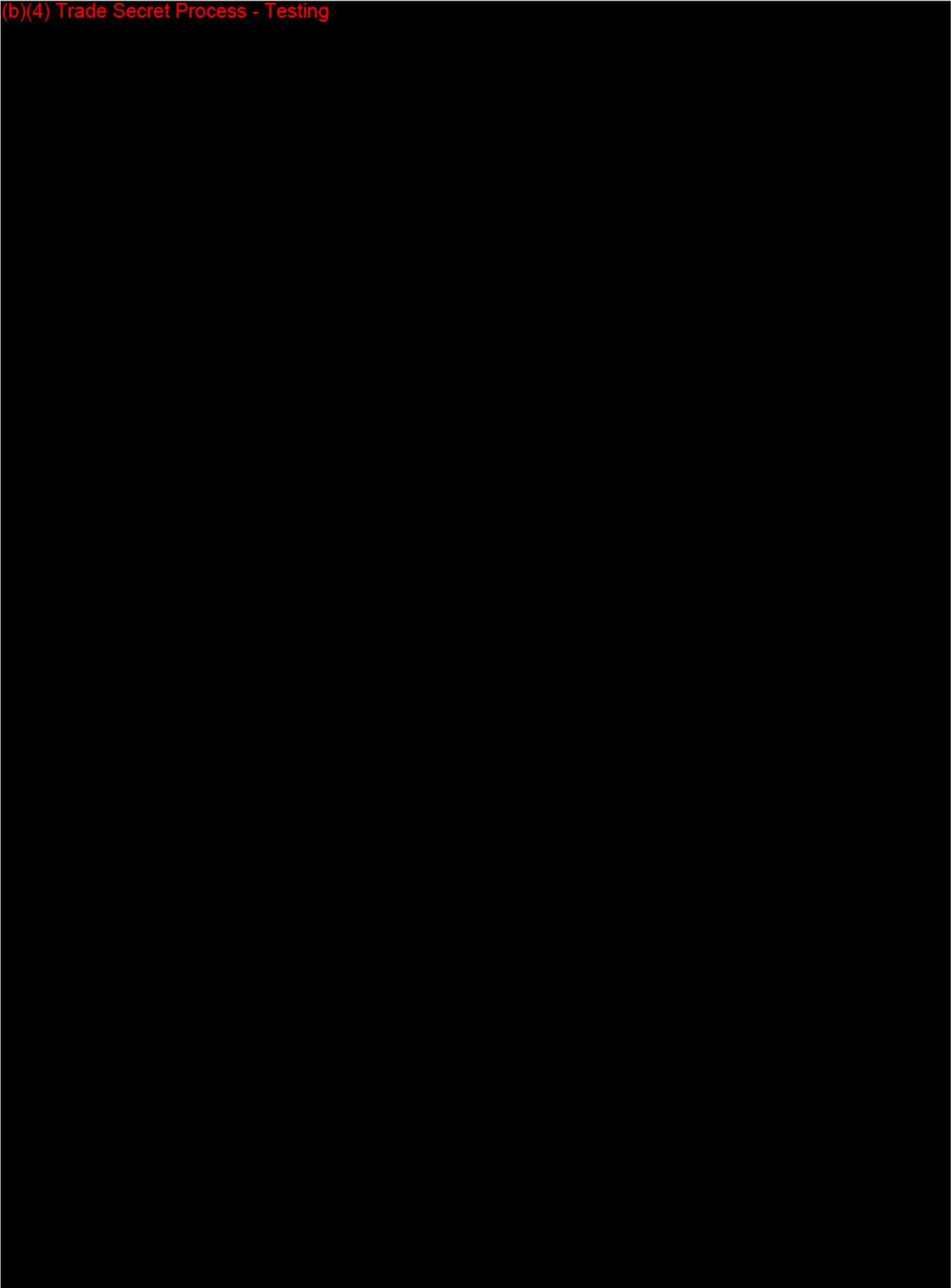
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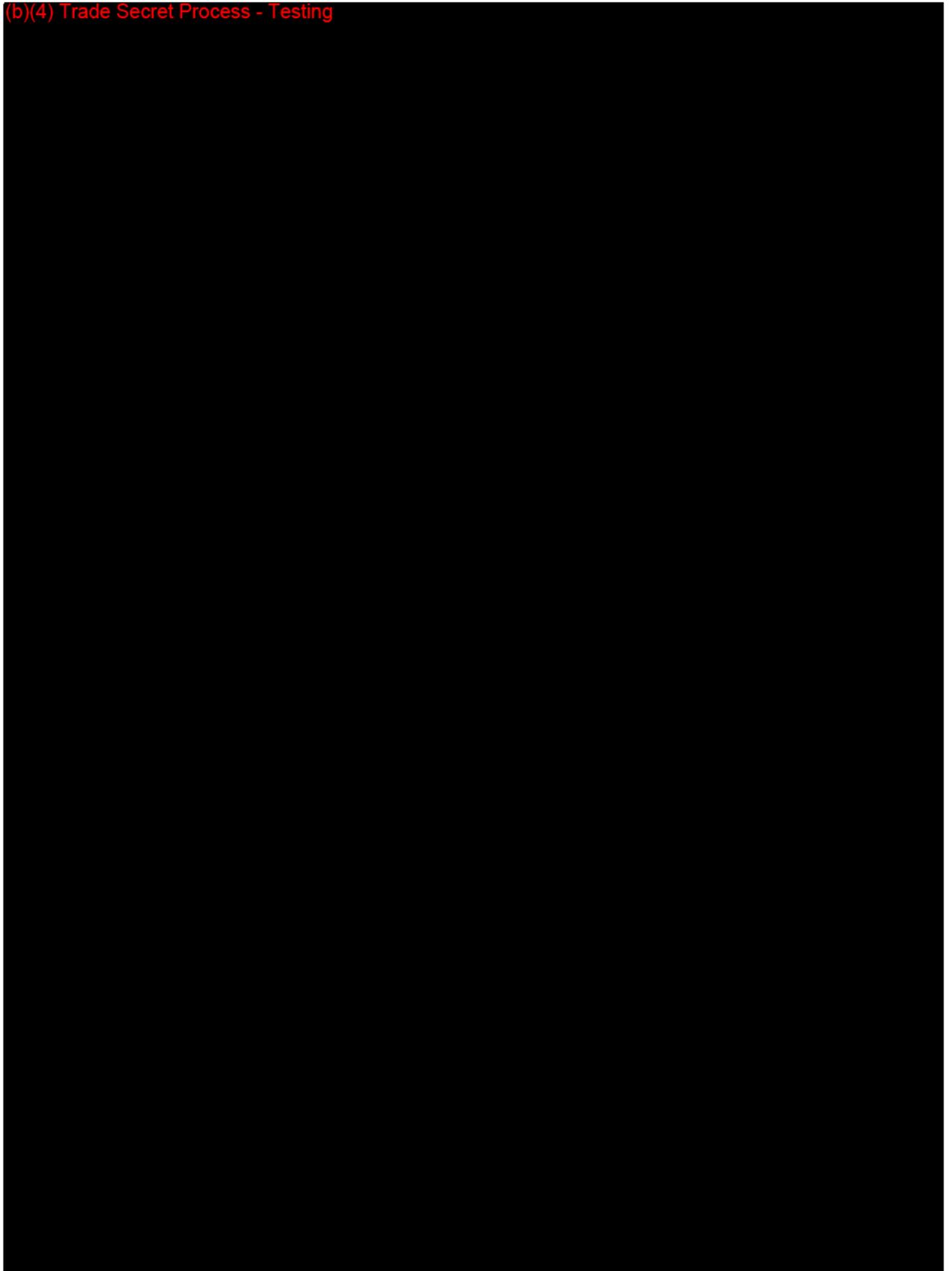
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(b)(4) Trade Secret Process - Testing



(b)(4) Trade Secret Process - Testing



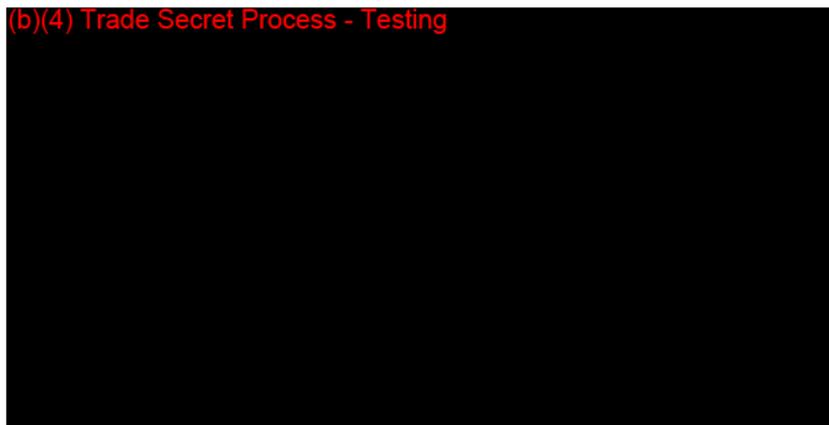
(b)(4) Trade Secret Process - Testing



1. Comparison Analysis:

The above data was analyzed according to CLSI EP12-A2 and presented in a 2x2 contingency tables (Table 13, 15, and 17). The sensitivity, specificity, and 95% confidence intervals (CI), are also presented (Tables 14, 16 and 18).

(b)(4) Trade Secret Process - Testing



(b)(4) Trade Secret Process - Testing

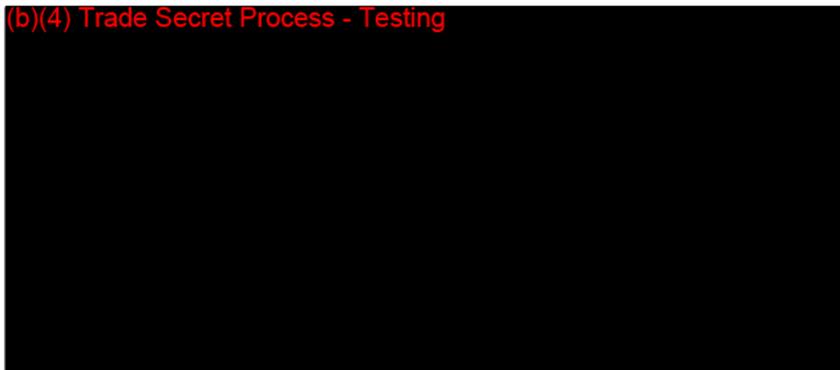


Table 17. CSF 2x2 Contingency Table: Culture/India Ink

		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	65	0
	Negative	0	99

Table 18. CSF Statistical Analysis: Culture/India Ink

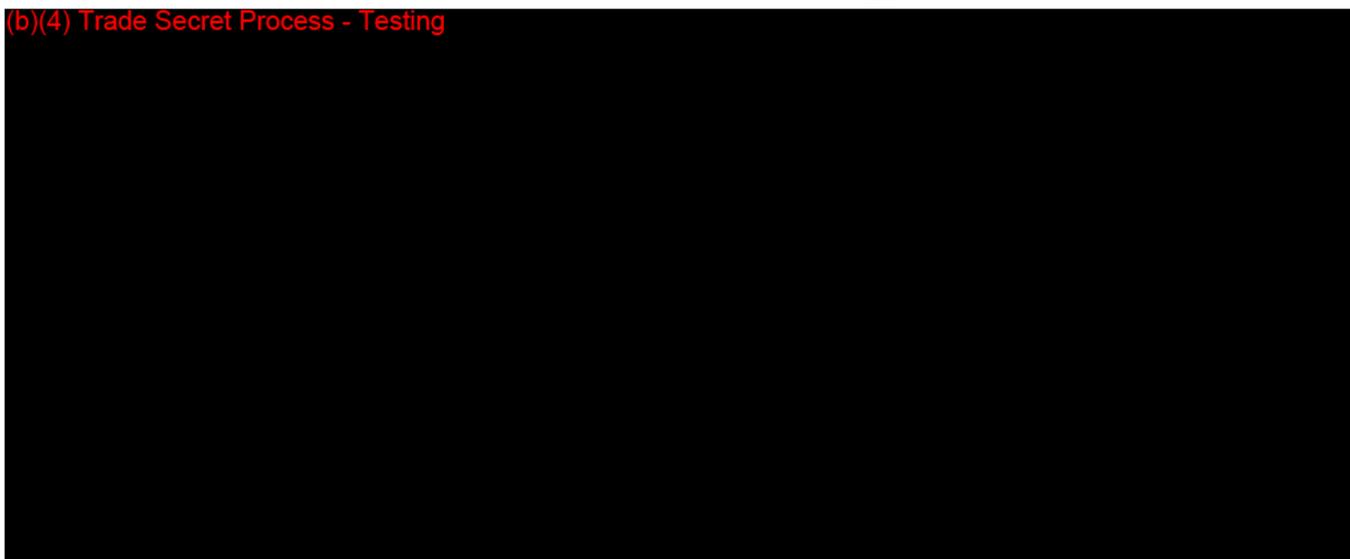
	Calculated	95% CI
Sensitivity	100%	94.4-100.0%
Specificity	100%	96.3-100%

2. Comparison Conclusions:

When the CrAg Lateral Flow Assay is compared to the gold standard in cryptococcosis diagnosis (culture or India Ink) using serum specimens, the new device is proven to be equivalent.

H. Other Method Comparisons – Semi-Quantitative Latex Agglutination

(b)(4) Trade Secret Process - Testing



(b)(4) Trade Secret Process - Testing



2. Semi-Quantitative Results:

Table 19. Semi-Quantitative Method Comparison Raw Data

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(b)(4) Trade Secret Process - Testing



3. Semi-Quantitative Method Comparison Analysis:

Cryptococcus Antigen Latex titer (LA) versus CrAg Lateral Flow Assay titer (LFA) (1:n) was plotted and regression analysis was performed (Figure 2).

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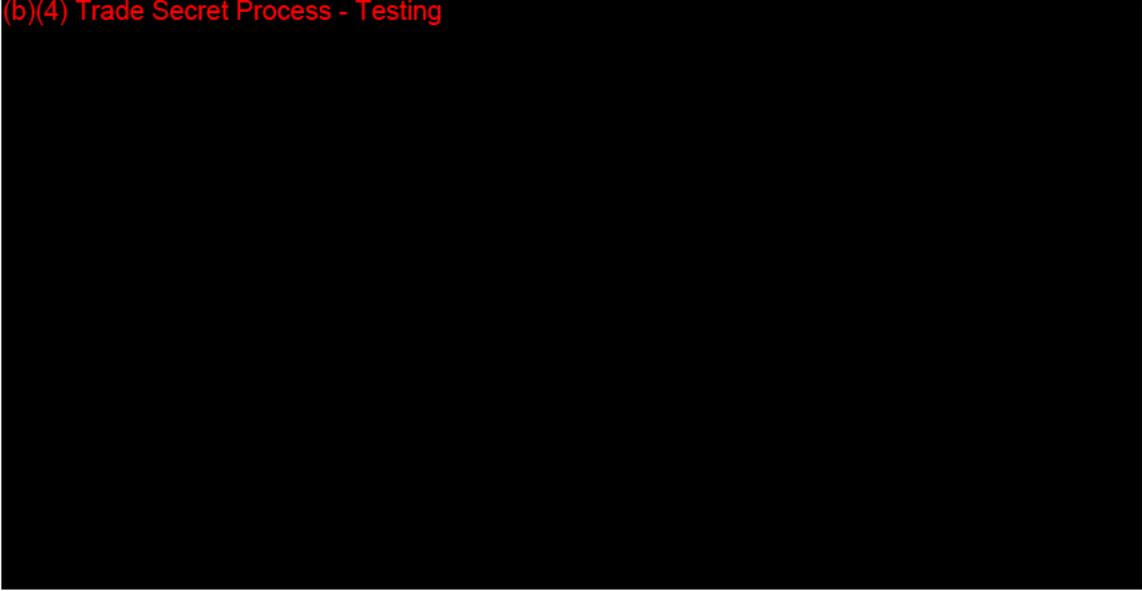


Figure 2. Crypto LA Titer vs LFA Titer

4. Semi-Quantitative Method Comparison Conclusions:

A strong correlation ($r^2 = 0.905$) exists when titers are determined on positive specimens using the CrAg Lateral Flow Assay and compared to the titers determined on the Latex-*Cryptococcus* Antigen Detection System.

Final Conclusions:

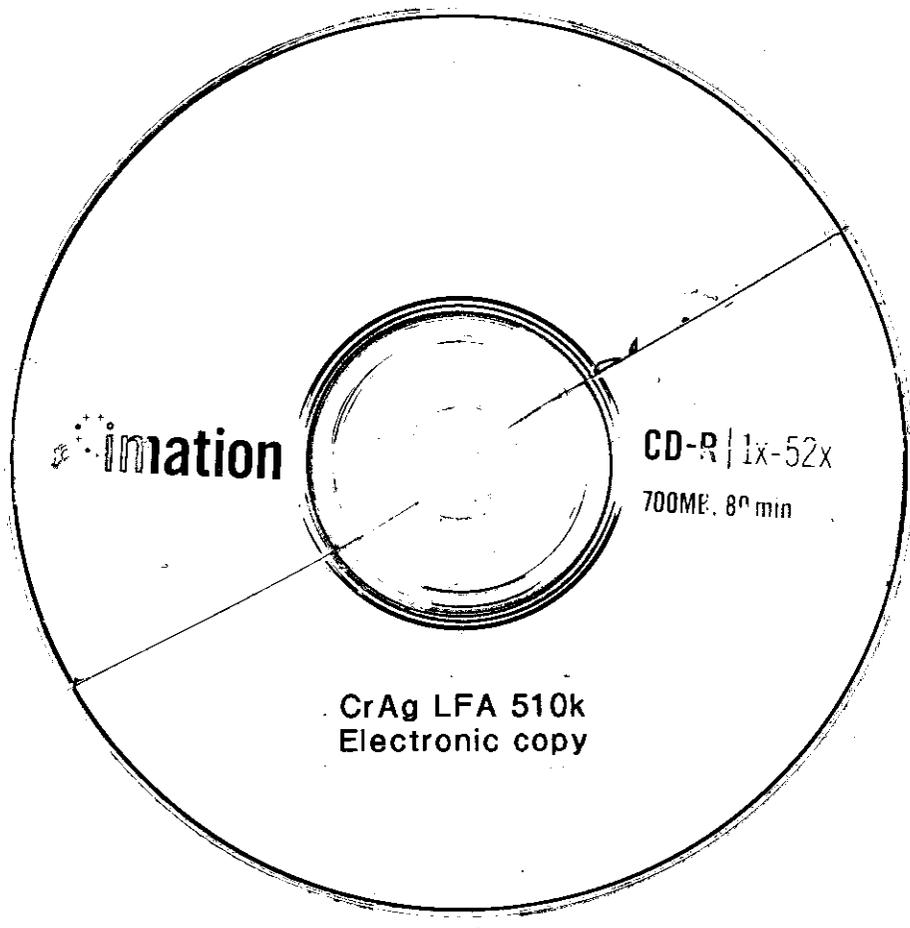
The information submitted in this premarket notification is complete and supports a substantial equivalence decision.

Section 19 Performance Testing - Animal

N/A

Section 20
Performance Testing - Clinical

N/A





COVER SHEET MEMORANDUM

From: Reviewer Name **Michael W. White**

Subject: 510(k) Number **K112422/S1**

To: The Record

Please list CTS decision code **CS**

- Refused to accept (Note: this is considered the first review cycle, See Screening Checklist http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_5631/Screening%20Checklist%207%202%2007.doc)
- Hold (Additional Information or Telephone Hold).
- Final Decision (SE, SE with Limitations, NSE (select code below), Withdrawn, etc.).

Not Substantially Equivalent (NSE) Codes

- NO NSE for lack of predicate
- NI NSE for new intended use
- NQ NSE for new technology that raises new questions of safety and effectiveness
- NP NSE for lack of performance data
- NM NSE requires PMA
- NS NSE no response
- NH NSE for another reason

Please complete the following for a final clearance decision (i.e., SE, SE with Limitations, etc.):		YES	NO
Indications for Use Page	<i>Attach IFU</i>	X	
510(k) Summary /510(k) Statement	<i>Attach Summary</i>	X	
Truthful and Accurate Statement.	<i>Must be present for a Final Decision</i>	X	
Is the device Class III?			X
If yes, does firm include Class III Summary?	<i>Must be present for a Final Decision</i>		
Does firm reference standards? (If yes, please attach form from http://www.fda.gov/opacom/morechoices/fdaforms/FDA-3654.pdf)		X	
Is this a combination product? (Please specify category _____, see http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_413b/CO_MBINATION%20PRODUCT%20ALGORITHM%20(REVISED%203-12-03).DOC)			X
Is this a reprocessed single use device? (Guidance for Industry and FDA Staff – MDUFMA - Validation Data in 510(k)s for Reprocessed Single-Use Medical Devices, http://www.fda.gov/cdrh/ode/guidance/1216.html)			X
Is this device intended for pediatric use only?			X
Is this a prescription device? (If both prescription & OTC, check both boxes.)		X	
Did the application include a completed FORM FDA 3674, Certification with Requirements of ClinicalTrials.gov Data Bank?		X	
Is clinical data necessary to support the review of this 510(k)? For United States-based clinical studies only : Did the application include a completed FORM FDA 3674, <i>Certification with Requirements of ClinicalTrials.gov Data Bank?</i> (If study was conducted in the United States, and FORM FDA 3674 was not included or incomplete, then applicant must be contacted to obtain completed form.)		X	
Does this device include an Animal Tissue Source?			X
All Pediatric Patients age<=21			

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K112422

B. Purpose for Submission:

To obtain substantial equivalence for a modification to add CSF to the original 510(k) device which detects Cryptococcal Antigen.

C. Measurand:

Capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*)

D. Type of Test:

Qualitative and semi-quantitative dipstick sandwich lateral flow immunochromatographic assay

E. Applicant:

Immuno-Mycologics, Inc.

F. Proprietary and Established Names:

CrAg Lateral Flow Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
GMD	II	866.3165	83-Microbiology

H. Intended Use:

1. Intended use:

The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of the capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum and cerebral spinal fluid (CSF).

2. Indication for use:

The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum, and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription use laboratory assay which can aid in the diagnosis of cryptococcosis

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Not applicable

I. Device Description:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay which detects cryptococcal antigen in cerebral spinal fluid (CSF). The assay consists of CrAg Lateral Flow test strips which have a gold-conjugated antibody and a gold-conjugated, anti-cryptococcal antibody deposited onto a sample membrane and anti-Crypto antibody and control-line capture antibody striped onto a membrane. The kit also includes a specimen diluent.

J. Substantial Equivalence Information:

1. Predicate Device name

Immuno-Mycologics' CrAg Lateral Flow Assay

2. Predicate K number:

K102286

Comparison with predicate:

Table 1: Comparison Between New Device and Predicate Device

SIMILARITIES		
Feature	CrAg LFA (New Device – K112422)	CrAg LFA (Serum Only) (K102286)
Indication For Use	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis
Device Description		
Technology	Lateral Flow Assay	Lateral Flow Assay
Instruments	None	None
Assay Components	Specimen diluent, lateral flow strips, built-in control, gold conjugated antibodies	Specimen diluent, lateral flow strips, built-in control, gold conjugated antibodies
Specimen Pre-Treatment	Dilution	Dilution
Detection Antibody	Anti-cryptococcal monoclonal antibody	Anti-cryptococcal monoclonal antibody
Storage Requirements	20-25°C	20-25°C
DIFFERENCES		
Feature	Cryptococcal Antigen Lateral Flow: New Device – K112422	Cryptococcal Antigen Lateral Flow: Serum Only – K102286
Intended Use		
Intended Use	Immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus species complex</i> (<i>Cryptococcus neoformans</i> and <i>Cryptococcus gatti</i>) in serum and CSF	Immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus species complex</i> (<i>Cryptococcus neoformans</i> and <i>Cryptococcus gatti</i>) in serum
Sample Matrix	CSF	Serum

K. Standard/Guidance Document Referenced (if applicable):

Not Applicable

L. Test Principle:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in CSF. For the qualitative procedure,

specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For the semi-quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the qualitative procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold-conjugated, anti-cryptococcal monoclonal antibodies and gold-conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it binds to the gold-conjugated, anti-cryptococcal antibodies. The gold-labeled antibody-antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti-cryptococcal monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold-labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold-conjugated control goat IgG antibody to move to the Control Line (C) which is immobilized bovine anti-goat IgG antibody. The immobilized anti-goat antibody will bind to the gold-conjugated goat IgG Control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line (Figure 1). If the control line fails to develop a line, then the test is not valid.

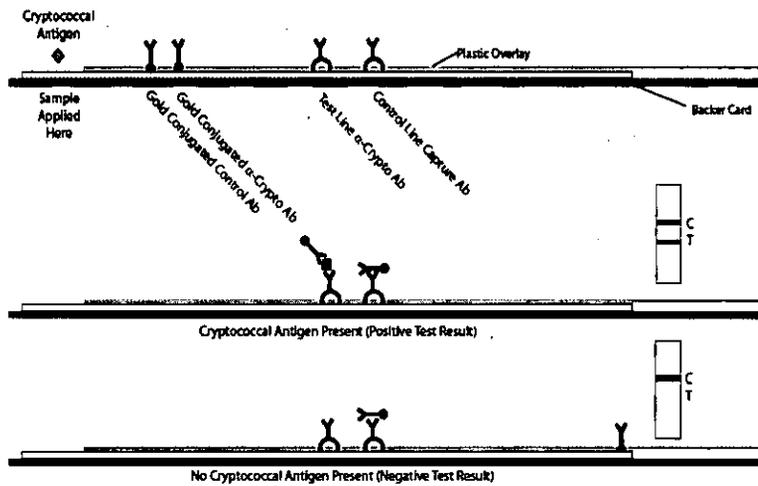


Figure1. CrAg Lateral Flow Assay Schematic

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The CrAg Lateral Flow Assay was evaluated for reproducibility and precision by spiking mock CSF samples with cryptococcal antigen to produce a panel consisting of a negative sample, a high-negative (C₅) sample, a low-positive sample and a moderate-positive sample. This panel was tested twice per day at three sites with a total of five operators over a five-day period in order to determine both the inter-lab and the intra-lab reproducibility and precision of the assay. The results of this study are shown in the tables below.

Table 2. Repeatability at 3 Different Sites

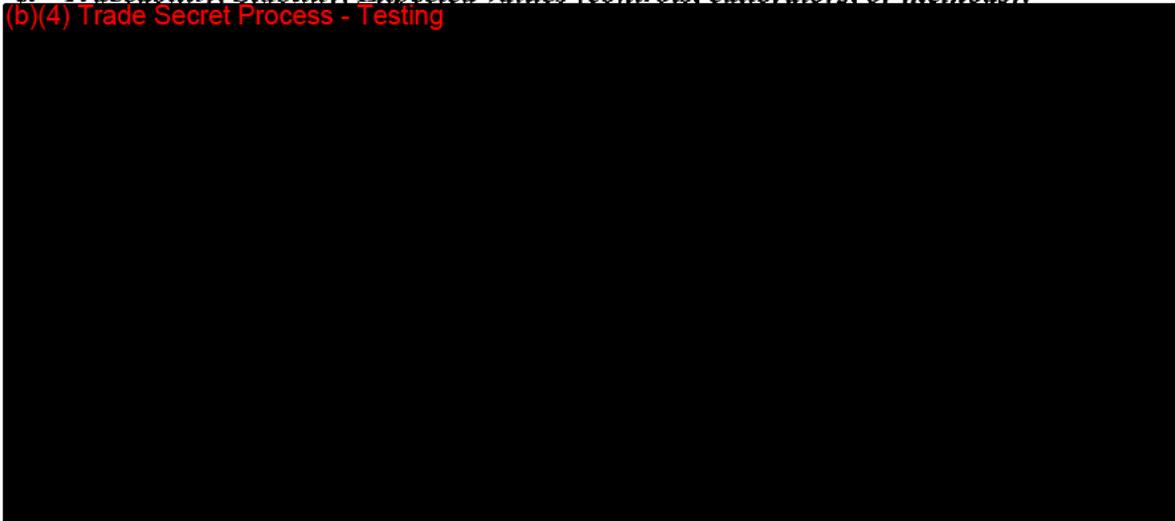
CSF PANEL	Site 1 % Pos	Site 2 % Pos	Site 3 % Pos	Overall % Pos
Negative	0% (0/30)	0% (0/30)	0% (0/15)	0% (0/75)
High Negative	10% (3/30)	0% (0/30)	0% (0/15)	3% (2/75)
Low Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)
Moderate Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)

b. Linearity/assay reportable range:

Not Applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

(b)(4) Trade Secret Process - Testing



d. Detection limit:

Detection Limit/Analytical Cut-off

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running varying concentrations of cryptococcal antigen diluted in LF Specimen Diluent. The concentration where 50% of the results were positive and 50% of the results were negative determined our analytical cut-off. The analytical cut-off is 1.25ng/ml.

Table 3. Analytical Cut-Off

Sample Concentration (ng/ml)	No. Positive	No. Tested	% Positive
0.50	0	24	0%
0.75	0	24	0%
1.00	4	24	17%
1.25	12	24	50%
1.50	21	24	88%
1.75	24	24	100%
2.00	24	24	100%
2.50	24	24	100%
3.00	24	24	100%

Analytical specificity:

Analytical specificity for the CrAg Lateral Flow Assay was determined by evaluating potentially cross-reacting medical conditions unrelated to cryptococcosis. Specimens were tested in triplicate. Percent positive was determined for each condition (Table 3).

Table 3. Analytical Specificity

Pathology	# of Samples	% Positive
Penicilliosis	5	0 % (0/5)
Sporothrichosis	6	0 % (0/6)
HAMA	5	0 % (0/5)
Syphilis	10	0 % (0/10)
Rubella	5	0 % (0/5)
Mycoplasmosis	10	0 % (0/10)
Toxoplasmosis	7	0 % (0/7)
CMV	10	0 % (0/10)
Blastomycosis	10	0 % (0/10)
Coccidiomycosis	10	0 % (0/10)
Histoplasmosis	10	0 % (0/10)
Candidiasis	10	0 % (0/10)
Aspergillus GM+	10	10 % (1/10)
Rheumatoid Factor*	10	0 % (0/10)

* Rheumatoid factor concentrations tested ranged from 112IU/ml to 6479IU/mls.

Additionally, cross-reactivity was assessed by testing crude culture filtrate antigens at a range of concentrations using the CrAg Lateral Flow Assay at high concentrations (>0.1 mg/ml), antigens from *Paracoccidioides brasiliensis* exhibited some cross-reactivity. Antigens from *Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus* did not exhibit cross-reactivity.

The assay was not evaluated for cross-reactivity against the following organisms or pathologies:

<i>Candida dubliniensis</i>	<i>Pneumocystis carinii</i>
<i>Candida tropicalis</i>	<i>Trichosporon beigeli</i>
<i>Candida parapsidosis</i>	<i>Zygomycetes</i>
<i>Candida krusei</i>	Antinuclear antibody +
<i>Candida glabrata</i>	Hepatitis A Virus
<i>Cladosporium trichoides</i>	Hepatitis C Virus
<i>Neisseria meningitidis</i>	<i>Staphylococcus</i> spp.
<i>Salmonella typhi</i>	<i>Streptococcus pneumonia</i>
<i>Mycobacterium tuberculosis</i>	<i>Streptococcus</i> spp.
<i>Enterovirus</i>	<i>Diphtheroid</i>
<i>Enterobacteriaceae</i>	<i>H. influenzae</i> type B
<i>Enterococcus</i> spp.	<i>Herpes simplex</i> viruses
<i>Epstein Barr</i>	<i>Listeria monocytogenes</i>
<i>Trichosporon beigeli</i>	Syneresis fluid condensation
	<i>Staphylococcus aureus</i>

The assay was not evaluated for potential interference related to specimen pretreatment with 2-mercaptoethanol or with specimens including the following substances: bloody CSF, cloudy CSF, white blood cells, xanthochromic CSF, bilirubin, protein, systemic lupus erythmatosus (SLE), sarcoidosis, or *N.memingitides*.

The effect of pronase on the CrAg LFA was determined by pronase-treating five Cryptococcal EIA positive specimens and five Cryptococcal EIA negative specimens. The samples were analyzed both untreated and pronase-treated. All treated, positives samples remained positive and all treated, negative samples remained negative. Therefore, pronase does not affect the CrAg LFA.

2. Comparison studies:

a. Method comparison with predicate device:

Not Applicable

b. Matrix comparison:

A matrix comparison study was performed on 86 paired serum and CSF specimen

that were collected prospectively. Percent agreement positive is 100%. Percent agreement negative is 88.9%, and overall agreement is 96.5%. Of the 3 discrepant results, one patient was diagnosed with cryptococcal meningitis approximately 5-6 week after the LFA was performed.

3. Clinical studies:

The CrAg Lateral Flow Assay was compared to the standard reference method for diagnoses of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay. These studies contained prospective specimens. A summary of the data collected is included in the following table.

CSF	CrAg LFA Assay		Culture/India Ink	
			Positive	Negative
		Positive	65	1
		Negative	0	77

CSF		Calculated	95% CI
	Sensitivity	100%	94.4% - 100%
	Specificity	98.7%	93.1% - 99.8%

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

(b)(4) Trade Secret Process - Testing

N. Proposed Labeling:

The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

P. Other Supportive Device and Instrument Information:

Q. Administrative Information:

1. Applicant contact information:

a. *Name of applicant:*

Immuno-Mycologics, Inc.

b. *Mailing address:*

2700 Technology Place.

Norman, OK 73071

c. *Phone #:* (405) 360-4669

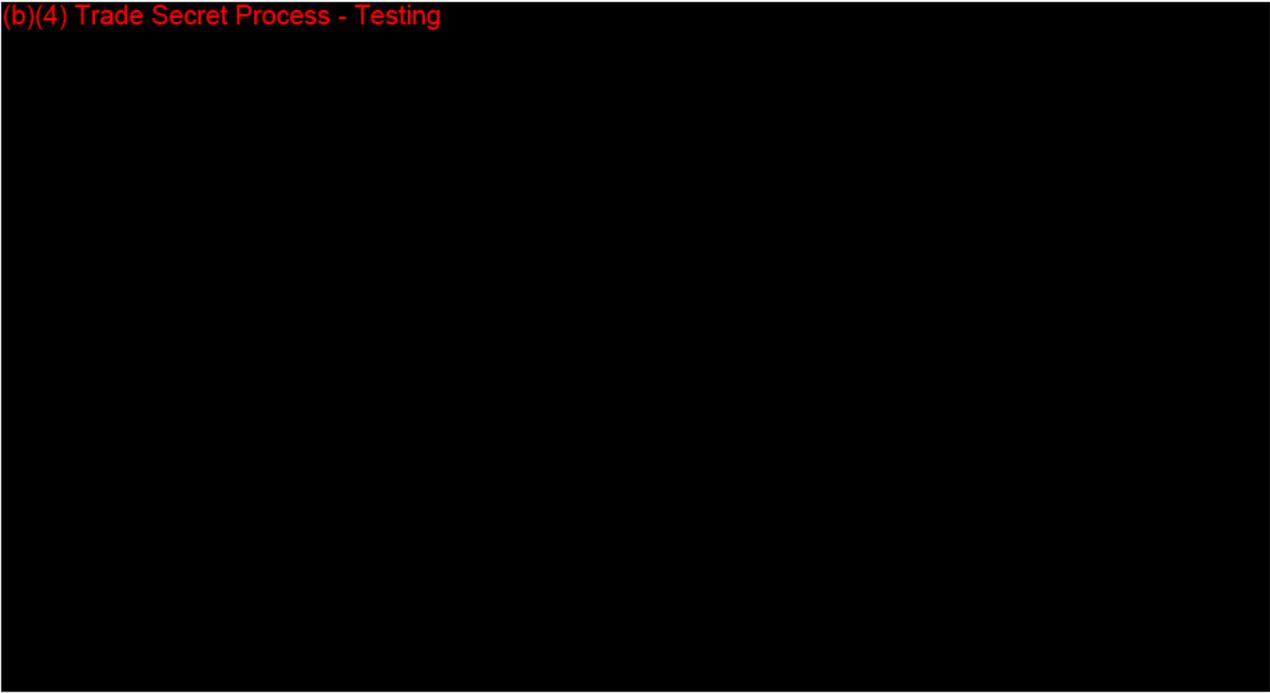
d. *Fax #:* (405)364-1058

e. *E-mail address (optional):* sean-bauman@immy.com

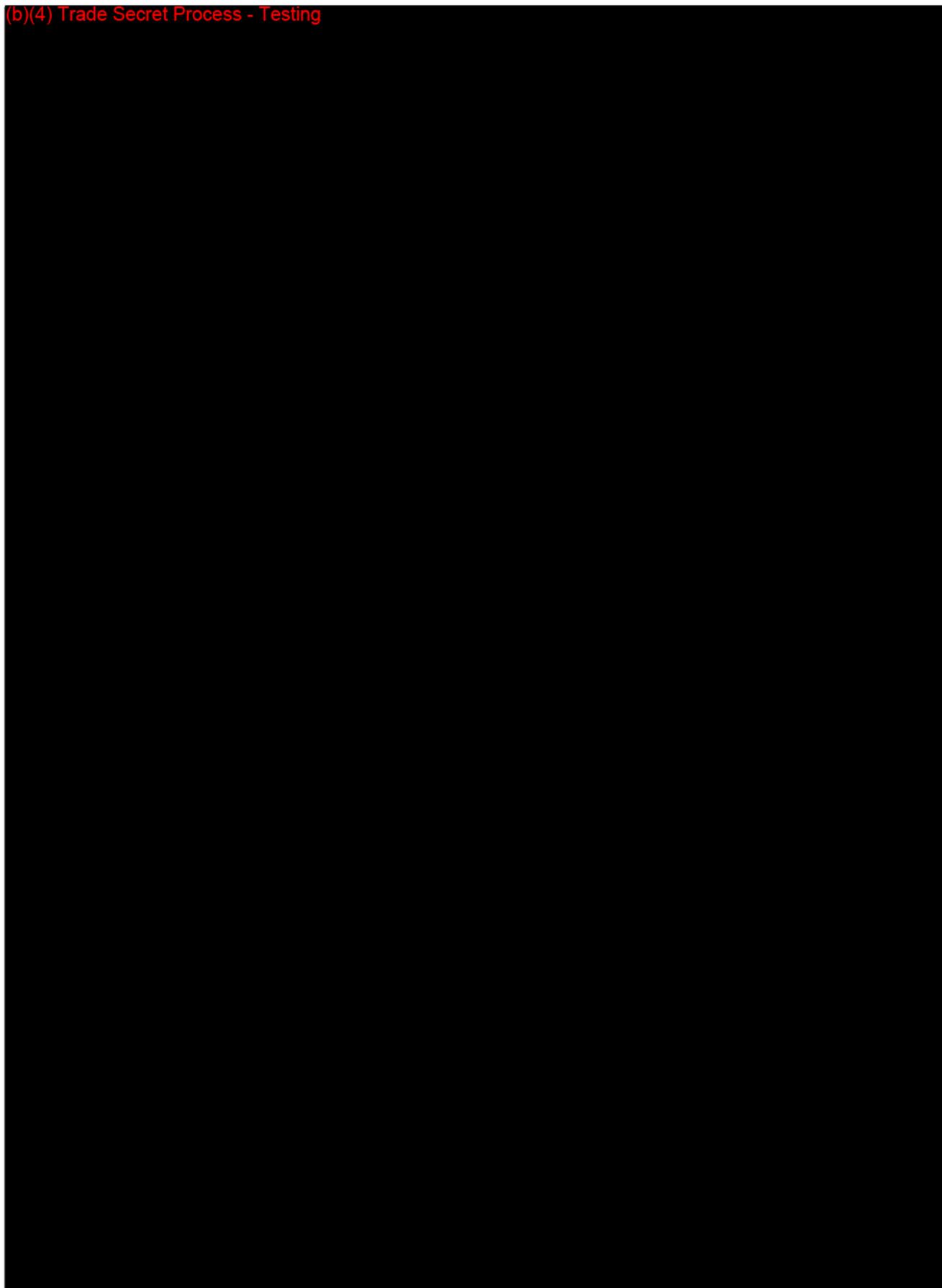
f. *Contact:* Sean Bauman

2. Review documentation:

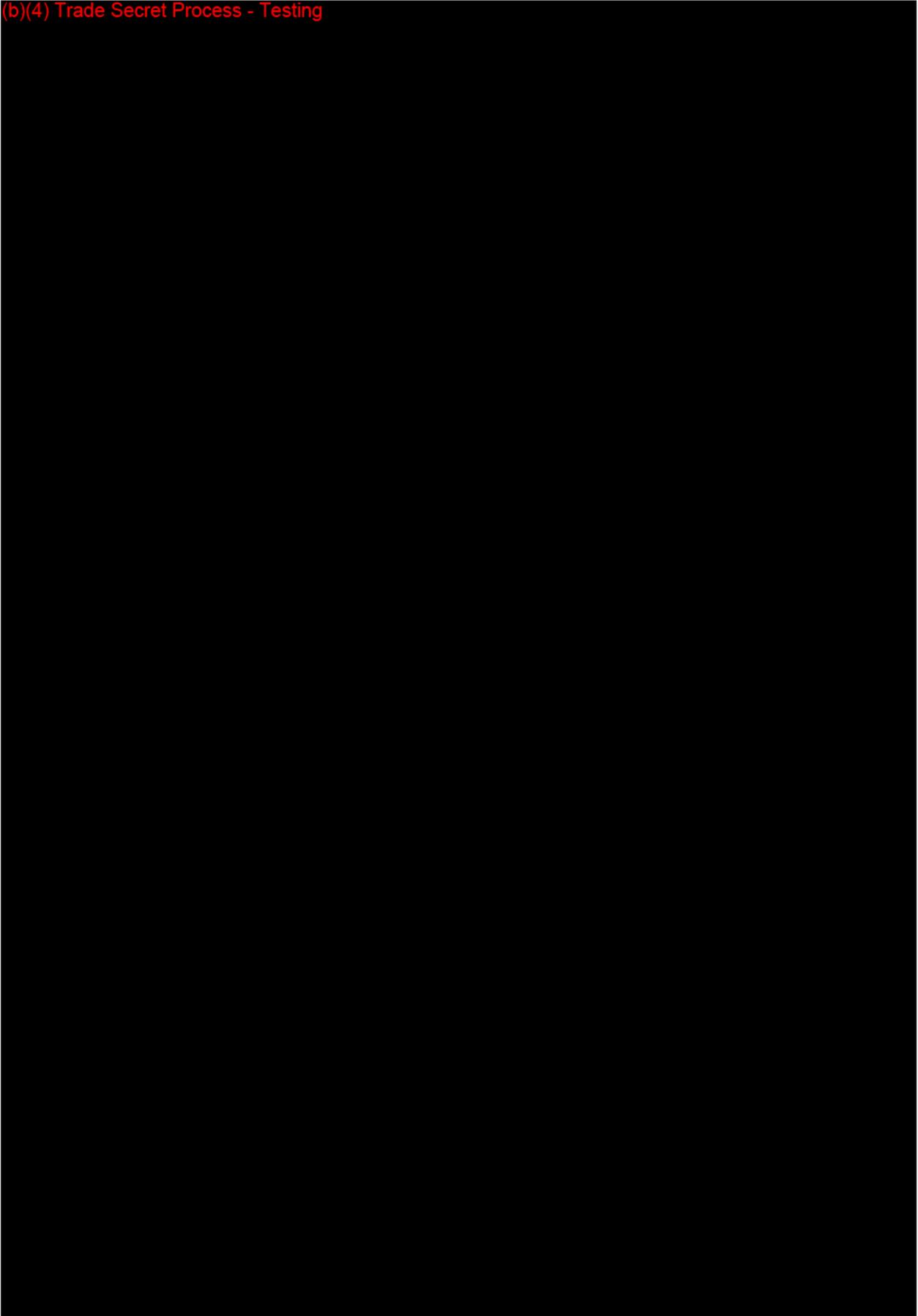
(b)(4) Trade Secret Process - Testing



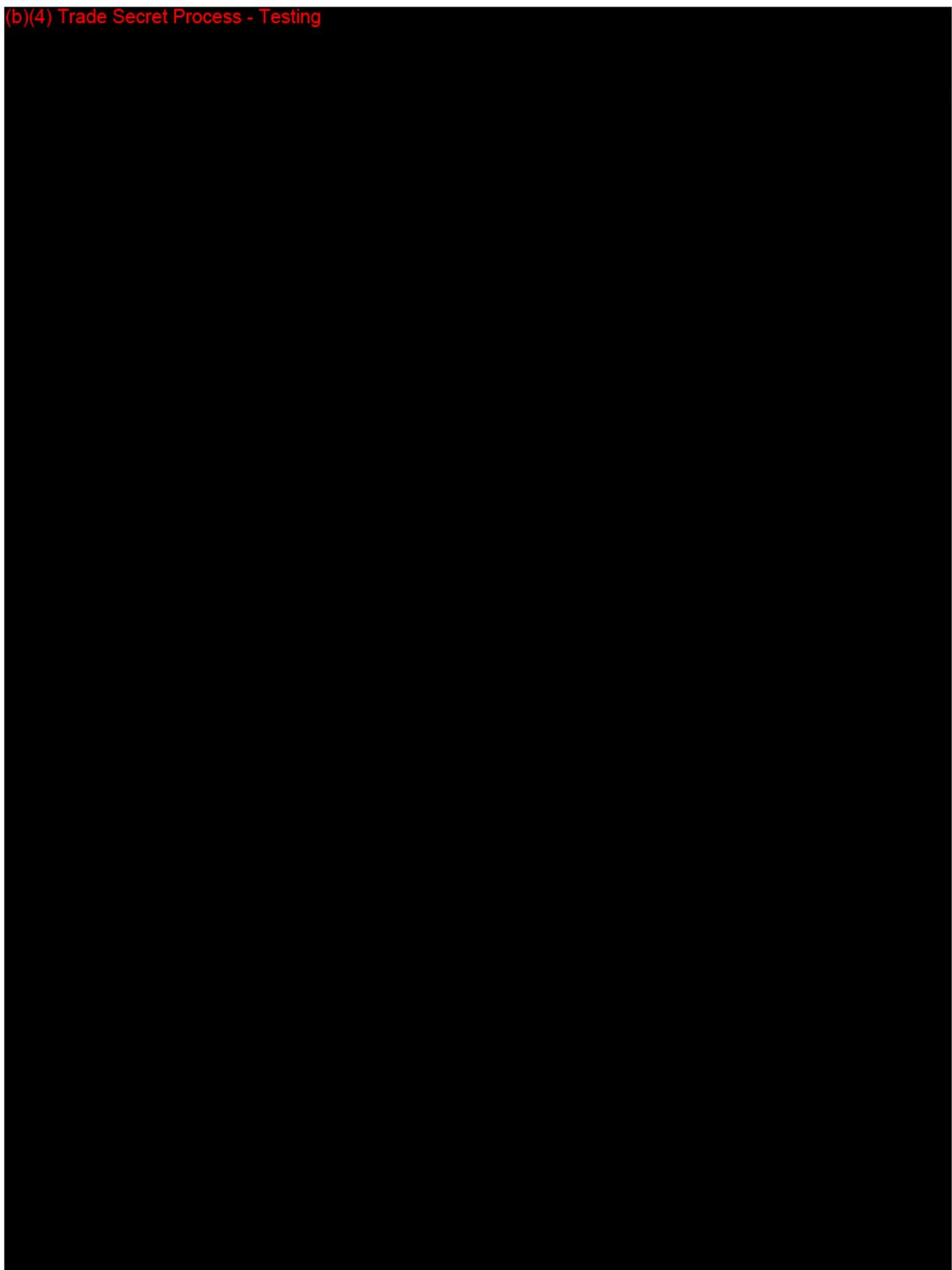
(b)(4) Trade Secret Process - Testing



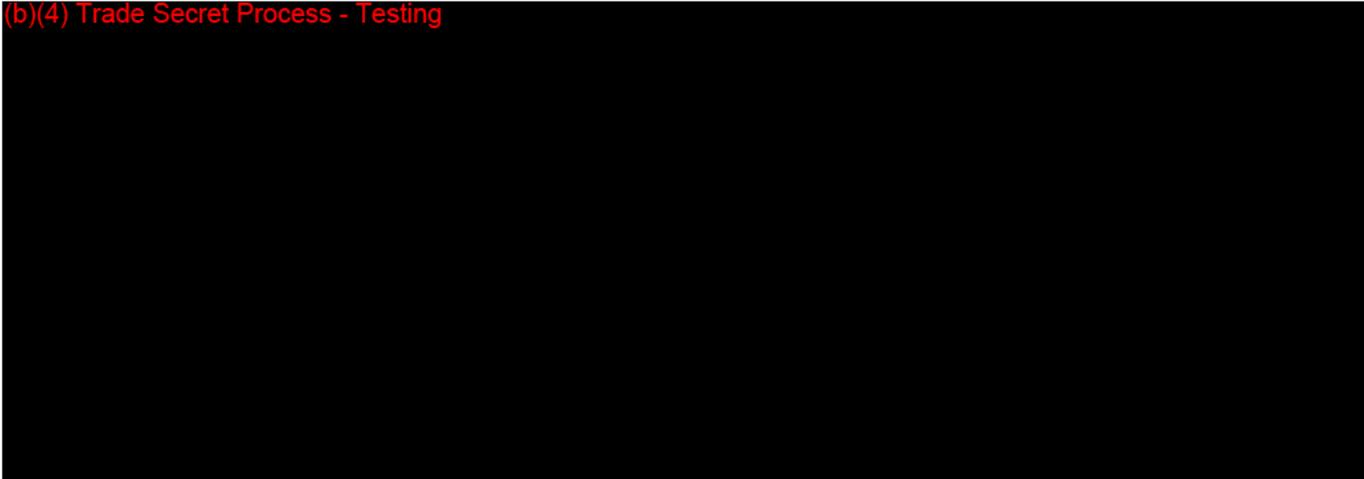
(b)(4) Trade Secret Process - Testing



(b)(4) Trade Secret Process - Testing



(b)(4) Trade Secret Process - Testing



Substantial Equivalence Discussion:

	Yes	No	
1. Same Indication Statement?	X		If YES = Go To 3
2. Do Differences Alter The Effect Or Raise New Issues of Safety Or Effectiveness?			If YES = Stop NSE
3. Same Technological Characteristics?	X		If YES = Go To 5
4. Could The New Characteristics Affect Safety Or Effectiveness?			If YES = Go To 6
5. Descriptive Characteristics Precise Enough?		X	If NO = Go To 8 If YES = Stop SE
6. New Types Of Safety Or Effectiveness Questions?			If YES = Stop NSE
7. Accepted Scientific Methods Exist?			If NO = Stop NSE
8. Performance Data Available?	X		If NO = Request Data
9. Data Demonstrate Equivalence?	X		Final Decision:

Note: See

http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_4148/FLOWCHART%20DECISION%20TREE%20.DOC for

Flowchart to assist in decision-making process. Please complete the following table and answer the corresponding questions. "Yes" responses to questions 2, 4, 6, and 9, and every "no" response requires an explanation.

e. Explain how descriptive characteristics are not precise enough:

(b)(4) Trade Secret Process - Testing

i. Explain how the performance data demonstrates that the device is or is not substantially equivalent:

(b)(4) Trade Secret Process - Testing



R. Reviewer Name and Signature:

Michael W. White
CDRH/OIVD/DMD



Product Code **GMD**
 Manufacturer **IMMUNO-MYCOLOGICS, INC.**
 Class **2**
 Regulation Device Classification **Cryptococcus neoformans serological reagents.**
 Device Name **ANTISERA, LATEX AGGLUTINATION, CRYPTOCOCCUS NEOFORMANS**
 Date Last Listed **12/09/2011**

CDRH Gen Docs with Manufacturer (None)

Premarket Reviews Completed (1)

<u>CTS</u>	K102286	CRAG LATERAL FLOW ASSAY (CRAG LFA)	SE	Michael White
<u>Image</u>	FDA.GOV	THE CRYPTOCOCCAL ANTIGEN LATERAL FLOW ASSAY (CRAG LFA) IS AN IMMUNOCHROMATOGRAPHIC TEST SYSTEM FOR THE QUALITATIVE OR SEMI-QUANTITATIVE DETECTION OF CAPSULAR POLYSACCHARIDE ANTIGENS OF CRYPTOCOCCUS SPECIES COMPLEX (CRYPTOCOCCUS NEOFORMANS AND CRYPTOCOCCUS GATTII) IN SERUM. THE CRAG LATERAL FLOW ASSAY IS A PRESCRIPTION-USE LABORATORY ASSAY, WHICH CAN AID IN THE DIAGNOSIS OF CRYPTOCOCCOSIS.		
		Microbiology	RLP	

Under Review, Withdrawn or Closed without Product Code (1)

<u>CTS</u>	K112422	CRAG LATERAL FLOW ASSAY (LFA)	On Hold	Michael White
<u>Image</u>	FDA.GOV	CRYPTOCOCCAL ANTIGEN LATERAL FLOW ASSAY		
		Microbiology	NOEL.GERALD	

Standards and Guidance (None)

MDR Summary (None)

		Total
	0	
Total		

MDR Analyst (1)

William Shackelford	william.shackelford@fda.hhs.gov
---------------------	--

MDR Distribution by Brand - Death or Injury (None)

Patient Problems (None)

Patient Outcomes (None)

Device Problems (None)

Manufacturer Evaluation Results (None)

24

Manufacturer Evaluation Conclusions (None)

Recalls (None)

Inspections (None)

CDRH Gen Docs without Manufacturer (None)

Rad Health Reports (None)

Rad Health Correspondence (None)

Rad Health Adverse Events (None)

Rad Health EIRs (None)

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TPLC disclaimers

We are continuing to improve and enhance the TPLC Universe and the TPLC sheets. When more data is available we will update this message.

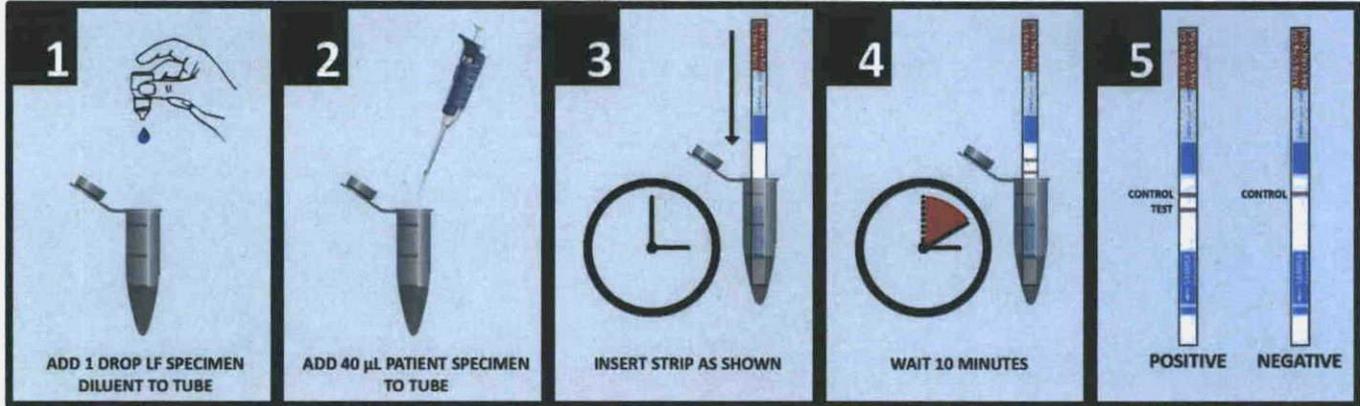
Please realize that in this initial release:

- Recall data is only available since October, 2009.
- In the Premarket Under Review section and the Recall section, the premarket submission or recall is only included if the product code is specified in CTS.
- The MDR count is a count of reports and contains duplicate reports.
- MDR track action or additional information letters is not available yet.
- EIR data related to inspections is not yet displayed.
- EIR data is accessed from CTS at this time, rather than FACTS.
- The TPLC name which consolidates variations on manufacturer names is not yet implemented.
- Publications are not yet linked in.
- Adverse events for radiation emitting products are submitted under 21 CFR 1002.20.
- Adverse events due to radiation problems for medical devices are submitted under 21 CFR 803.

We are working hard to address these issues and many can be addressed before the next release.



QUALITATIVE – BASIC PROCEDURE



INTENDED USE

The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of the capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription-use laboratory assay which can aid in the diagnosis of cryptococcosis.

SUMMARY and EXPLANATION of the Test

Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) (4). Individuals with impaired cell-mediated immunity are at greatest risk of infection (8). Cryptococcosis is one of the most common opportunistic infections in AIDS patients (6). Detection of cryptococcal antigen (CrAg) in serum and CSF has been extensively utilized with very high sensitivity and specificity (1-3).

BIOLOGICAL PRINCIPLES

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay. Specimens and specimen diluent are added into an appropriate reservoir, such as a test tube, and the lateral flow device is placed into the reservoir. The test uses specimen wicking to capture gold-conjugated, anti-CrAg monoclonal antibodies and gold-conjugated control antibodies deposited on the test membrane. If CrAg is present in the specimen, then it binds to the gold-conjugated, anti-CrAg antibodies. The gold-labeled antibody-antigen complex continues to wick up the membrane where it will interact with the test line, which has immobilized anti-CrAg monoclonal antibodies. The gold-labeled antibody-antigen complex forms a sandwich at the test line causing a visible line to form. With proper flow and reagent reactivity, the wicking of any specimen, positive or negative, will cause the gold-conjugated control antibody to move to the control line. Immobilized antibodies at the control line will bind to the gold-conjugated control antibody and form a visible control line. Positive test results create two lines (test and control). Negative test results form only one line (control). If a control line fails to develop then the test is not valid.

WARNINGS and PRECAUTIONS

For in Vitro Diagnostic Use only.

REAGENT PRECAUTIONS

1. Specific standardization is necessary to produce our high-quality reagents and materials. The user assumes full responsibility for any modification to the procedures published herein.

2. When handling patient specimens, adequate measures should be taken to prevent exposure to etiologic agents potentially present in the specimens.
3. Always wear gloves when handling reagents in this kit as some reagents are preserved with 0.095% (w/w) sodium azide. Sodium azide should never be flushed down the drain as this chemical may react with lead or copper plumbing to form potentially explosive metal azides. Excess reagents should be discarded in an appropriate waste receptacle.

REAGENTS

1. LF Specimen Diluent (2.5 mL, REF GLF025): Glycine-buffered saline containing blocking agents and a preservative
2. CrAg LF Test Strips (50 strips in desiccant vial, REF LFCR50)
3. CrAg Positive Control (1 mL, REF CB1020): Glycine-buffered saline spiked with cryptococcal antigen (strain 184A – clinical isolate from Tulane University (Infection & Immunity, June 1983, p. 1052-1059))
4. Package insert

MATERIALS NOT PROVIDED

1. Pipettor (40-µL and 80-µL)
2. Timer
3. Disposable micro-centrifuge tubes, test tubes, or a micro-titer plate

REAGENT PREPARATIONS

The entire kit should be at room temperature (22-25 °C) before and during use.

REAGENT STABILITY AND STORAGE

All reagents included in this kit should be stored at room temperature (22-25°C) until the expiration dates listed on the reagent labels.

Unused test strips should be stored in the LF test strip vial with the desiccant cap firmly attached.

SPECIMEN COLLECTION & PREPARATION

For optimal results, sterile non-hemolyzed serum should be used. Collect CSF specimens aseptically following accepted procedures. If a delay is encountered in specimen processing, storage at 2-8°C for up to 72 hours is permissible. Specimens may be stored for longer periods at <-20°C, provided they are not repeatedly thawed and refrozen. Specimens in transit should be maintained at 2-8°C or <-20°C.

PROCEDURE

REFER TO REAGENTS SECTION FOR A LIST OF MATERIALS PROVIDED.

Qualitative Procedure

1. Add 1 drop of LF Specimen Diluent (REF GLF025) to an appropriate reservoir (disposable micro-centrifuge tube, test tubes, or micro-titer plate, etc.).
2. Add 40 µL of specimen to the container and mix.
3. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip (REF LFCR50) into the specimen.
4. Wait 10 minutes.
5. Read and record the results (See READING THE TEST).

Semi-Quantitative Titration Procedure

1. Prepare dilutions starting with an initial dilution of 1:5, followed by 1:2 serial dilutions to 1:2560.
2. Place 10 micro-centrifuge or test tubes in an appropriate rack and label them 1-10 (1:5 through 1:2560). Additional dilutions may be necessary if the specimen is positive at 1:2560.
3. Add 4 drops of LF Specimen Diluent (REF GLF025) to tube #1.
4. Add 2 drops of LF Specimen Diluent to each of the tubes labeled 2-10.
5. Add 40 µL of specimen to tube #1 and mix well.
6. Transfer 80 µL of specimen from tube #1 to tube #2 and mix well. Continue this dilution procedure through tube #10. Discard 80 µL from tube 10 for a final tube volume of 80 µL.
7. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip into the specimen in each of the 10 tubes.
8. Wait 10 minutes.
9. Read and record the results (See READING THE TEST).

READING THE TEST

Read the reactions. The presence of two lines (test and control), regardless of the intensity of the test line, indicates a positive result.

For the semi-quantitative titration procedure, the patient's titer should be reported as the highest dilution that yields a positive result.

A single control line indicates a negative result. If the control line does not appear, the results are invalid and the test should be repeated.

REPRODUCIBILITY AND PRECISION

The CrAg Lateral Flow Assay was evaluated for reproducibility and precision by spiking serum and mock CSF with cryptococcal antigen to produce a panel consisting of a negative sample, a high-negative (C_s) sample, a low-positive sample and a moderate-positive sample. This panel was tested twice per day at three sites with a total of five operators over a five-day period in order to determine both the inter-lab and the intra-lab reproducibility and precision of the assay. The results of this study are shown in the tables below.

SERUM PANEL	Site 1 % Pos	Site 2 % Pos	Site 3 % Pos	Overall % Pos
Negative	0% (0/30)	0% (0/30)	0% (0/15)	0% (0/75)
High Negative	7% (2/30)	0% (0/30)	0% (0/15)	3% (2/75)
Low Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)
Moderate Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)

CSF PANEL	Site 1 % Pos	Site 2 % Pos	Site 3 % Pos	Overall % Pos
Negative	0% (0/30)	0% (0/30)	0% (0/15)	0% (0/75)
High Negative	10% (3/30)	0% (0/30)	0% (0/15)	3% (2/75)
Low Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)
Moderate Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)

BIBLIOGRAPHY

1. Doering, T. L. 2009. Annu. Rev. Microbiol. 63:223-247.
2. Goodman, J. S., L. Kaufman, and M. G. Koenig. 1971. N. Engl. J. Med. 285:434-436.
3. Kozel, T. R. 1995. Trends Microbiol. 3:295-299.
4. Lin, X. and J. Heitman. 2006. T. Annu. Rev. Microbiol. 60:69-105.
5. Kambugu, A., D.B. Meya, J. Rhein, M. O'Brien, E.N. Janoff, A.R. Ronald, M.R. Kanya, H. Mayanja-Kizza, M.A. Sande, P.R. Bohjanen, and D.R. Boulware. 2008. Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. Clin.Infect.Dis. 46: 1694-1701.
6. Park, B. J., K. A. Wannemuehler, B. J. Marston, N. Govender, P. G. Pappas, and T. M. Chiller. 2009. AIDS 23:525-530.
7. Rolles, M., Butler, E., von Hohenberg, M., Nabeta, H., Kwizera, R., Rajasingham, R., Bahr, N., Bohjanen, P., Meya, D., and Boulware, D. Evaluation of a novel point-of-care lateral flow assay to detect cryptococcal antigen in plasma and CSF. Conference on Retroviruses and Opportunistic Infections (CROI) Poster # 953. 2012
8. Zhou, Q. and W. J. Murphy. 2006. Immunol. Res. 35:191-208.

Immuno-Mycologics, Inc.
2700 Technology Place
Norman OK 73071 U.S.A.
(405) 360-4669/ (800) 654-3639
Fax: (405) 364-1058
E-mail: info@immv.com
WEB: www.immv.com



MDSS
Schiffgraben 41
30175 Hannover, Germany

International Symbol Usage



Storage
20-25 C



Lot Number



Manufactured by



Reference
Number



Expiration Date



In Vitro
Diagnostics



Conforms to European
Union Requirements



Sufficient for "#"
Tests



Protect from Humidity

Interactive Review

White, Michael

From: White, Michael
Sent: Wednesday, August 31, 2011 6:37 PM
To: 'Sean Bauman'
Cc: Hojvat, Sally A; Poole, Freddie M.
Subject: 510(k) submission (k112422), Cryptococcal Antigen Lateral Flow Assay

Dear Dr. Bauman,

The CDRH/OIVD/DMD is recently in receipt of your 510(k) submission (k112422), Cryptococcal Antigen Lateral Flow Assay. I have been assigned as your lead scientific reviewer and look forward to working with you during the review process. Would it be acceptable to contact you directly as the representative of your company either through email, fax, and/or phone for any issues that might arise? If so, please confirm in writing or via email.

If available, please email to me the electronic copy of your submission (or any parts of it that you have available in electronic form, preferably in MS Word Format). This will make the review of the submission more efficient and therefore more expeditious.

Finally, I expect this to be an interactive process and should you have any questions or concerns please do not hesitate to contact me.

Sincerely,

Michael

Michael W. White
Microbiologist/Regulatory Scientist
FDA/Center for Devices and Radiological Health (CDRH)
Office of In-Vitro Diagnostic Device Evaluation and Safety
Division of Microbiology Devices
10903 New Hampshire Avenue
Building 66, Room 5537
Silver Spring, MD 20993-0002
Phone: 301-796-6121
FAX: 301-8478512
Email: michael.white@fda.hhs.gov

White, Michael

From: Sean Bauman [Sean-Bauman@immy.com]
Sent: Wednesday, March 28, 2012 5:23 PM
To: White, Michael
Cc: Joy M. Pelfrey; Brandon Neary; Poole, Freddie M.; Sean Bauman
Subject: Fwd: Indications for Use statement
Attachments: 004_Indications for Use Statement.pdf; ATT00001.htm

Hi Michael,
Attached is the revised indications for use statement.
Kind regards,
Sean

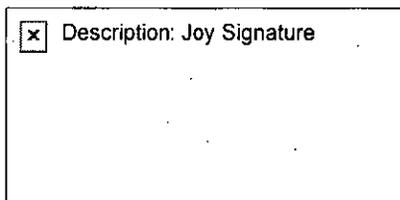
Sean K. Bauman, PhD
President & CEO
IMMY

www.immy.com
405.360.4669
Sean-Bauman@immy.com

Sent from my iPhone

Begin forwarded message:

From: "Joy M. Pelfrey" <Joy-Pelfrey@immy.com>
Date: March 28, 2012 4:18:21 PM CDT
To: Sean Bauman <Sean-Bauman@immy.com>
Subject: Indications for Use statement



www.immy.com
405.360.4669
Joy-Pelfrey@immy.com

REVIEW MEMO

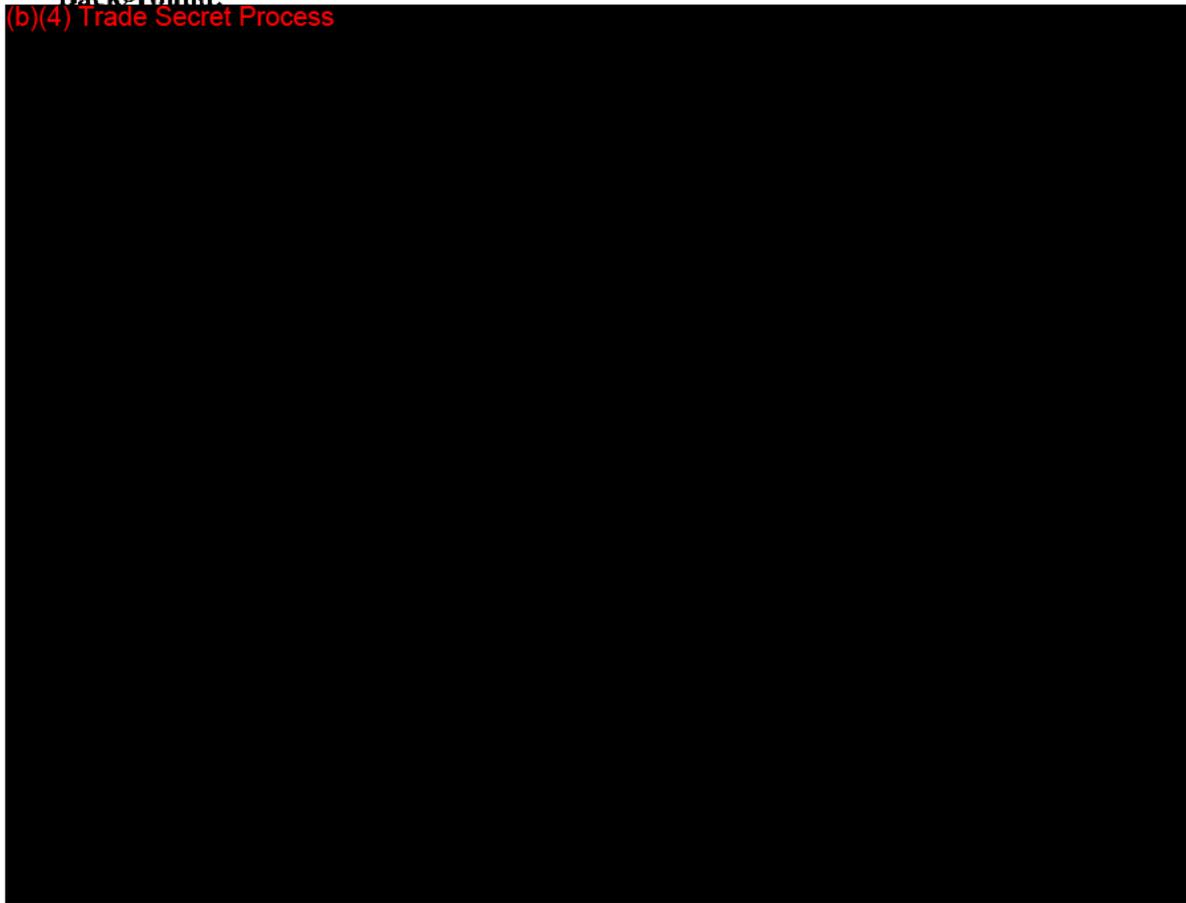
To: Sean K. Bauman, Ph.D. President and CEO
Subject: CrAg Lateral Flow Assay
From: Michael W. White, Scientific Reviewer Division of Microbiology Devices, Office of In Vitro Diagnostic Device Evaluation and Safety, CDRH
Date: 3/21/2012
Company: Immuno-Mycologics, Inc.
Telephone: (405) 364-1058

Re: K112422

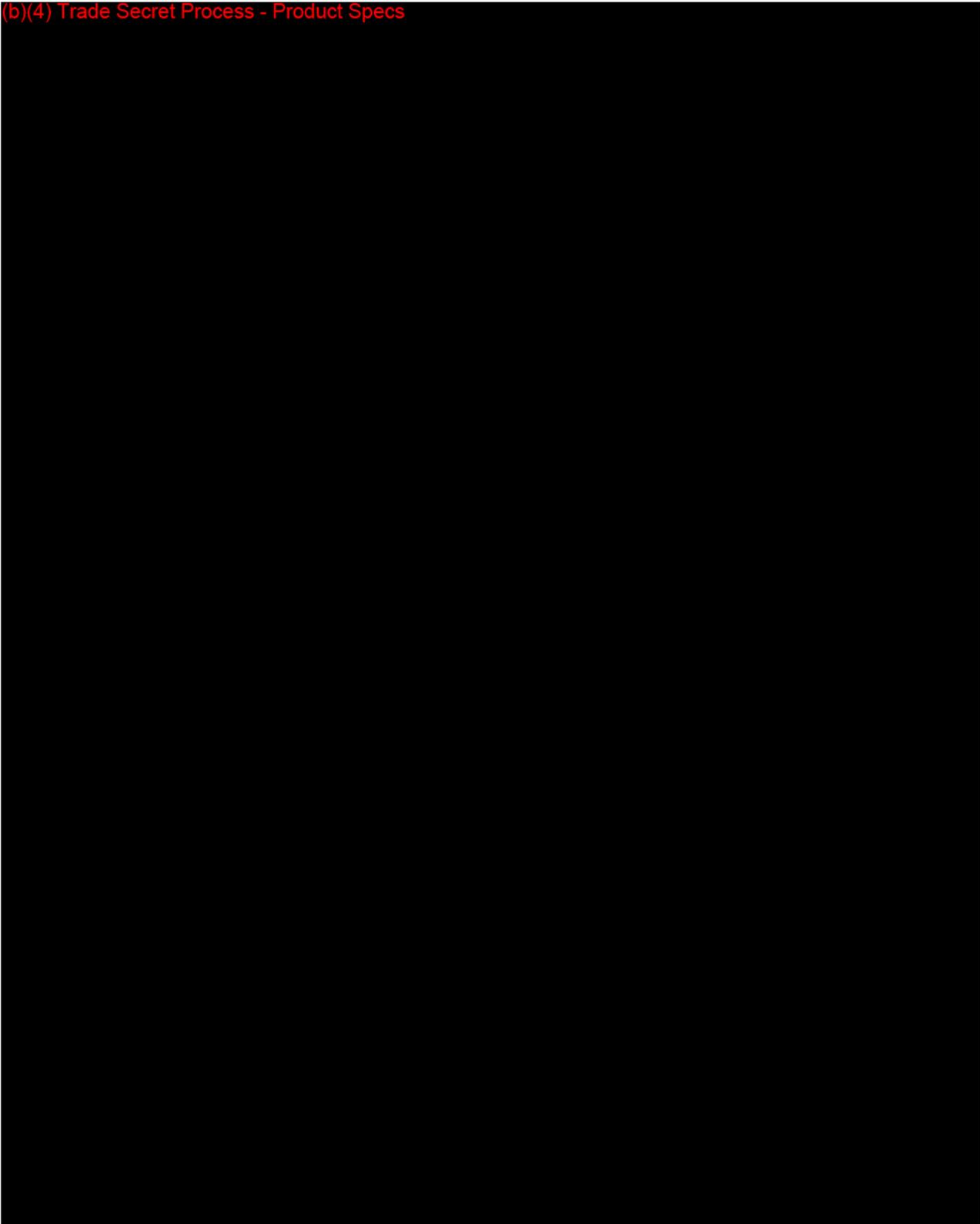
Through: Freddie Poole, Associate Director and Sally Hojvat, Ph.D., Director, Microbiology Division, OIVD, CDRH.

Background:

(b)(4) Trade Secret Process



(b)(4) Trade Secret Process - Product Specs



(b)(4) Trade Secret Process - Product Specs



Michael W. White
Scientific Reviewer

White, Michael

From: Sean Bauman [Sean-Bauman@immy.com]
Sent: Thursday, March 22, 2012 2:50 PM
To: White, Michael; Hojvat, Sally A; Poole, Freddie M.
Cc: Brandon Neary; Joy M. Pelfrey; Sean Bauman
Subject: Fwd: CrAg Lateral Flow Assay (K112422)
Attachments: (b)(4) Trade Secret Process - Product Specs

Dear Michael,

(b)(4) Trade Secret Process - Product Specs

Kind regards,
Sean

Sean K. Bauman, PhD
President & CEO
IMMY

www.immy.com
405.360.4669
Sean-Bauman@immy.com

Sent from my iPhone

Begin forwarded message:

From: "Joy M. Pelfrey" <Joy-Pelfrey@immy.com>
Date: March 22, 2012 11:46:35 AM EDT
To: Sean Bauman <Sean-Bauman@immy.com>, Brandon Neary <brandon-neary@immy.com>
Subject: RE: CrAg Lateral Flow Assay (K112422)

(b)(4) Trade Secret Process - Product Specs

From: Sean Bauman

36

3/27/2012

Sent: Thursday, March 22, 2012 9:57 AM
To: Joy M. Pelfrey; Brandon Neary
Subject: Fwd: CrAg Lateral Flow Assay (K112422)

(b)(4) Trade Secret Process - Product Specs

Sean K. Bauman, PhD
President & CEO
IMMY

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Sean-Bauman@immy.com

Sent from my iPhone

Begin forwarded message:

From: "White, Michael" <Michael.White@fda.hhs.gov>
To: "Sean Bauman" <Sean-Bauman@immy.com>
Cc: "Poole, Freddie M." <Freddie.Poole@fda.hhs.gov>, "Hojvat, Sally A" <Sally.Hojvat@fda.hhs.gov>
Subject: CrAg Lateral Flow Assay (K112422)

Dear Dr. Bauman,

(b)(4) Trade Secret Process - Product Specs

Best regards,
Michael

White, Michael

From: Sean Bauman [Sean-Bauman@immy.com]
Sent: Monday, March 26, 2012 3:48 PM
To: White, Michael
Cc: Brandon Neary; Joy M. Pelfrey; Hojvat, Sally A; Poole, Freddie M.
Subject: Re: CrAg Lateral Flow Assay (K112422)

Dear Michael

(b)(4) Trade Secret Process - Product Specs

Kind regards,
Sean

Sean K. Bauman, PhD
President & CEO
IMMY

www.immy.com
405.360.4669
Sean-Bauman@immy.com

Sent from my iPhone

On Mar 23, 2012, at 11:03 AM, "White, Michael" <Michael.White@fda.hhs.gov> wrote:

Dear Dr. Bauman,

(b)(4) Trade Secret Process - Product Specs

Best regards,
Michael

From: Sean Bauman [mailto:Sean-Bauman@immy.com]
Sent: Thursday, March 22, 2012 2:50 PM
To: White, Michael; Hojvat, Sally A; Poole, Freddie M.
Cc: Brandon Neary; Joy M. Pelfrey; Sean Bauman
Subject: Fwd: CrAg Lateral Flow Assay (K112422)

Dear Michael,

(b)(4) Trade Secret Process - Product Specs

Kind regards,
Sean

38

Sean K. Bauman, PhD
President & CEO
IMMY

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Sent from my iPhone

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From: "Joy M. Pelfrey" <Joy-Pelfrey@immy.com>
Date: March 22, 2012 11:46:35 AM EDT
To: Sean Bauman <Sean-Bauman@immy.com>, Brandon Neary <brandon-neary@immy.com>
Subject: RE: CrAg Lateral Flow Assay (K112422)

(b)(4) Trade Secret Process - Product Specs



From: Sean Bauman
Sent: Thursday, March 22, 2012 9:57 AM
To: Joy M. Pelfrey; Brandon Neary
Subject: Fwd: CrAg Lateral Flow Assay (K112422)

(b)(4) Trade Secret Process - Product Specs

Sean K. Bauman, PhD
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Sean-Bauman@immy.com
Sent from my iPhone

Begin forwarded message:

From: "White, Michael" <Michael.White@fda.hhs.gov>
To: "Sean Bauman" <Sean-Bauman@immy.com>
Cc: "Poole, Freddie M." <Freddie.Poole@fda.hhs.gov>, "Hojvat, Sally A" <Sally.Hojvat@fda.hhs.gov>

Subject: CrAg Lateral Flow Assay (K112422)

Dear Dr. Bauman,

(b)(4) Trade Secret Process - Product Specs



Best regards,
Michael

40

REVIEW MEMO

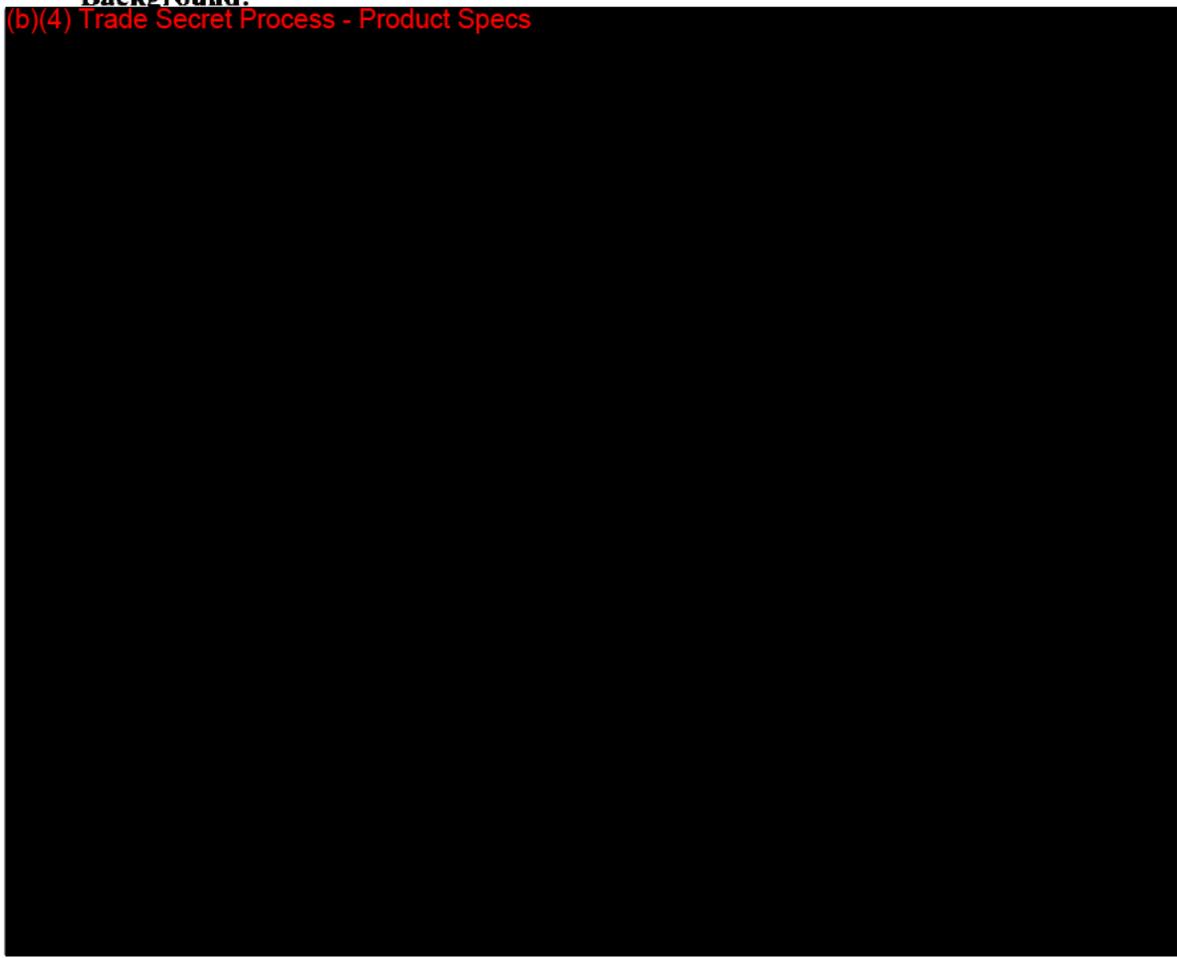
To: Sean K. Bauman, Ph.D. President and CEO
Subject: CrAg Lateral Flow Assay
From: Michael W. White, Scientific Reviewer Division of
Microbiology Devices, Office of In Vitro Diagnostic
Device Evaluation and Safety, CDRH
Date: 2/9/2012
Company: Immuno-Mycologics, Inc.
Telephone: (405) 364-1058

Re: K112422

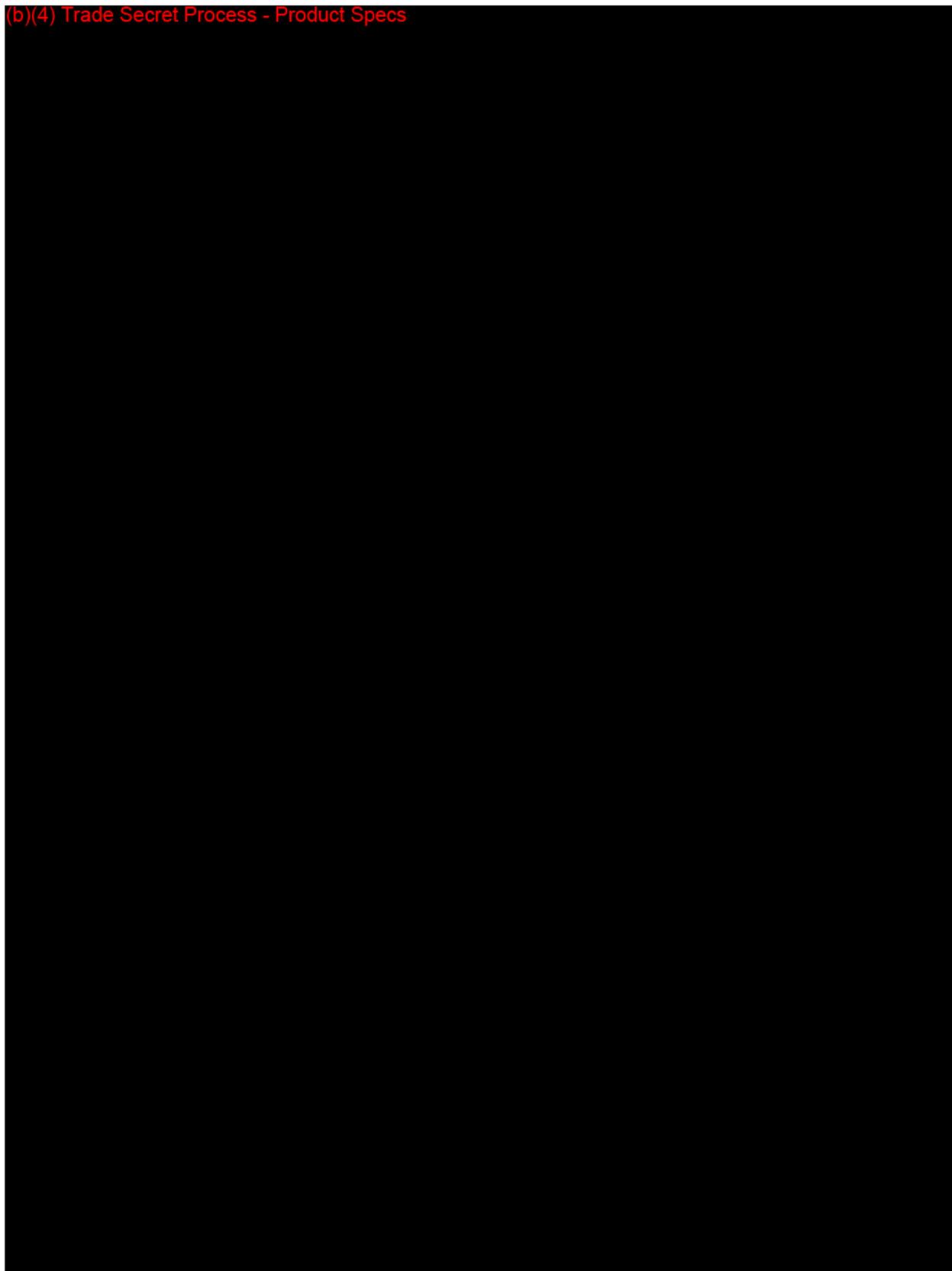
Through: Freddie Poole, Associate Director and Sally Hojvat, Ph.D., Director,
Microbiology Division, OIVD, CDRH.

Background:

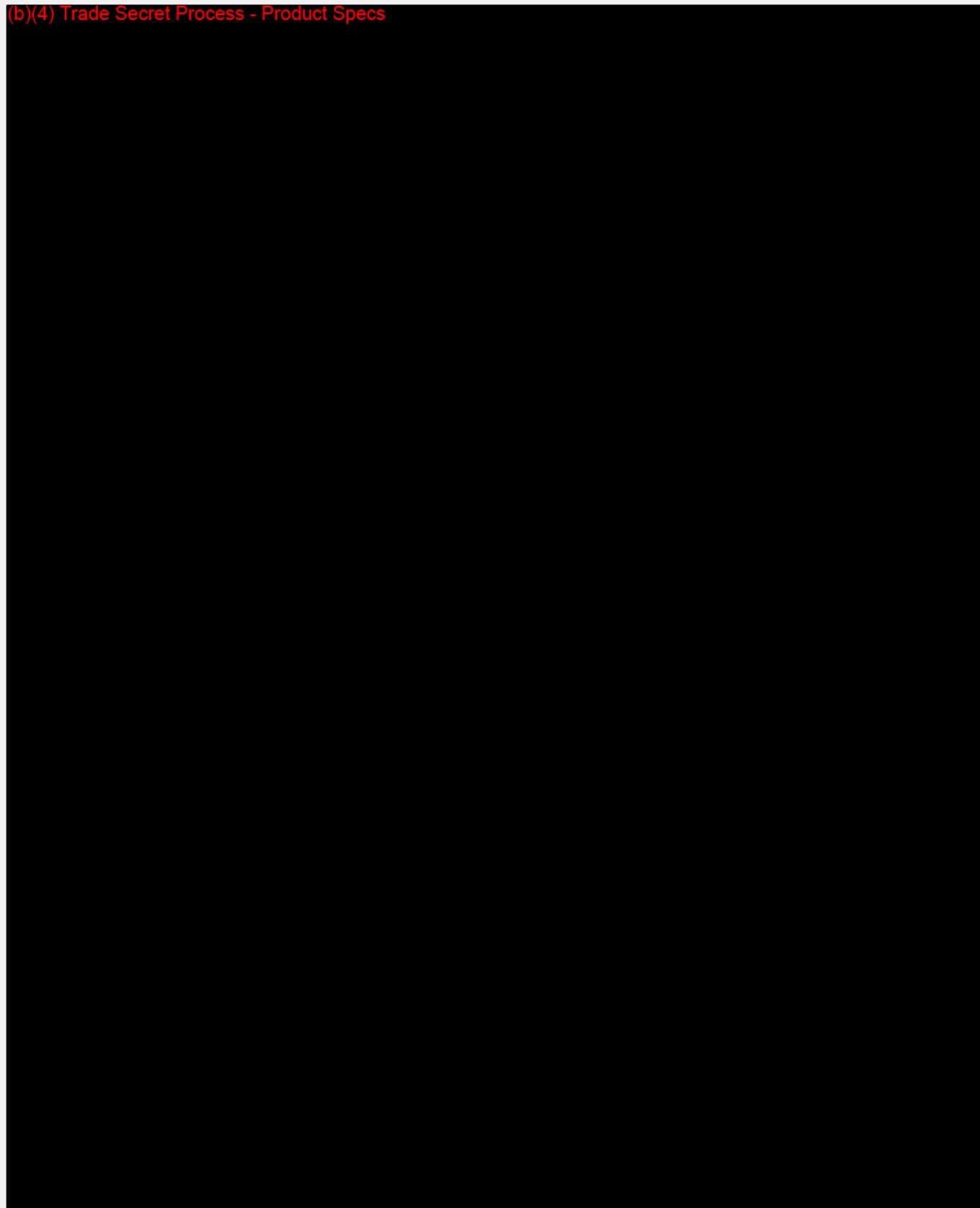
(b)(4) Trade Secret Process - Product Specs



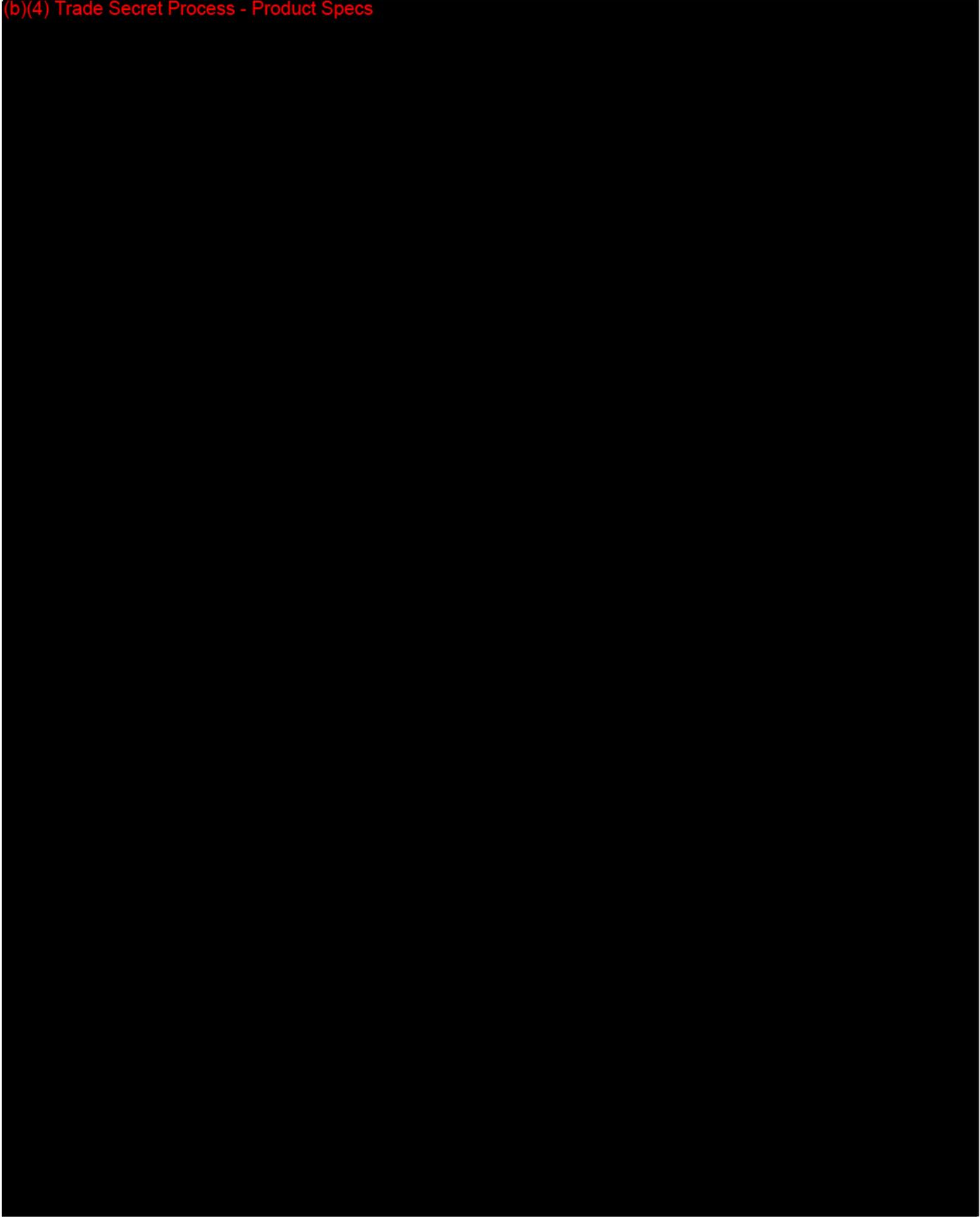
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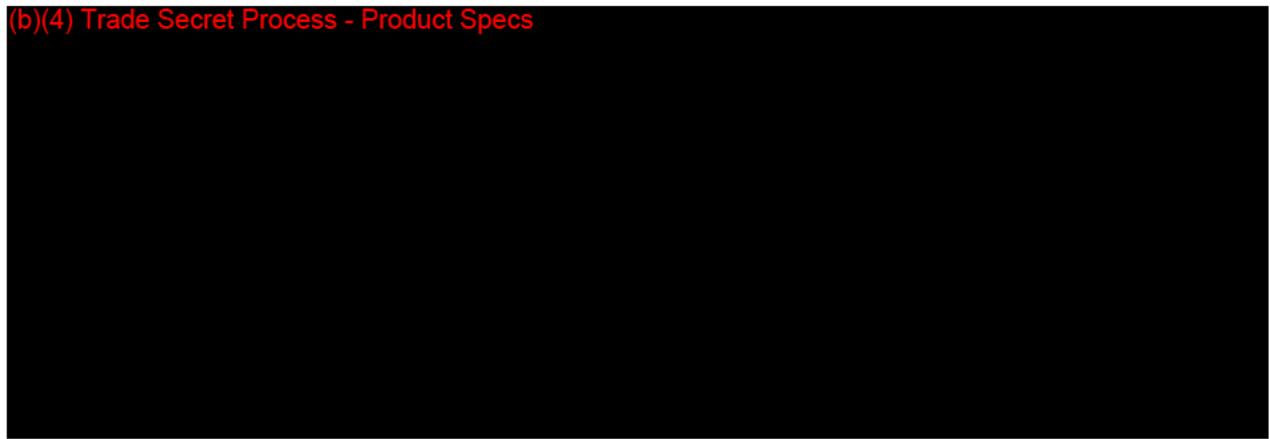
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(b)(4) Trade Secret Process - Product Specs



(b)(4) Trade Secret Process - Product Specs





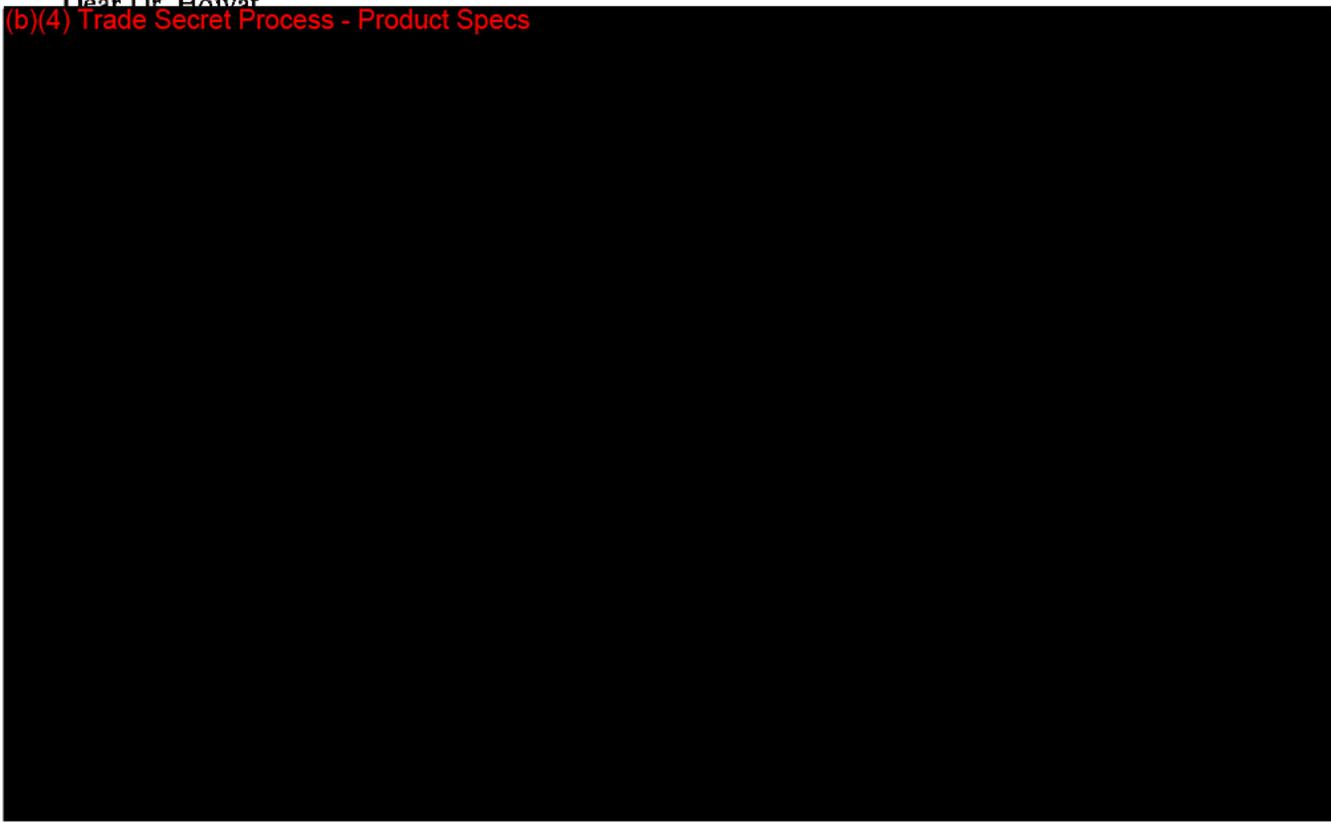
February 10, 2012

Sally Hojvat, M.Sc., Ph.D.
Director, Division of Microbiology Devices
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health
Food and Drug Administration
Document Mail Center – WO66-0609
10903 New Hampshire Avenue
Silver Springs, MD 20993-0002

Re: K112422, CrAg Lateral Flow Assay

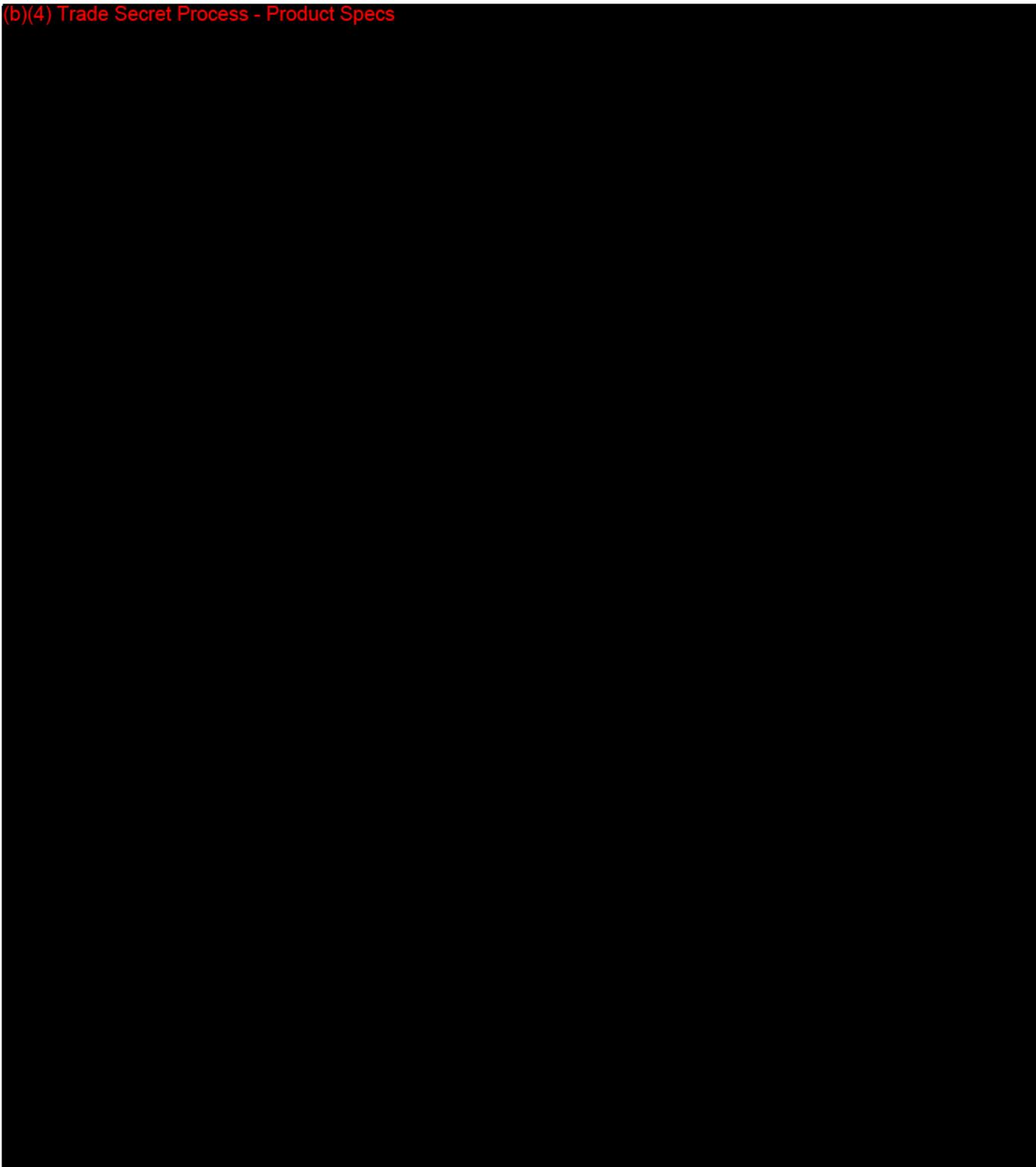
Dear Dr. Hojvat

(b)(4) Trade Secret Process - Product Specs



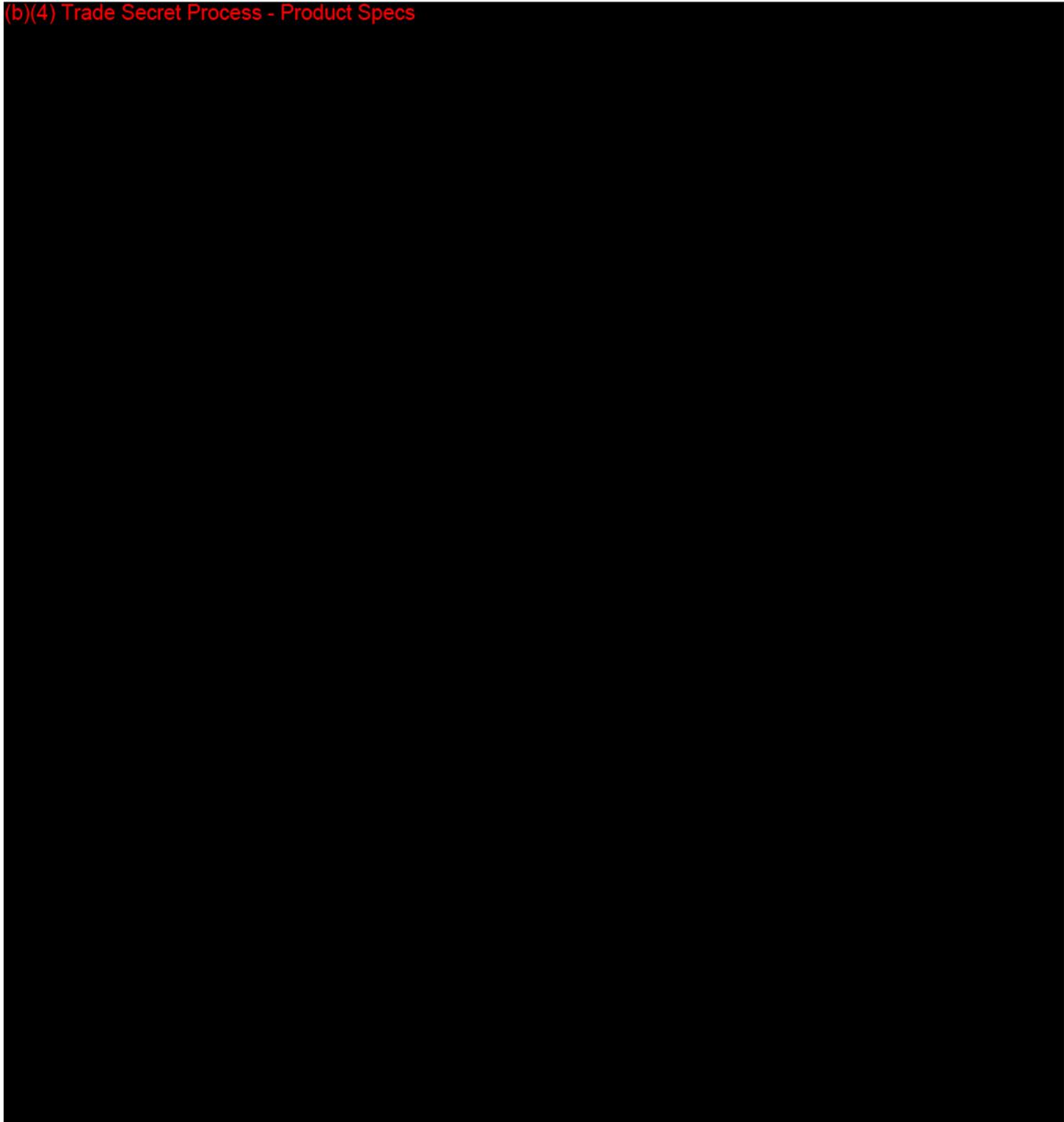


(b)(4) Trade Secret Process - Product Specs





(b)(4) Trade Secret Process - Product Specs





(b)(4) Trade Secret Process - Product Specs

Kind regards,

Sean K. Bauman, Ph.D.

Sean K. Bauman, PhD
President & CEO

REVIEW MEMO

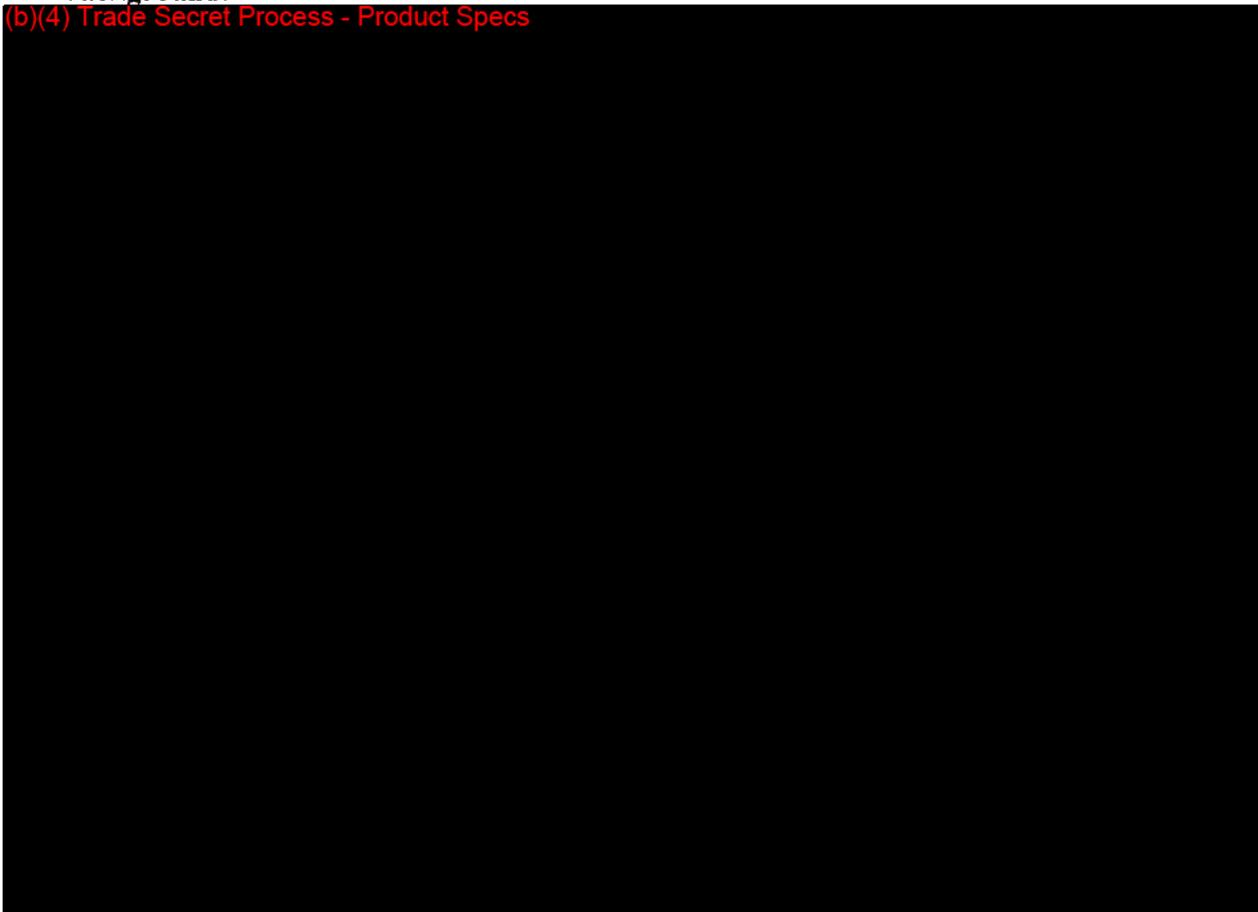
To: Scan K. Bauman, Ph.D. President and CEO
Subject: CrAg Lateral Flow Assay
From: Michael W. White, Scientific Reviewer Division of
Microbiology Devices, Office of In Vitro Diagnostic
Device Evaluation and Safety, CDRH
Date: 12/16/2011
Company: Immuno-Mycologies, Inc.
Telephone: (405) 364-1058

Re: K112422

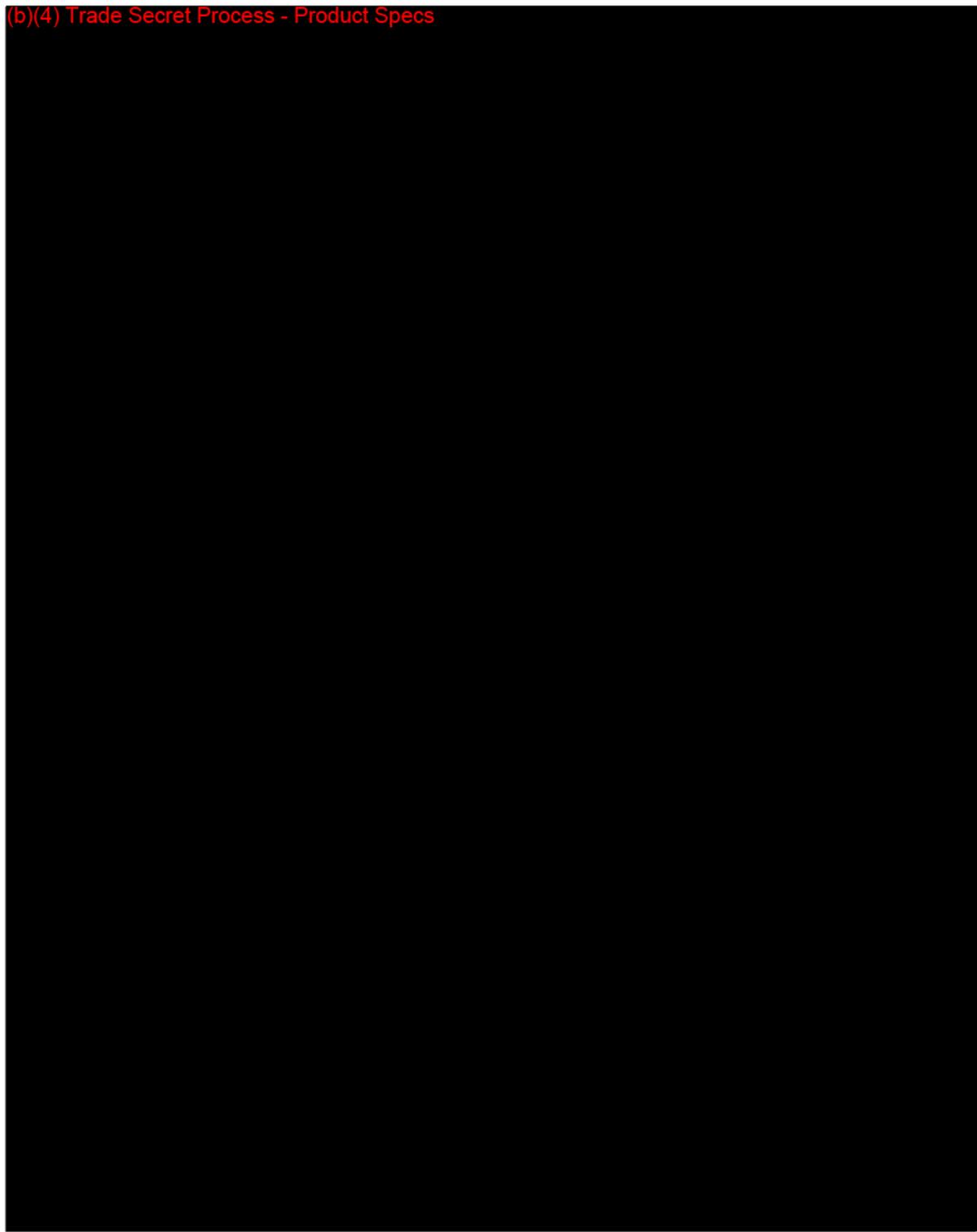
Through: Freddie Poole, Associate Director and Sally Hojvat, Ph.D., Director,
Microbiology Division, OIVD, CDRH.

Background:

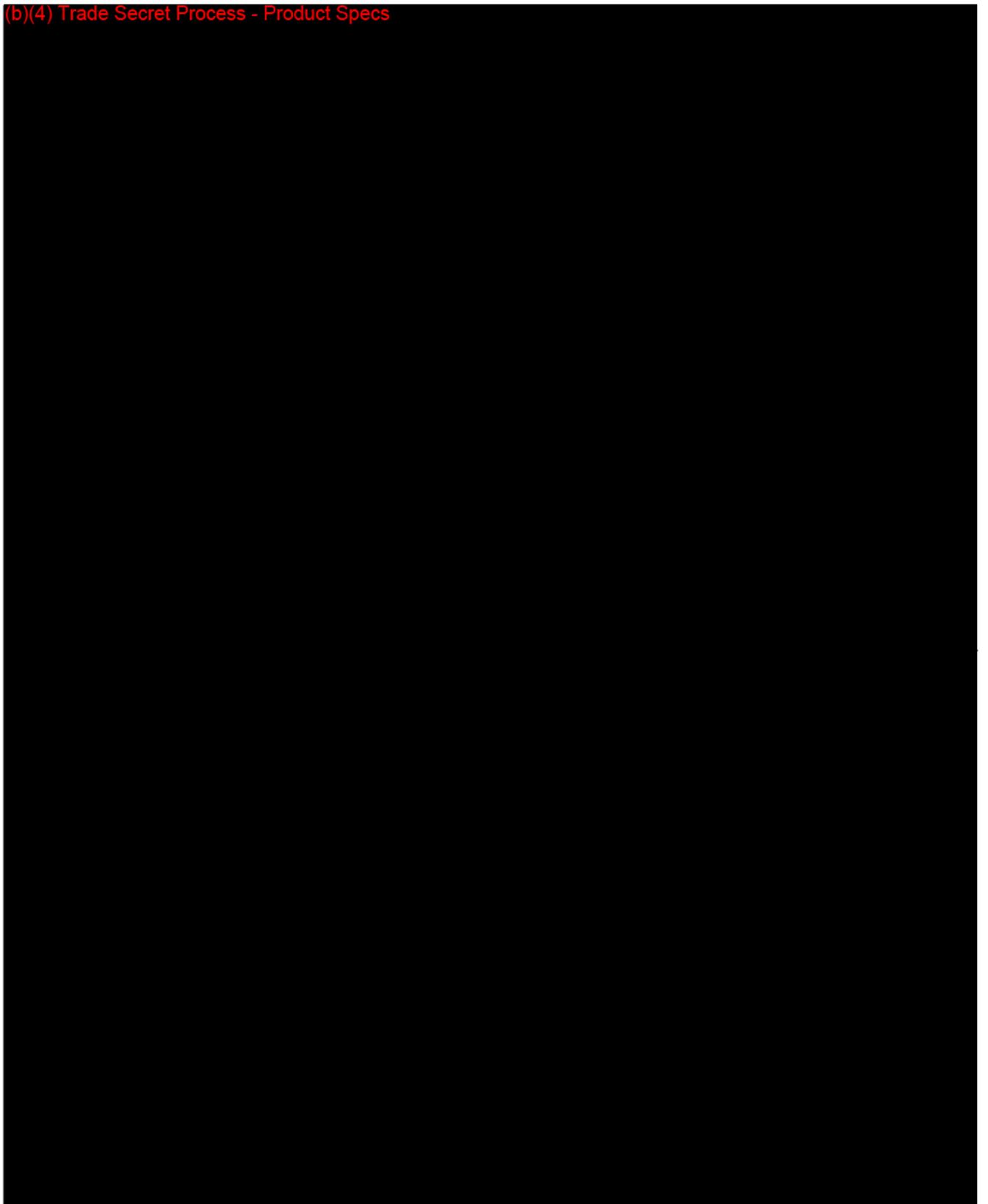
(b)(4) Trade Secret Process - Product Specs



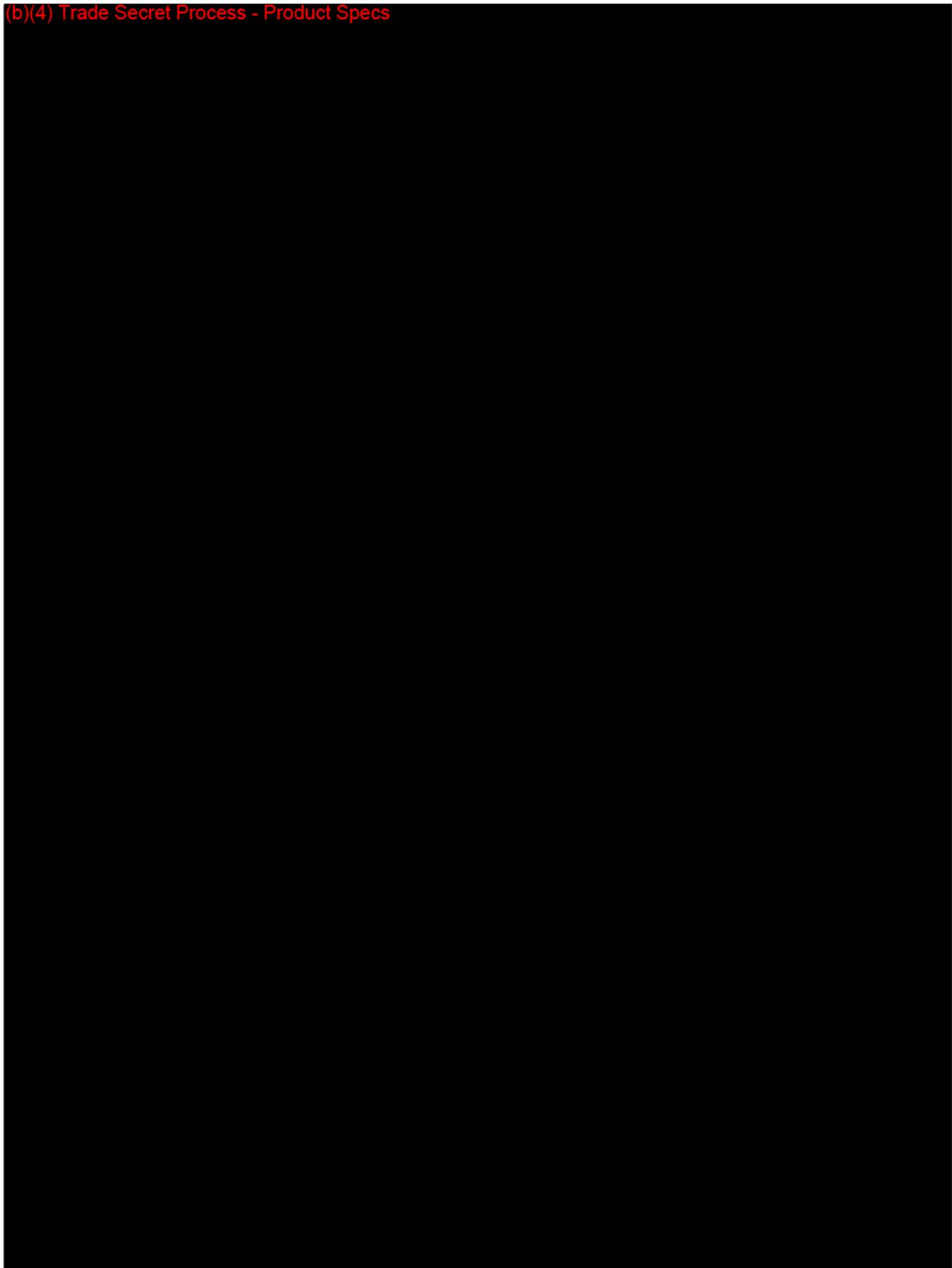
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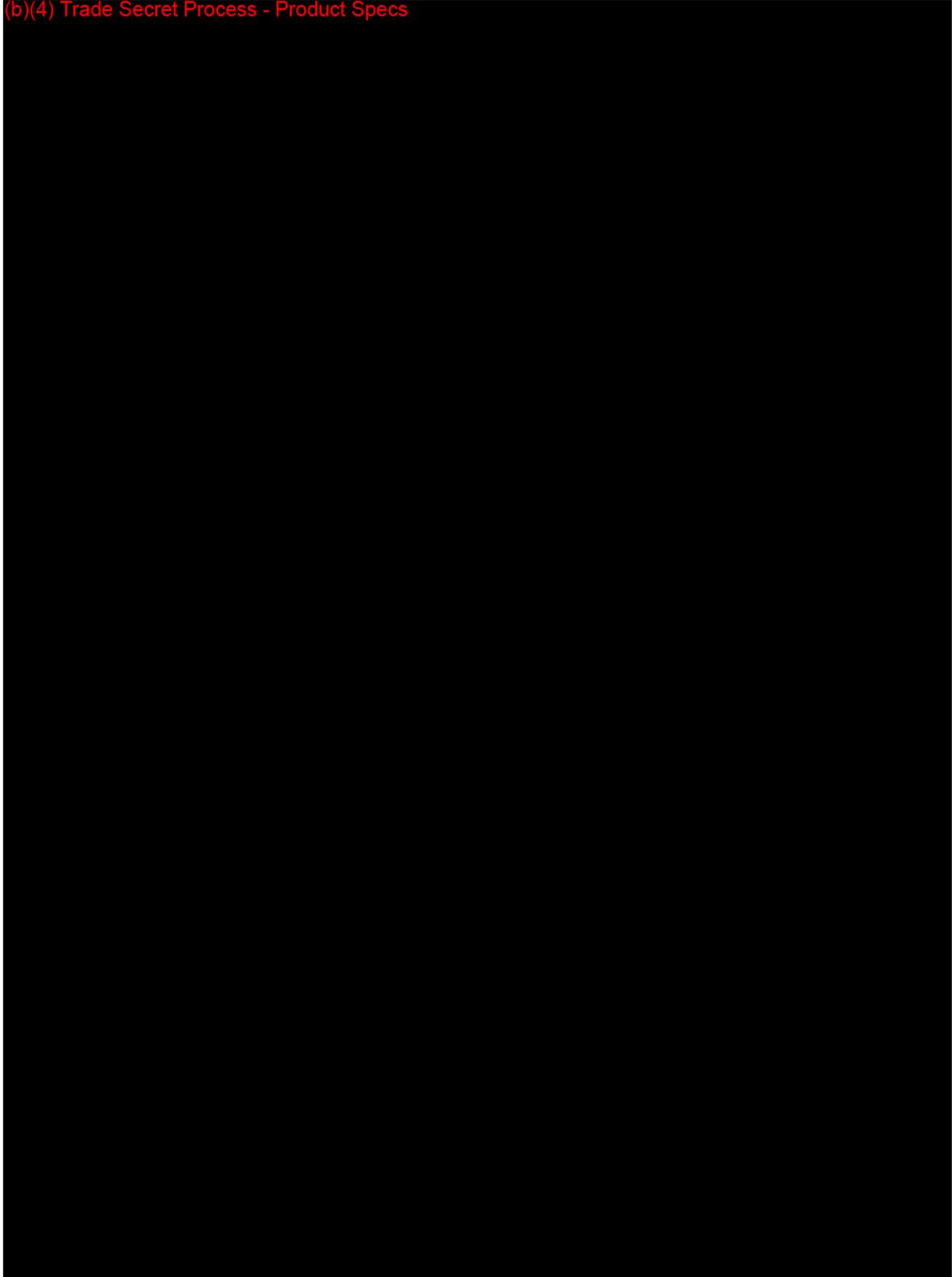
(b)(4) Trade Secret Process - Product Specs



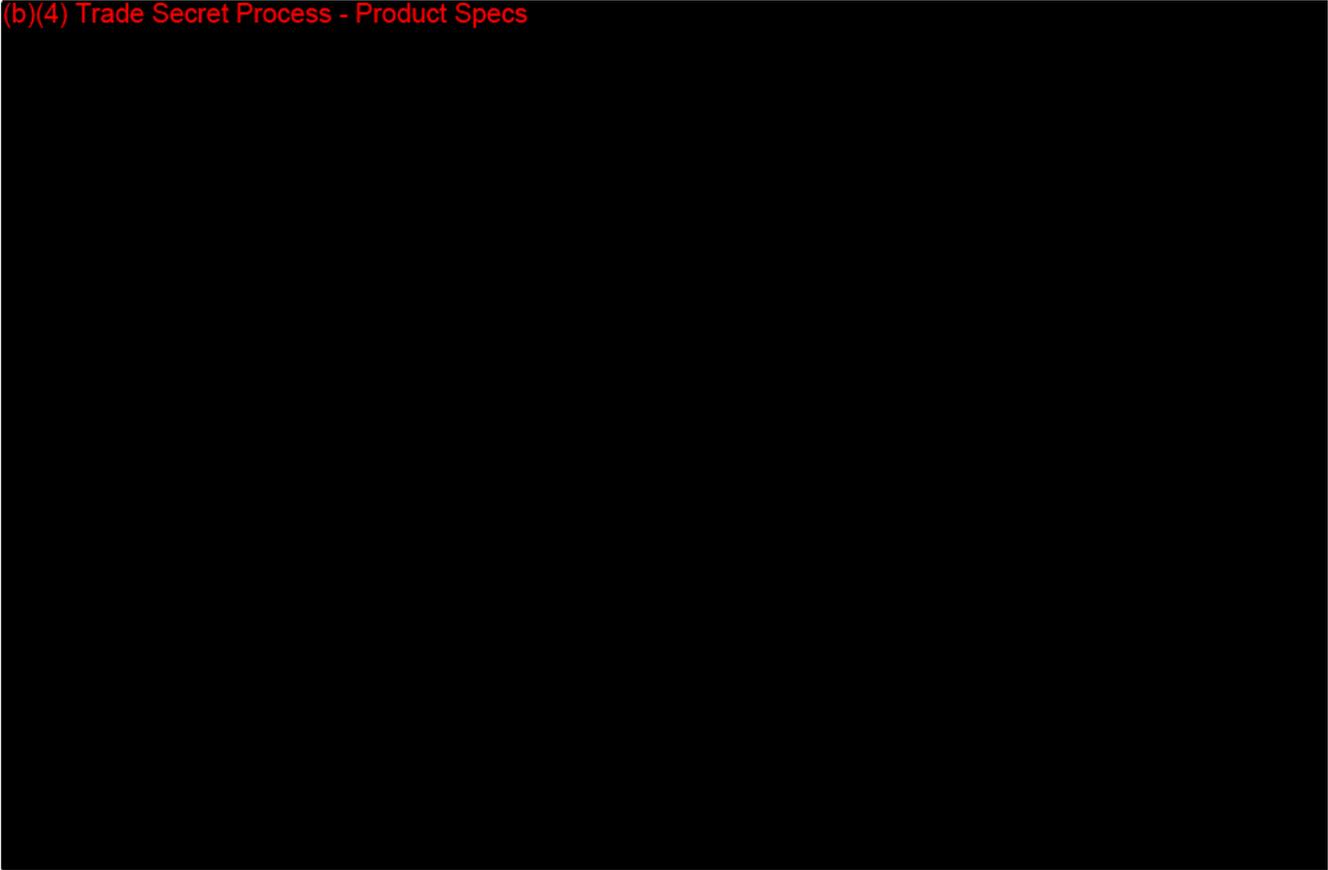
(b)(4) Trade Secret Process - Product Specs



(b)(4) Trade Secret Process - Product Specs



(b)(4) Trade Secret Process - Product Specs



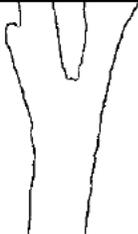
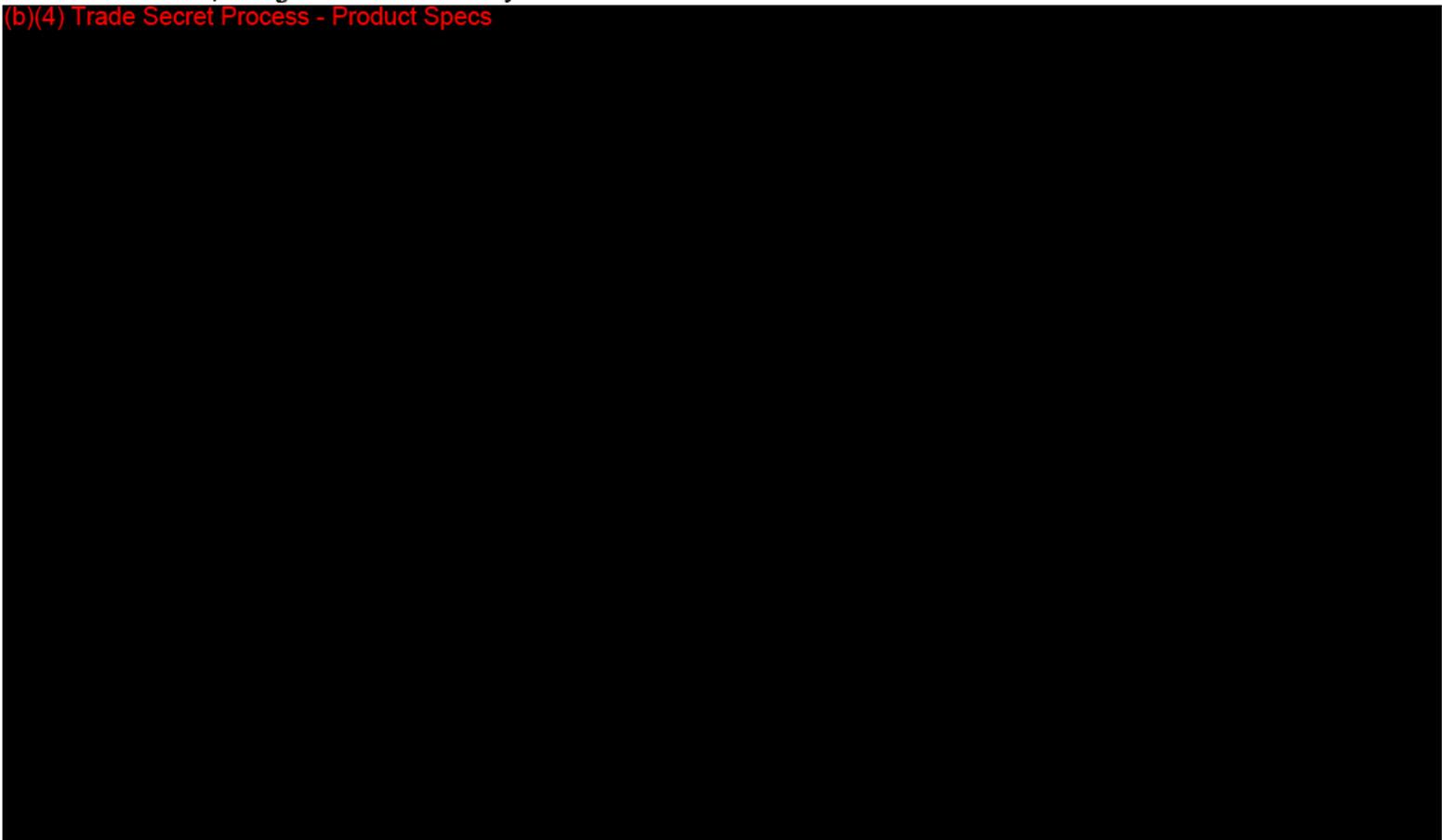


December 21, 2011

Sally Hojvat, M.Sc., Ph.D.
Director, Division of Microbiology Devices
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health
Food and Drug Administration
Document Mail Center – WO66-0609
10903 New Hampshire Avenue
Silver Springs, MD 20993-0002

Re: K112422, CrAg Lateral Flow Assay

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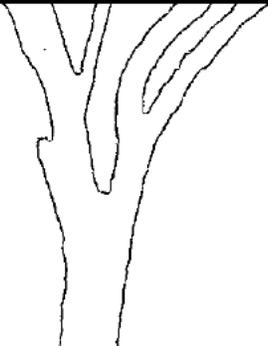
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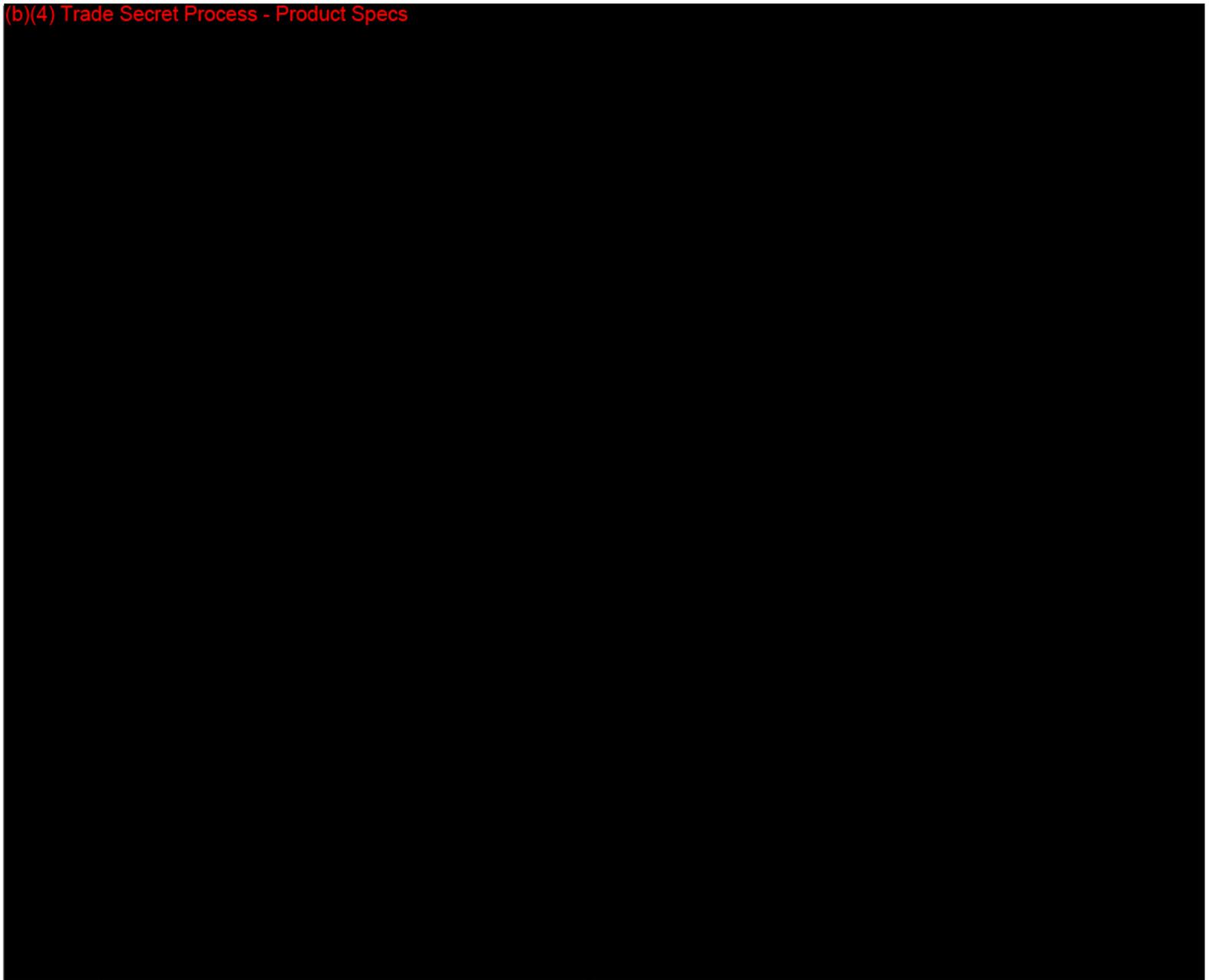
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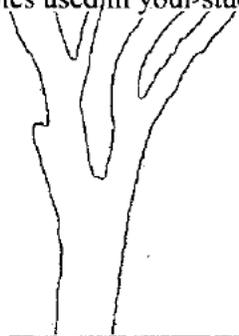
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samples used in your study.



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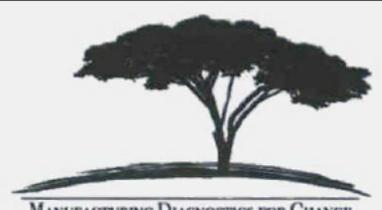
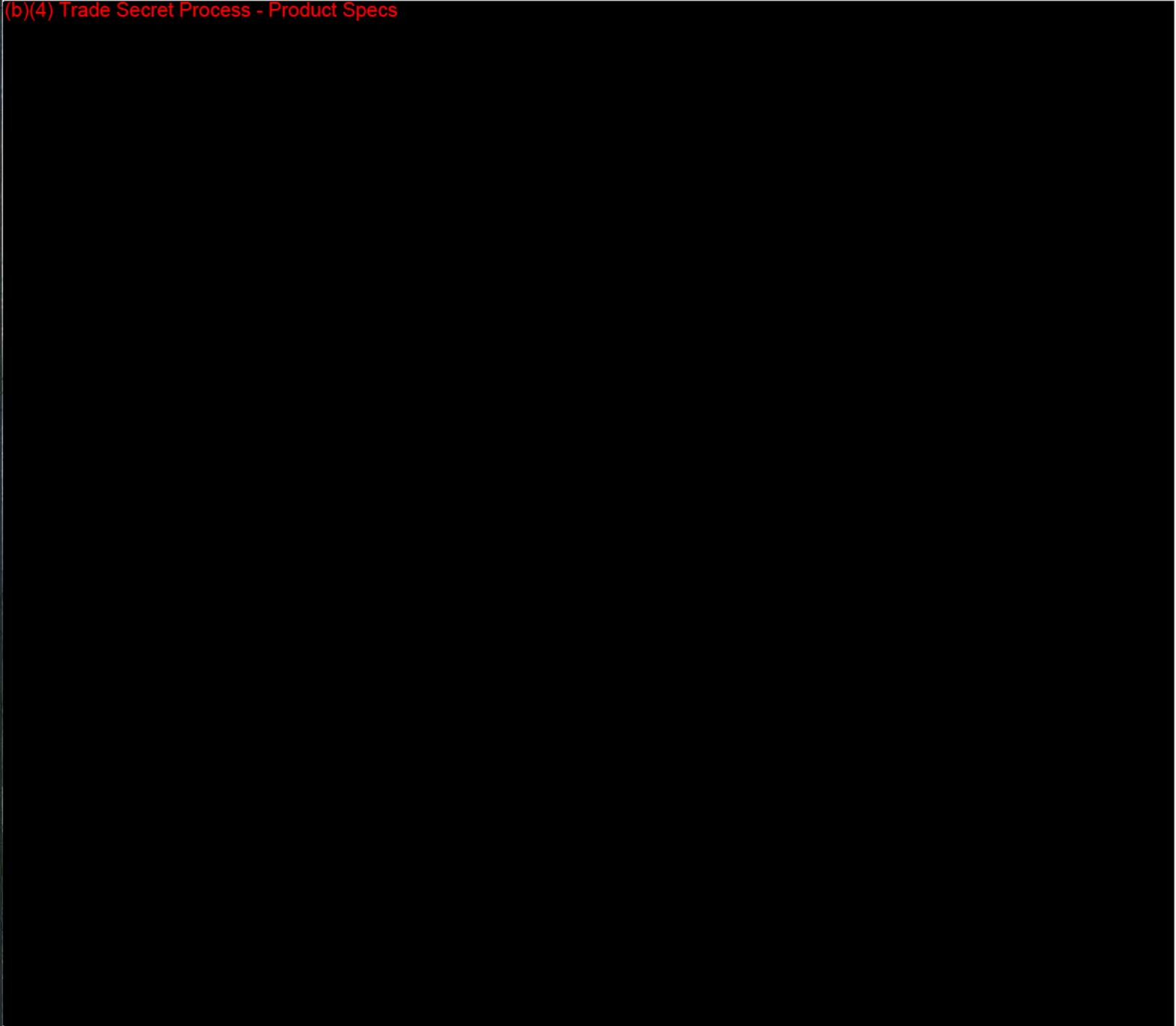


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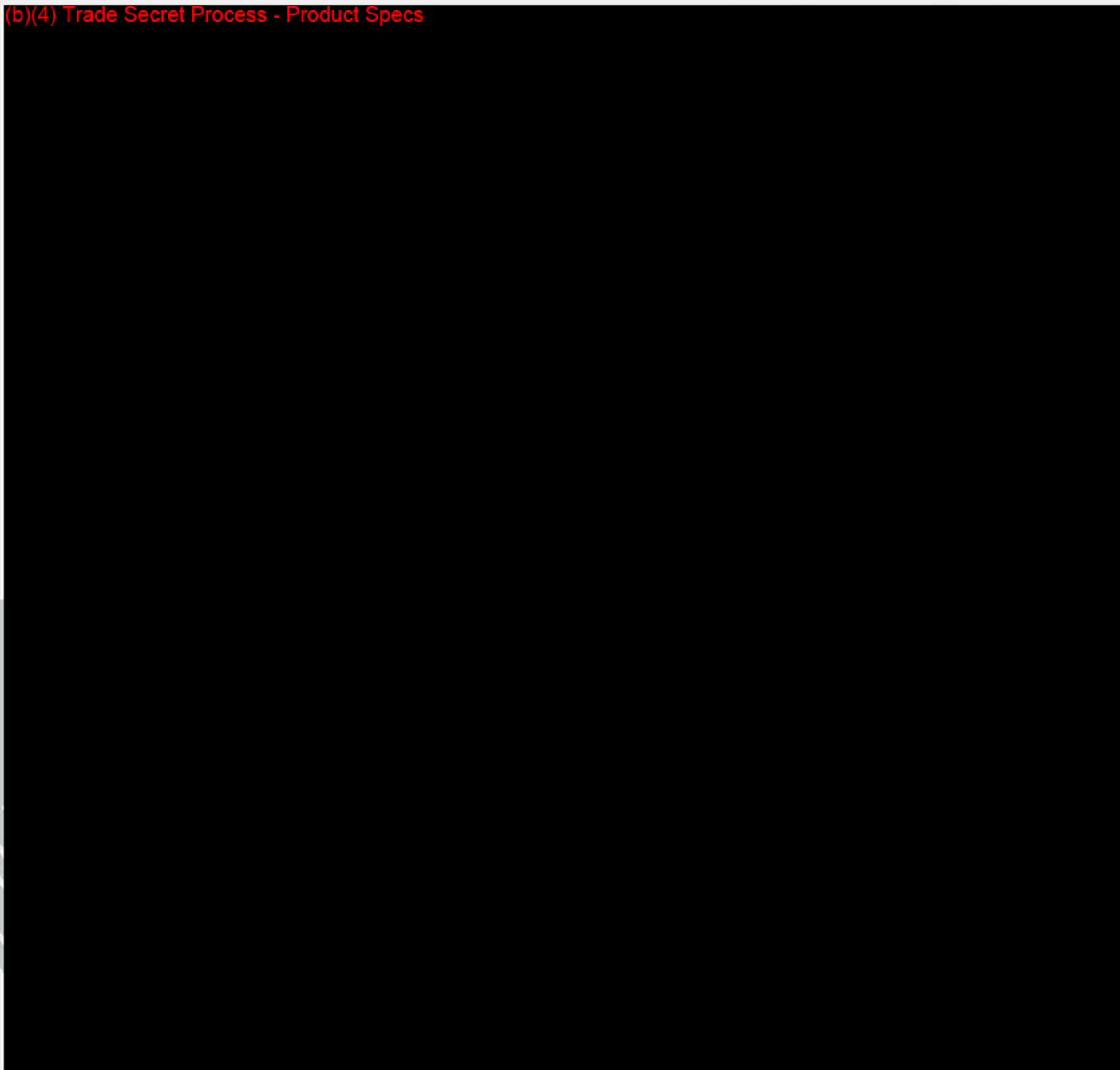
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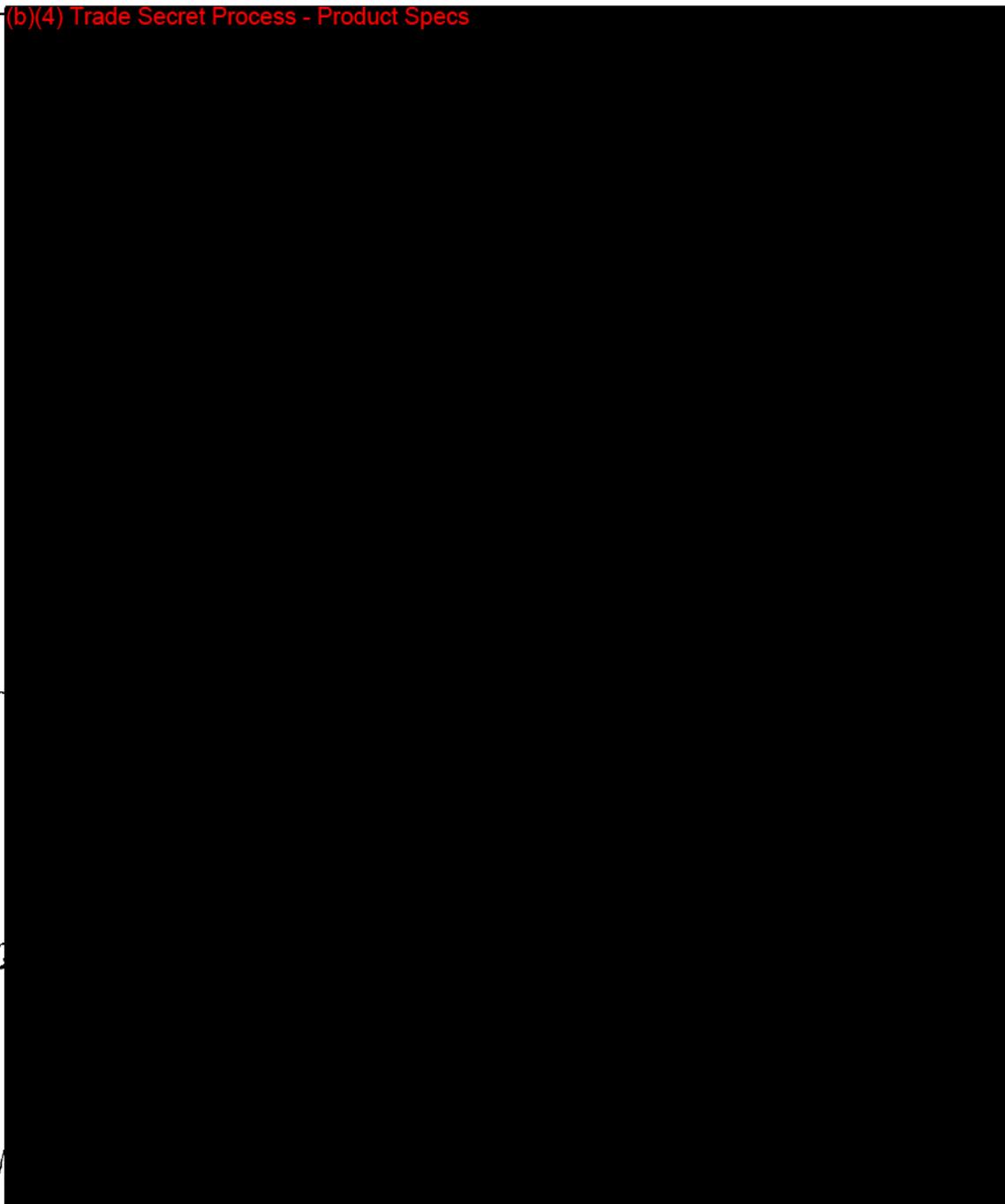
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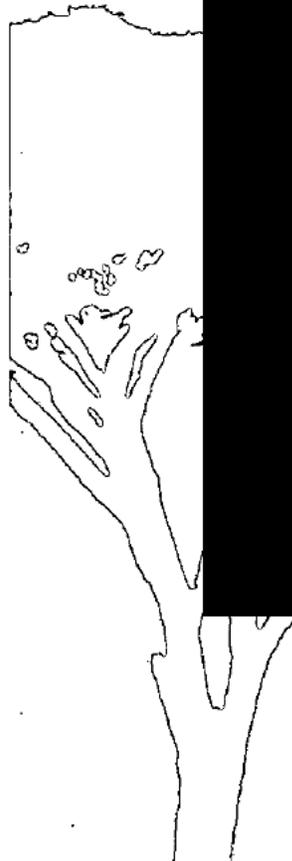
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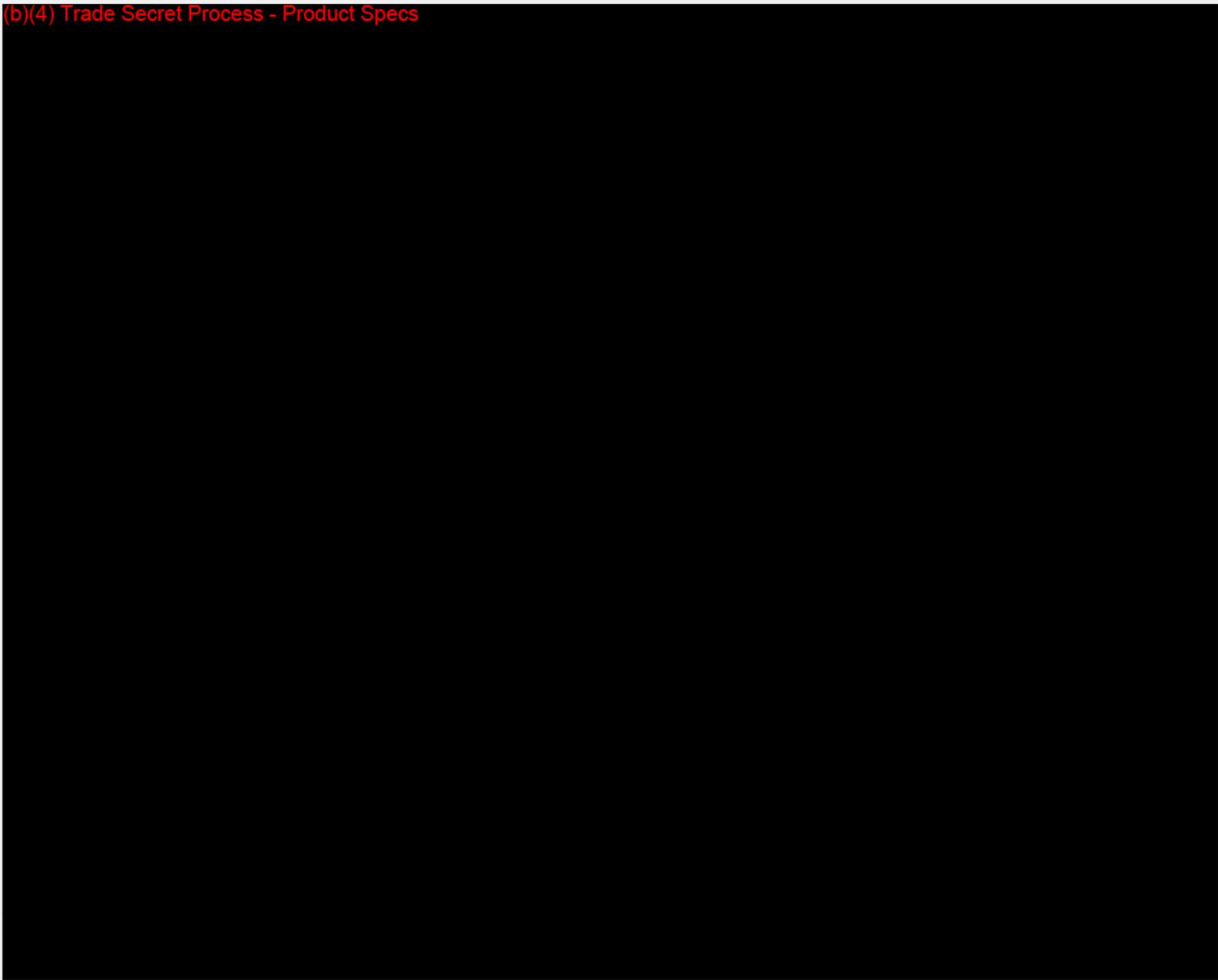
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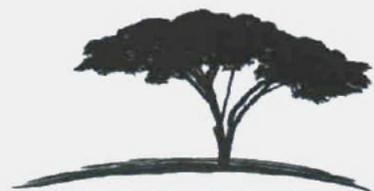
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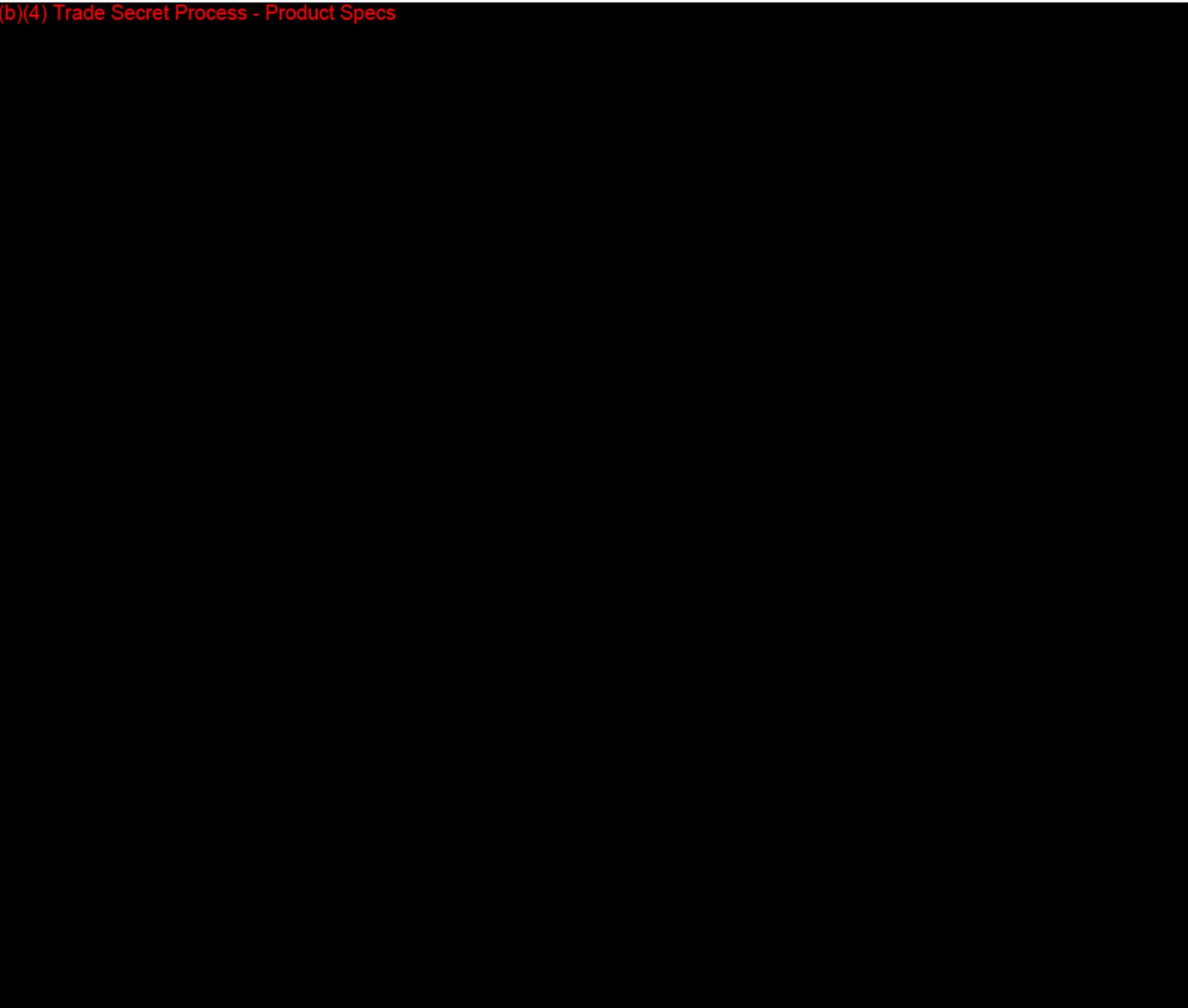
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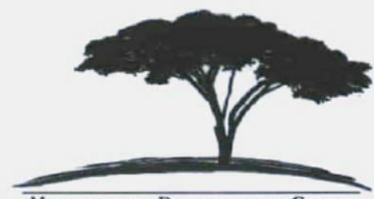
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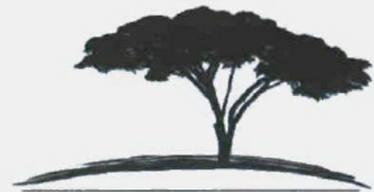
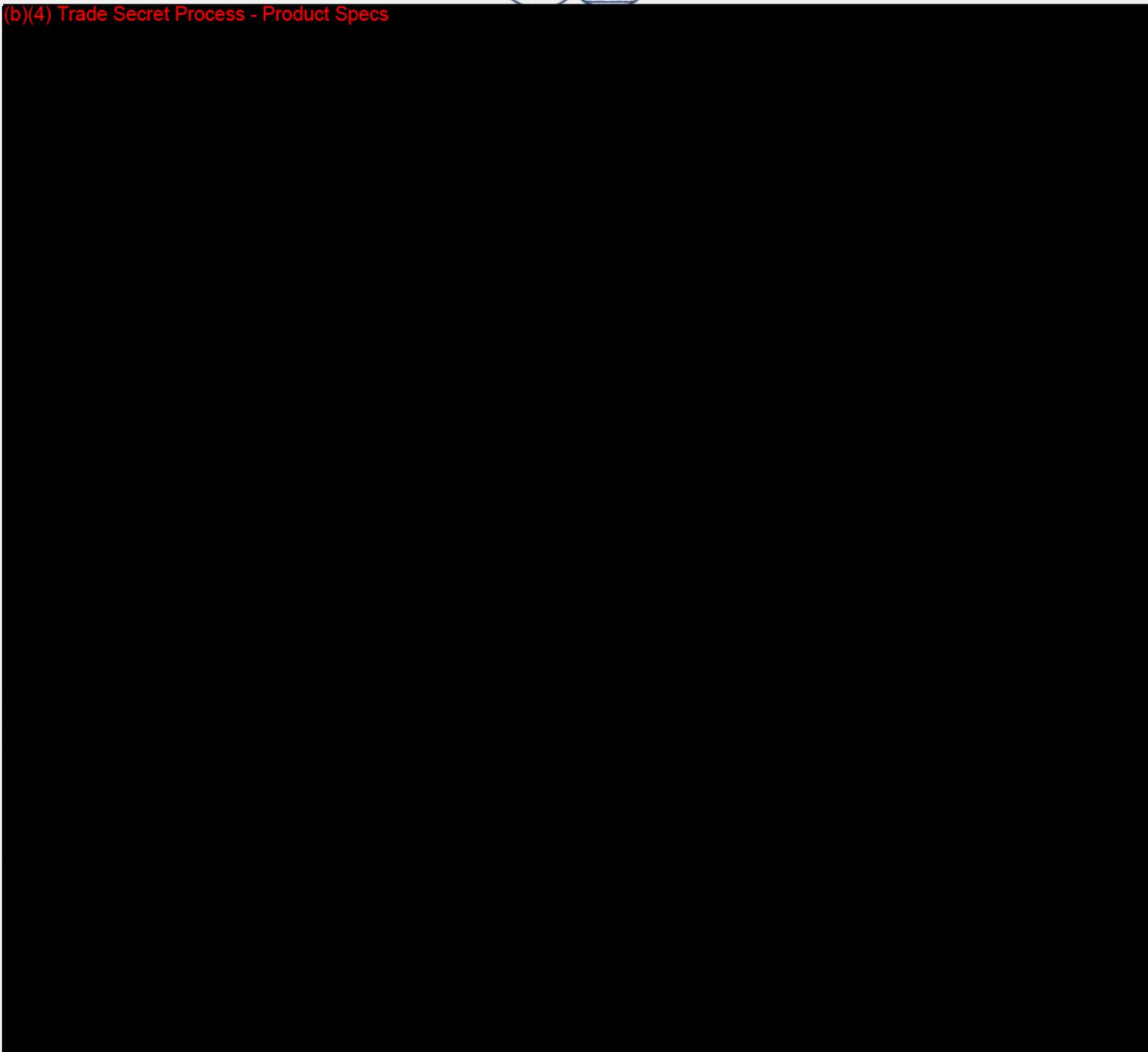
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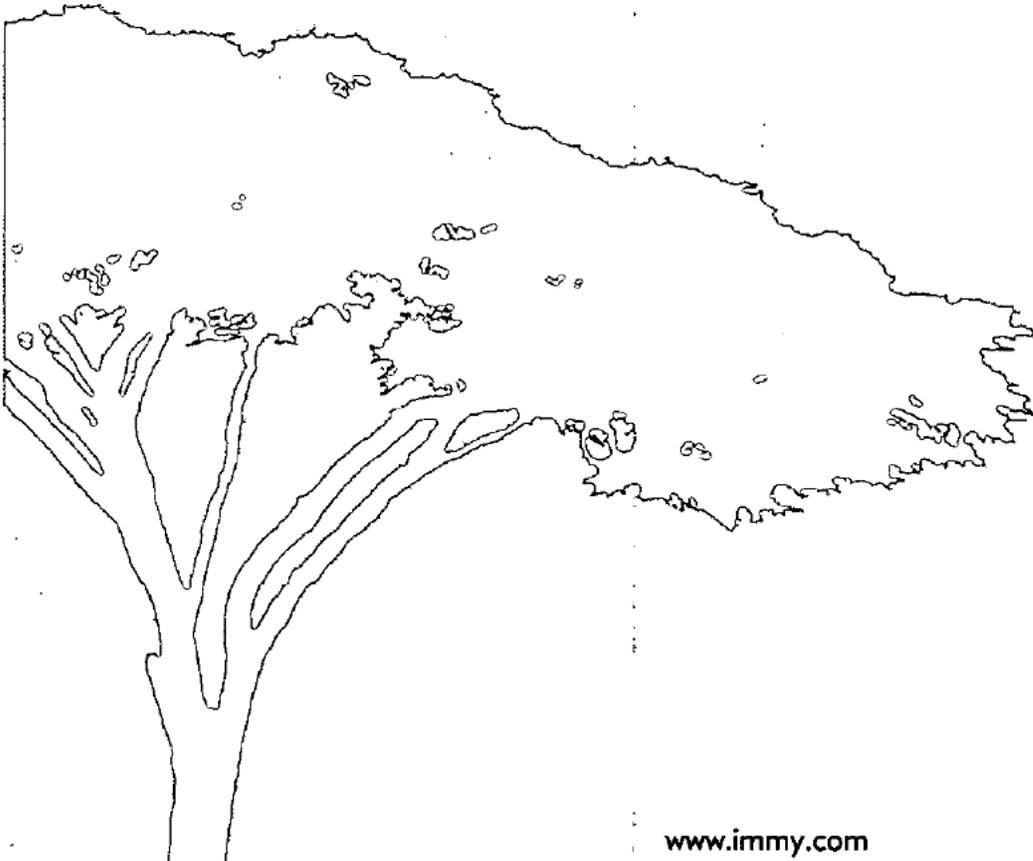


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Kind regards,

Sean K. Bauman, Ph.D.

Sean K. Bauman, Ph.D.
President and CEO



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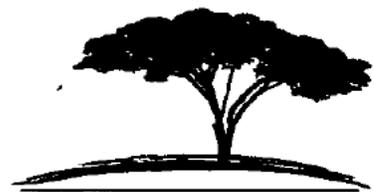
November 17, 2011

Sally Hojvat, M.Sc., Ph.D.
Director, Division of Microbiology Devices
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health
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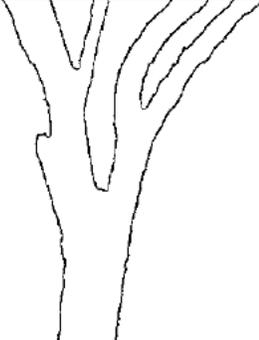
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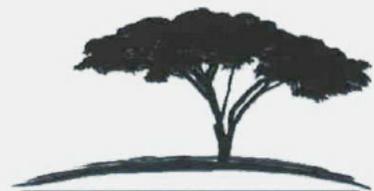
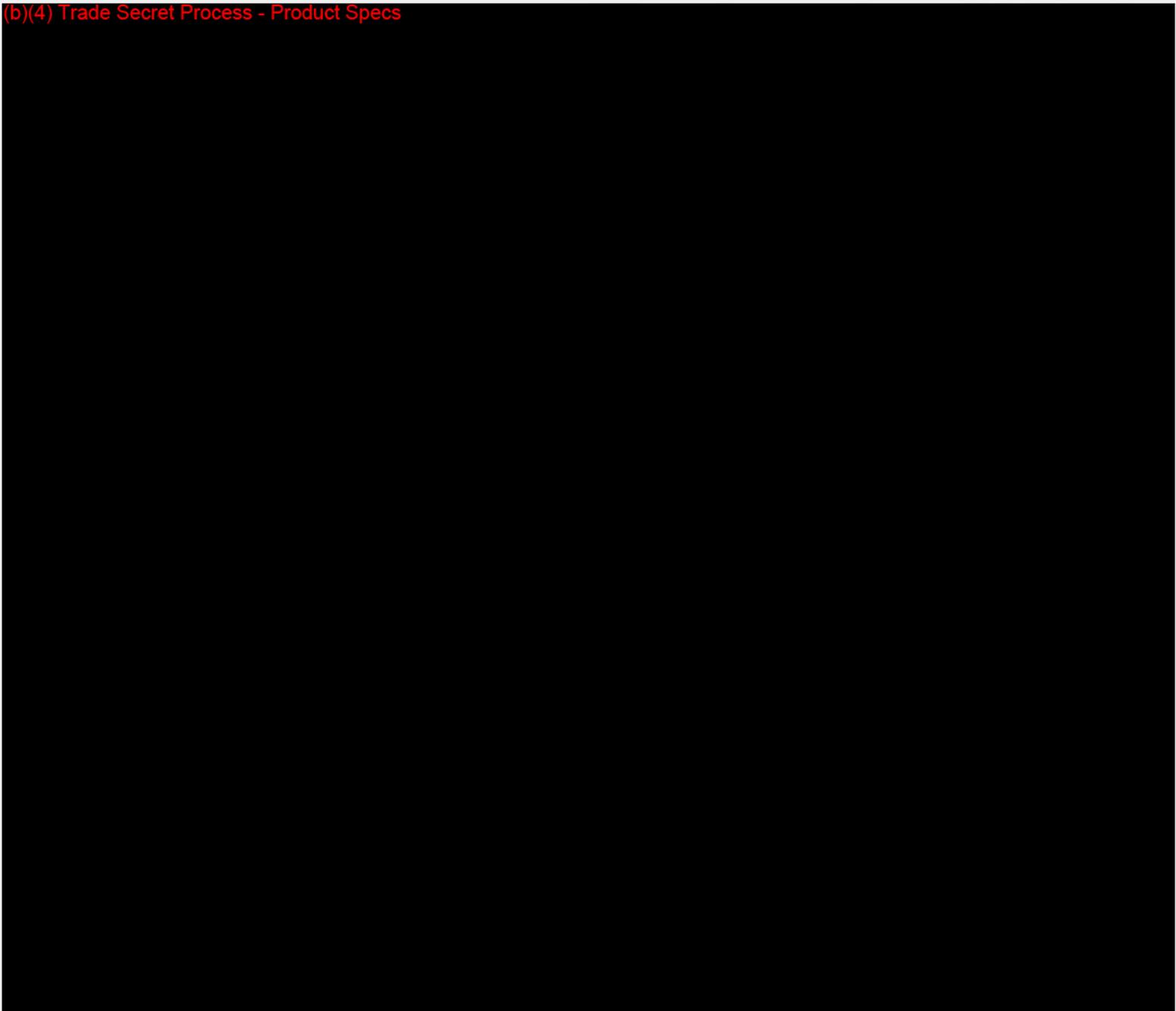
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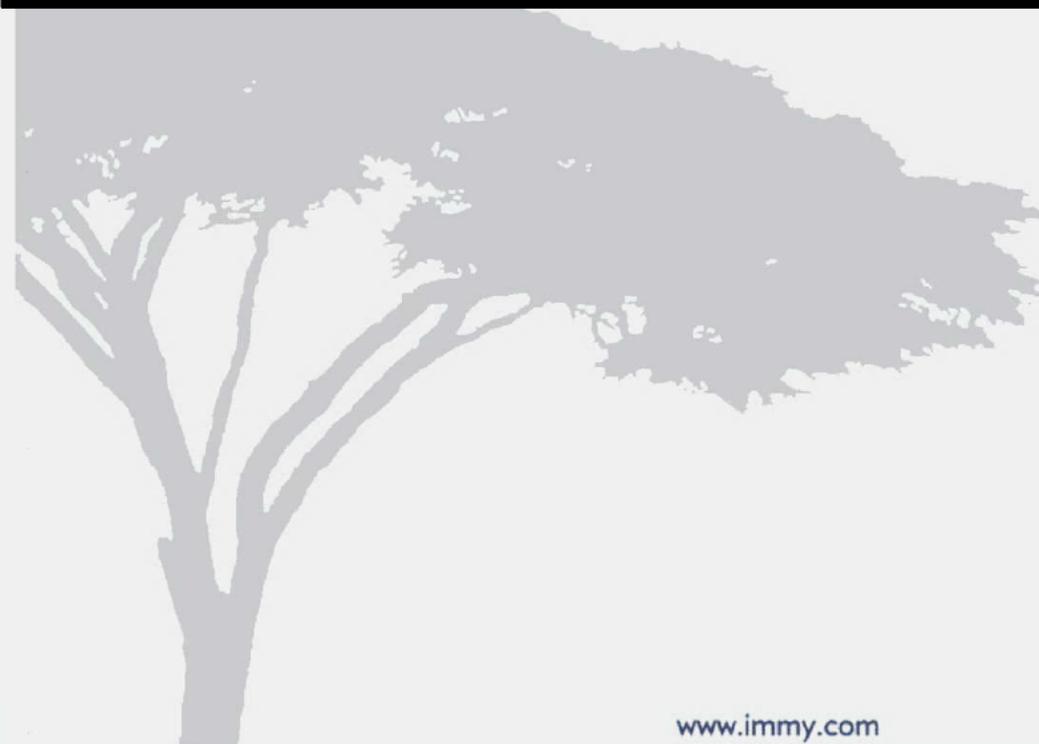
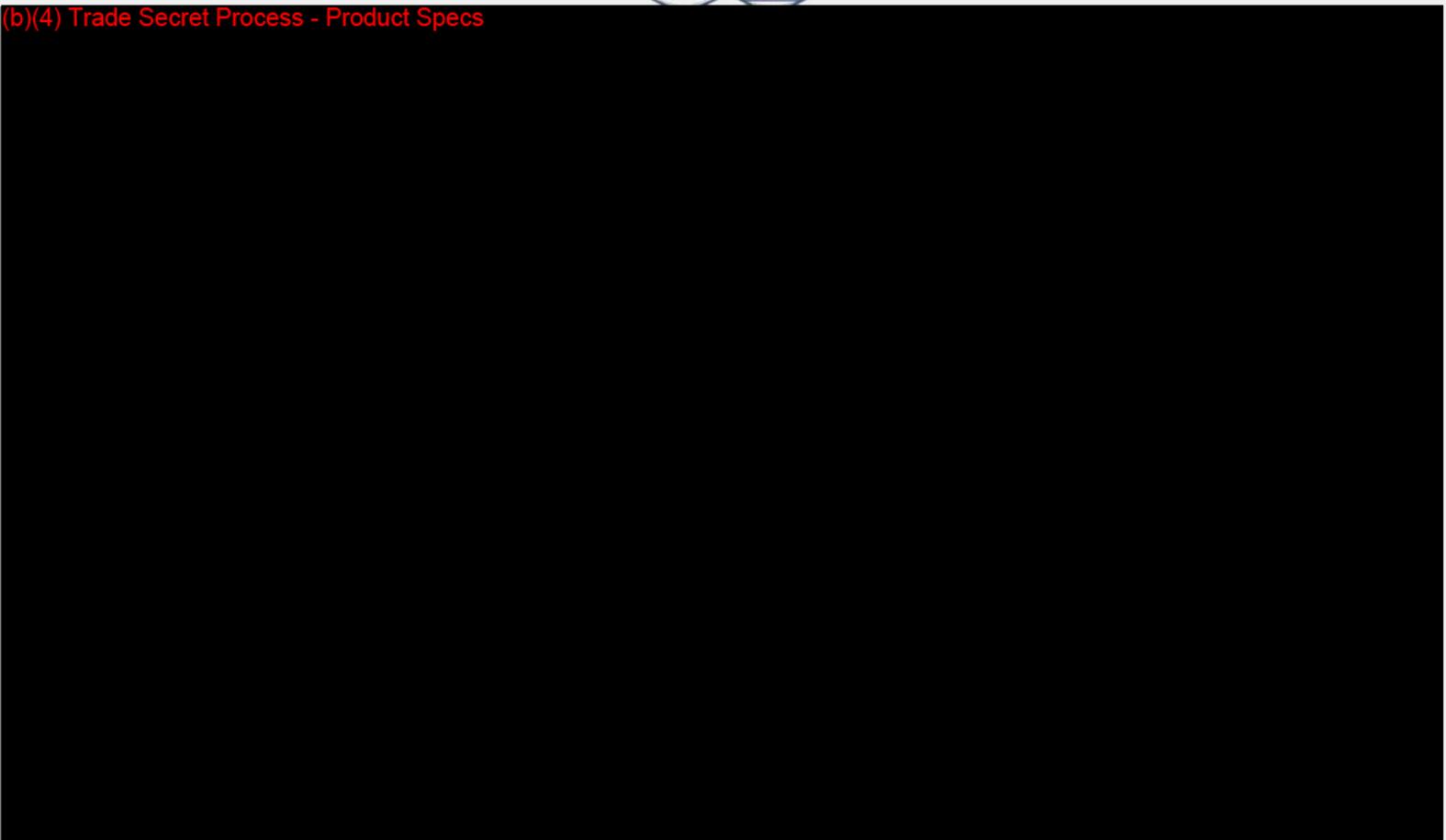
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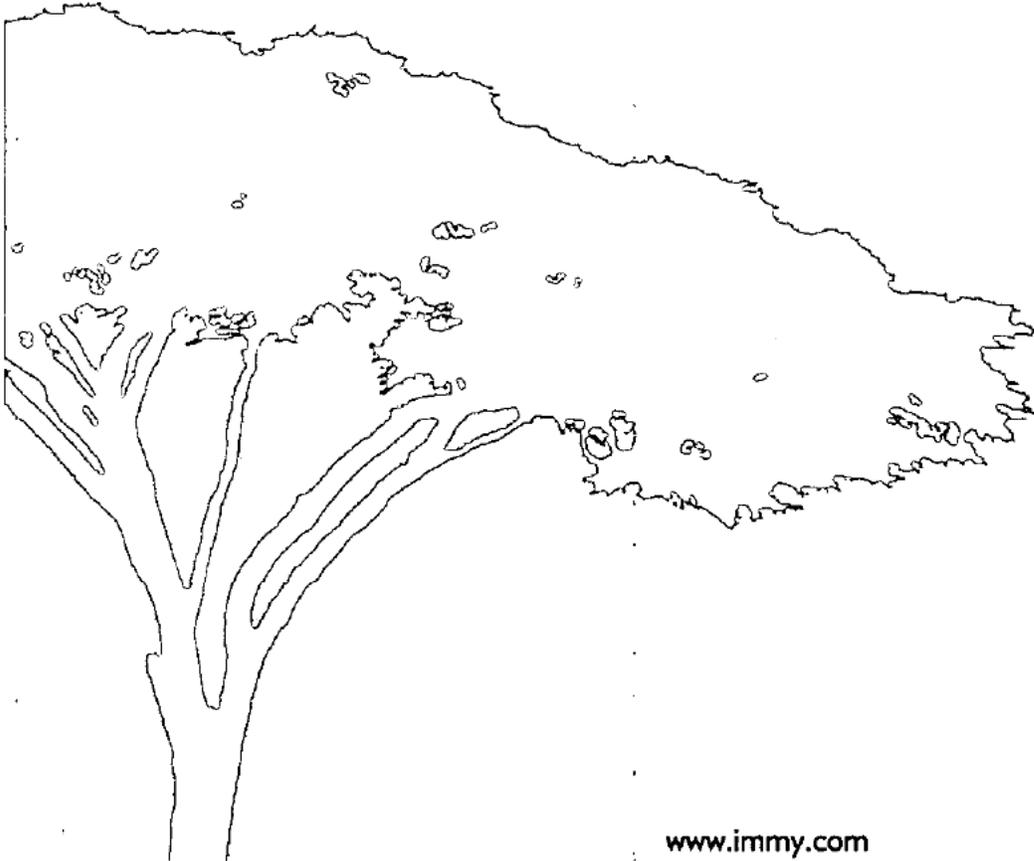


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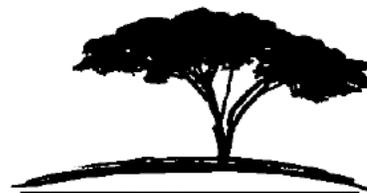
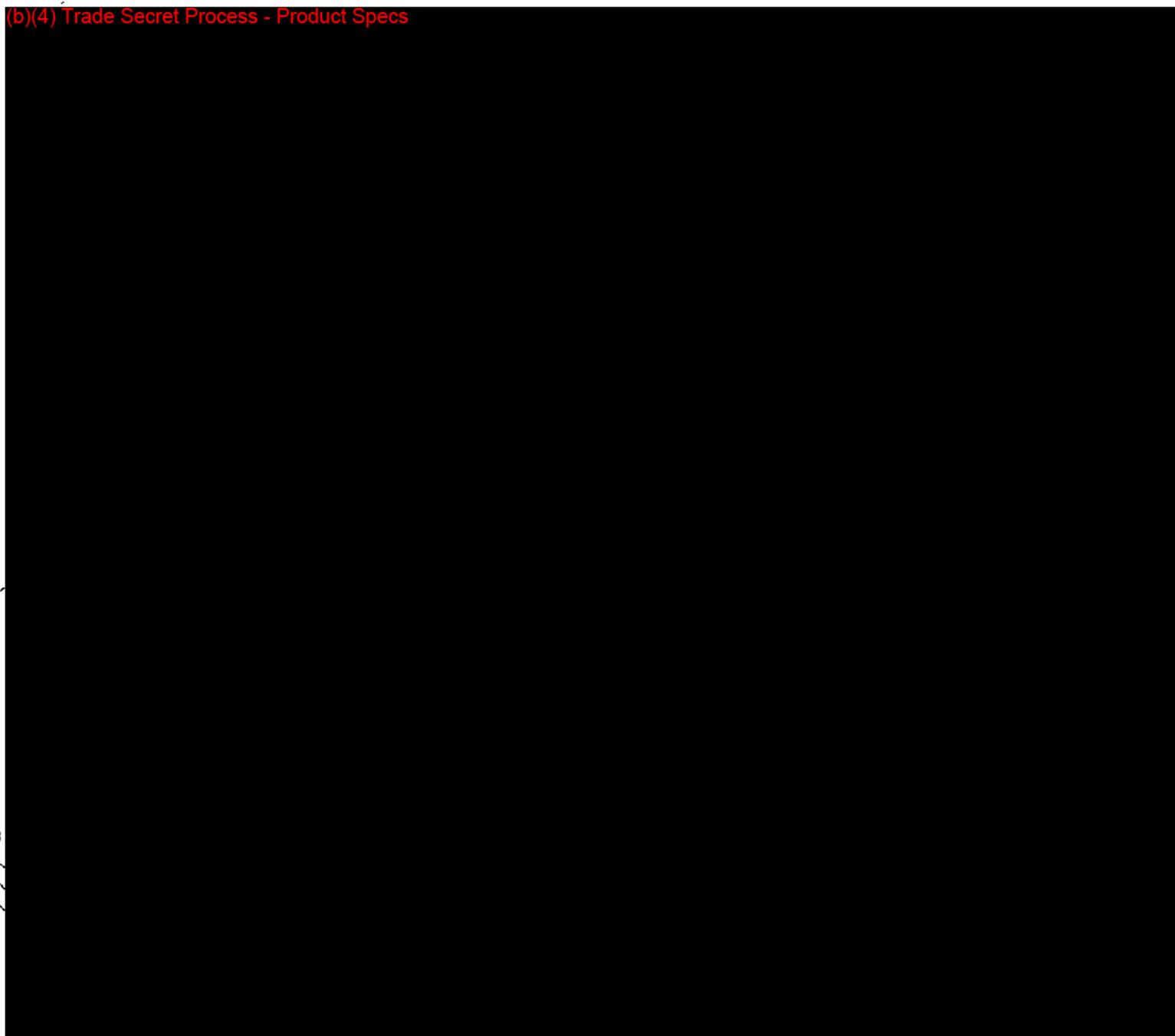
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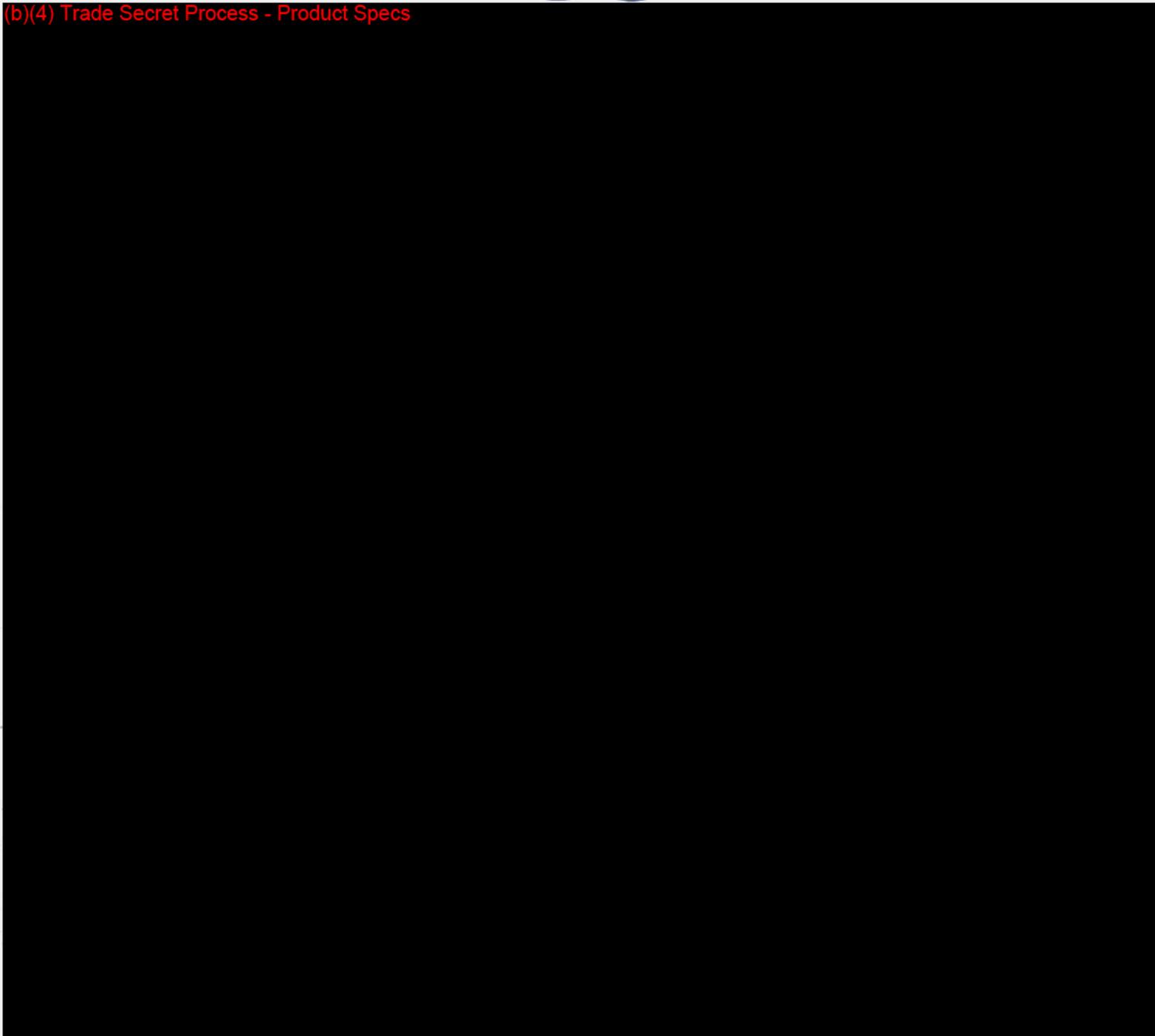
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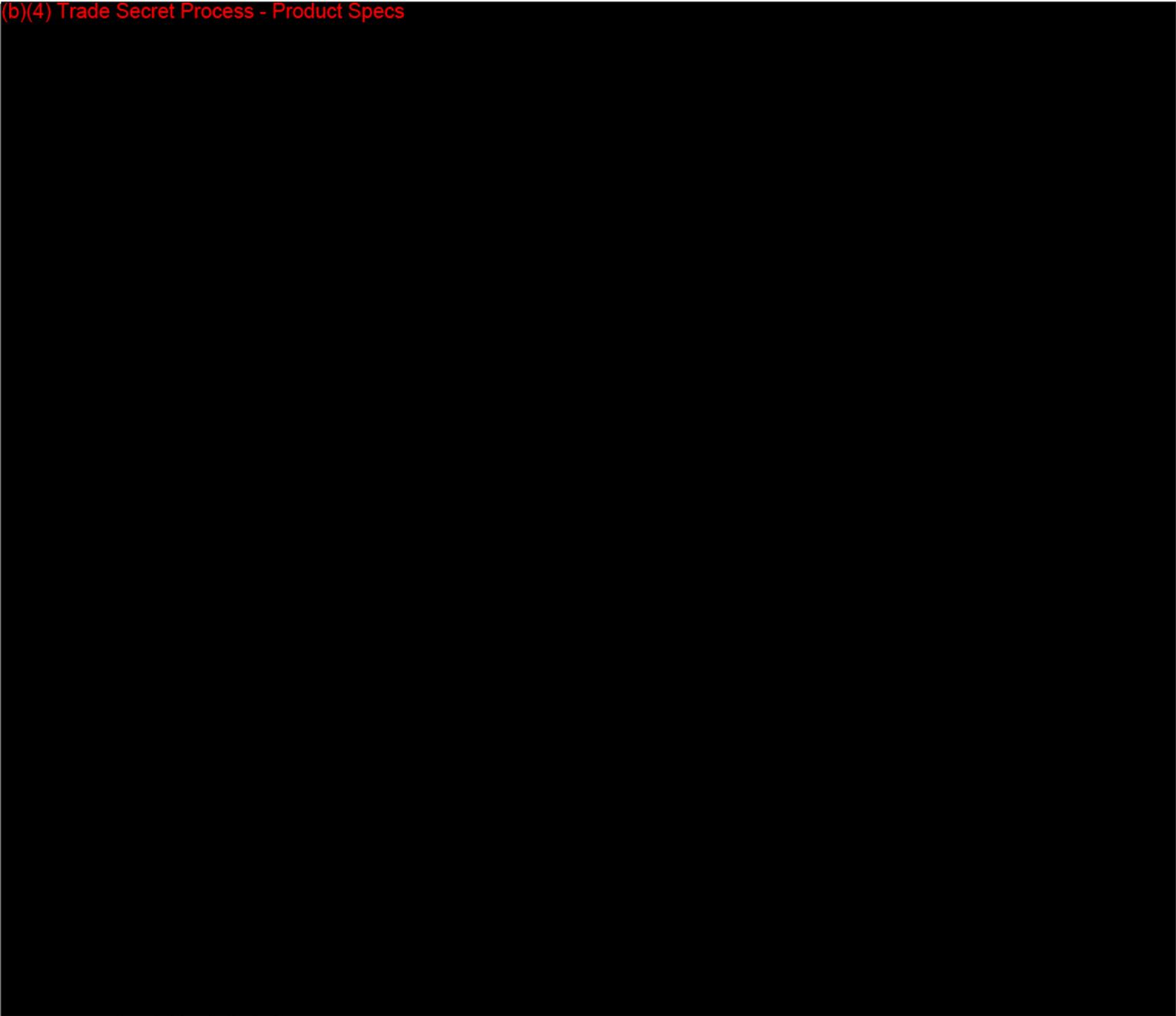
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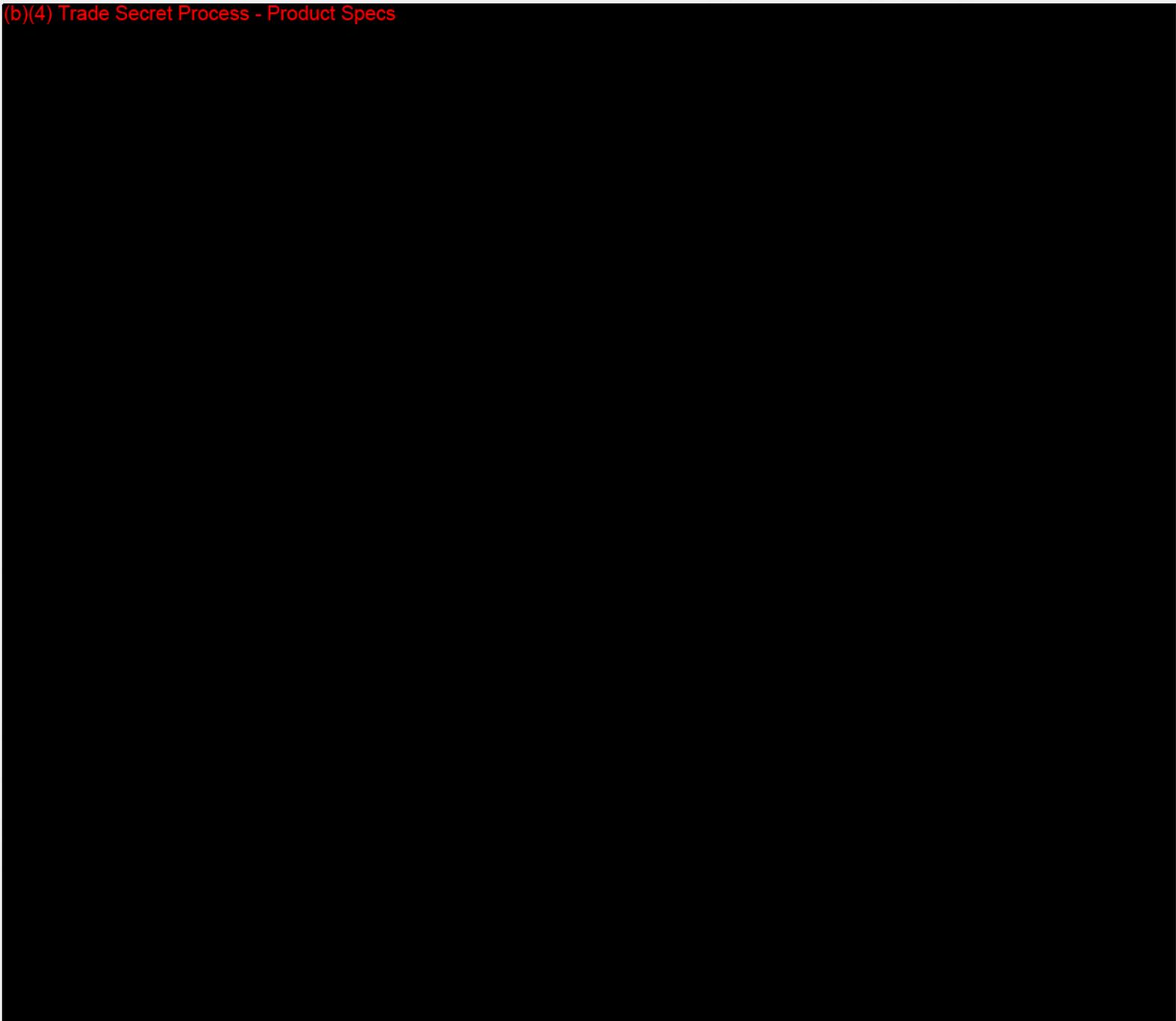


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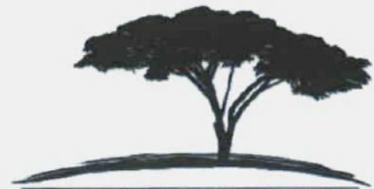
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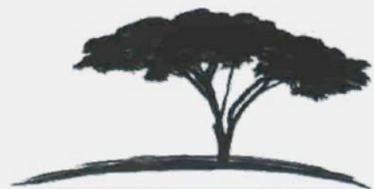
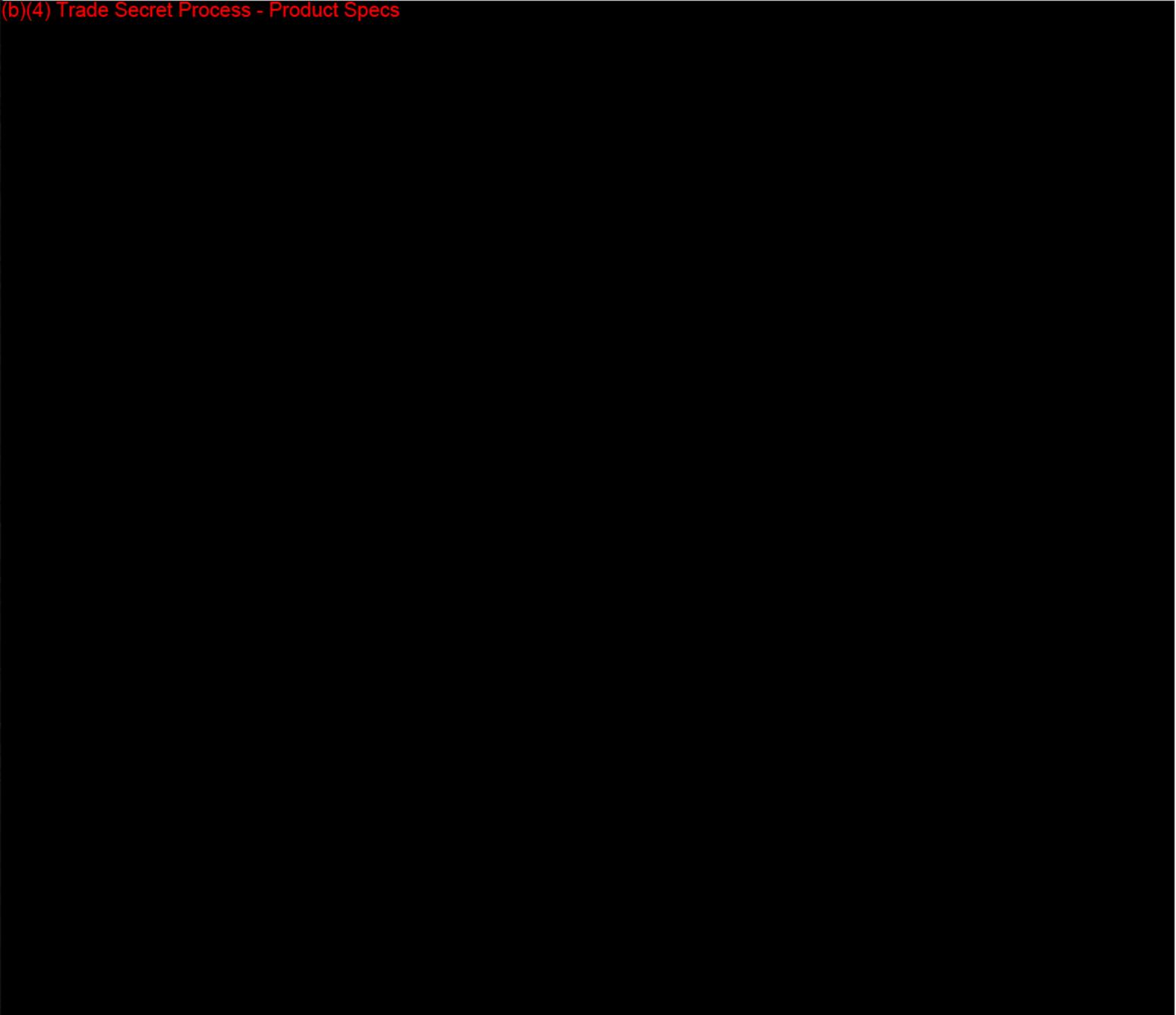
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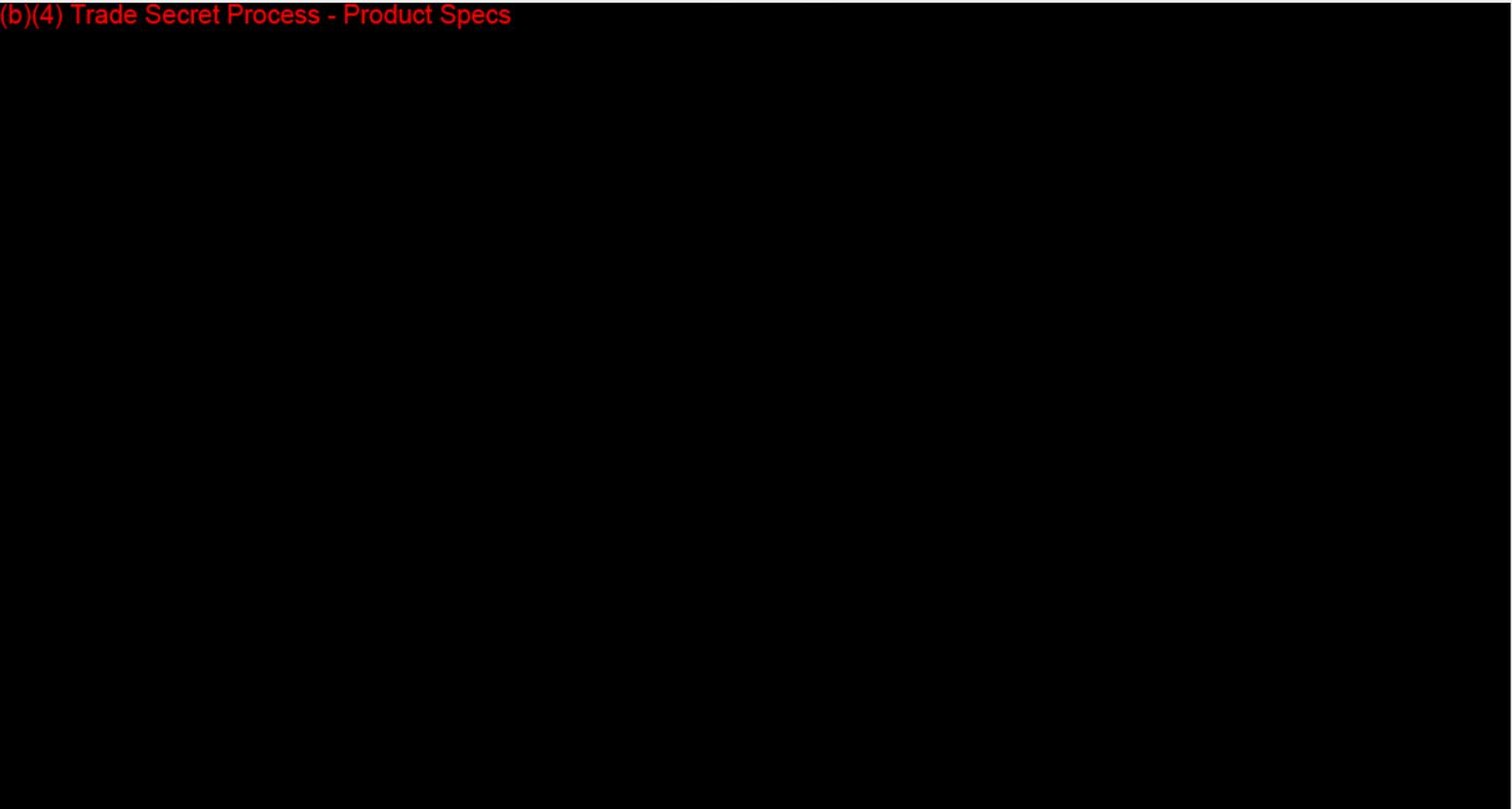
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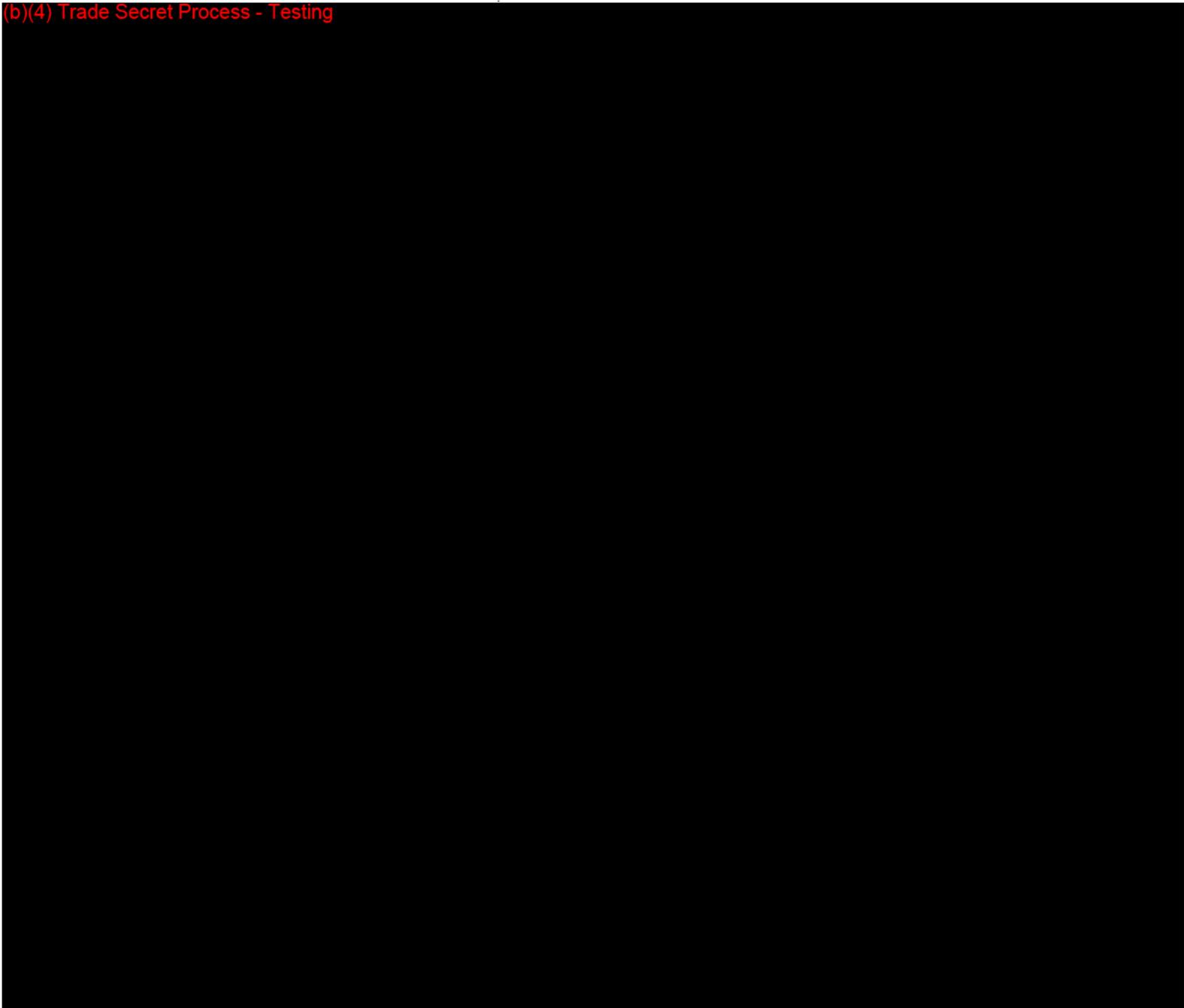
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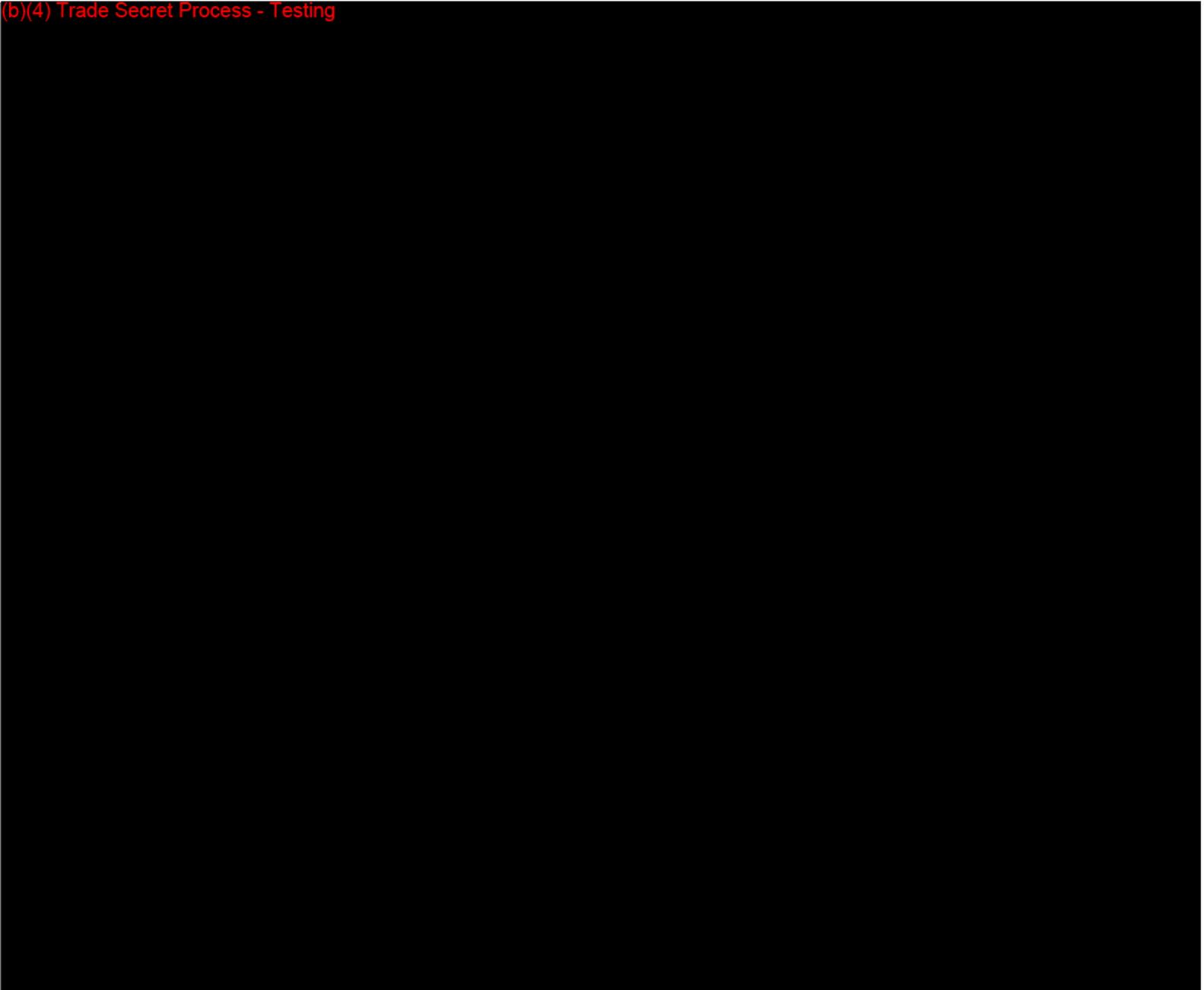
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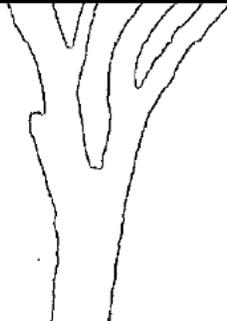
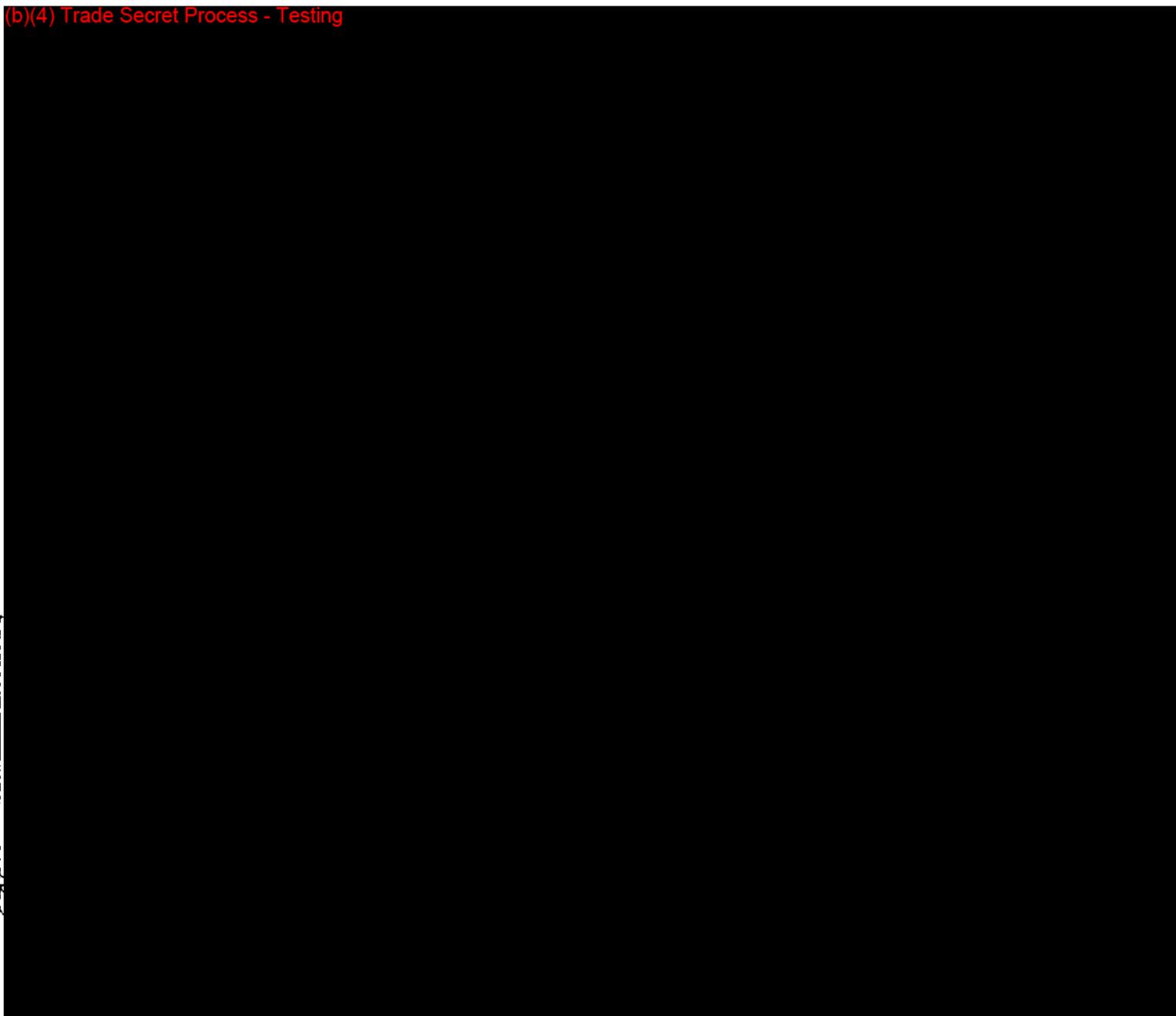


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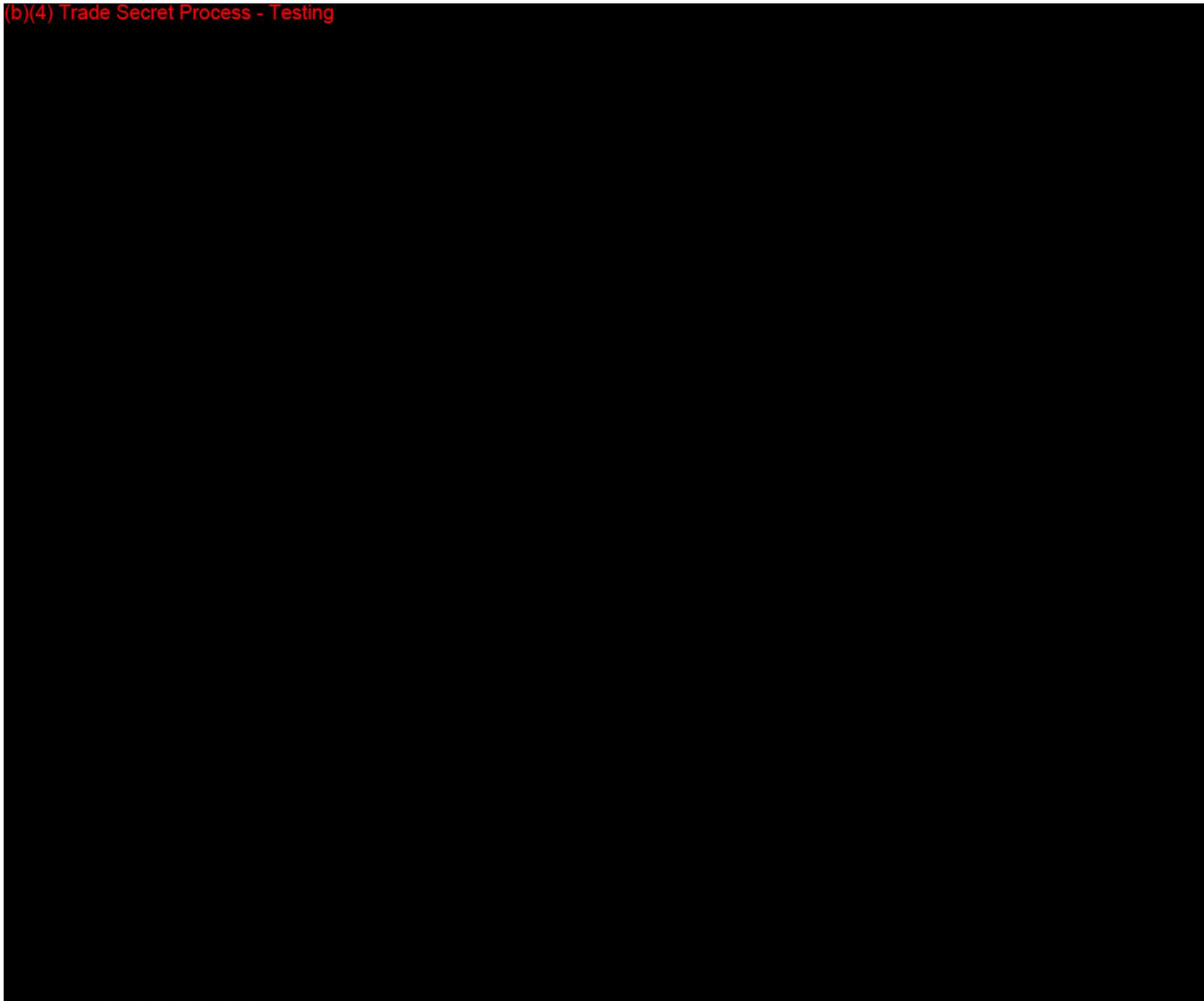
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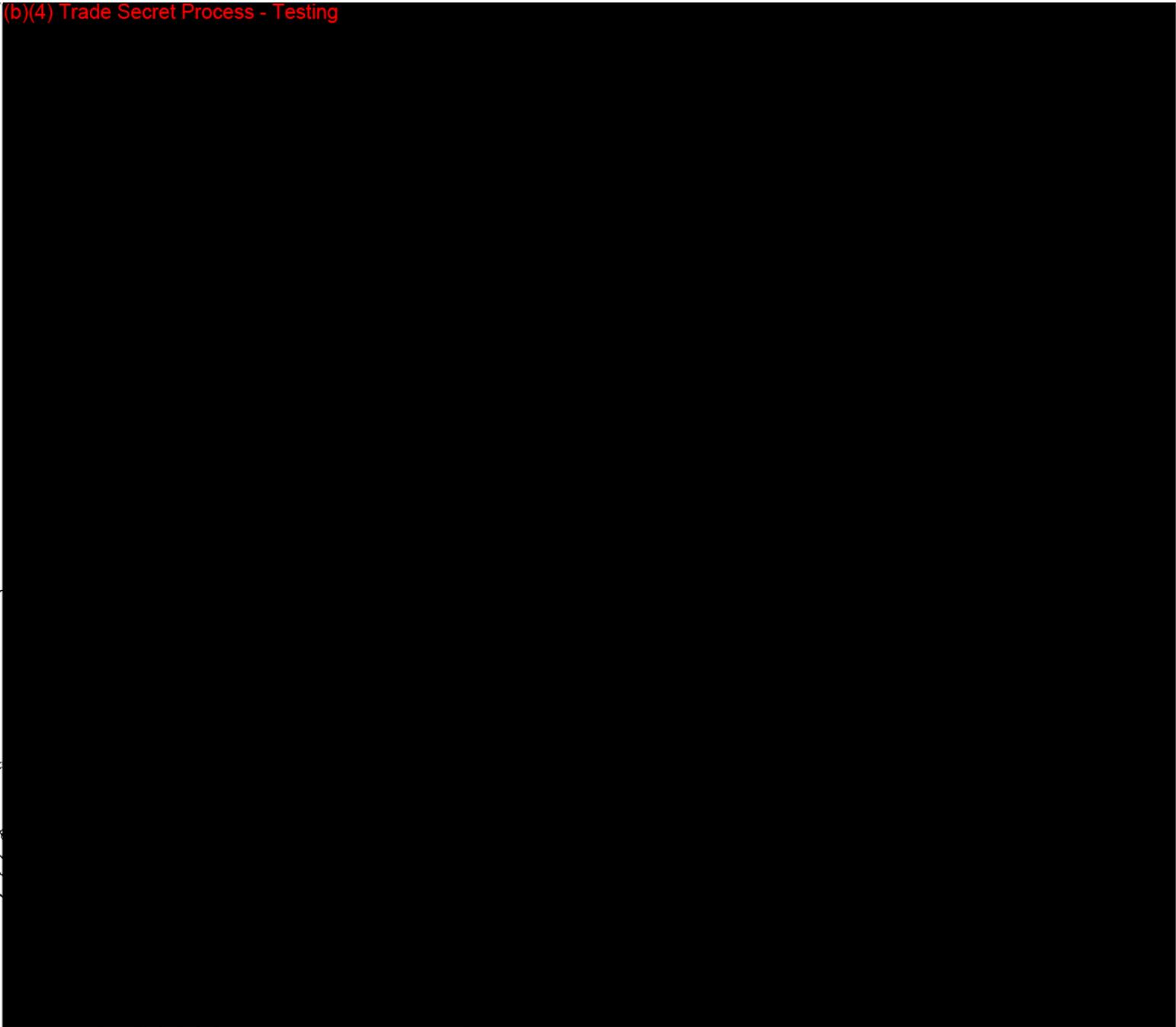
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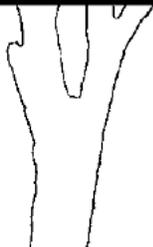
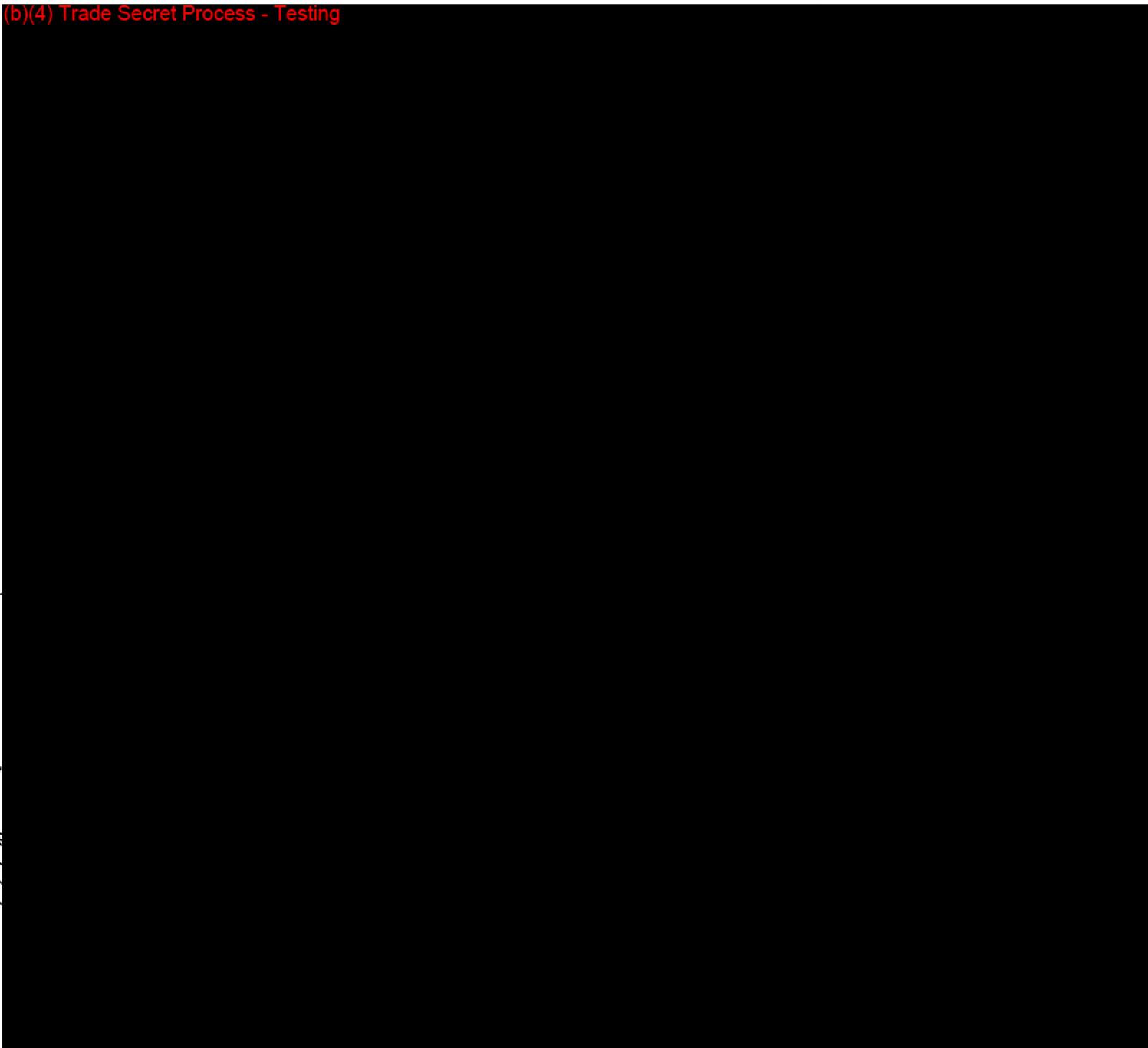


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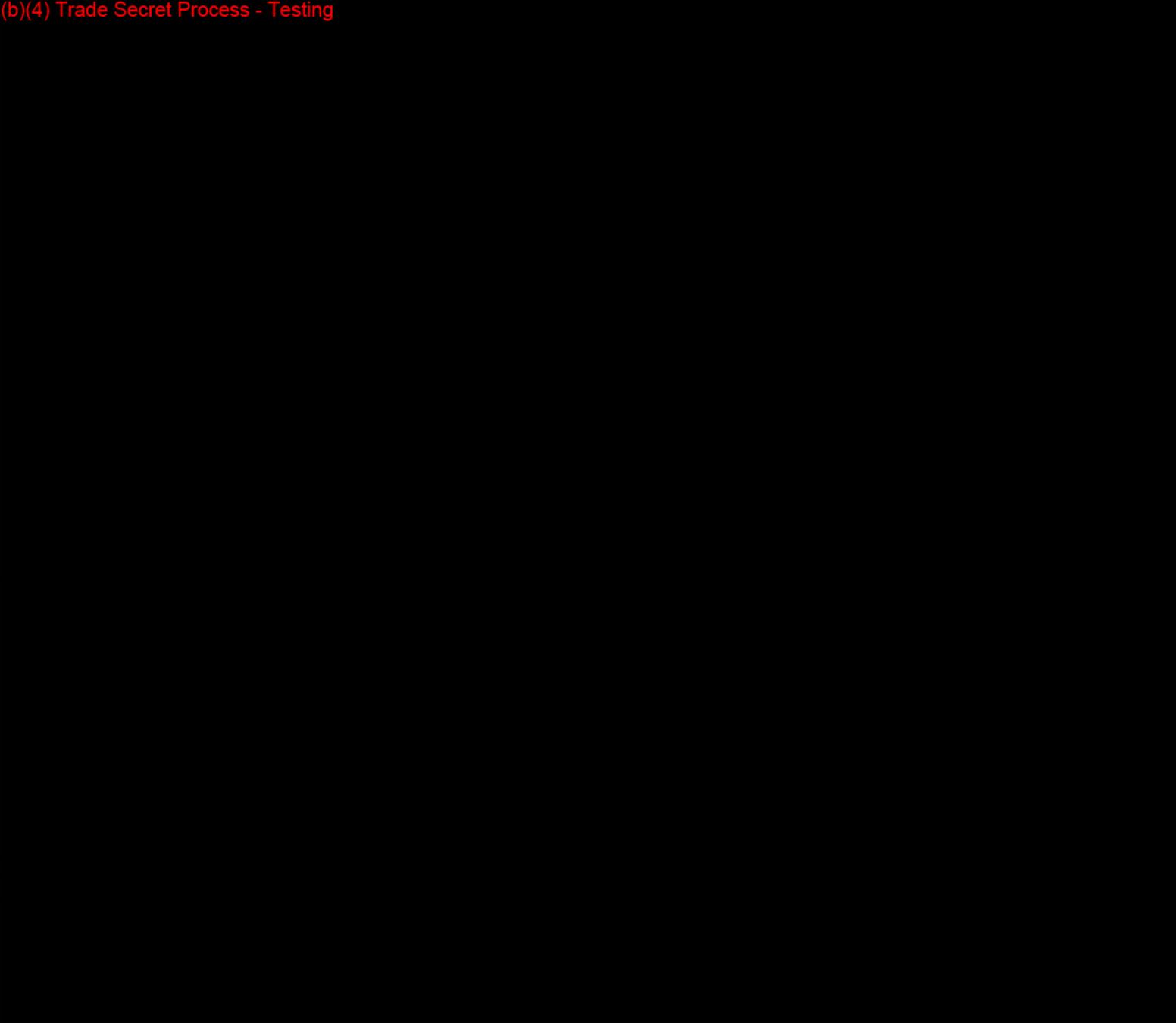
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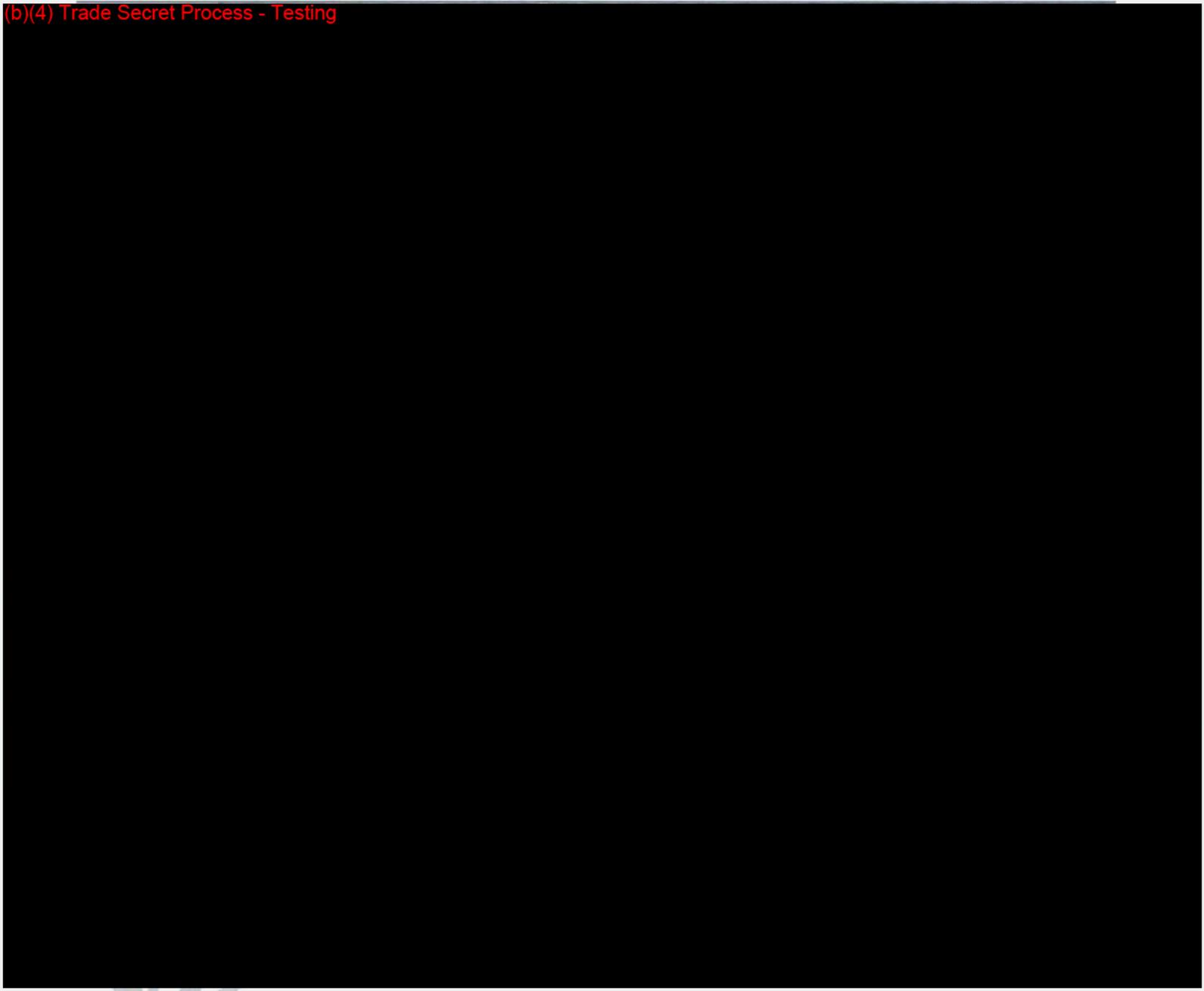


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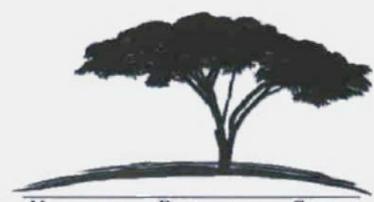
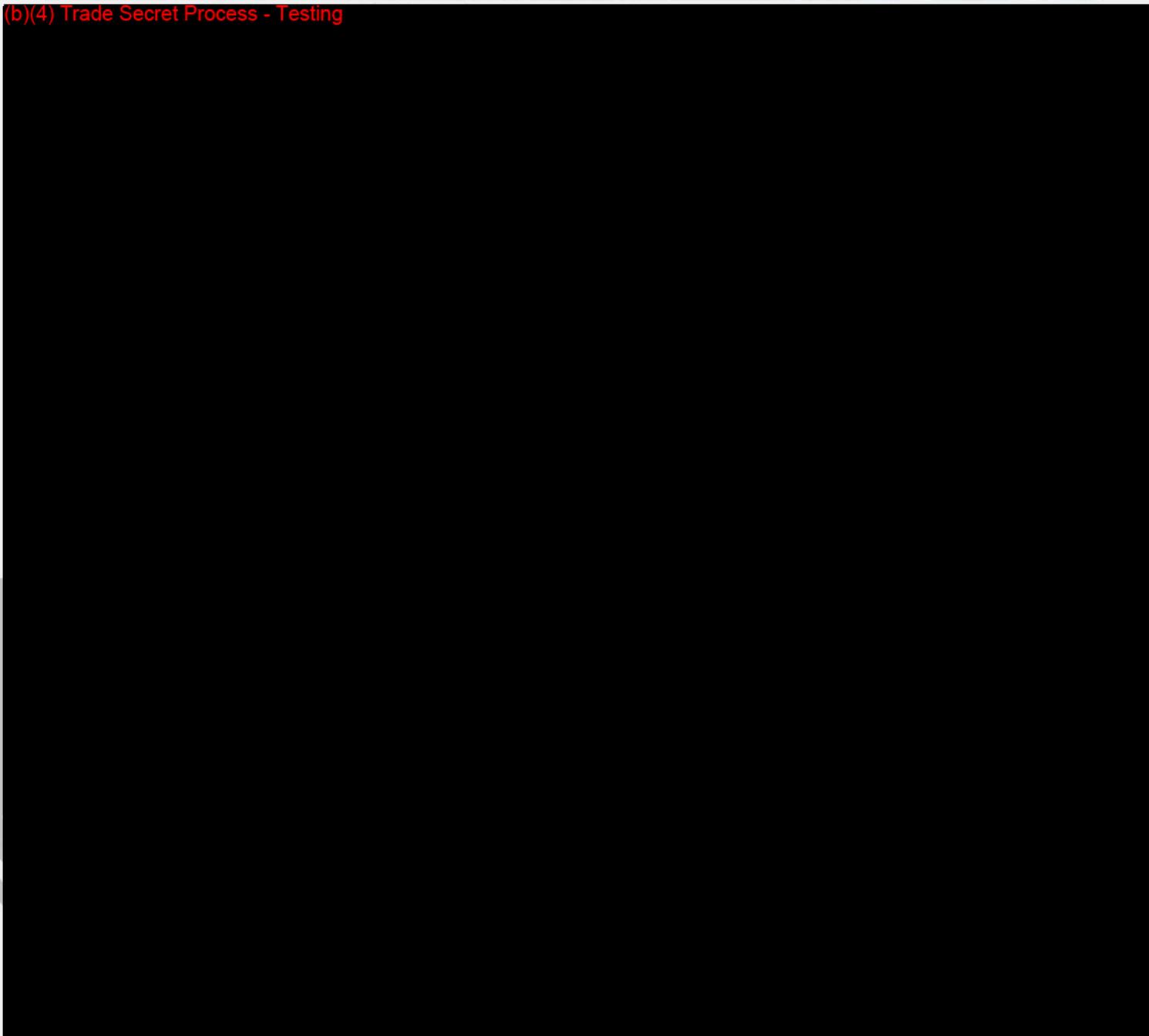
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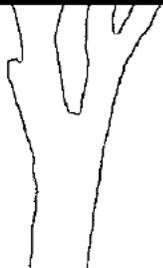
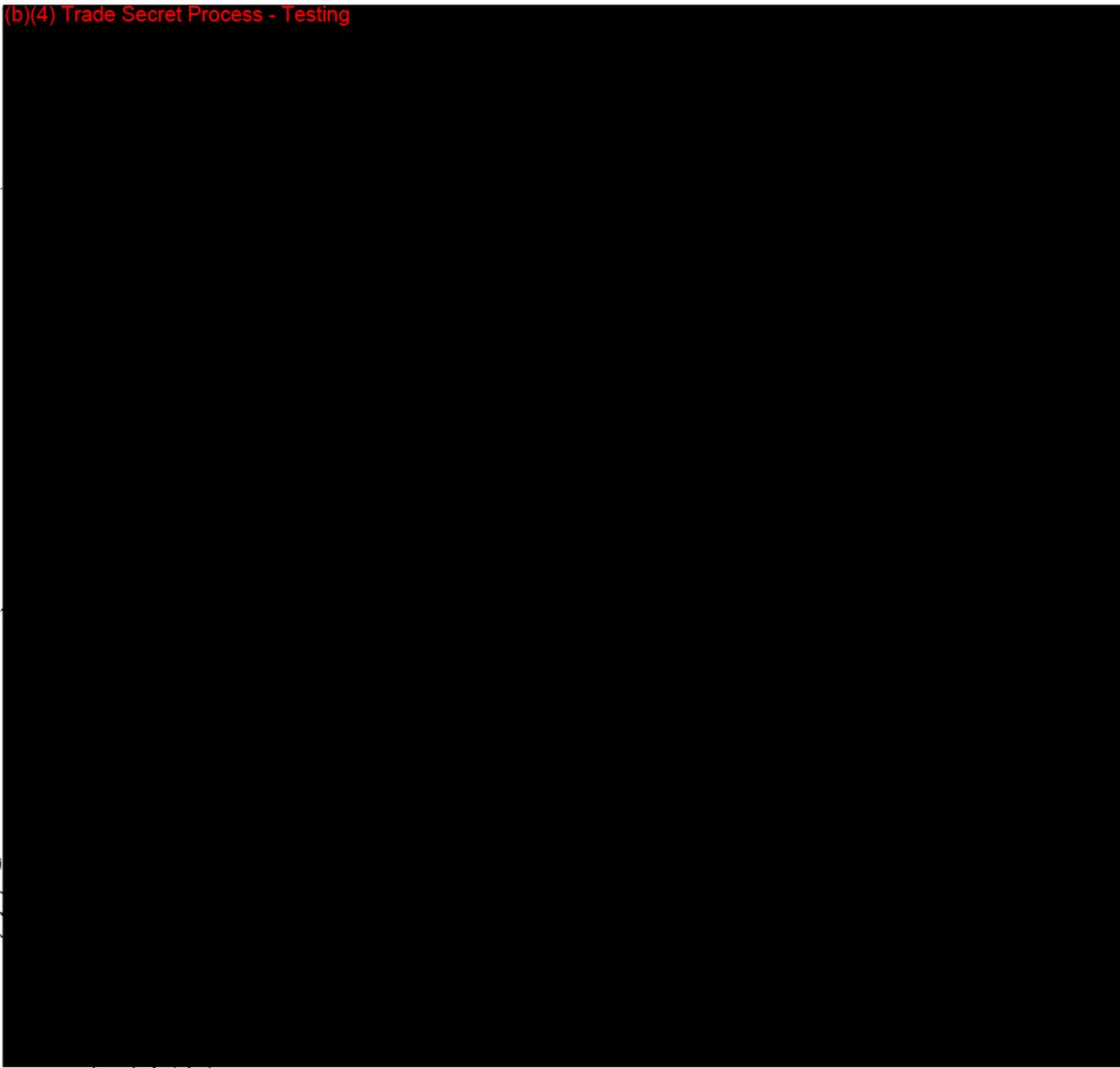
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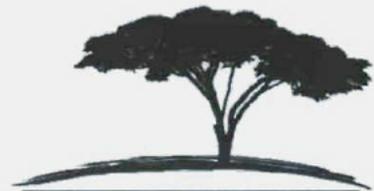
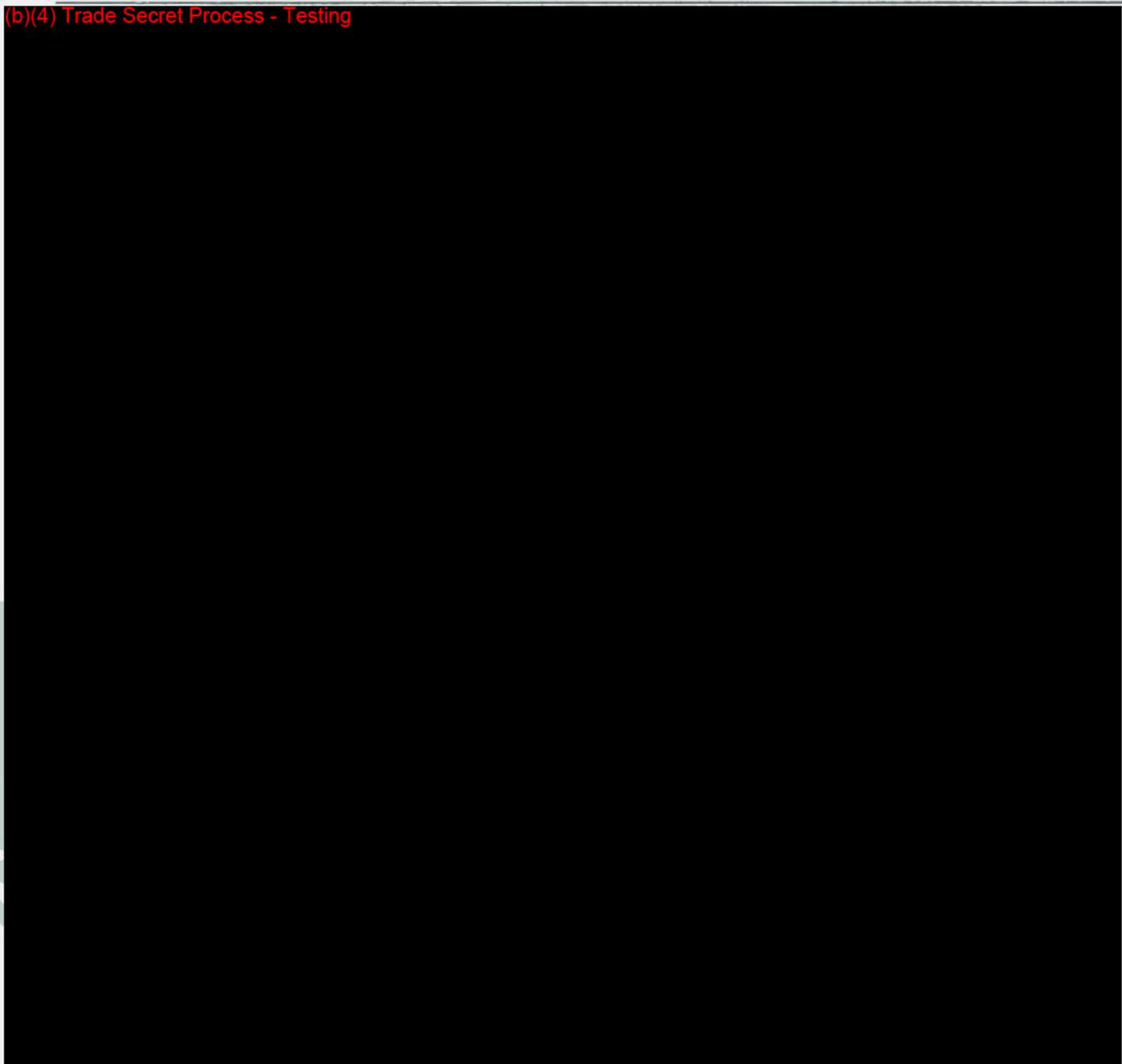
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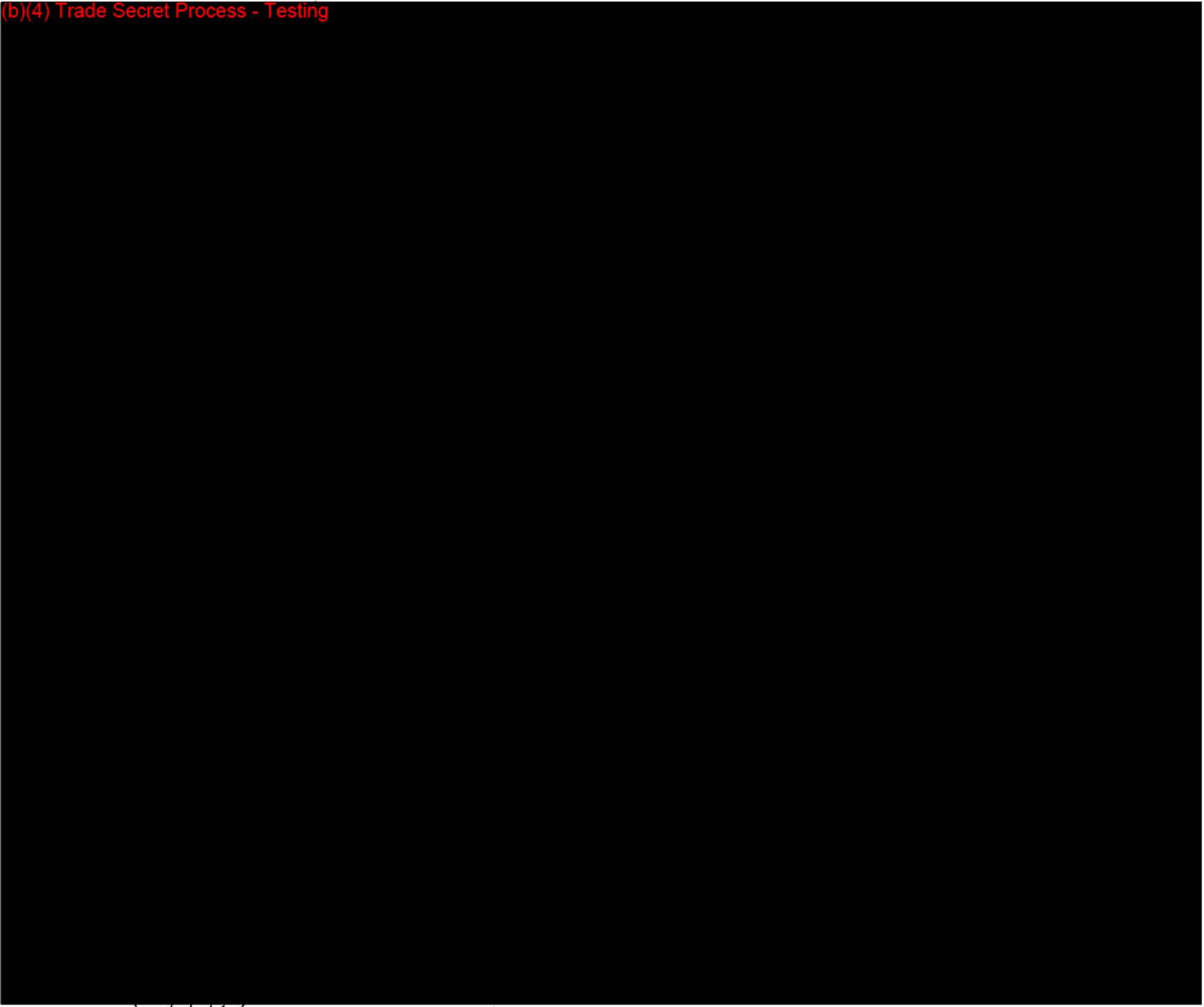
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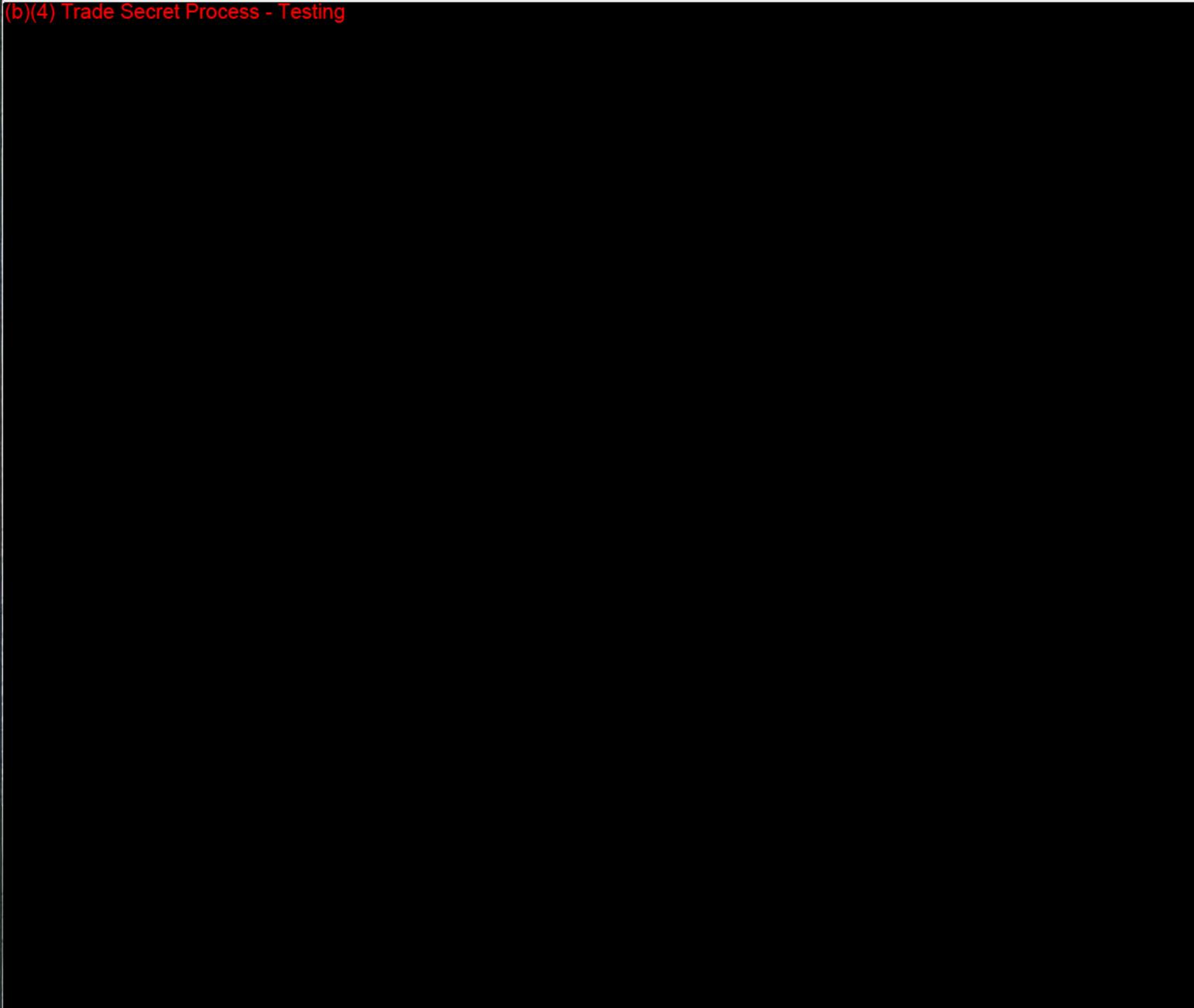


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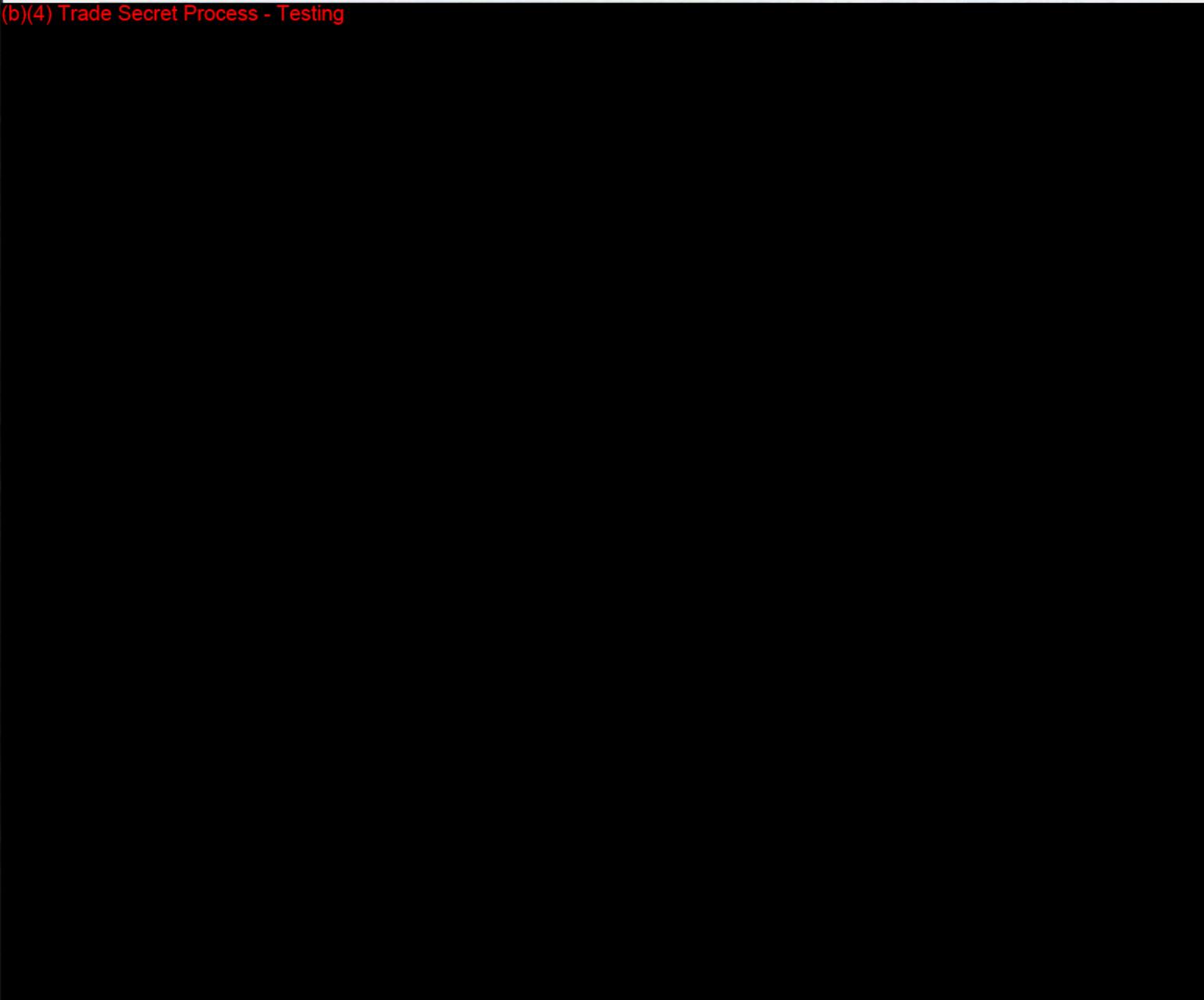
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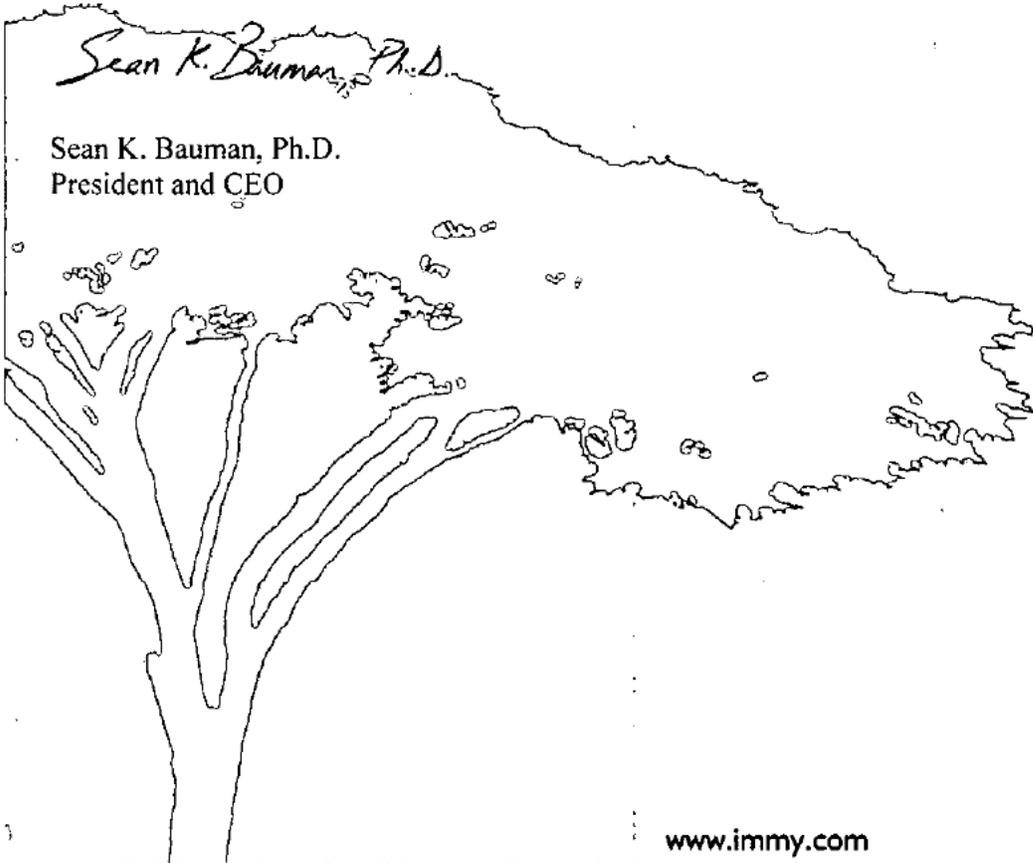
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Kind regards,

Sean K. Bauman, Ph.D.

Sean K. Bauman, Ph.D.
President and CEO



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COVER SHEET MEMORANDUM

From: Reviewer Name Michael White
Subject: 510(k) Number K112422
To: The Record

Please list CTS decision code AI

- Refused to accept (Note: this is considered the first review cycle, See Screening Checklist http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_5631/Screening%20Checklist%207%202%2007.doc)
- Hold (Additional Information or Telephone Hold).
- Final Decision (SE, SE with Limitations, NSE (select code below), Withdrawn, etc.).

Not Substantially Equivalent (NSE) Codes

- NO NSE for lack of predicate
- NI NSE for new intended use
- NQ NSE for new technology that raises new questions of safety and effectiveness
- NP NSE for lack of performance data
- NM NSE requires PMA
- NS NSE no response
- NH NSE for another reason

Please complete the following for a final clearance decision (i.e., SE, SE with Limitations, etc.):		YES	NO
Indications for Use Page	Attach IFU		
510(k) Summary /510(k) Statement	Attach Summary		
Truthful and Accurate Statement.	Must be present for a Final Decision		
Is the device Class III?			
If yes, does firm include Class III Summary?	Must be present for a Final Decision		
Does firm reference standards? (If yes, please attach form from http://www.fda.gov/opacom/morechoices/fdaforms/FDA-3654.pdf)			
Is this a combination product? (Please specify category _____, see http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_413b/COMBINATION%20PRODUCT%20ALGORITHM%20(REVISED%203-12-03).DOC)			
Is this a reprocessed single use device? (Guidance for Industry and FDA Staff – MDUFMA - Validation Data in 510(k)s for Reprocessed Single-Use Medical Devices, http://www.fda.gov/cdrh/ode/guidance/1216.html)			
Is this device intended for pediatric use only?			
Is this a prescription device? (If both prescription & OTC, check both boxes.)			
Did the application include a completed FORM FDA 3674, Certification with Requirements of ClinicalTrials.gov Data Bank?			
Is clinical data necessary to support the review of this 510(k)?			
For United States-based clinical studies only: Did the application include a completed FORM FDA 3674, Certification with Requirements of ClinicalTrials.gov Data Bank? (If study was conducted in the United States, and FORM FDA 3674 was not included or incomplete, then applicant must be contacted to obtain completed form.)			
Does this device include an Animal Tissue Source?			
All Pediatric Patients age <=21			

Neonate/Newborn (Birth to 28 days)

Infant (29 days - < 2 years old)

Child (2 years - < 12 years old)

Adolescent (12 years - < 18 years old)

Transitional Adolescent A (18 - < 21 years old) Special considerations are being given to this group, different from adults age ≥ 21 (different device design or testing, different protocol procedures, etc.)

Transitional Adolescent B (18 - ≤ 21 ; No special considerations compared to adults $\Rightarrow 21$ years old)

Nanotechnology

Is this device subject to the Tracking Regulation? (Medical Device Tracking Guidance, <http://www.fda.gov/cdrh/comp/guidance/169.html>)

Contact OC.

Regulation Number

Class*

Product Code

(*If unclassified, see 510(k) Staff)

Additional Product Codes: _____

Review:

Freddie L. Cook
(Branch Chief)

BACB

(Branch Code)

11/09/11
(Date)

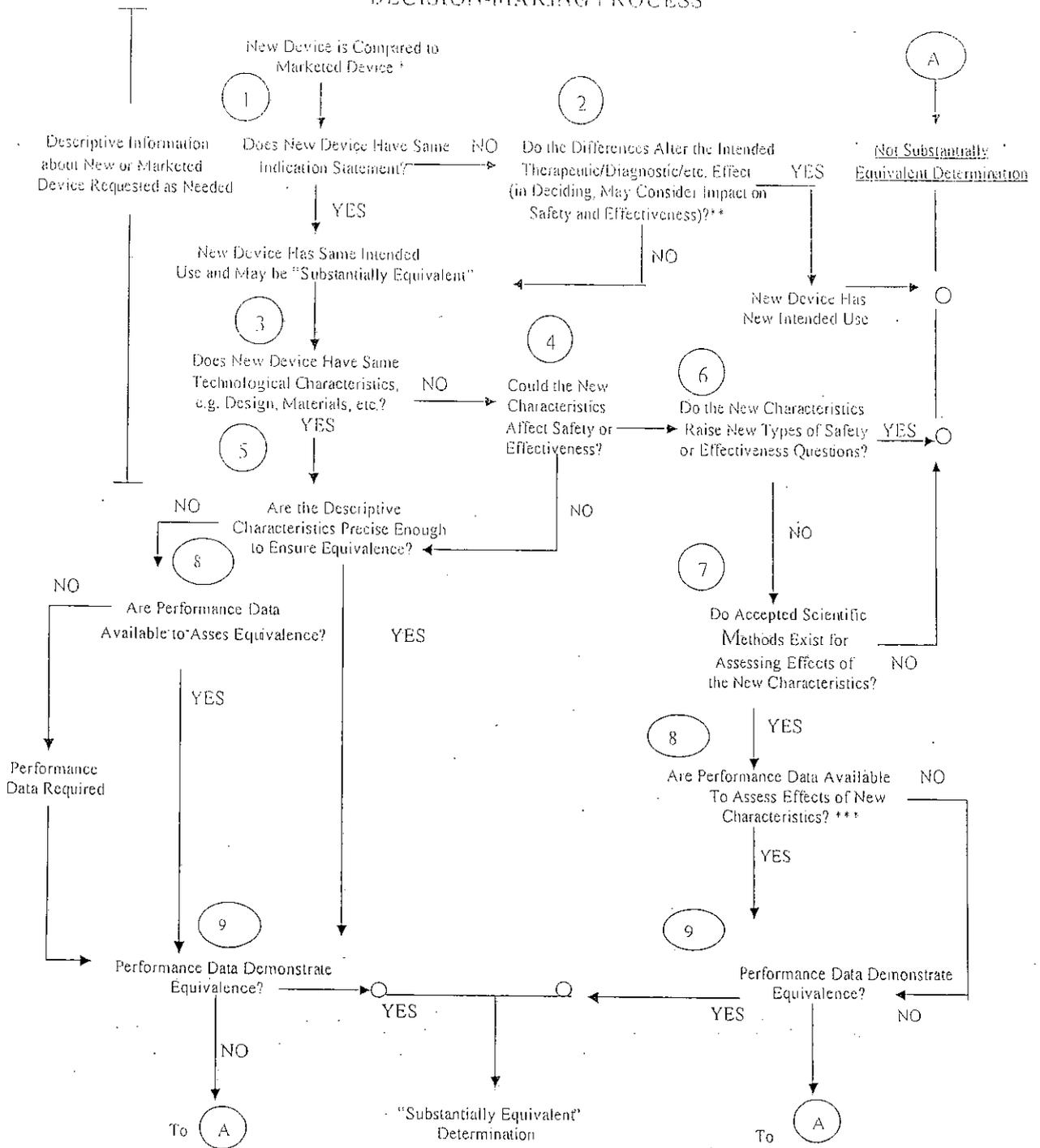
Final Review:

John H. Davis
(Division Director)

>

11/9/11
(Date)

510(k) "SUBSTANTIAL EQUIVALENCE" DECISION-MAKING PROCESS



* 510(k) Submissions compare new devices to marketed devices. FDA requests additional information if the relationship between marketed and "predicate" (pre-Amendments or reclassified post-Amendments) devices is unclear.

** This decision is normally based on descriptive information alone, but limited testing information is sometimes required.

*** Data may be in the 510(k), other 510(k)s, the Center's classification files, or the literature.

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K112422

B. Purpose for Submission:

To obtain substantial equivalence for an original 510(k) for a device which detects Cryptococcal Antigen.

C. Measurand:

Capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*)

D. Type of Test:

Qualitative and semi-quantitative dipstick sandwich lateral flow immunochromatographic assay

E. Applicant:

Immuno-Mycologies, Inc.

F. Proprietary and Established Names:

CrAg Lateral Flow Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
GMD	II	866.3165	83-Microbiology

H. Intended Use:

1. Intended use:

The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum, (b)(4) Trade and cerebral spinal fluid (CSF).

2. Indication for use:

The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum, (b)(4) Trade [REDACTED] and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription use laboratory assay which can aid in the diagnosis of cryptococcosis

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Not applicable

I. Device Description:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay which detects cryptococcal antigen in serum, (b)(4) Trade [REDACTED], and cerebral spinal fluid (CSF). The assay consists of CrAg Lateral Flow test strips which have a gold-conjugated antibody and a gold-conjugated, anti-cryptococcal antibody deposited onto a sample membrane and anti-Crypto antibody and control-line capture antibody striped onto a membrane. Also in the kit is a specimen diluent.

J. Substantial Equivalence Information:

1. Predicate Device names

Immuno-Mycologics' CrAg Lateral Flow Assay

2. Predicate K number:

K102286

Comparison with predicate:

Table 1: Comparison Between New Device and Predicate Device

SIMILARITIES		
Feature	CrAg LFA (New Device)	CrAg LFA (Serum Only) (K102286)
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum
Indication For Use	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis	Prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis
Device Description		
Technology	Lateral Flow Assay	Lateral Flow Assay
Sample Matrix	Serum	Serum
Instruments	None	None
Assay Components	Specimen diluent, lateral flow strips, built-in control, gold conjugated antibodies	Positive control, negative control, latex cards, latex conjugated antibodies
Specimen Pre-Treatment	Dilution	Dilution
Detection Antibody	Anti-cryptococcal monoclonal antibody	Anti-cryptococcal monoclonal antibody
Storage Requirements	20-25°C	20-25°C
DIFFERENCES		
Feature	Cryptococcal Antigen Lateral Flow Assay	Latex- <i>Cryptococcus</i> Antigen Detection System
Intended Use	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum, (b)(4), and CSF

K. Standard/Guidance Document Referenced (if applicable):

Not Applicable

L. Test Principle:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in serum, (b)(4) Trade, and CSF. For the qualitative procedure, specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For the semi-quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the qualitative procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then

placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold-conjugated, anti-cryptococcal monoclonal antibodies and gold-conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it binds to the gold-conjugated, anti-cryptococcal antibodies. The gold-labeled antibody-antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti-cryptococcal monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold-labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold-conjugated control goat IgG antibody to move to the Control Line (C) which is immobilized bovine anti-goat IgG antibody. The immobilized anti-goat antibody will bind to the gold-conjugated goat IgG Control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line (Figure 1). If the control line fails to develop a line, then the test is not valid.

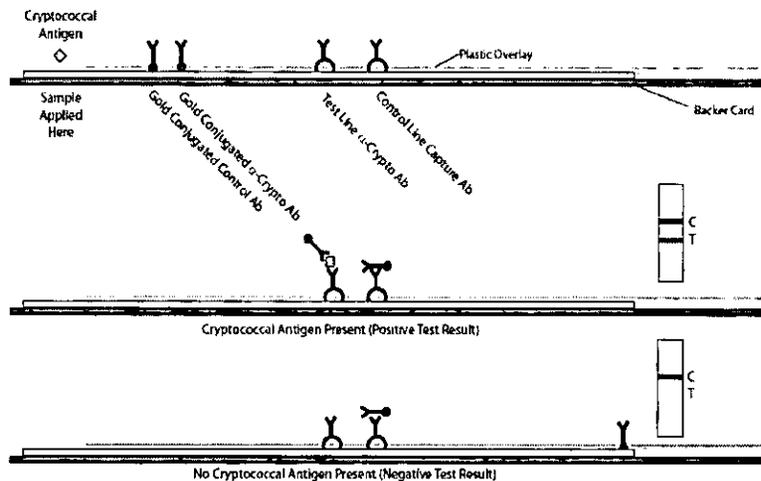


Figure1. CrAg Lateral Flow Assay Schematic

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Serum:

Repeatability and reproducibility with serum specimens were determined by spiking a serum specimen pool that was negative by the IMMY Latex-Cryptococcus Antigen Detection System with cryptococcal antigen at four concentrations: Negative, high negative (C_5), low positive (near C_{95}), and medium positive. The

samples were analyzed on the CrAg Lateral Flow Assay in triplicate on five different days, at three different sites with a total of five different operators, on one lot, according to EP5-A2. One site was internal (Site 1) and the remaining two were a US reference laboratory (Site 2) and a US hospital laboratory (Site 3). For repeatability, percent positive and percent negative detected were calculated for each site (Table 2). For reproducibility, overall percent positive and percent negative detected were calculated by combining the data from all three sites (last two rows of Table 2).

(b)(4) Trade Secret Process - Product Specs

Table 2. Repeatability at 3 Different Sites

Sample	Serum							
	1		2		3		4	
	Med. Pos.		Low Pos		High Neg		Neg	
Neg/Pos	-	+	-	+	-	+	-	+
Site 1	0	30	0	30	28	2	30	0
Percent %	0	100	0	100	93	7	100	0
Site 2	0	30	0	30	30	0	30	0
Percent %	0	100	0	100	100	0	100	0
Site 3	0	15	0	15	15	0	15	0
Percent %	0	100	0	100	100	0	100	0
Total No.	0	75	0	75	73	2	75	0
Percent %	0	100	0	100	97	3	100	0

b. *Linearity/assay reportable range:*

Not Applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

d. *Detection limit:*

Detection Limit/Analytical Cut-off

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running varying concentrations of cryptococcal antigen diluted in LF Specimen Diluent. The concentration where 50% of the results were positive and 50% of the results were negative determined our analytical cut-off. The analytical cut-off is 1.25ng/ml.

Table 3. Analytical Cut-Off

Sample Concentration (ng/ml)	No. Positive	No. Tested	% Positive
0.50	0	24	0%
0.75	0	24	0%
1.00	4	24	17%
1.25	12	24	50%
1.50	21	24	88%
1.75	24	24	100%
2.00	24	24	100%
2.50	24	24	100%
3.00	24	24	100%
3.50	24	24	100%
4.00	24	24	100%

Analytical specificity:

Analytical specificity for the CrAg Lateral Flow Assay was determined by running potentially cross-reacting medical conditions unrelated to cryptococcosis. A total of 118 serum specimens were tested in triplicate. Percent positive was determined for each condition (Table 3).

Table 3. Analytical Specificity

Pathology	# of Samples	% Positive
Penicilliosis	5	0 % (0/5)
Sporothrichosis	6	0 % (0/6)
HAMA	5	0 % (0/5)
Syphilis	10	0 % (0/10)
Rubella	5	0 % (0/5)
Mycoplasmosis	10	0 % (0/10)
Toxoplasmosis	7	0 % (0/7)
CMV	10	0 % (0/10)
Blastomycosis	10	0 % (0/10)
Coccidiomycosis	10	0 % (0/10)
Histoplasmosis	10	0 % (0/10)
Candidiasis	10	0 % (0/10)
Aspergillus GM+	10	10 % (1/10)
Rheumatoid Factor*	10	0 % (0/10)

* Rheumatoid factor concentrations tested ranged from 112IU/ml to 6479IU/mls.

Additionally, cross-reactivity was assessed by testing crude culture filtrate antigens at a range of concentrations using the CrAg Lateral Flow Assay. At high concentrations (>0.1 mg/ml), antigens from *Paracoccidioides brasiliensis* exhibited some cross-reactivity. Antigens from *Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus* did not exhibit cross-reactivity.

Interference testing was also performed on five icteric, five hemolyzed, and five lipemic serum specimens. Each specimen was spiked with cryptococcal antigen at three times the C95 concentration. All specimens were then tested at IMMY, on one lot of CrAg Lateral Flow assay in triplicate: spiked and unspiked. Percent positivity was determined for each condition. All of the unspiked specimens had negative results on the CrAg Lateral Flow Assay. All spiked specimens were positive, thus, these types of serum specimens do not interfere with the CrAg Lateral Flow Assay. However, it is possible that hemolyzed samples could lead to false negatives due to the high background color on the strip.

The effect of pronase on the CrAg LFA was determined by pronase-treating 5 Cryptococcal EIA positive specimens and 5 Cryptococcal EIA negative specimens.

The samples were analyzed both untreated and pronase-treated. All treated, positives samples remained positive and all treated, negative samples remained negative. Therefore, pronase does not affect the CrAg LFA.

Due to specimen availability, the following conditions were not tested in the CrAg Lateral Flow Assay: *Candida dubliniensis*, *Candida tropicalis*, *Candida parapsidosis*, *Candida krusei*, *Candida glabrata*, *Cladosporium trichoides*, *Neisseria meningitidis*, *Salmonella typhi*, *Pneumocystis carinii*, *Trichosporon beigelii*, *Zygomycetes*, (b)(4) Trade Secret Process - Product Specs, ANA+, HAV, HCV, *Staph*, and *Strep*.

f. Assay cut-off:

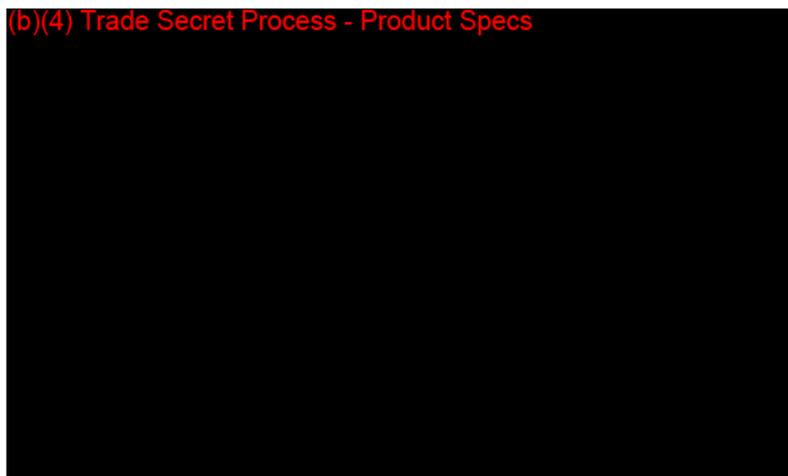
High dose hook effect concentrations with serum specimens were determined by spiking negative serum that were negative by the IMMY Latex-*Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at various concentrations between 20 and 500ug/ml. Each concentration was tested in triplicate at IMMY on one lot of CrAg Lateral Flow Assay, according to the package insert. It was determined that serum specimens with a cryptococcal antigen concentration higher than 200ug/ml can produce a high dose hook effect and therefore may produce a false negative result.

2. Comparison studies:

a. Method comparison with predicate device:

The CrAg Lateral Flow Assay was compared to the gold standard diagnoses of cryptococcosis (culture and/or India ink) to evaluate the sensitivity and specificity of the assay in (b)(4) Trade and CSF. These studies contained a mix of both prospective and retrospective specimens. A summary of the data collected is included in tables 4-9 below.

(b)(4) Trade Secret Process - Product Specs



(b)(4) Trade Secret Process - Product Specs



Table 8. CSF 2x2 Contingency Table: Culture/India Ink

		Culture/India Ink	
		Positive	Negative
CrAg	Positive	65	0
LFA Assay	Negative	0	99

Table 9. CSF Statistical Analysis: Culture/India Ink

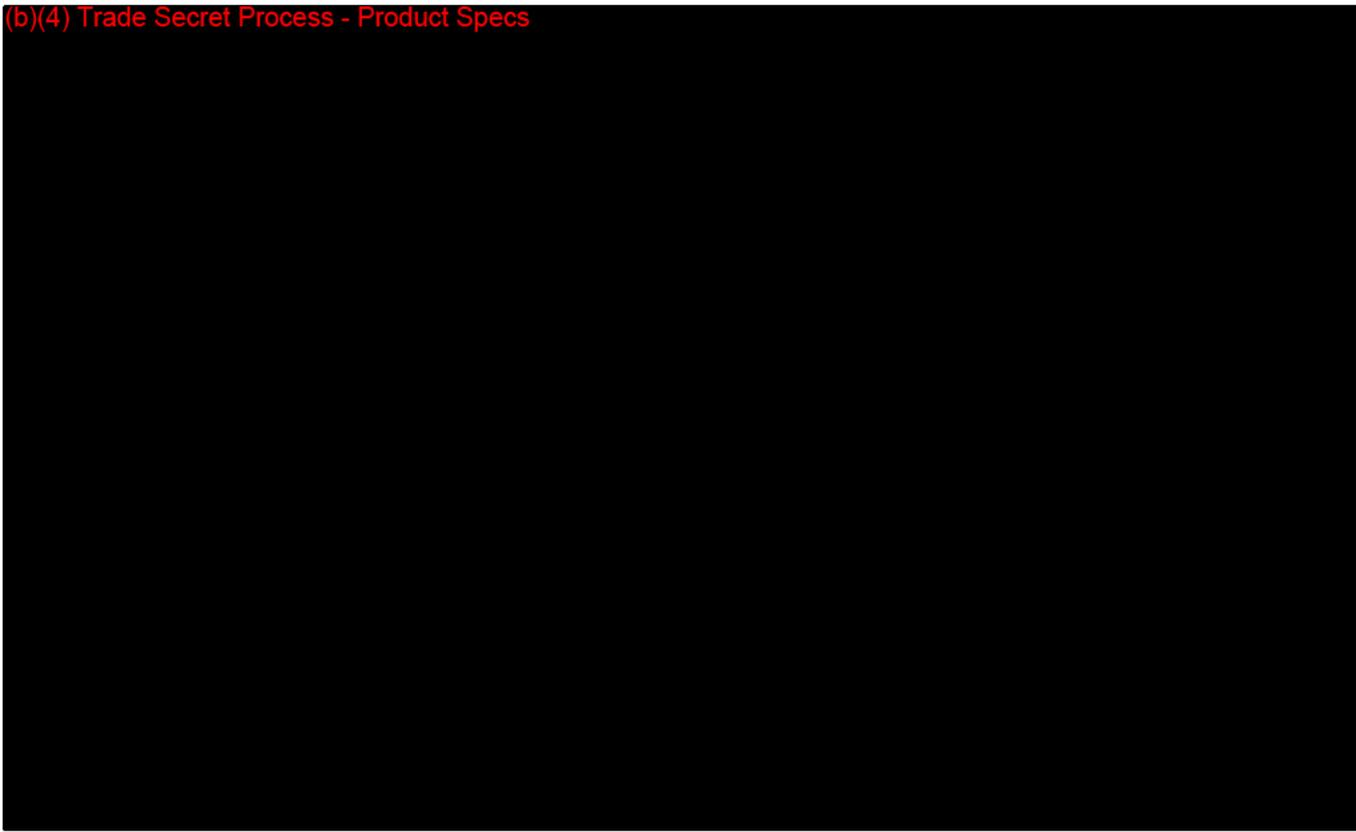
	Calculated	95% CI
Sensitivity	100%	94.4-100.0%
Specificity	100%	96.3-100%

b. Matrix comparison:

(b)(4) Trade Secret Process - Product Specs



(b)(4) Trade Secret Process - Product Specs



3. Clinical studies:

Not Applicable

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

Not Applicable

N. Proposed Labeling:

(b)(4) Trade Secret Process - Product Specs



O. Conclusion:

(b)(4) Trade Secret Process - Product Specs



P. Other Supportive Device and Instrument Information:

Q. Administrative Information:

1. Applicant contact information:

a. *Name of applicant:*

Immuno-Mycologics, Inc.

b. *Mailing address:*

2700 Technology Place.

Norman, OK 73071

c. *Phone #:* (405) 360-4669

d. *Fax #:* (405)364-1058

e. *E-mail address (optional):* sean-bauman@immy.com

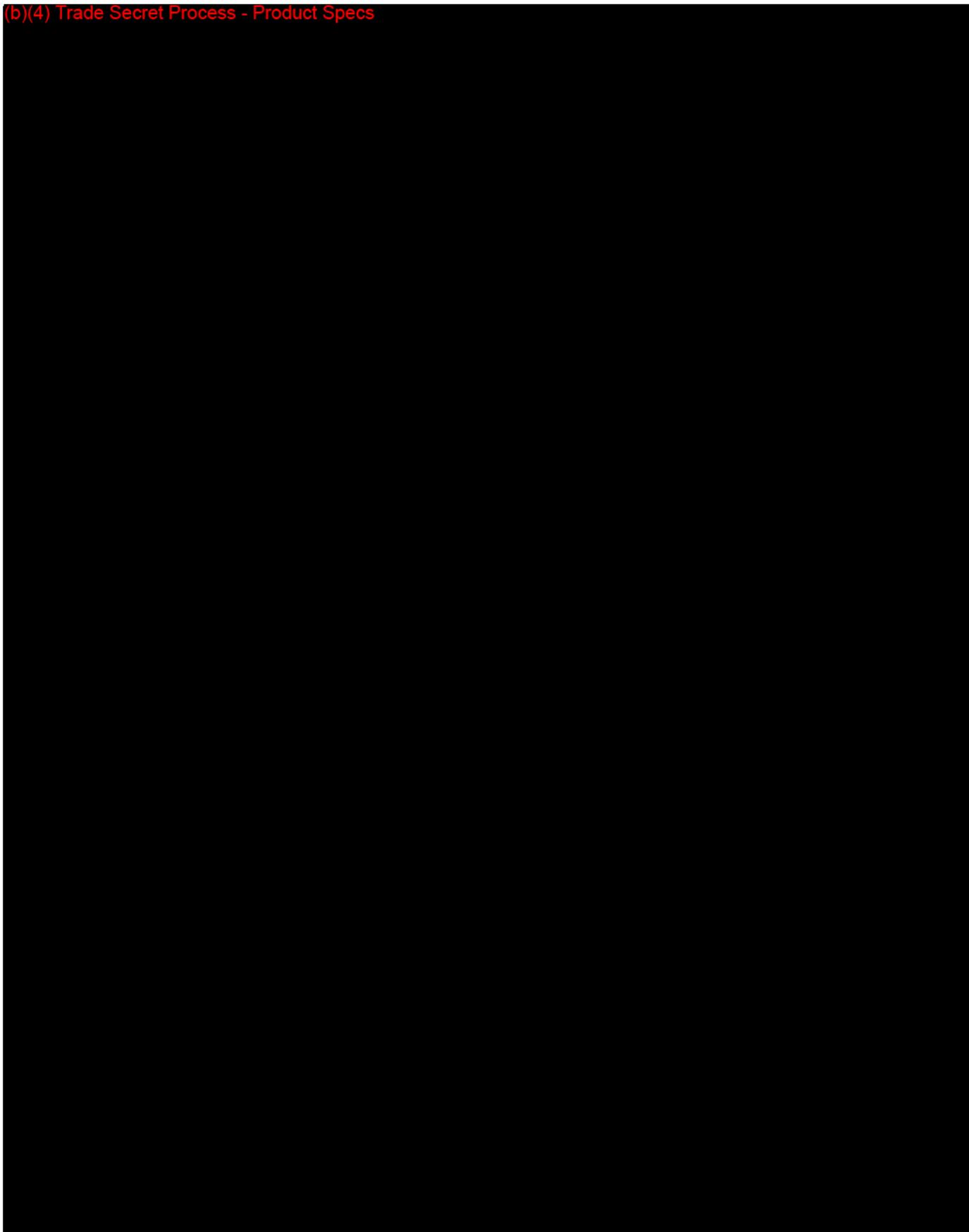
f. *Contact:* Sean Bauman

2. Review documentation:

(b)(4) Trade Secret Process - Product Specs

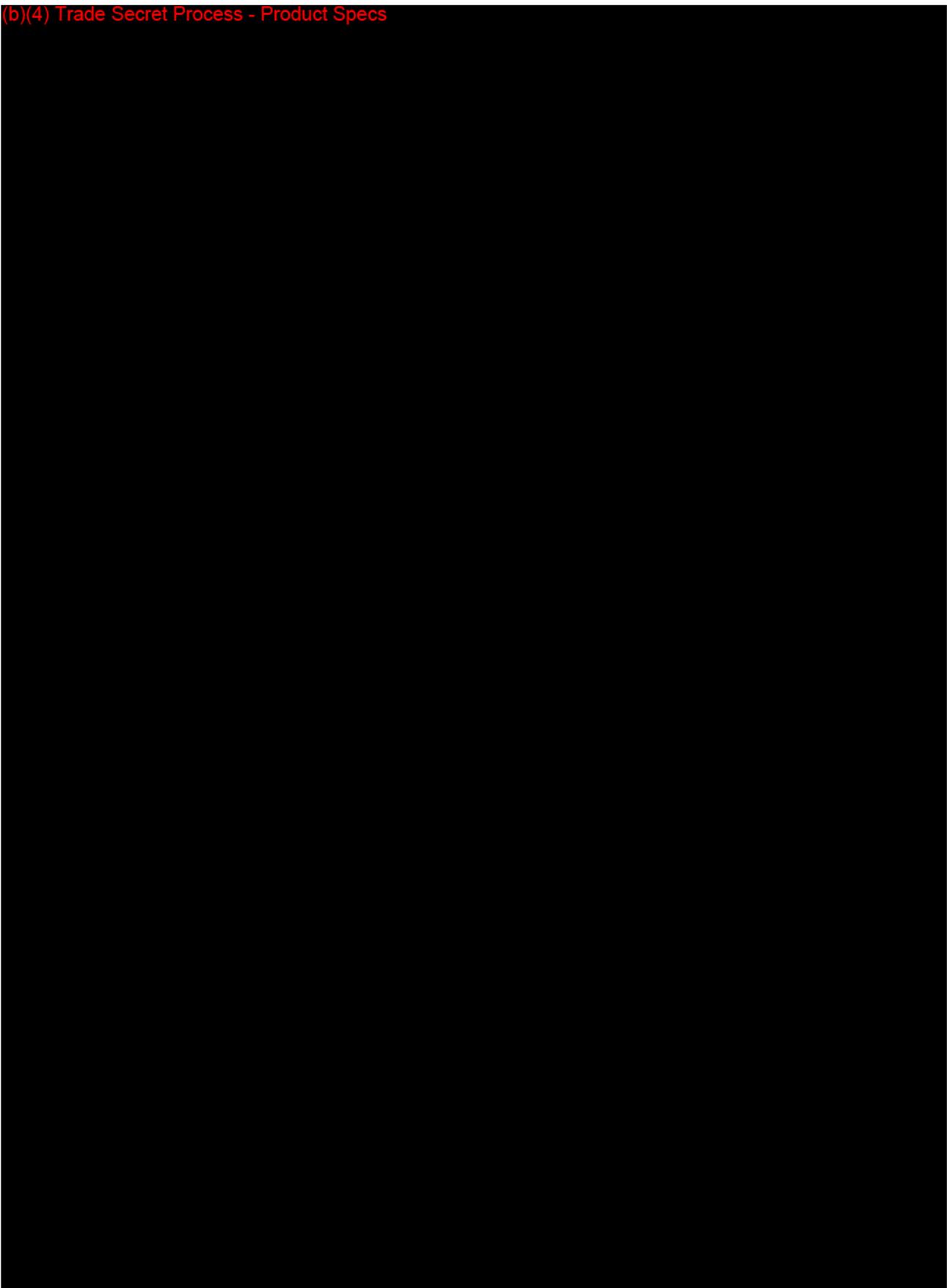


(b)(4) Trade Secret Process - Product Specs

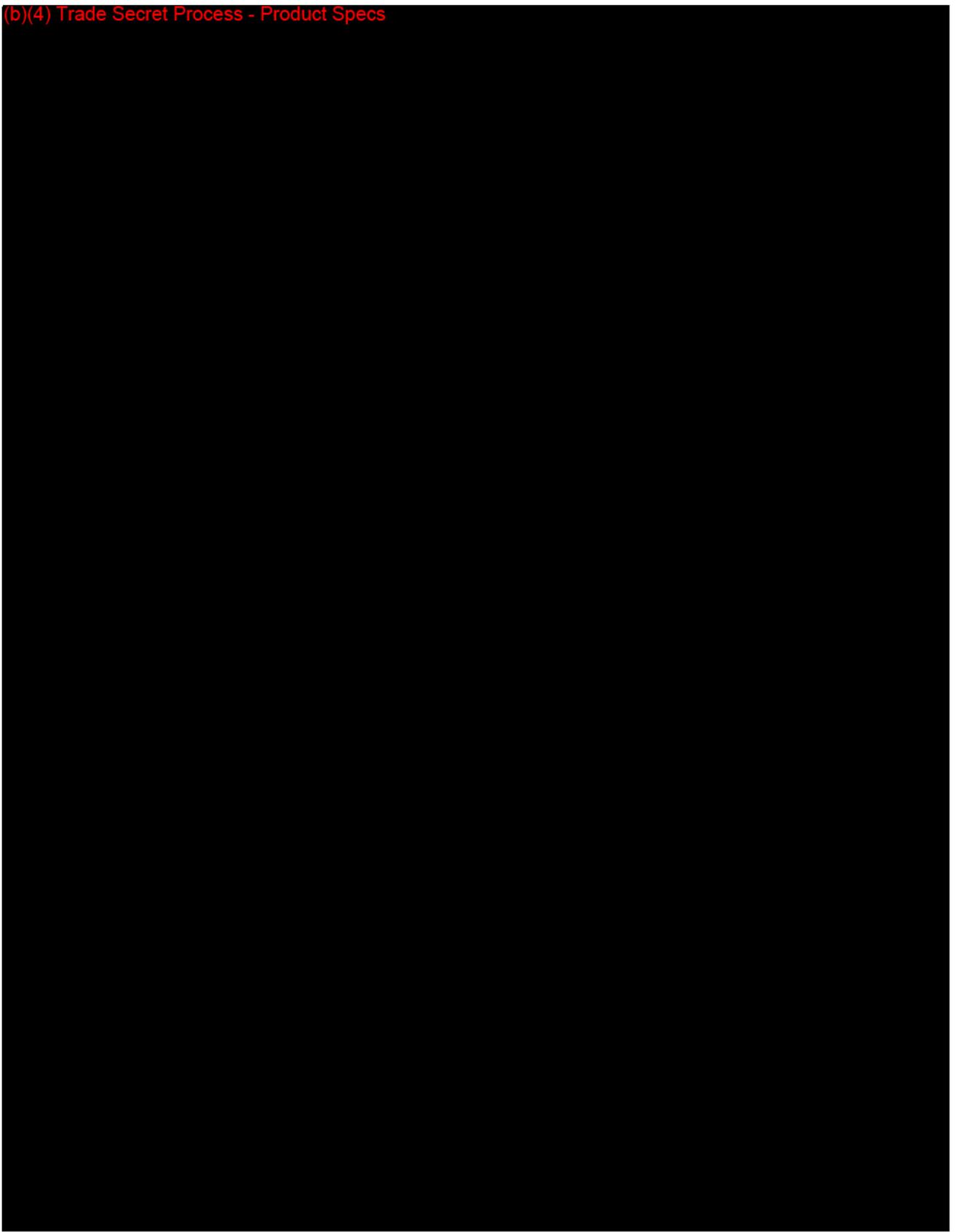


114

(b)(4) Trade Secret Process - Product Specs



(b)(4) Trade Secret Process - Product Specs



(b)(4) Trade Secret Process - Product Specs



Substantial Equivalence Discussion:

	Yes	No	
1. Same Indication Statement?			If YES = Go To 3
2. Do Differences Alter The Effect Or Raise New Issues of Safety Or Effectiveness?			If YES = Stop NSE
3. Same Technological Characteristics?			If YES = Go To 5
4. Could The New Characteristics Affect Safety Or Effectiveness?			If YES = Go To 6
5. Descriptive Characteristics Precise Enough?			If NO = Go To 8 If YES = Stop SE
6. New Types Of Safety Or Effectiveness Questions?			If YES = Stop NSE
7. Accepted Scientific Methods Exist?			If NO = Stop NSE
8. Performance Data Available?			If NO = Request Data
9. Data Demonstrate Equivalence?			Final Decision:

Note: See

http://erom.fda.gov/eRoomReq/Files/CDRH3/CDRHPreMarketNotification510kProgram/0_4148/FLOWCHART%20DECISION%20TREE%20.DOC for Flowchart to assist in decision-making process. Please complete the following table and answer the corresponding questions. "Yes" responses to questions 2, 4, 6, and 9, and every "no" response requires an explanation.

e. Explain how descriptive characteristics are not precise enough:

(b)(4) Trade Secret Process - Product Specs



i. Explain how the performance data demonstrates that the device is or is not substantially equivalent:

R. Reviewer Name and Signature:

Michael W. White
CDRH/OIVD/DMD

Consult Review Memorandum

Center for Devices and Radiological Health
Public Health Service
Food and Drug Administration



To: Michael White, Division of Microbiology Devices, Office of In Vitro Diagnostic Device Evaluation and Safety, CDRH, (HFZ-440)

From: Noel Gerald, Division of Microbiology Devices, Office of In Vitro Diagnostic Device Evaluation and Safety, CDRH, (HFZ-440)

For: Immuno-Mycologics, Inc, 2700 Technology Place, Norman, OK 73071

Date: September 29, 2011

Re: Traditional 510(k) for the Cryptococcal Antigen Lateral Flow Assay (K112422)

Through: Freddie Poole, Associate Director and Sally Hojvat, Director, Microbiology Division, OIVD, CDRH

BACKGROUND and PROPOSED INTENDED USE

The detection of cryptococcal antigen in serum and cerebral spinal fluid (CSF) samples has been used to aid in the diagnosis of cryptococcosis. The sponsor Immuno-Mycologics, Inc previously received clearance for the use of the Cryptococcal Antigen Lateral Flow Assay (CrAg LFA, K102286) as a qualitative or semi-quantitative immunochromatographic test to detect cryptococcal antigen in serum samples. In the current 510(k) submission, the sponsor proposes to expand the intended use of the CrAg Lateral Flow Assay to include (b)(4) Trade, and CSF samples. The scope of this review includes the data and labeling included in support of the new sample matrices.

Indications for Use:

The Cryptococcal Antigen Lateral Flow Assay (CrAg LFA) is an immunochromatographic test system for the qualitative or semi-quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum, (b)(4) Trade, and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription-use laboratory assay, which can aid in the diagnosis of cryptococcosis.

Device Description:

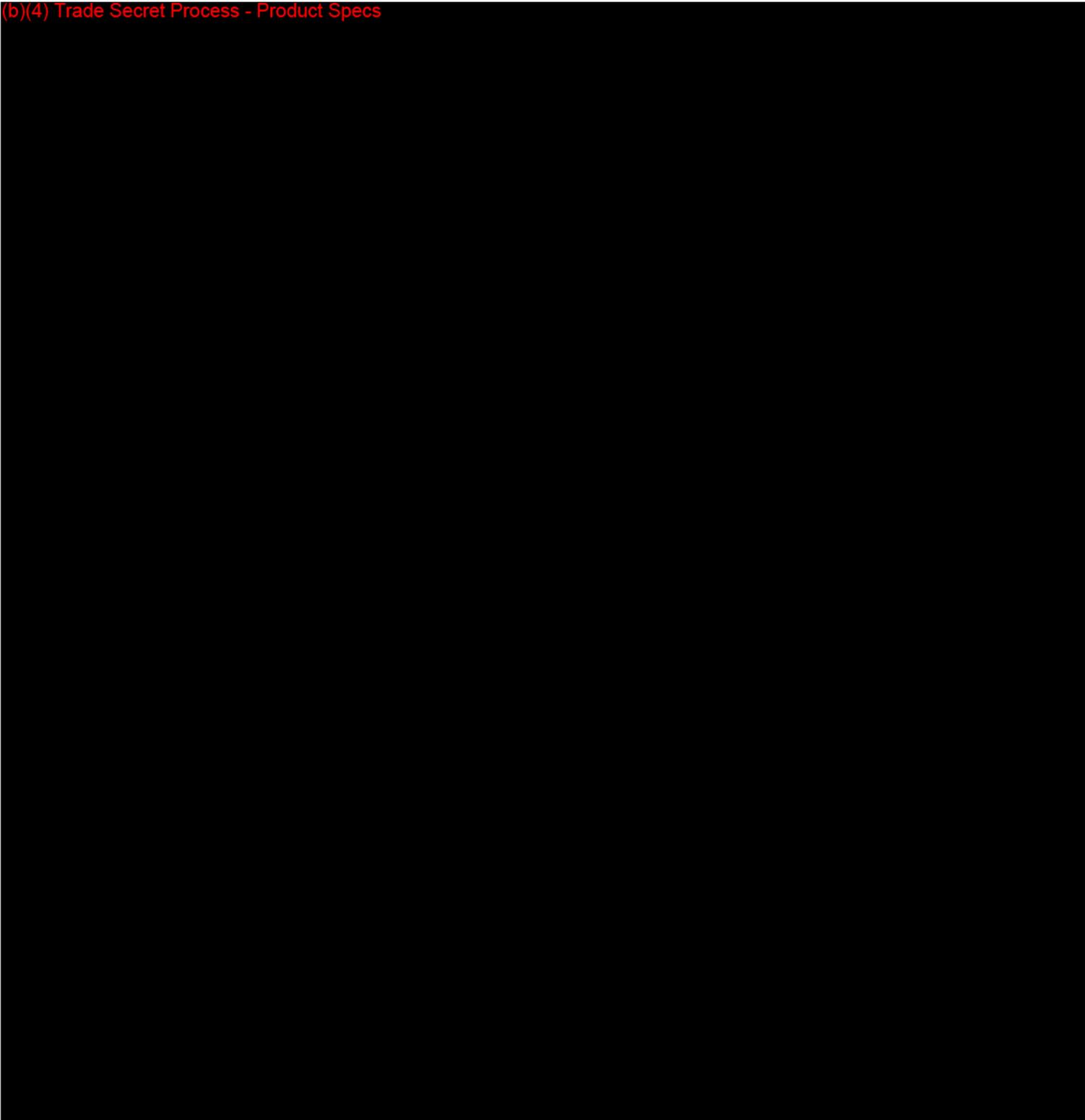
The CrAg LFA is a dipstick sandwich immunochromatographic assay. A specimen is diluted in Specimen Diluent and the lateral flow dipstick test membrane is placed in contact with the specimen. Capsular polysaccharide cryptococcal antigens are captured by gold-conjugated monoclonal antibodies, and the antibody-antigen complexes flow up the test membrane. The antibody-antigen complexes are captured by immobilized anti-cryptococcal antibodies at the test line site (T) and develop a visible line

K112422 Cryptococcal Antigen Lateral Flow Assay

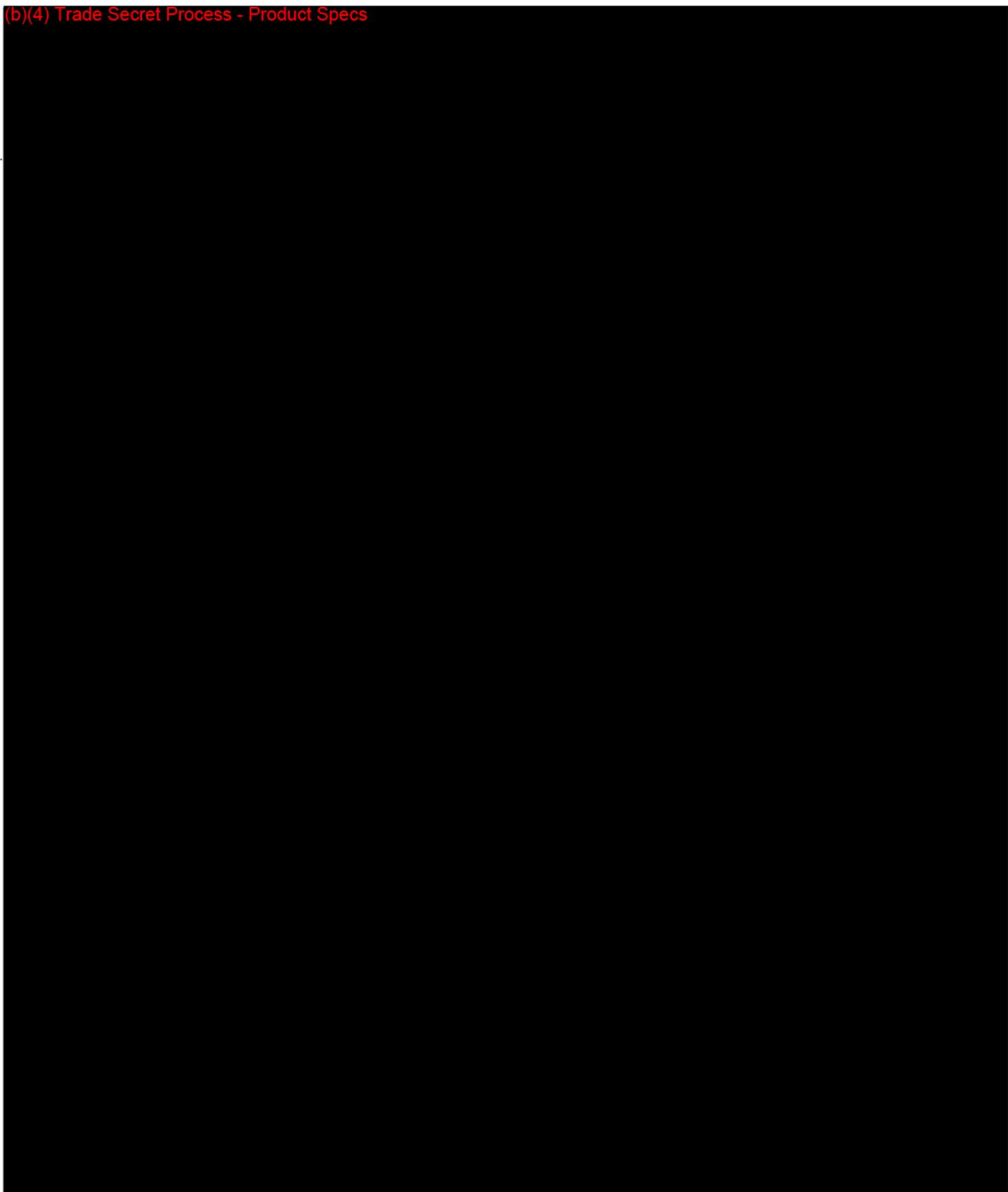
on the strip. A gold-conjugated goat IgG antibody that is captured by immobilized bovine anti-goat IgG antibody at the control line site (C) serves as an internal control for test function.

Reviewer Comments:

(b)(4) Trade Secret Process - Product Specs

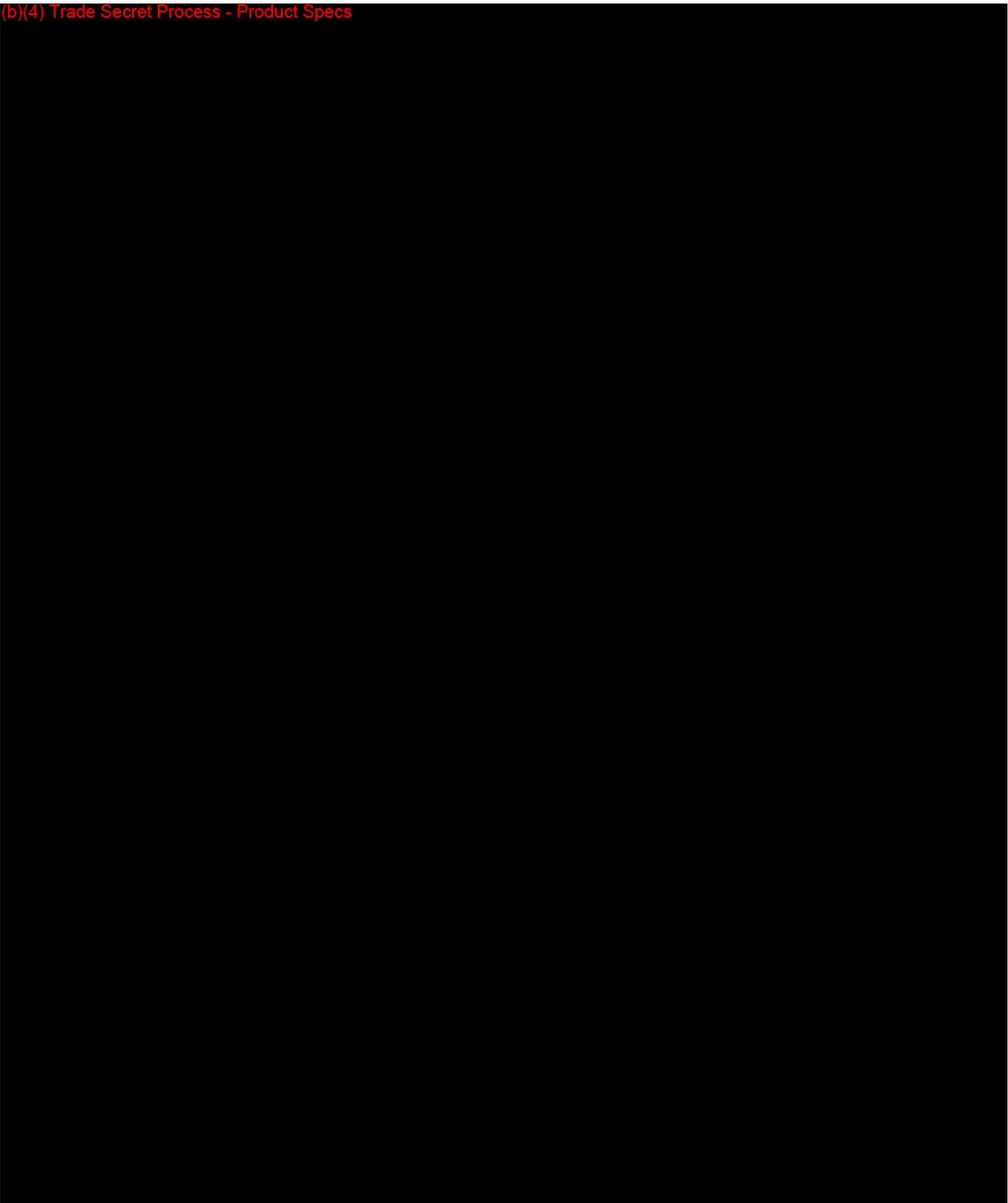


(b)(4) Trade Secret Process - Product Specs

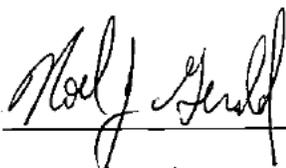
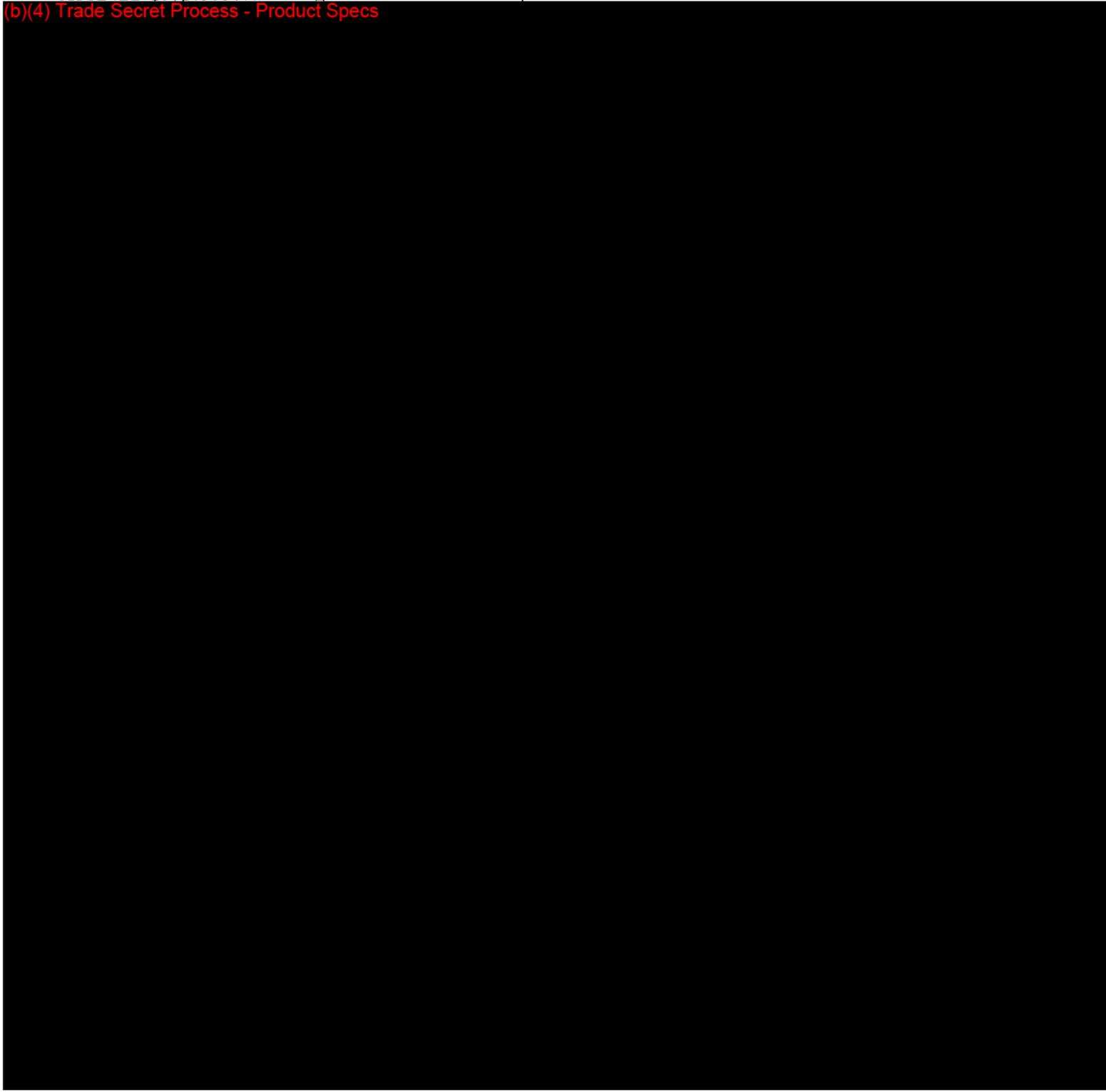


¹ Lindsley, M.D., et al. "Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis," Clin. Infect. Dis. 53(4):321-325. 2011

(b)(4) Trade Secret Process - Product Specs



(b)(4) Trade Secret Process - Product Specs



Noel J. Gerald
Scientific Reviewer

mz/omd



IMMY

K112422/SI

U.S. Food and Drug Administration
Center for Devices and Radiological Health (CDRH)
Document Mail Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, Maryland 20993-0002

FDA/CDRH/DCC

MAR 27 2012

RECEIVED

K34

Submission Date: 03/26/2012

Re: Cryptococcal Antigen Lateral Flow Assay (CrAg Lateral Flow Assay) (K112422)

Please find enclosed our traditional 510(k) submission for the Cryptococcal Antigen Lateral Flow Assay (CrAg Lateral Flow Assay), which expands the intended use of K102286 to include cerebral spinal fluid (CSF).

Submission Type	Traditional 510(k) for an expanded intended use
Submitter	Immuno-Mycologics, Inc (Registration # 1627497; Owner Operator # 9916020)
Contact Person	Sean K. Bauman, Ph.D., President/CEO 2700 Technology Place Norman, OK 73071 Phone: (800) 654-3639 Fax: (405) 364-1058 Sean-Bauman@immy.com
Continued Confidentiality	Yes
Common Name	Cryptococcal Antigen Lateral Flow Assay
Trade Name	CrAg Lateral Flow Assay (CrAg LFA)
Class	II
Classification Regulation	866.3165
Review Panel	Microbiology
Predicate Device	CrAg Lateral Flow Assay (CrAg LFA) (K102286)
Special Controls	No applicable mandatory performance standards or special controls exist for this device

Kind regards,

Sean K. Bauman, Ph.D.

President/CEO

Enclosures: CrAg Lateral Flow Assay 510(k) paper copy and electronic copy (CD)



DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

**Certification of Compliance, under 42 U.S.C. § 282(j)(5)(B), with
Requirements of ClinicalTrials.gov Data Bank (42 U.S.C. § 282(j))**

(For submission with an application/submission, including amendments, supplements, and resubmissions, under §§ 505, 515, 520(m), or 510(k) of the Federal Food, Drug, and Cosmetic Act or § 351 of the Public Health Service Act.)

SPONSOR / APPLICANT / SUBMITTER INFORMATION

1. NAME OF SPONSOR/APPLICANT/SUBMITTER Sean K. Bauman, PhD, President and CEO	2. DATE OF THE APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES Aug 24, 2011
3. ADDRESS (Number, Street, State, and ZIP Code) 2700 Technology Place Norman, OK 73071	4. TELEPHONE AND FAX NUMBERS (Include Area Code) (Tel.) 405-360-4669 (Fax) 405-364-1058

PRODUCT INFORMATION

5. **FOR DRUGS/BIOLOGICS:** Include Any/All Available Established, Proprietary and/or Chemical/Biochemical/Blood/Cellular/Gene Therapy Product Name(s)
FOR DEVICES: Include Any/All Common or Usual Name(s), Classification, Trade or Proprietary or Model Name(s) and/or Model Number(s)
(Attach extra pages as necessary)

Cryptococcal Antigen Lateral Flow Assay

CrAg Lateral Flow Assay (CrAg LFA)

APPLICATION / SUBMISSION INFORMATION

6. TYPE OF APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES
 IND NDA ANDA BLA PMA HDE 510(k) PDP Other

7. INCLUDE IND/NDA/ANDA/BLA/PMA/HDE/510(k)/PDP/OTHER NUMBER (If number previously assigned)

8. SERIAL NUMBER ASSIGNED TO APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES

CERTIFICATION STATEMENT / INFORMATION

9. CHECK ONLY ONE OF THE FOLLOWING BOXES (See instructions for additional information and explanation)

A. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, do not apply because the application/submission which this certification accompanies does not reference any clinical trial.

B. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, do not apply to any clinical trial referenced in the application/submission which this certification accompanies.

C. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, apply to one or more of the clinical trials referenced in the application/submission which this certification accompanies and that those requirements have been met.

10. IF YOU CHECKED BOX C, IN NUMBER 9, PROVIDE THE NATIONAL CLINICAL TRIAL (NCT) NUMBER(S) FOR ANY "APPLICABLE CLINICAL TRIAL(S)," UNDER 42 U.S.C. § 282(j)(1)(A)(i), SECTION 402(j)(1)(A)(i) OF THE PUBLIC HEALTH SERVICE ACT, REFERENCED IN THE APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES (Attach extra pages as necessary)

NCT Number(s):

The undersigned declares, to the best of her/his knowledge, that this is an accurate, true, and complete submission of information. I understand that the failure to submit the certification required by 42 U.S.C. § 282(j)(5)(B), section 402(j)(5)(B) of the Public Health Service Act, and the knowing submission of a false certification under such section are prohibited acts under 21 U.S.C. § 331, section 301 of the Federal Food, Drug, and Cosmetic Act.
Warning: A willfully and knowingly false statement is a criminal offense, U.S. Code, title 18, section 1001.

11. SIGNATURE OF SPONSOR/APPLICANT/SUBMITTER OR AN AUTHORIZED REPRESENTATIVE (Sign) 	12. NAME AND TITLE OF THE PERSON WHO SIGNED IN NO. 11 (Name) Sean K. Bauman, Ph.D. (Title) President and CEO
13. ADDRESS (Number, Street, State, and ZIP Code) (of person identified in Nos. 11 and 12) 2700 Technology Place Norman, OK 73071	14. TELEPHONE AND FAX NUMBERS (Include Area Code) (Tel.) 405-360-4669 (Fax) 405-364-1058
	15. DATE OF CERTIFICATION Aug 22, 2011

DEPARTMENT OF HEALTH AND HUMAN SERVICES FOOD AND DRUG ADMINISTRATION MEDICAL DEVICE USER FEE COVER SHEET		PAYMENT IDENTIFICATION NUMBER: (b)(4) Trade Write the Payment Identification number on your check.
A completed cover sheet must accompany each original application or supplement subject to fees. If payment is sent by U.S. mail or courier, please include a copy of this completed form with payment. Payment and mailing instructions can be found at: http://www.fda.gov/oc/mdufma/coversheet.html		
1. COMPANY NAME AND ADDRESS (include name, street address, city state, country, and post office code) IMMUNO MYCOLOGICS INC 2700 TECHNOLOGY PLACE NORMAN OK 73071 US 1.1 EMPLOYER IDENTIFICATION NUMBER (EIN) (b)(4)	2. CONTACT NAME Sean Bauman 2.1 E-MAIL ADDRESS sean-bauman@immy.com 2.2 TELEPHONE NUMBER (include Area code) 405-360-4669 2.3 FACSIMILE (FAX) NUMBER (Include Area code)	
3. TYPE OF PREMARKET APPLICATION (Select one of the following in each column; if you are unsure, please refer to the application descriptions at the following web site: http://www.fda.gov/oc/mdufma) <u>Select an application type:</u> <input checked="" type="checkbox"/> Premarket notification(510(k)); except for third party <input type="checkbox"/> 513(g) Request for Information <input type="checkbox"/> Biologics License Application (BLA) <input type="checkbox"/> Premarket Approval Application (PMA) <input type="checkbox"/> Modular PMA <input type="checkbox"/> Product Development Protocol (PDP) <input type="checkbox"/> Premarket Report (PMR) <input type="checkbox"/> Annual Fee for Periodic Reporting (APR) <input type="checkbox"/> 30-Day Notice		
3.1 Select a center <input checked="" type="checkbox"/> CDRH <input type="checkbox"/> CBER 3.2 Select one of the types below <input checked="" type="checkbox"/> Original Application <u>Supplement Types:</u> <input type="checkbox"/> Efficacy (BLA) <input type="checkbox"/> Panel Track (PMA, PMR, PDP) <input type="checkbox"/> Real-Time (PMA, PMR, PDP) <input type="checkbox"/> 180-day (PMA, PMR, PDP)		
4. ARE YOU A SMALL BUSINESS? (See the instructions for more information on determining this status) <input type="checkbox"/> YES, I meet the small business criteria and have submitted the required qualifying documents to FDA <input checked="" type="checkbox"/> NO, I am not a small business 4.1 If Yes, please enter your Small Business Decision Number:		
5. FDA WILL NOT ACCEPT YOUR SUBMISSION IF YOUR COMPANY HAS NOT PAID AN ESTABLISHMENT REGISTRATION FEE THAT IS DUE TO FDA. HAS YOUR COMPANY PAID ALL ESTABLISHMENT REGISTRATION FEES THAT ARE DUE TO FDA? <input checked="" type="checkbox"/> YES (All of our establishments have registered and paid the fee, or this is our first device, and we will register and pay the fee within 30 days of FDA's approval/clearance of this device.) <input type="checkbox"/> NO (If "NO," FDA will not accept your submission until you have paid all fees due to FDA. This submission will not be processed; see http://www.fda.gov/cdrh/mdufma for additional information)		
6. IS THIS PREMARKET APPLICATION COVERED BY ANY OF THE FOLLOWING USER FEE EXCEPTIONS? IF SO, CHECK THE APPLICABLE EXCEPTION. <input type="checkbox"/> This application is the first PMA submitted by a qualified small business, including any affiliates <input type="checkbox"/> This biologics application is submitted under section 351 of the Public Health Service Act for a product licensed for further manufacturing use only <input type="checkbox"/> The sole purpose of the application is to support conditions of use for a pediatric population <input type="checkbox"/> The application is submitted by a state or federal government entity for a device that is not to be distributed commercially		
7. IS THIS A SUPPLEMENT TO A PREMARKET APPLICATION FOR WHICH FEES WERE WAIVED DUE TO SOLE USE IN A PEDIATRIC POPULATION THAT NOW PROPOSES CONDITION OF USE FOR ANY ADULT POPULATION? (If so, the application is subject to the fee that applies for an original premarket approval application (PMA)). <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO		
PAPERWORK REDUCTION ACT STATEMENT Public reporting burden for this collection of information is estimated to average 18 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to the address below. Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, 1350 Piccard Drive, 4th Floor Rockville, MD 20850 [Please do NOT return this form to the above address, except as it pertains to comments on the burden estimate.]		
8. USER FEE PAYMENT AMOUNT SUBMITTED FOR THIS PREMARKET APPLICATION (b)(4) Trade Secret Process -		

22-Aug-2011

Online Payment

Step 3: Confirm Payment

1 | 2 | 3

Thank you.
Your transaction has been successfully completed.

Pay.gov Tracking Information

Application Name: FDA User Fees
Pay.gov Tracking ID: (b)(4)
Agency Tracking ID: (b)(4)
Transaction Date and Time: 08/22/2011 10:37 EDT

Payment Summary

Address Information	Account Information	Payment Information
<p>Account Holder IMMUNO Name: MYCOLOGICS INC 2700 Technology Billing Address: Place Billing Address 2: City: Norman State / Province: OK Zip / Postal Code: 73071 Country: USA</p>	<p>Card Type: (b)(4) Trade Card Number: *(b)(4)</p>	<p>Payment Amount: (b)(4) Transaction Date and Time: 08/22/2011 10:37 EDT</p>

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0900262180fe752e.pdf

System attempted to attach the file. Please look at attachments to open this file manually.



U.S. Food and Drug Administration
Center for Devices and Radiological Health (CDRH)
Document Mail Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, Maryland 20993-0002

Submission Date: 03/26/2012

Re: Cryptococcal Antigen Lateral Flow Assay (CrAg Lateral Flow Assay) (K112422)

Please find enclosed our traditional 510(k) submission for the Cryptococcal Antigen Lateral Flow Assay (CrAg Lateral Flow Assay), which expands the intended use of K102286 to include cerebral spinal fluid (CSF).

Submission Type	Traditional 510(k) for an expanded intended use
Submitter	Immuno-Mycologics, Inc (Registration # 1627497; Owner Operator # 9916020)
Contact Person	Sean K. Bauman, Ph.D., President/CEO 2700 Technology Place Norman, OK 73071 Phone: (800) 654-3639 Fax: (405) 364-1058 Sean-Bauman@immy.com
Continued Confidentiality	Yes
Common Name	Cryptococcal Antigen Lateral Flow Assay
Trade Name	CrAg Lateral Flow Assay (CrAg LFA)
Class	II
Classification Regulation	866.3165
Review Panel	Microbiology
Predicate Device	CrAg Lateral Flow Assay (CrAg LFA) (K102286)
Special Controls	No applicable mandatory performance standards or special controls exist for this device

Kind regards,

A handwritten signature in blue ink that reads "Sean Bauman".

Sean K. Bauman, Ph.D.

President/CEO

Enclosures: CrAg Lateral Flow Assay 510(k) paper copy and electronic copy (CD)



Indications for Use Statement

510(k) Number (if known): K112422

Device Name: CrAg Lateral Flow Assay

Indications for Use:

The Cryptococcal Antigen Lateral Flow Assay (CrAg LFA) is an immunochromatographic test system for the qualitative or semi quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription use laboratory assay, which can aid in the diagnosis of cryptococcosis.

Prescription Use _____ AND/OR Over The Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Division Sign Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k):

www.immy.com | 2700 Technology Pl Norman, OK 73071 USA | 405.360.4669



510(k) Summary CrAg Lateral Flow Assay

This 510(k) summary is submitted in accordance with 21 CFR §807.92

Owner: Immuno Mycologics, Inc.
2700 Technology Place
Norman, OK 73071
Tel: 405 360 4669
Fax: 405 364 1058
Contact: Dr. Sean K. Bauman, President & CEO
Sean.Bauman@immy.com

Prepared: March 26, 2012

Trade Name: CrAg Lateral Flow Assay

Common Name: Cryptococcal Antigen Lateral Flow Immunoassay

Regulation: 866.3165

Predicate Device: Immuno Mycologics' CrAg Lateral Flow Assay (K102286)

Intended Use: The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) in serum and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription use laboratory assay, which can aid in the diagnosis of Cryptococcosis.

Device Description:

Explanation:

Detection of cryptococcal antigen in serum and CSF has been used for over forty years to aid in the diagnosis of cryptococcosis with very high sensitivity and specificity (9,14,15). Current guidelines for the management of cryptococcal disease partially base treatment recommendations on cryptococcal antigen presence and more specifically on cryptococcal antigen titers (16).

Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) (5,6,12,13). Individuals with impaired cell mediated

immune (CMI) function due to acquired immunodeficiency syndrome (AIDS) (19), lymphoproliferative disorders (18), steroid therapy (8), and organ transplantation (7) are at increased risk of cryptococcosis. AIDS accounts for 80-90% of cryptococcal infections (11). The incidence of cryptococcosis in AIDS patients in the United States is estimated to be 5-10% (11), while the incidence of cryptococcosis in other parts of the world, such as Africa, is as high as 30% (3). Cryptococcosis is the fourth most common opportunistic, life-threatening infection among AIDS patients (10).

Description:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in serum and CSF. For the qualitative procedure, specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For the semi-quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the qualitative procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold conjugated, anti-cryptococcal monoclonal antibodies and gold conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it binds to the gold-conjugated, anti-cryptococcal antibodies. The gold-labeled antibody-antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti-cryptococcal monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold-labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold-conjugated control antibody to move to the Control Line (C) which is immobilized bovine anti-goat IgG antibody. The immobilized anti-goat antibody will bind to the gold-conjugated goat IgG control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line (Figure 1). If the control line fails to develop a line, then the test is not valid.

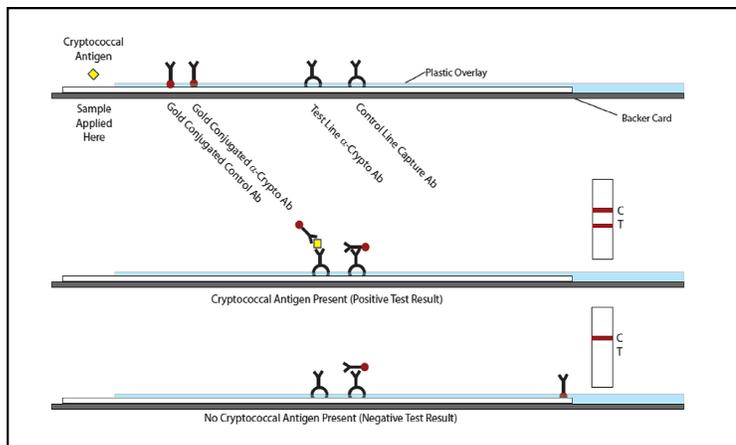


Figure 1. CrAg Lateral Flow Assay Schematic

Technological Characteristics Summary

A comparison between the CrAg LFA and the CrAg LFA (K102286 Serum only) is presented in Table 1.

Table 1. Comparison with Predicate Device

SIMILARITIES		
Feature	CrAg LFA (New Device)	CrAg LFA (Serum Only) (K102286)
Intended Use	Intended Use	
Intended Use	Immunochromatographic test system for the qualitative or semi quantitative detection of the capsular polysaccharide antigens of <i>Cryptococcus</i> species complex (<i>Cryptococcus neoformans</i> and <i>Cryptococcus gattii</i>) in serum	Immunochromatographic test system for the qualitative or semi quantitative detection of the capsular polysaccharide antigens of <i>Cryptococcus</i> species complex (<i>Cryptococcus neoformans</i> and <i>Cryptococcus gattii</i>) in serum
Indication For Use	Prescription use laboratory assay, which can aid in the diagnosis of cryptococcosis	Prescription use laboratory assay, which can aid in the diagnosis of cryptococcosis
Device Description		
Technology	Lateral Flow Assay	Lateral Flow Assay
Sample Matrix	Serum	Serum
Instruments	None	None
Assay Components	Specimen diluent, lateral flow strips, built in control, gold conjugated antibodies	Positive control, negative control, latex cards, latex conjugated antibodies
Specimen Pre Treatment	Dilution	Dilution
Detection Antibody	Anti cryptococcal monoclonal antibody	Anti cryptococcal monoclonal antibody
Storage Requirements	20 25°C	20 25°C
DIFFERENCES		
Feature	Cryptococcal Antigen Lateral Flow Assay	Latex- <i>Cryptococcus</i> Antigen Detection System
Intended Use	Intended Use	
Intended Use	Test for the qualitative or semi quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum and CSF
Indication For Use	No differences	No differences

Performance Summary

A. Precision Studies (Repeatability & Reproducibility)

Serum repeatability and reproducibility results can be found in the predicate device 510(k) (K102286)

Repeatability and reproducibility with CSF specimens were determined by spiking a mock CSF that was negative by the IMMY Latex *Cryptococcus* Antigen Detection System with cryptococcal antigen at four concentrations: Negative, high negative (C₅), low positive (near C₉₅), and medium positive. The samples were analyzed on the CrAg Lateral Flow Assay in triplicate on five different days, at three different sites with a total of five different operators, on one lot, according to EP5 A2. One site was internal (Site 1) and the remaining two were a US reference laboratory (Site 2) and a US hospital laboratory (Site 3). For repeatability, percent positive and percent negative detected were calculated for each site (Table 2). For reproducibility, overall percent positive and percent negative detected were calculated by combining the data from all three sites (last two rows of Table 2).

Table 2. Repeatability at 3 Different Sites

Sample	CSF							
	1		2		3		4	
	Med. Pos	Low Pos	High Neg	Neg				
Neg/Pos		+		+		+		+
Site 1	0	30	0	30	27	3	30	0
Percent %	0	100	0	100	90	10	100	0
Site 2	0	30	0	30	30	0	30	0
Percent %	0	100	0	100	100	0	100	0
Site 3	0	15	0	15	15	0	15	0
Percent %	0	100	0	100	100	0	100	0
Total No.	0	75	0	75	72	3	75	0
Percent %	0	100	0	100	96	4	100	0

B. Analytical Sensitivity (lower limits of the assay/analytical cut off)

Serum analytical sensitivity can be found in the predicate device 510(k) (K102286)

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running 24 replicates of varying concentrations of cryptococcal antigen diluted in mock CSF on one lot of kits, according to EP12 A2. The analytical cut off was defined as the concentration where 50% of the results were positive and 50% of the results were negative. The analytical cut off is 1.25ng/ml.

C. Analytical Specificity (cross reactivity)

Serum analytical specificity can be found in the predicate device 510(k) (K102286)

Due to specimen availability, the following CSF conditions were not tested in the CrAg Lateral Flow Assay: *S. pneumonia*, *Enterovirus*, *Enterobacteriaceae*, *Streptococcus* spp., *Staphylococcus* spp., *diphtheroid*, *H. influenzae* type B, *N. meningitidis*, *Enterococcus* spp., *Epstein Barr*, *Herpes simplex virus* Type 1 and 2, *Listeria monocytogenes*, *Trichosporon beigeli*, and samples with syneresis fluid condensation.

This assay was not evaluated for potential interference related to specimen pretreatment with 2 mercaptoethanol or with specimens including the following substances or conditions: bloody CSF, cloudy CSF, white blood cells, xanthochromic CSF, bilirubin, protein, systemic lupus erythmatosus (SLE), sarcoidosis, or *N. meningitides*.

D. Linearity

N/A

E. High Dose Hook Effect

High dose hook effect concentrations with specimens were determined by spiking negative serum that was negative by the IMMY Latex *Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at various concentrations between 20 and 500ug/ml. Each concentration was tested in triplicate at IMMY on one lot of CrAg Lateral Flow Assay, according to the package insert. It was determined that serum specimens with a cryptococcal antigen concentration higher than 200ug/ml can produce a high dose hook effect and therefore may produce a false negative result.

F. Method Comparisons

Predicate Device Method Comparison

Not Applicable

Other Method Comparison – Culture/India Ink (Gold Standards)

Serum method comparison to gold standards can be found in the predicate device 510(k) (K102286)

The CrAg Lateral Flow Assay was compared to the gold standard for the diagnosis of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay in CSF. These studies contained a mix of both prospective and retrospective specimens. A summary of the data collected is included in Tables 3 and 4 below:

Table 3. CSF 2x2 Contingency Table: Culture/India Ink

		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	65	0
	Negative	0	99

Table 4. CSF Statistical Analysis: Culture/India Ink

	Calculated	95% CI
Sensitivity	100%	94.4-100.0%
Specificity	100%	96.3-100%

Conclusion

The information submitted in this premarket notification is complete and supports a substantial equivalence decision.

Premarket Notification Truthful And Accurate Statement

[As Required by 21 CFR 807.87(k)]

I certify that, in my capacity as President and CEO of Immuno-Mycologics, Inc., I believe to the best of my knowledge, that all data and information submitted in the premarket notification are truthful and accurate and that no material fact has been omitted.



(Signature)

Sean K. Bauman

(Typed Name)

3/1/2012

(Date)

*(Premarket Notification [510(k)] Number)

*For a new submission, leave the 510(k) number blank.

Must be signed by a responsible person of the firm required to submit the premarket notification [e.g., not a consultant for the 510(k) submitter].



Section 07
Class III Summary and Certification

N/A

CERTIFICATION: FINANCIAL INTERESTS AND ARRANGEMENTS OF CLINICAL INVESTIGATORS

TO BE COMPLETED BY APPLICANT

With respect to all covered clinical studies (or specific clinical studies listed below (if appropriate)) submitted in support of this application, I certify to one of the statements below as appropriate. I understand that this certification is made in compliance with 21 CFR part 54 and that for the purposes of this statement, a clinical investigator includes the spouse and each dependent child of the investigator as defined in 21 CFR 54.2(d).

Please mark the applicable checkbox.

- (1) As the sponsor of the submitted studies, I certify that I have not entered into any financial arrangement with the listed clinical investigators (enter names of clinical investigators below or attach list of names to this form) whereby the value of compensation to the investigator could be affected by the outcome of the study as defined in 21 CFR 54.2(a). I also certify that each listed clinical investigator required to disclose to the sponsor whether the investigator had a proprietary interest in this product or a significant equity in the sponsor as defined in 21 CFR 54.2(b) did not disclose any such interests. I further certify that no listed investigator was the recipient of significant payments of other sorts as defined in 21 CFR 54.2(f).

Clinical Investigators	(b) (6)	(b) (6)
	(b) (6)	
	(b) (6)	

- (2) As the applicant who is submitting a study or studies sponsored by a firm or party other than the applicant, I certify that based on information obtained from the sponsor or from participating clinical investigators, the listed clinical investigators (attach list of names to this form) did not participate in any financial arrangement with the sponsor of a covered study whereby the value of compensation to the investigator for conducting the study could be affected by the outcome of the study (as defined in 21 CFR 54.2(a)); had no proprietary interest in this product or significant equity interest in the sponsor of the covered study (as defined in 21 CFR 54.2(b)); and was not the recipient of significant payments of other sorts (as defined in 21 CFR 54.2(f)).
- (3) As the applicant who is submitting a study or studies sponsored by a firm or party other than the applicant, I certify that I have acted with due diligence to obtain from the listed clinical investigators (attach list of names) or from the sponsor the information required under 54.4 and it was not possible to do so. The reason why this information could not be obtained is attached.

NAME Sean K. Bauman	TITLE President and CEO
FIRM/ORGANIZATION Immuno-Mycologics, Inc.	
SIGNATURE 	DATE (mm/dd/yyyy) 3/1/2012

Paperwork Reduction Act Statement

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. Public reporting burden for this collection of information is estimated to average 1 hour per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the necessary data, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information to the address to the right:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
1350 Piccard Drive, 420A
Rockville, MD 20850



Section 09
Declarations of Conformity and Summary Reports

N/A



Executive Summary

Trade Name: CrAg Lateral Flow Assay

Common Name: Cryptococcal Antigen Lateral Flow Assay

Regulation: 866.3165

Predicate Device: CrAg Lateral Flow Assay (K102286)

Intended Use: The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) in serum and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription use laboratory assay, which can aid in the diagnosis of Cryptococcosis.

Device Description:

Explanation:

Detection of cryptococcal antigen in serum and CSF has been used for over forty years to aid in the diagnosis of cryptococcosis with very high sensitivity and specificity (6,11,12). Current guidelines for the management of cryptococcal disease partially base treatment recommendations on cryptococcal antigen presence and more specifically on cryptococcal antigen titers (13).

Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gatti*) (2,3,9,10). Individuals with impaired cell mediated immune (CMI) function due to acquired immunodeficiency syndrome (AIDS) (15), lymphoproliferative disorders (14), steroid therapy (5), and organ transplantation (4) are at increased risk of Cryptococcosis. AIDS accounts for 80-90% of Cryptococcal infections (8). The incidence of cryptococcosis in AIDS patients in the United States is estimated to be 5-10% (8) while the incidence of Cryptococcosis in other parts of the world, such as Africa, is as high as 30% (1). Cryptococcosis is the fourth most common opportunistic, life threatening infection among AIDS patients (7).

Description:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in serum and CSF. For qualitative procedure, specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For semi quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the screening procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold conjugated, anti cryptococcal antigen antibodies and gold conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it picks up and binds to the gold-conjugated, anti cryptococcal antigen antibodies. The gold labeled antibody antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti cryptococcal antigen monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold conjugated control goat IgG antibody to move to the Control Line (C) which is immobilized bovine anti goat IgG antibody. The immobilized anti goat antibody will bind to the gold conjugated goat IgG Control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line (Figure 1). If the control line fails to develop a line, then the test is not valid.

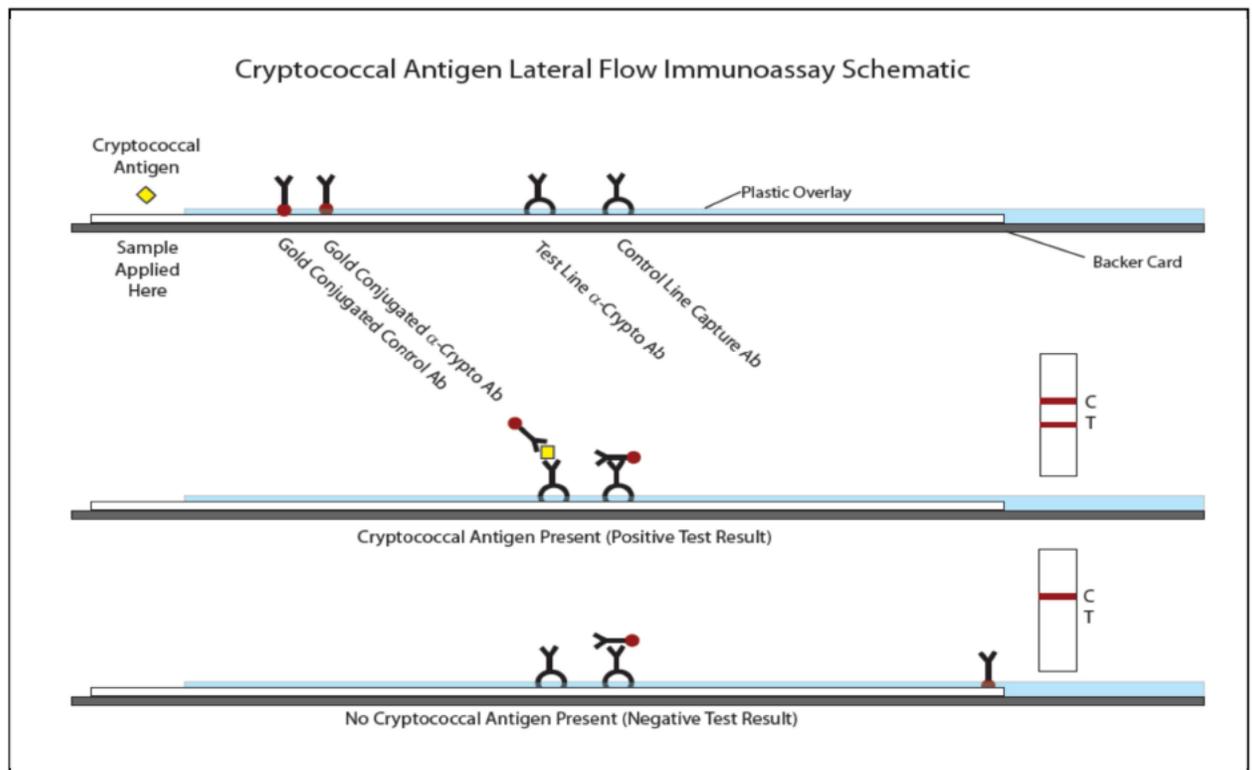


Figure 1. Cryptococcal Antigen Lateral Flow Immunoassay Schematic

Technological Characteristics Summary

A comparison between the Cryptococcal Antigen Lateral Flow Immunoassay and the Latex *Cryptococcus* Antigen Detection System is presented in Table 1.

Table 1. Comparison between Cryptococcal Antigen LFI and *Cryptococcus* Antigen Detection System

SIMILARITIES		
Feature	CrAg LFA	CrAg LFA (Serum Only) (K102286)
Intended Use		
Intended Use	Immunochromatographic test system for the qualitative or semi quantitative detection of the capsular polysaccharide antigens of <i>Cryptococcus</i> species complex (<i>Cryptococcus neoformans</i> and <i>Cryptococcus gattii</i>) in serum	Immunochromatographic test system for the qualitative or semi quantitative detection of the capsular polysaccharide antigens of <i>Cryptococcus</i> species complex (<i>Cryptococcus neoformans</i> and <i>Cryptococcus gattii</i>) in serum
Indication For Use	Prescription use laboratory assay, which can aid in the diagnosis of cryptococcosis	Prescription use laboratory assay, which can aid in the diagnosis of cryptococcosis
Device Description		
Technology	Lateral Flow Assay	Lateral Flow Assay
Sample Matrix	Serum	Serum
Instruments	None	None
Assay Components	Specimen diluent, lateral flow strips, built in control, gold conjugated antibodies	Positive control, negative control, latex cards, latex conjugated antibodies
Specimen Pre Treatment	Dilution	Dilution
Detection Antibody	Anti cryptococcal monoclonal antibody	Anti cryptococcal monoclonal antibody
Storage Requirements	20 25°C	20 25°C
DIFFERENCES		
Feature	Cryptococcal Antigen Lateral Flow Assay	Latex- <i>Cryptococcus</i> Antigen Detection System
Intended Use		
Intended Use	Test for the qualitative or semi quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum and CSF
Indication For Use	No differences	No differences

This comparison supports substantial equivalence in many ways. The only difference between the two devices is the sample matrix.

Performance Summary

A. Precision Studies (Repeatability & Reproducibility)

Serum repeatability and reproducibility results can be found in the predicate device 510(k) (K102286)

Repeatability and reproducibility with CSF specimens were determined by spiking a mock CSF that was negative by the IMMY Latex *Cryptococcus* Antigen Detection System with cryptococcal antigen at four concentrations: Negative, high negative (C₅), low positive (near C₉₅), and medium positive. The samples were analyzed on the CrAg Lateral Flow Assay in triplicate on five different days, at three different sites with a total of five different operators, on one lot, according to EP5 A2. One site was internal (Site 1) and the remaining two were a US reference laboratory (Site 2) and a US hospital laboratory (Site 3). For repeatability, percent positive and percent negative detected were calculated for each site (Table 2). For reproducibility, overall percent positive and percent negative detected were calculated by combining the data from all three sites (last two rows of Table 2).

Table 2. Repeatability at 3 Different Sites

Sample	CSF							
	1		2		3		4	
	Med. Pos	Low Pos	High Neg	Neg				
Neg/Pos		+		+		+		+
Site 1	0	30	0	30	27	3	30	0
Percent %	0	100	0	100	90	10	100	0
Site 2	0	30	0	30	30	0	30	0
Percent %	0	100	0	100	100	0	100	0
Site 3	0	15	0	15	15	0	15	0
Percent %	0	100	0	100	100	0	100	0
Total No.	0	75	0	75	72	3	75	0
Percent %	0	100	0	100	96	4	100	0

As expected, the high negative (C₅) gave a positive result nearly 4% of the time. All other samples performed 100% as expected across all sites, all runs, and all operators.

B. Analytical Sensitivity (lower limits of the assay/analytical cut off)

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running 24 replicates of varying concentrations of cryptococcal antigen diluted in mock CSF on one lot of kits, according to EP12 A2. The analytical cut off was defined as the concentration where 50% of the results were positive and 50% of the results were negative. The analytical cut off is 1.25ng/ml (Figure 2 and Table 3).



Figure 2. Analytical Cut-off Analysis – Percent Positive versus Concentration

Table 3. Analytical Cut-off Analysis

Sample Concentration (ng/ml)	No. Positive	No. Tested	% Positive
0.50	0	24	0%
0.75	0	24	0%
1.00	4	24	17%
1.25	12	24	50%
1.50	21	24	88%
1.75	24	24	100%
2.00	24	24	100%
2.50	24	24	100%
3.00	24	24	100%
3.50	24	24	100%
4.00	24	24	100%

C. Analytical Specificity/Interference

Analytical Specificity

Serum analytical specificity can be found in the predicate device 510(k) (K102286)

Due to specimen availability, the following CSF conditions were not tested in the CrAg Lateral Flow Assay: *S. pneumonia*, *Enterovirus*, *Enterobacteriaceae*, *Streptococcus* spp., *Staphylococcus* spp., *diphtheroid*, *H. influenzae* type B, *N. meningitidis*, *Enterococcus* spp., *Epstein Barr*, *Herpes simplex virus* Type 1 and 2, *Listeria monocytogenes*, *Trichosporon beigelii*, and samples with syneresis fluid condensation.

This assay was not evaluated for potential interference related to specimen pretreatment with 2 mercaptoethanol or with specimens including the following substances or

conditions: bloody CSF, cloudy CSF, white blood cells, xanthochromic CSF, bilirubin, protein, systemic lupus erythmatosus (SLE), sarcoidosis, or *N. meningitides*.

D. Linearity

N/A

E. High Dose Hook Effect

High dose hook effect concentrations with serum specimens were determined by spiking negative serum that were negative by the IMMY Latex *Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at various concentrations between 20 and 500ug/ml. Each concentration was tested in triplicate at IMMY on one lot of CrAg Lateral Flow Assay, according to the package insert. It was determined that serum specimens with a cryptococcal antigen concentration higher than 200ug/ml can produce a high dose hook effect and therefore may produce a false negative result.

F. Method Comparison

Predicate Device Method Comparison

Not Applicable

Other Method Comparison – Culture/India Ink (Gold Standards)

Serum method comparison to gold standards can be found in the predicate device 510(k) (K102286)

The CrAg Lateral Flow Assay was compared to the gold standard for the diagnosis of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay in CSF. These studies contained a mix of both prospective and retrospective specimens. A summary of the data collected is included in Tables 4 and 5 below:

Table 4. CSF 2x2 Contingency Table: Culture/India Ink

		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	65	0
	Negative	0	99

Table 5. CSF Statistical Analysis: Culture/India Ink

	Calculated	95% CI
Sensitivity	100%	94.4-100.0%
Specificity	100%	96.3-100%

Summary and Conclusion

The information submitted in this premarket notification is complete and supports a substantial equivalence decision.

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510(k) Device Description CrAg Lateral Flow Assay

Description and Performance Specifications:

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in serum and cerebral spinal fluid (CSF). For the qualitative procedure, specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For the semi quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the qualitative procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold conjugated, anti cryptococcal monoclonal antibodies and gold conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it binds to the gold-conjugated, anti cryptococcal antibodies. The gold labeled antibody antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti cryptococcal monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold conjugated control goat IgG antibody to move to the Control Line (C) which is immobilized bovine anti goat IgG antibody. The immobilized anti goat antibody will bind to the gold conjugated goat IgG Control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line (Figure 1). If the control line fails to develop a line, then the test is not valid.

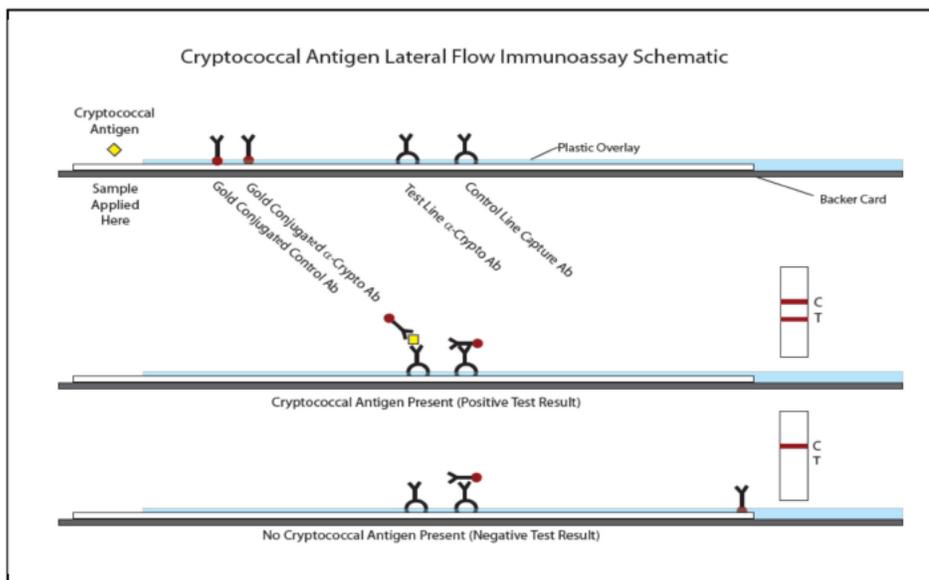


Figure 1. CrAg Lateral Flow Assay Schematic

The kit components and specifications are described in Table 1.

Table 1. CrAg Lateral Flow Assay Components

Kit Component	Component		Specification
	Reference Number	Volume/Kit	
LF Specimen Diluent	GLF070	2.5 ml	Creates a negative result on the CrAg LF Test Strips
CrAg LF Test Strips	LFCR01	50 strips	1 line with negative control and 2 lines with <i>Cryptococcus</i> positive specimens



**510(k) Substantial Equivalence Determination
Decision Summary
CrAg Lateral Flow Assay**

A. 510(k) Number
K112422

B. Purpose for Submission
To expand the Intended Use Statement to include cerebral spinal fluid (CSF) specimens.

C. Measurand
Capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*)

D. Type of Test
Qualitative and semi quantitative dipstick sandwich lateral flow immunochromatographic assay

E. Applicant
Immuno Mycologics, Inc.
2700 Technology Place
Norman, OK 73071
Tel. 405.360.4669
Fax. 405.364.1058

F. Proprietary and Established Names:
CrAg Lateral Flow Assay

G. Regulatory Information

- a. Regulation Section:**
866.3165
- b. Classification**
Class II
- c. Product Code**
GMD
- d. Panel**
Microbiology

H. Intended Use

a. Intended Use

The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi quantitative detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum and cerebral spinal fluid (CSF).

b. Indication for Use

The CrAg Lateral Flow Assay is a prescription use laboratory assay, which can aid in the diagnosis of cryptococcosis.

I. Device Description

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay which detects cryptococcal antigen in serum and cerebral spinal fluid (CSF). The assay consists of CrAg Lateral Flow test strips which have a gold conjugated antibody and a gold conjugated, anti cryptococcal antibody deposited onto a sample membrane and anti Crypto antibody and control line capture antibody striped onto a membrane. Also in the kit is a specimen diluent.

J. Substantial Equivalence Information

a. Predicate Device Name

Immuno Mycologics’ CrAg Lateral Flow Assay (serum only)

b. Predicate 510(k) Number

K102286

c. Comparison with predicate

Table 1: Comparison Between New Device and Predicate Device

SIMILARITIES		
Feature	CrAg LFA (New Device)	CrAg LFA (Serum Only) (K102286)
Intended Use	Immunochromatographic test system for the qualitative or semi quantitative detection of the capsular polysaccharide antigens of <i>Cryptococcus</i> species complex (<i>Cryptococcus neoformans</i> and <i>Cryptococcus gattii</i>) in serum	Immunochromatographic test system for the qualitative or semi quantitative detection of the capsular polysaccharide antigens of <i>Cryptococcus</i> species complex (<i>Cryptococcus neoformans</i> and <i>Cryptococcus gattii</i>) in serum
Indication For Use	Prescription use laboratory assay, which can aid in the diagnosis of cryptococcosis	Prescription use laboratory assay, which can aid in the diagnosis of cryptococcosis
Device Description		
Technology	Lateral Flow Assay	Lateral Flow Assay
Sample Matrix	Serum	Serum

Instruments	None	None
Assay Components	Specimen diluent, lateral flow strips, built in control, gold conjugated antibodies	Positive control, negative control, latex cards, latex conjugated antibodies
Specimen Pre Treatment	Dilution	Dilution
Detection Antibody	Anti cryptococcal monoclonal antibody	Anti cryptococcal monoclonal antibody
Storage Requirements	20 25°C	20 25°C
DIFFERENCES		
Feature	Cryptococcal Antigen Lateral Flow Assay	Latex-<i>Cryptococcus</i> Antigen Detection System
Intended Use		
Intended Use	Test for the qualitative or semi quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum	Test for the qualitative or semi quantitative detection of capsular polysaccharide antigens of <i>Cryptococcus</i> in serum and CSF
Indication For Use	No differences	No differences

K. Standard/Guidance Document Referenced (if applicable)

Not Applicable

Predicate: Not Applicable

L. Test Principle

Immunochromatographic Assay

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay, which detects cryptococcal antigen in serum and CSF. For the qualitative procedure, specimens are diluted 1:2 in 1x Specimen Diluent and analyzed. For the semi quantitative procedure, specimens are diluted 1:5 in 1x Specimen Diluent followed by 1:2 serial dilutions. All dilutions are then analyzed as in the qualitative procedure. Specimens are placed into an appropriate reservoir, such as a test tube or microtiter plate, and the lateral flow device is then placed into the reservoir allowing the specimen to come into contact with the test membrane. The test uses specimen wicking to capture gold conjugated, anti cryptococcal monoclonal antibodies and gold conjugated control antibodies that are deposited onto a membrane. If cryptococcal antigen is present in the specimen, it binds to the gold-conjugated, anti cryptococcal antibodies. The gold labeled antibody antigen complex will continue to wick up the membrane where it will interact with the Test Line (T). The Test Line is immobilized anti cryptococcal monoclonal antibodies. If the specimen contains cryptococcal antigen, a sandwich is created with the gold labeled antibodies and the immobilized antibodies, causing a visible line to develop at the test line site (T). If proper flow occurs and the reagents are reactive at the time of use, the wicking of any specimen, positive or negative, will cause the gold conjugated control goat IgG antibody to move to the

Control Line (C) which is immobilized bovine anti goat IgG antibody. The immobilized anti goat antibody will bind to the gold conjugated goat IgG Control antibody and will cause a visible line to develop (C). A positive test result will create two lines, while a negative test result will create one line. If the control line fails to develop a line, then the test is not valid.

Predicate: Same

M. Performance Characteristics

a. Analytical Performance

i. Precision

Serum repeatability and reproducibility results can be found in the predicate device 510(k) (K102286)

Repeatability and reproducibility with CSF specimens were determined by spiking a mock CSF that was negative by the IMMY Latex *Cryptococcus* Antigen Detection System with cryptococcal antigen at four concentrations: Negative, high negative (C₅), low positive (near C₉₅), and medium positive. The samples were analyzed on the CrAg Lateral Flow Assay in triplicate on five different days, at three different sites with a total of five different operators, on one lot, according to EP5 A2. One site was internal (Site 1) and the remaining two were a US reference laboratory (Site 2) and a US hospital laboratory (Site 3). For repeatability, percent positive and percent negative detected were calculated for each site (Table 2). For reproducibility, overall percent positive and percent negative detected were calculated by combining the data from all three sites (last two rows of Table 2).

Table 2. Repeatability at 3 Different Sites

Sample	CSF							
	1		2		3		4	
	Med. Pos	Low Pos	High Neg	Neg				
Neg/Pos		+		+		+		+
Site 1	0	30	0	30	27	3	30	0
Percent %	0	100	0	100	90	10	100	0
Site 2	0	30	0	30	30	0	30	0
Percent %	0	100	0	100	100	0	100	0
Site 3	0	15	0	15	15	0	15	0
Percent %	0	100	0	100	100	0	100	0
Total No.	0	75	0	75	72	3	75	0
Percent %	0	100	0	100	96	4	100	0

As expected, the high negative (C₅) gave a positive result nearly 4% of the time. All other samples performed 100% as expected across all sites, all runs, and all operators.

ii. Linearity

N/A

Predicate: N/A

iii. Traceability/Stability/Expected Values (controls, calibrators, or methods)

(b)(4) Trade Secret Process - Product Specs



Predicate: Same

iv. Detection Limit/Analytical Cut-off

Analytical sensitivity for serum can be found in the predicate device 510(k) (K102286)

Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running varying concentrations of cryptococcal antigen diluted in mock CSF on one lot of kits, according to EP12 A2. Test results determined that the analytical cut off is 1.25ng/ml (Table 3).

Table 3. Analytical Cut-Off

Sample Concentration (ng/ml)	No. Positive	No. Tested	% Positive
0.50	0	24	0%
0.75	0	24	0%
1.00	4	24	17%
1.25	12	24	50%
1.50	21	24	88%

1.75	24	24	100%
2.00	24	24	100%
2.50	24	24	100%
3.00	24	24	100%
3.50	24	24	100%
4.00	24	24	100%

v. Analytical Specificity/Interference

Analytical Specificity

Serum analytical specificity can be found in the predicate device 501(k) (K102286)

Due to specimen availability, the following CSF conditions were not tested in the CrAg Lateral Flow Assay: *S. pneumonia*, *Enterovirus*, *Enterobacteriaceae*, *Streptococcus* spp., *Staphylococcus* spp., *diphtheroid*, *H. influenzae* type B, *N. meningitidis*, *Enterococcus* spp., *Epstein Barr*, *Herpes simplex virus* Type 1 and 2, *Listeria monocytogenes*, *Trichosporon beigelii*, and samples with syneresis fluid condensation.

This assay was not evaluated for potential interference related to specimen pretreatment with 2 mercaptoethanol or with specimens including the following substances or conditions: bloody CSF, cloudy CSF, white blood cells, xanthochromic CSF, bilirubin, protein, systemic lupus erythmatosus (SLE), sarcoidosis, or *N. meningitides*.

vi. High Dose Hook Effect

High dose hook effect concentrations with serum specimens were determined by spiking negative serum that were negative by the IMMY Latex *Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at various concentrations between 20 and 500ug/ml. Each concentration was tested in triplicate at IMMY on one lot of CrAg Lateral Flow Assay, according to the package insert. It was determined that serum specimens with a cryptococcal antigen concentration higher than 200ug/ml can produce a high dose hook effect and therefore may produce a false negative result.

b. Comparison Studies

Matrix Comparison

(b)(4) Trade Secret Process - Product Specs

Table 4. Matrix Comparison 2x2 Contingency Table

(b)(4) Trade Secret Process - Product Specs

Table 5. Matrix Comparison Statistical Analysis

(b)(4) Trade Secret Process - Product Specs

Other Method Comparison – Culture/India Ink (Gold Standards)

The CrAg Lateral Flow Assay was compared to the gold standard diagnosis of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay in (b)(4) CSF. These studies contained a mix of both prospective and retrospective specimens. A summary of the data collected is included in Tables 5-10 below:

Serum method comparison to gold standards can be found in the predicate device 510(k) (K102286)

The CrAg Lateral Flow Assay was compared to the gold standard for the diagnosis of cryptococcosis (culture and/or India Ink) to evaluate the sensitivity and specificity of the assay in CSF. These studies contained a mix of both prospective and retrospective specimens. A summary of the data collected is included in Tables 6 and 7 below:

Table 6. CSF 2x2 Contingency Table: Culture/India Ink

		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	65	0
	Negative	0	99

Table 7. CSF Statistical Analysis: Culture/India Ink

	Calculated	95% CI
Sensitivity	100%	94.4-100.0%
Specificity	100%	96.3-100%

Predicate Device:

Table 8. Serum 2x2 Contingency Table: Culture/India Ink

	Culture/India Ink	
	Positive	Negative
CrAg LFA Assay		
Positive	91	0
Negative	0	123

Table 9. Serum Statistical Analysis: Culture/India Ink

	Calculated	95% CI
Sensitivity	100%	96.0%-100%
Specificity	100%	97.0%-100%

c. Clinical Studies

Not Applicable

d. Clinical Cut-Off

Not Applicable

e. Expected Values/Reference Range

Not Applicable

N. Proposed Labeling

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusions

The submitted information in the premarket notification is complete and supports a substantial equivalence decision.

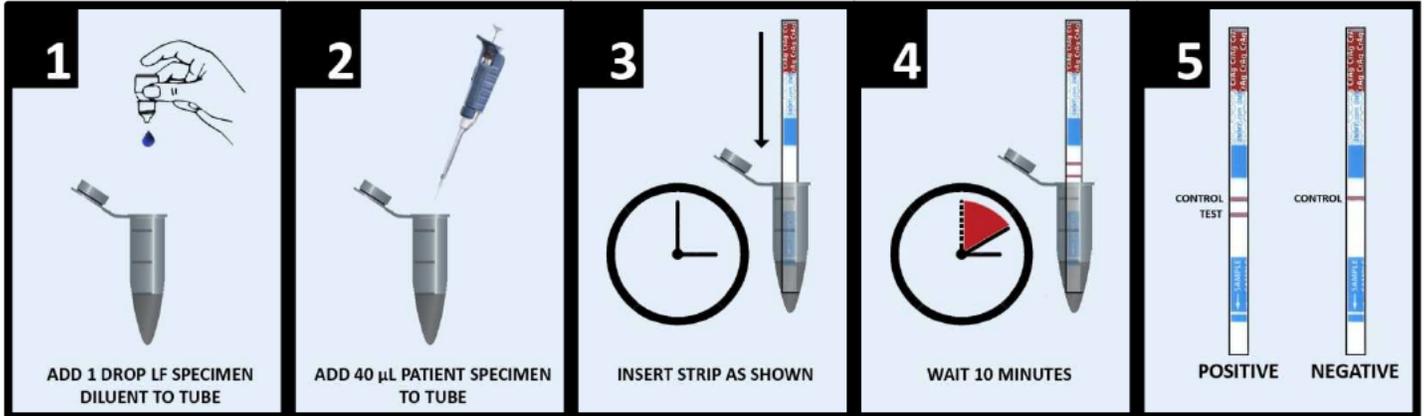


CrAg Lateral Flow Assay

For the Detection of Cryptococcal Antigen – REF CR2003



QUALITATIVE – BASIC PROCEDURE



INTENDED USE

The CrAg Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of the capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum and cerebral spinal fluid (CSF).

The CrAg Lateral Flow Assay is a prescription-use laboratory assay which can aid in the diagnosis of cryptococcosis.

SUMMARY and EXPLANATION of the Test

Cryptococcosis is caused by both species of the *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) (4). Individuals with impaired cell-mediated immunity are at greatest risk of infection (8). Cryptococcosis is one of the most common opportunistic infections in AIDS patients (6). Detection of cryptococcal antigen (CrAg) in serum and CSF has been extensively utilized with very high sensitivity and specificity (1-3).

BIOLOGICAL PRINCIPLES

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay. Specimens and specimen diluent are added into an appropriate reservoir, such as a test tube, and the lateral flow device is placed into the reservoir. The test uses specimen wicking to capture gold-conjugated, anti-CrAg monoclonal antibodies and gold-conjugated control antibodies deposited on the test membrane. If CrAg is present in the specimen, then it binds to the gold-conjugated, anti-CrAg antibodies. The gold-labeled antibody-antigen complex continues to wick up the membrane where it will interact with the test line, which has immobilized anti-CrAg monoclonal antibodies. The gold-labeled antibody-antigen complex forms a sandwich at the test line causing a visible line to form. With proper flow and reagent reactivity, the wicking of any specimen, positive or negative, will cause the gold-conjugated control antibody to move to the control line. Immobilized antibodies at the control line will bind to the gold-conjugated control antibody and form a visible control line. Positive test results create two lines (test and control). Negative test results form only one line (control). If a control line fails to develop then the test is not valid.

WARNINGS and PRECAUTIONS

For in Vitro Diagnostic Use only.

REAGENT PRECAUTIONS

1. Specific standardization is necessary to produce our high-quality reagents and materials. The user assumes full responsibility for any modification to the procedures published herein.

2. When handling patient specimens, adequate measures should be taken to prevent exposure to etiologic agents potentially present in the specimens.
3. Always wear gloves when handling reagents in this kit as some reagents are preserved with 0.095% (w/w) sodium azide. Sodium azide should never be flushed down the drain as this chemical may react with lead or copper plumbing to form potentially explosive metal azides. Excess reagents should be discarded in an appropriate waste receptacle.

REAGENTS

1. LF Specimen Diluent (2.5 mL, REF GLF025): Glycine-buffered saline containing blocking agents and a preservative
2. CrAg LF Test Strips (50 strips in desiccant vial, REF LFCR50)
3. CrAg Positive Control (1 mL, REF CB1020): Glycine-buffered saline spiked with cryptococcal antigen (strain 184A clinical isolate from Tulane University (Infection & Immunity, June 1983, p. 1052-1059))
4. Package insert

MATERIALS NOT PROVIDED

1. Pipettor (40-µL and 80-µL)
2. Timer
3. Disposable micro-centrifuge tubes, test tubes, or a micro-titer plate

REAGENT PREPARATIONS

The entire kit should be at room temperature (22-25 °C) before and during use.

REAGENT STABILITY AND STORAGE

All reagents included in this kit should be stored at room temperature (22-25°C) until the expiration dates listed on the reagent labels.

Unused test strips should be stored in the LF test strip vial with the desiccant cap firmly attached.

SPECIMEN COLLECTION & PREPARATION

For optimal results, sterile non-hemolyzed serum should be used. Collect CSF specimens aseptically following accepted procedures. If a delay is encountered in specimen processing, storage at 2-8°C for up to 72 hours is permissible. Specimens may be stored for longer periods at <-20°C, provided they are not repeatedly thawed and refrozen. Specimens in transit should be maintained at 2-8°C or <-20°C.

PROCEDURE

REFER TO REAGENTS SECTION FOR A LIST OF MATERIALS PROVIDED.

Qualitative Procedure

1. Add 1 drop of LF Specimen Diluent (REF GLF025) to an appropriate reservoir (disposable micro-centrifuge tube, test tubes, or micro-titer plate, etc.).
2. Add 40 µL of specimen to the container and mix.
3. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip (REF LFCR50) into the specimen.
4. Wait 10 minutes.
5. Read and record the results (See READING THE TEST).

Semi-Quantitative Titration Procedure

1. Prepare dilutions starting with an initial dilution of 1:5, followed by 1:2 serial dilutions to 1:2560.
2. Place 10 micro-centrifuge or test tubes in an appropriate rack and label them 1-10 (1:5 through 1:2560). Additional dilutions may be necessary if the specimen is positive at 1:2560.
3. Add 4 drops of LF Specimen Diluent (REF GLF025) to tube #1.
4. Add 2 drops of LF Specimen Diluent to each of the tubes labeled 2-10.
5. Add 40 µL of specimen to tube #1 and mix well.
6. Transfer 80 µL of specimen from tube #1 to tube #2 and mix well. Continue this dilution procedure through tube #10. Discard 80 µL from tube 10 for a final tube volume of 80 µL.
7. Submerge the white end of a Cryptococcal Antigen Lateral Flow Test Strip into the specimen in each of the 10 tubes.
8. Wait 10 minutes.
9. Read and record the results (See READING THE TEST).

READING THE TEST

Read the reactions. The presence of two lines (test and control), regardless of the intensity of the test line, indicates a positive result.

For the semi-quantitative titration procedure, the patient's titer should be reported as the highest dilution that yields a positive result.

A single control line indicates a negative result. If the control line does not appear, the results are invalid and the test should be repeated.

REPRODUCIBILITY AND PRECISION

The CrAg Lateral Flow Assay was evaluated for reproducibility and precision by spiking serum and mock CSF with cryptococcal antigen to produce a panel consisting of a negative sample, a high-negative (C_s) sample, a low-positive sample and a moderate-positive sample. This panel was tested twice per day at three sites with a total of five operators over a five-day period in order to determine both the inter-lab and the intra-lab reproducibility and precision of the assay. The results of this study are shown in the tables below.

SERUM PANEL	Site 1 % Pos	Site 2 % Pos	Site 3 % Pos	Overall % Pos
Negative	0% (0/30)	0% (0/30)	0% (0/15)	0% (0/75)
High Negative	7% (2/30)	0% (0/30)	0% (0/15)	3% (2/75)
Low Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)
Moderate Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)

CSF PANEL	Site 1 % Pos	Site 2 % Pos	Site 3 % Pos	Overall % Pos
Negative	0% (0/30)	0% (0/30)	0% (0/15)	0% (0/75)
High Negative	10% (3/30)	0% (0/30)	0% (0/15)	3% (2/75)
Low Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)
Moderate Positive	100% (30/30)	100% (30/30)	100% (15/15)	100% (75/75)

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WEB: www.immy.com



MDSS
Schiffgraben 41
30175 Hannover, Germany

International Symbol Usage

 Storage
20-25 C

 LOT Lot Number

 Manufactured by

 REF Reference
Number
In Vitro
Diagnostics

 Expiration Date

 Conforms to European
Union Requirements

 Protect from Humidity

 Sufficient for "H"
Tests

Section 13 Proposed Labeling

I. Kit Label:

CrAg Lateral Flow Assay

For the Detection of Cryptococcal Antigen

IVD

REF

CR2003

50 Tests

LOT

SAMPLE

2011-06

Kit Contents		
REF	Description	Qty
LFCR01	CrAg Lateral Flow Test Strips	50 strips
GLF070	LF Specimen Diluent	2.5 ml
PKGINS	Package Insert	N/A

Immuno-Mycologics, Inc. - 2700 Technology PI - Norman OK 73071 USA - (800)654-3639

1

ADD 1 DROP LF SPECIMEN
DILUENT TO TUBE

2

ADD 40 µL PATIENT SPECIMEN
TO TUBE

3

INSERT STRIP AS SHOWN

4

WAIT 10 MINUTES

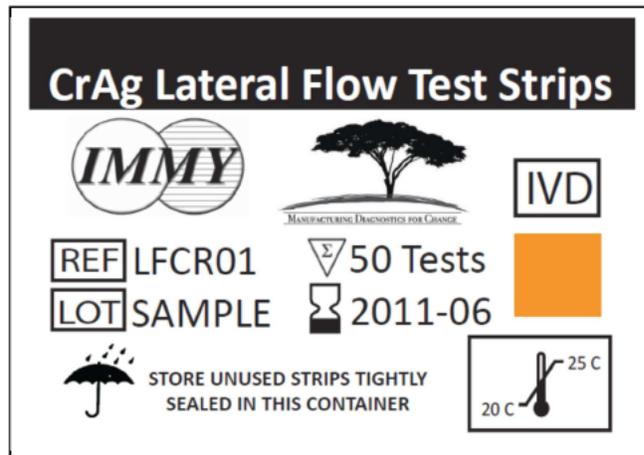
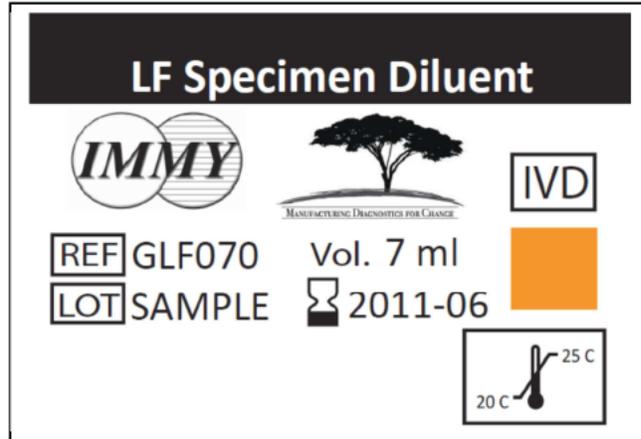
5

CONTROL
TEST
CONTROL
TEST

POSITIVE
NEGATIVE

Section 13 Proposed Labeling

II. Kits Components' Labels

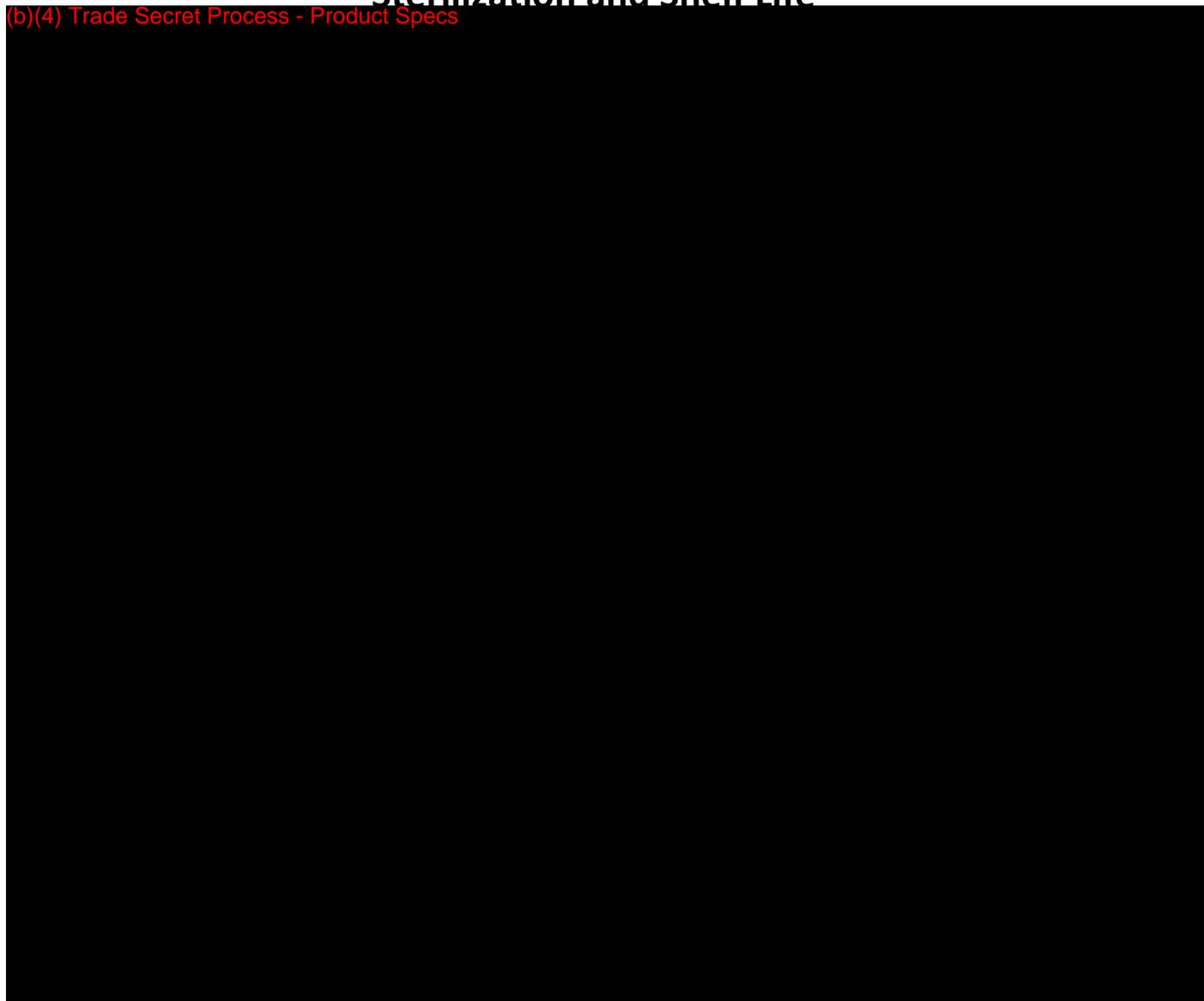


III. Package Insert (see next 3 pages)



Sterilization and Shelf Life

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Results:

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Conclusions: All data support an 18 month shelf life for the CrAg Lateral Flow Test Kit.



Section 15 Biocompatibility

N/A



Section 16 Software

N/A



Section 17

Electromagnetic Compatibility and Electrical Safety

N/A



Performance Testing - Bench CrAg Lateral Flow Assay

A. Precision Studies (Repeatability & Reproducibility)

Test Objective: To determine assay inter and intra lab precision.
Test Articles Used: Negative serum spiked with cryptococcal antigen
Test Methods: CLSI EP5 A2
Study Endpoint: Percent negative and percent positive

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1. Precision Test Methods:

Repeatability and reproducibility with serum specimens were determined by spiking a serum specimen pool that was negative by the IMMY Latex *Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at four concentrations: negative, high negative (C₅), low positive (near C₉₅), and medium positive. The samples were analyzed on the CrAg Lateral Flow Assay in triplicate on five different days, at three different sites with a total of five different operators, on one lot, according to CLSI EP5 A2. One site was internal (Site 1) and the remaining two were a US reference laboratory (Site 2) and a US hospital laboratory (Site 3). A test was considered positive when two lines formed (Test and Control) and negative when one line was formed (Control). A test was considered invalid if the Control Line failed to develop.

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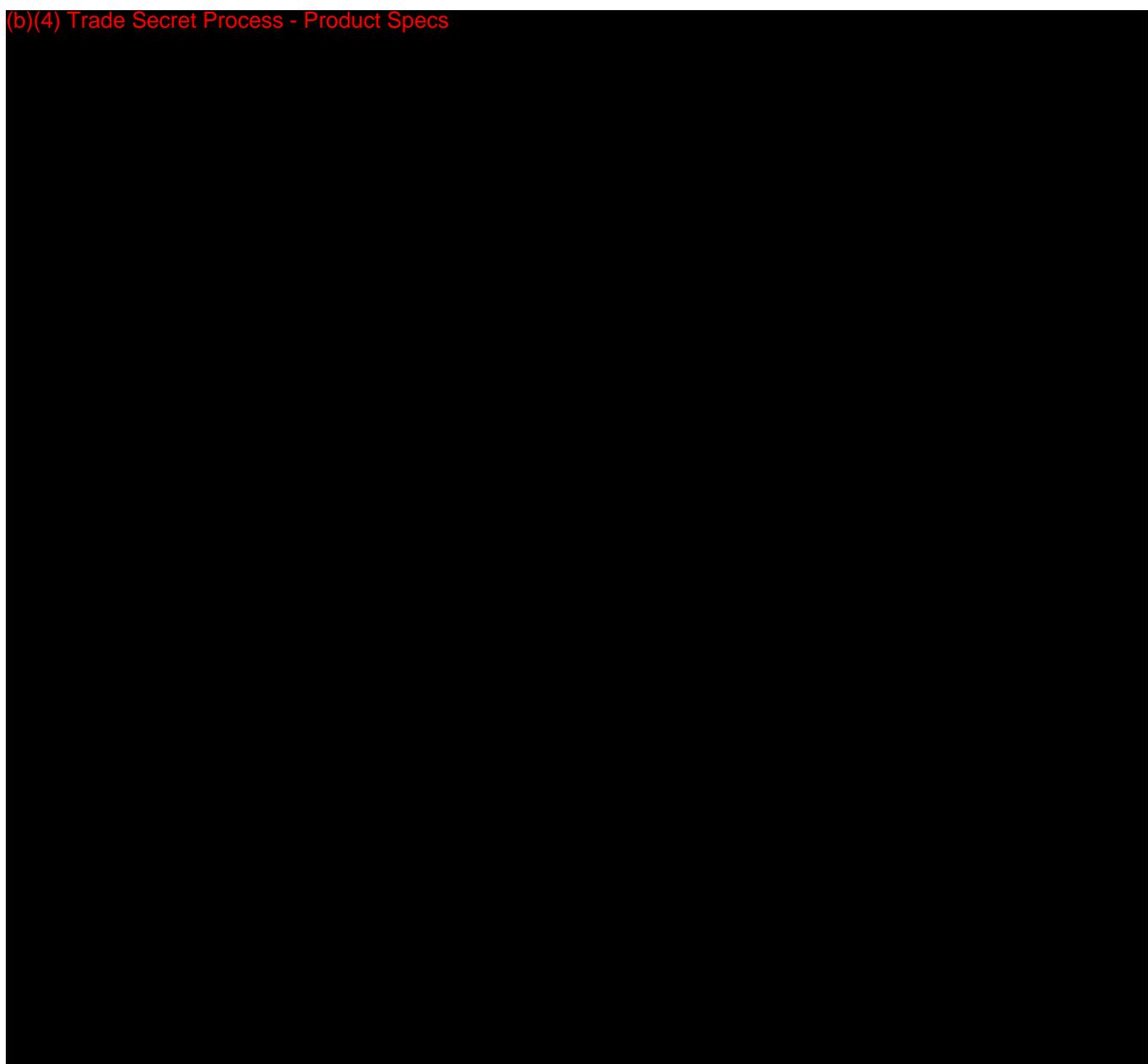


(b)(4) Trade Secret Process - Product Specs

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2. Precision Results:

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3. Precision Analysis:

For repeatability, percent positives detected and percent negatives detected were calculated for each site (Table 2). For reproducibility, overall percent positives detected were calculated by combining the data from all three sites (Table 3).

Table 2. Repeatability

Sample	Serum							
	Med. Pos		Low Pos		High Neg		Neg	
Neg/Pos		+		+		+		+
Site 1	0	30	0	30	28	2	30	0
Percent %	0	100	0	100	93	7	100	0
Site 2	0	30	0	30	30	0	30	0
Percent %	0	100	0	100	100	0	100	0
Site 3	0	15	0	15	15	0	15	0
Percent %	0	100	0	100	100	0	100	0

Table 3. Reproducibility

Sample	Serum							
	Med. Pos		Low Pos		High Neg		Neg	
Neg/Pos		+		+		+		+
Total No.	0	75	0	75	73	2	75	0
Percent %	0	100	0	100	97	3	100	0

4. Precision Conclusions:

Percent negatives on negative specimens are greater than 90%, percent positives on low positive samples are greater than 90%, and percent positives on medium positive specimen are greater than 95%. Therefore, the assay is precise with serum samples across operators, days, and sites.

B. Analytical Sensitivity (Lower Limits of the Assay/Analytical Cut-Off)

Test Objective: To determine the lower limits of the assay
 Test Articles Used: Cryptococcal antigen spiked into LF Specimen Diluent
 Test Methods: CLSI EP12 A2
 Study Endpoint: C₅₀ (Concentration where sample runs positive 50% of the time)
 Acceptance Criteria: N/A

1. Analytical Sensitivity Methods:

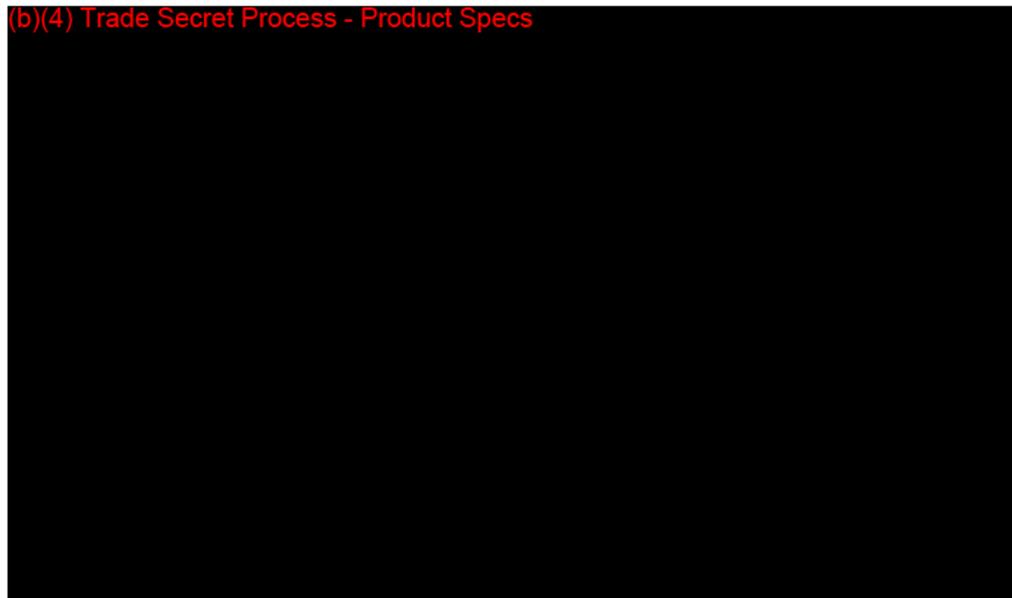
Analytical sensitivity for the CrAg Lateral Flow Assay was estimated by running 24 replicates of varying concentrations of cryptococcal antigen diluted in Lateral Flow Specimen Diluent, on one lot of kits. The analytical cut off was defined as the concentration where 50% of the results were positive and 50% of the results were negative (Table 4 and Figure 1).

2. Analytical Sensitivity Results/Analysis:

Table 4. Analytical Cut-Off

Sample Concentration (ng/ml)	No. Positive	No. Tested	% Positive
0.50	0	24	0%
0.75	0	24	0%
1.00	4	24	17%
1.25	12	24	50%
1.50	21	24	88%
1.75	24	24	100%
2.00	24	24	100%
2.50	24	24	100%
3.00	24	24	100%
3.50	24	24	100%
4.00	24	24	100%

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3. Analytical Sensitivity Conclusions:

Analysis shows that the analytical sensitivity (analytical cut off) is 1.25 ng/ml.

C. Analytical Specificity/Interference

Test Objective: To determine assay cross reactivity
 Test Articles Used: Specimens representative of potentially cross reacting medical conditions unrelated to cryptococcosis; specimens containing potentially interfering substances.
 Test Methods: See below
 Study Endpoint: Percent positive
 Acceptance Criteria: (b)(4) Trade Secret Process - Product Specs



1. Analytical Specificity

a. Analytical Specificity Methods:

Analytical specificity for the CrAg Lateral Flow Assay was determined by running potentially cross reacting medical conditions unrelated to cryptococcosis. The following specimens were run in triplicate on one lot of the CrAg Lateral Flow Assay. A total of 118 serum specimen and 15 fungal culture filtrates were tested. (b)(4) Trade Secret Process - Product Specs).

b. Analytical Specificity Results/Analysis:

Table 5. Analytical Specificity Analysis

Pathology	No. of Specimens	No. of Replicates Per Specimen	Total Tests	Total Number of Positives	% Positive (No Pos/Total)
Non-Fungal Pathologies					
HAMA (Human anti Mouse antibody) Positive	5	3	15	0	0%
Syphilis	10	3	30	0	0%
Rubella	5	3	15	0	0%
Mycoplasma	10	3	30	0	0%
Toxoplasmosis	7	3	21	0	0%
CMV Infection	10	3	30	0	0%
Rheumatoid factor*	10	3	30	0	0%

Total	57	3	171	0	0%
Fungal Pathologies					
Penicilliosis	5	3	15	0	0 %
Sporotrichosis	6	3	18	0	0%
Blastomycosis	10	3	30	0	0%
Coccidioidomycosis	10	3	30	0	0%
Histoplasmosis	10	3	30	0	0%
Candidiasis	10	3	30	0	0%
Aspergillosis **	10	3	30	3	10%
<i>Aspergillus terreus</i> Culture Filtrate	3	3	9	0	0%
<i>Aspergillus niger</i> Culture Filtrate	3	3	9	0	0%
<i>Aspergillus flavus</i> Culture Filtrate	3	3	9	0	0%
<i>Aspergillus fumigatus</i> Culture Filtrate	3	3	9	0	0%
<i>Paracoccidioides brasiliensis</i> Culture Filtrate	3	3	9	6	67%
Total	76	3	228	9	3.9%

* Rheumatoid factor concentrations tested ranged from 112 IU/ml to 6479 IU/ml.

** The three positives were the result of three replicates of the same specimen, which was positive.

c. Analytical Specificity Conclusions:

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the following statement is included in the CrAg Lateral Flow Assay Package Insert's Limitations of the Procedure section:

"At high concentrations, *Paracoccidioides brasiliensis* antigens can exhibit cross reactivity."

Rheumatoid factor is known to cause false positive results in the Cryptococcal latex agglutination method. Rheumatoid factor did not cause false positives in the CrAg Lateral Flow Assay between the range of 112 IU/ml and 6479 IU/ml. The normal reference range for rheumatoid factor is 0-14 IU/ml.

1. Interference

a. Interference Methods:

In addition to the cross reactivity study, interference testing was also performed on five icteric, five hemolyzed, and five lipemic serum specimens. Each specimen was spiked with cryptococcal antigen at three times the C_{95} concentration. All specimens were then tested at IMMY, on one lot of CrAg Lateral Flow assay in triplicate: spiked and unspiked. The effect of pronase on the CrAg LFA was determined by pronase treating 5 Meridian EIA positive specimens and 5 Meridian EIA negative specimens. The samples were analyzed both untreated and pronase treated. Percent positivity was determined for each condition (Table 6).

b. Interference Results/Analysis:

Table 6. Interference Analysis

Interfering Condition	No. of Specimens	No. of Replicates/Specimen	Total Tests	Total Positive	Total Positive
Iceteric – unspiked	5	3	15	0	0%
Iceteric spiked	5	3	15	15	100%
Hemolyzed unspiked	5	3	15	0	0%
Hemolyzed – spiked	5	3	15	15	100%
Lipemic – unspiked	5	3	15	0	0%
Lipemic – spiked	5	3	15	15	100%
CrAg Pos – untreated	5	3	15	15	100%
CrAg Pos – pronase tx	5	3	15	15	100%
CrAg Neg – untreated	5	3	15	0	0%
CrAg Neg – pronase tx	5	3	15	0	0%

c. Interference Conclusions:

All of the unspiked icteric, hemolyzed, and lipemic specimens had negative results on the CrAg Lateral Flow Assay. All spiked specimens were positive, thus, these types of serum specimens do not interfere with the CrAg Lateral Flow Assay. However, it is possible that

hemolyzed samples could lead to false negatives due to the high background color on the strip. As such, a statement is included in the Limitations of the Procedure section of the package insert:

“Hemolyzed serum samples could lead to false negative results due to the high background color on the strip.”

Pronase does not affect the CrAg LFA results.

D. High Dose Hook Effect

Test Objective:	To determine if specimens with high concentrations of cryptococcal antigen will produce a negative result on the CrAg Lateral Flow Assay
Test Articles Used:	Human serum specimens spiked with cryptococcal antigen at various concentrations, CrAg Lateral Flow Assay
Test Methods:	See Below
Study Endpoint:	The concentration of cryptococcal antigen well above the analytical sensitivity that fails to produce a positive result in the CrAg Lateral Flow Assay.

1. High Dose Hook Effect Methods

High dose hook effect concentrations with serum specimens were determined by spiking a serum specimen pool that was negative by the IMMY Latex *Cryptococcus* Antigen Detection System and CrAg Lateral Flow Assay, with cryptococcal antigen at various concentrations between 20 and 500ug/ml. Each concentration was tested in triplicate at IMMY on one lot of CrAg Lateral Flow Assay, according to the package insert. Line data can be found in Table 7.

2. High Dose Hook Effect Results/Analysis

Table 7. High Dose Hook Effect

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3. High Dose Hook Effect Conclusions

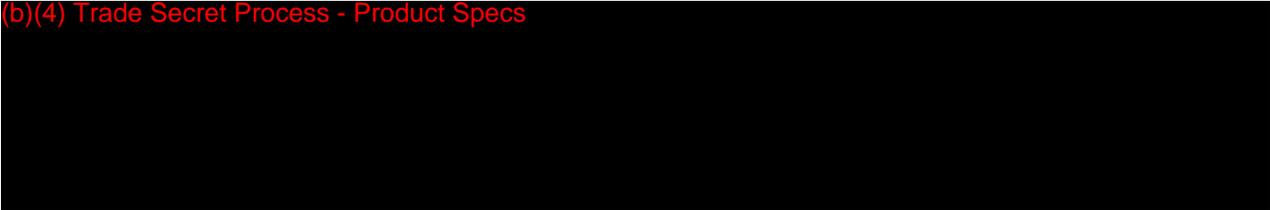
(b)(4) Trade Secret Process - Product Specs




 Therefore, any serum specimen containing more than 200ug/ml of cryptococcal antigen may produce a false negative result in the CrAg Lateral Flow Assay. As such, a statement will be included in the package insert as a limitation of the procedure.

E. Freeze/Thaw Studies

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1. Freeze/Thaw Study Methods:

(b)(4) Trade Secret Process - Product Specs



2. Freeze/Thaw Study Results

(b)(4) Trade Secret Process - Product Specs



(b)(4) Trade Secret Process - Product Specs

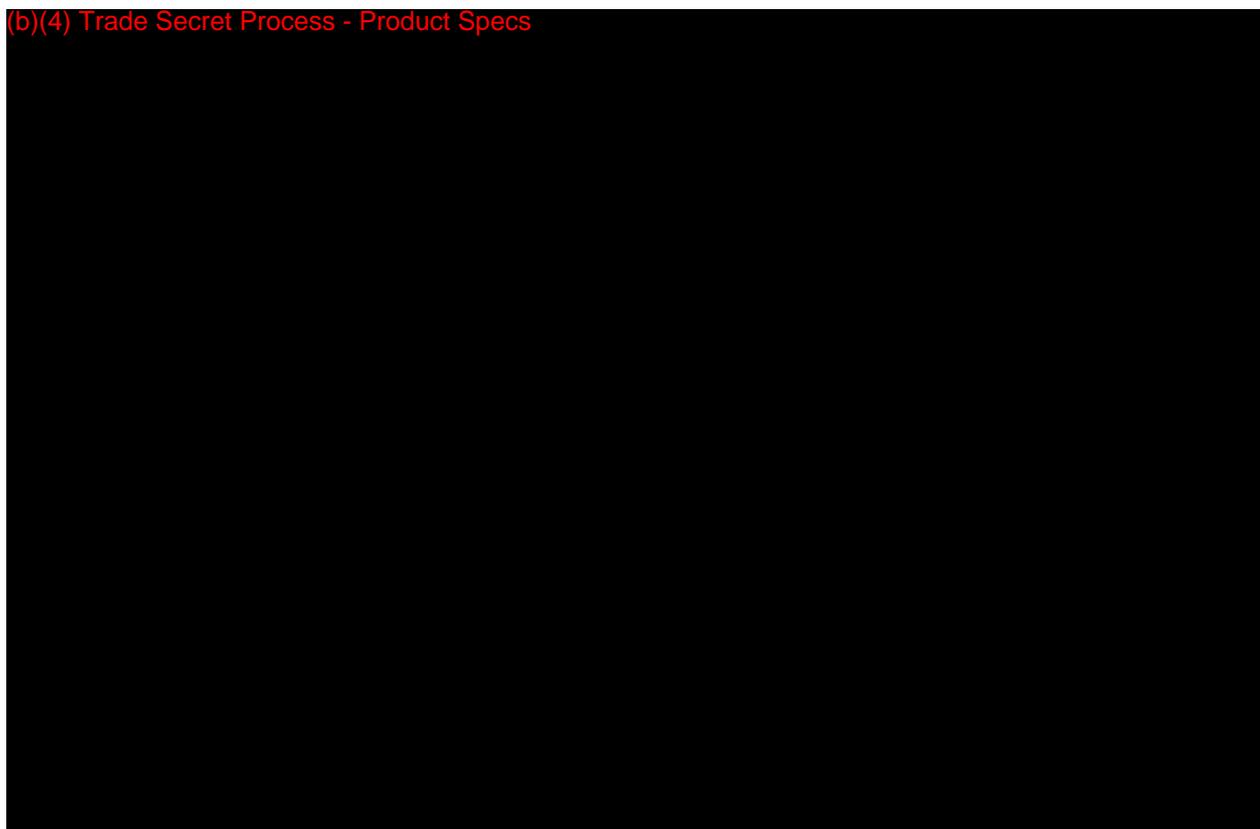
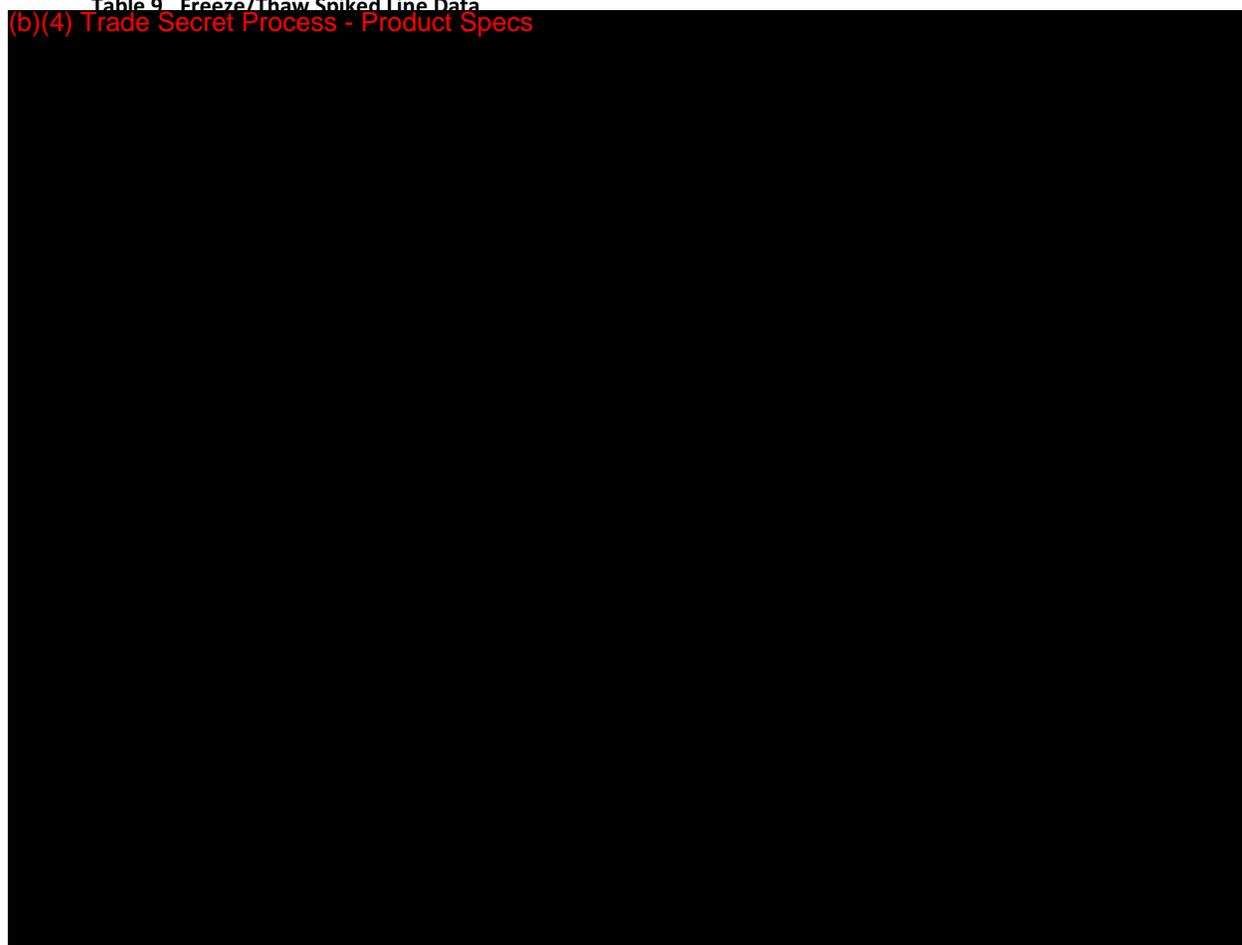
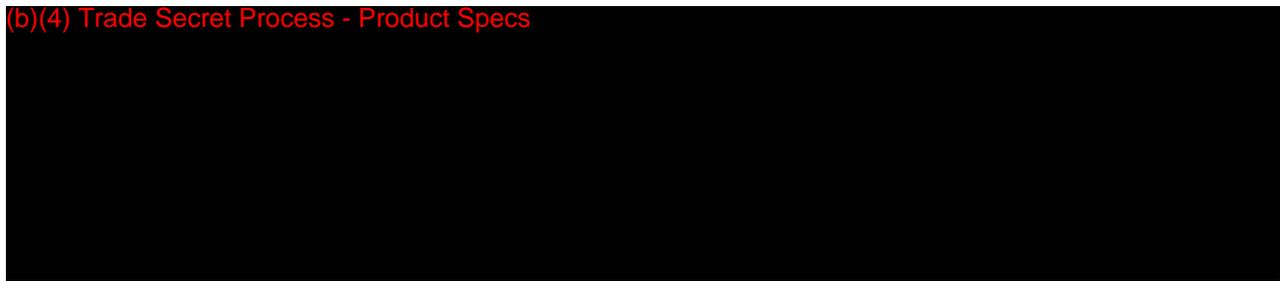


Table 9. Freeze/Thaw Spiked Line Data

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3. Freeze/Thaw Study Conclusion:

There is 100% agreement (95 100%, 95% CI) between frozen and fresh specimens. Therefore, frozen specimens are equivalent to fresh specimens.

F. Method Comparisons – Culture/India Ink

Test Objective:	To establish the new device's sensitivity and specificity
Test Articles Used:	Culture confirmed human (b)(4) Trade Secret CSF specimens tested collected retrospectively and prospectively.
Test Methods:	EP12 A2
Study Endpoint:	Sensitivity and Specificity
Acceptance Criteria:	Sensitivity values greater than 90% and specificity values greater than 90%.

1. Comparison Methods:

(b)(4) Trade Secret Process - Product Specs

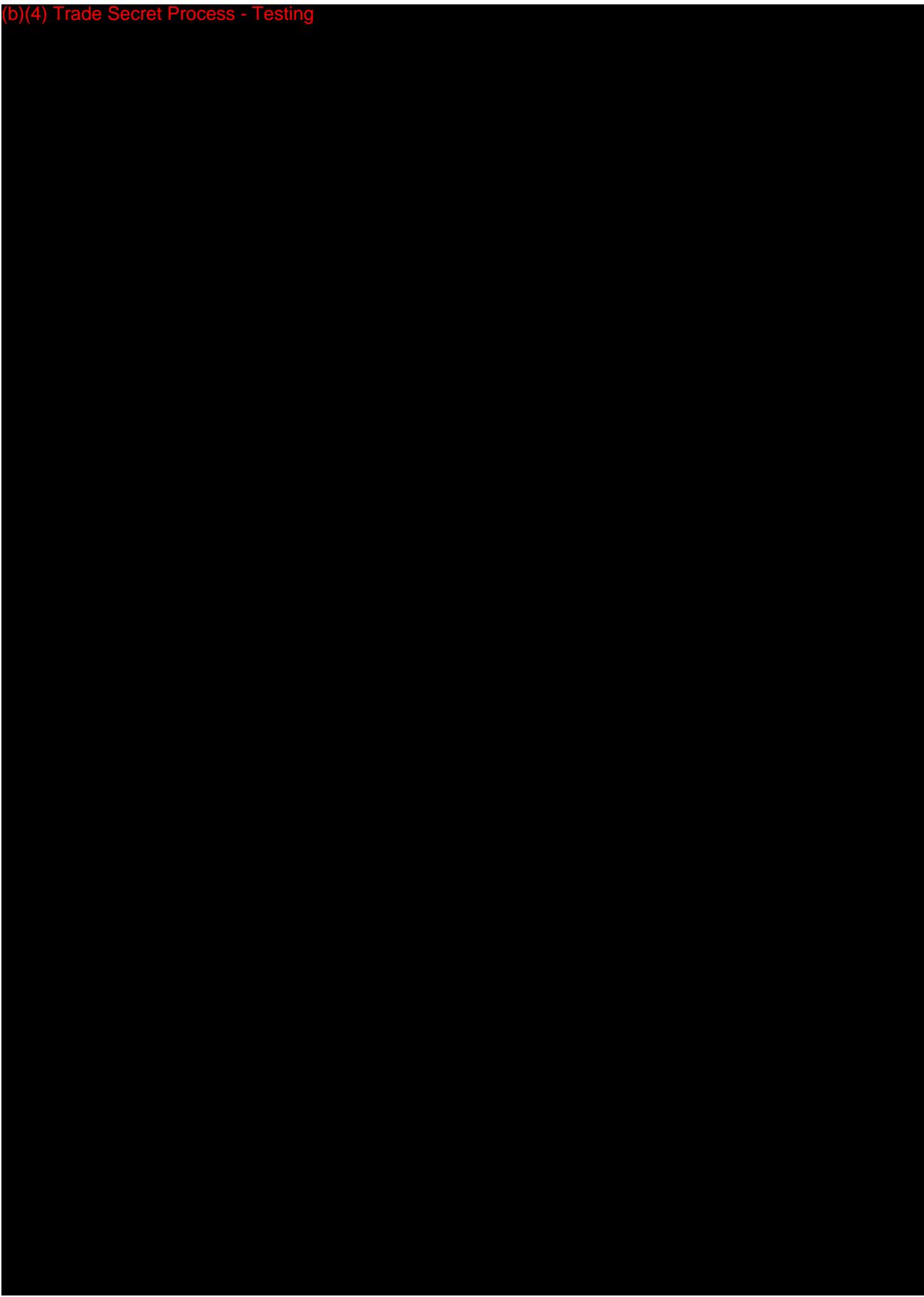


2. Comparison Results:

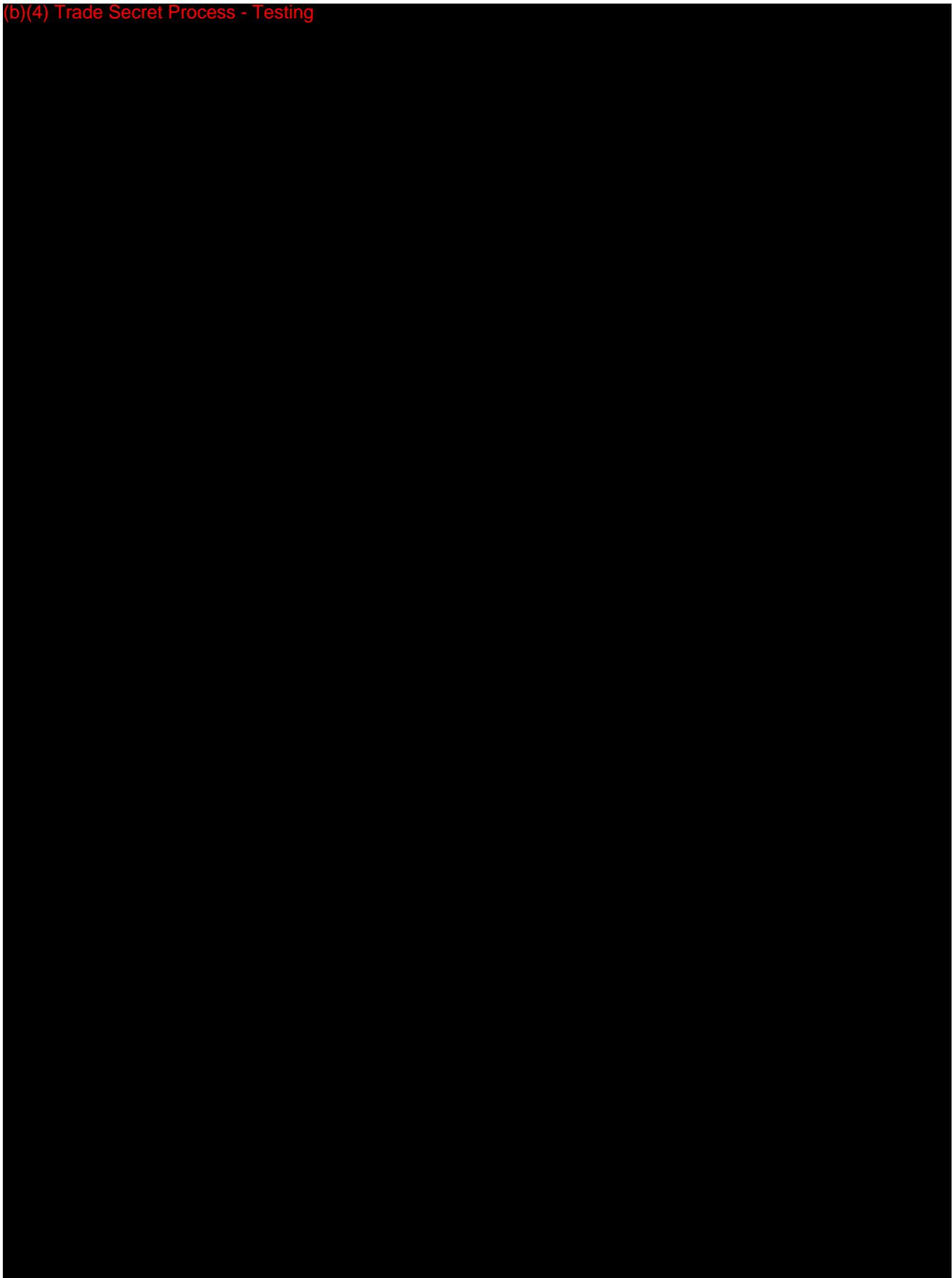
(b)(4) Trade Secret Process - Product Specs



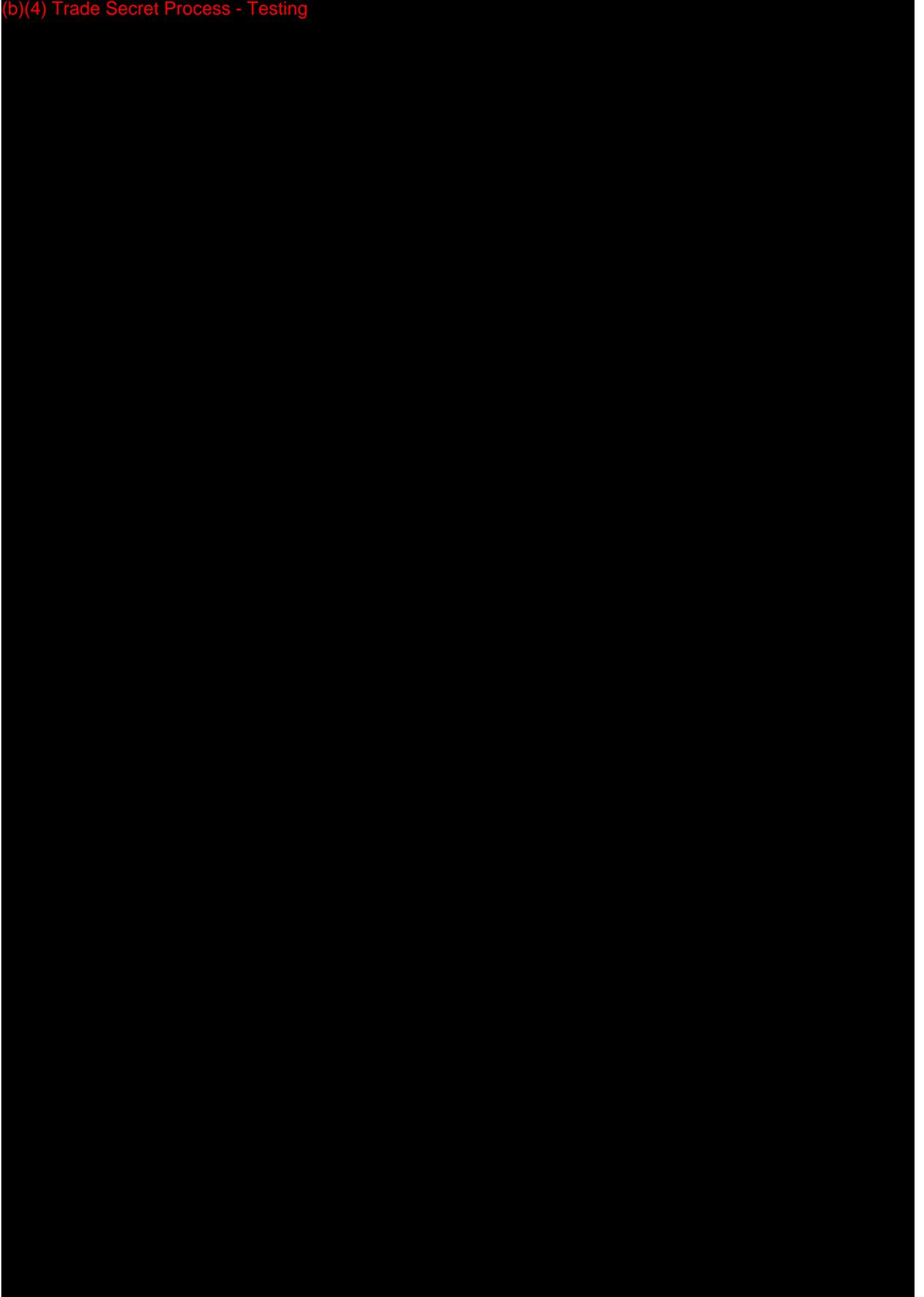
(b)(4) Trade Secret Process - Testing



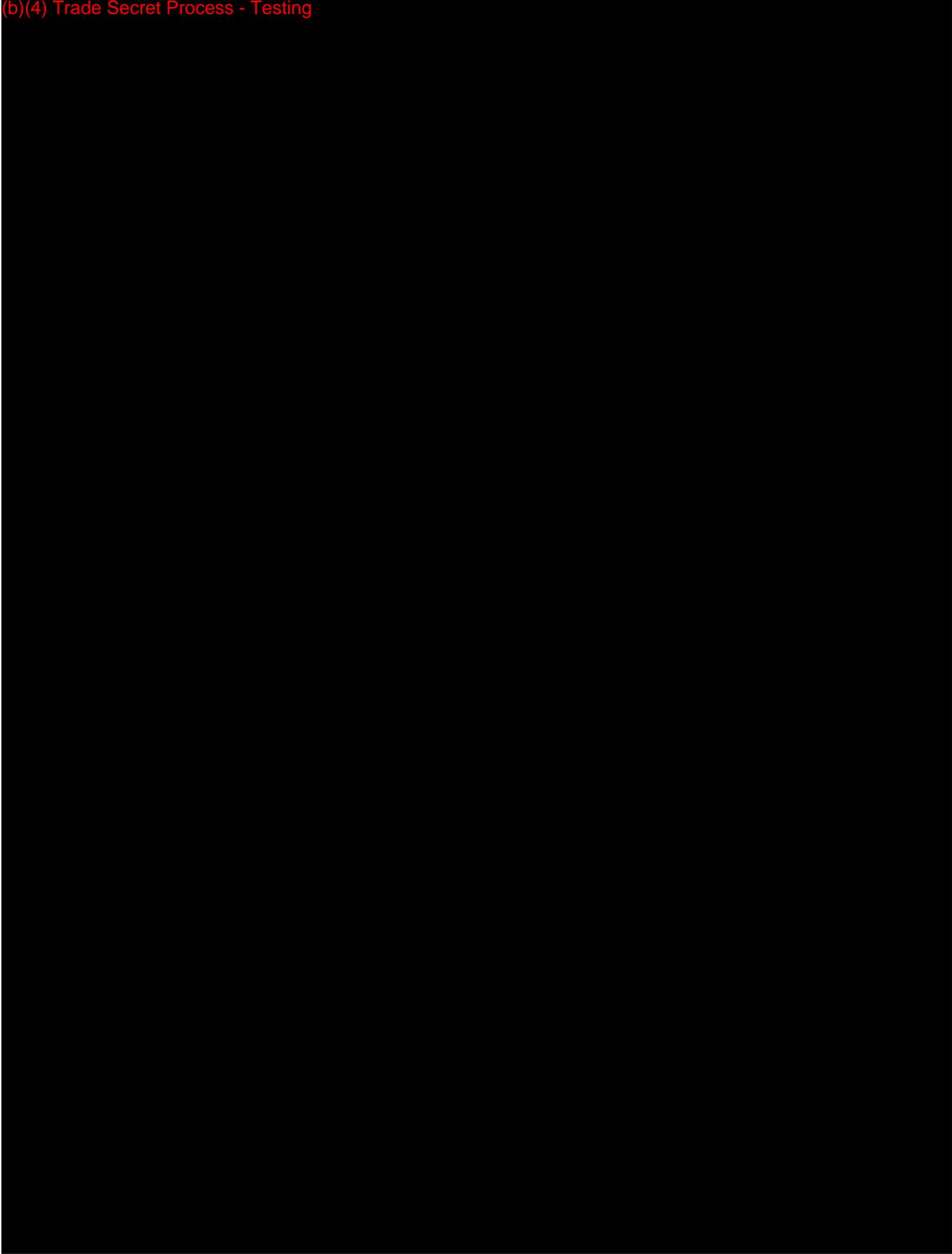
(b)(4) Trade Secret Process - Testing



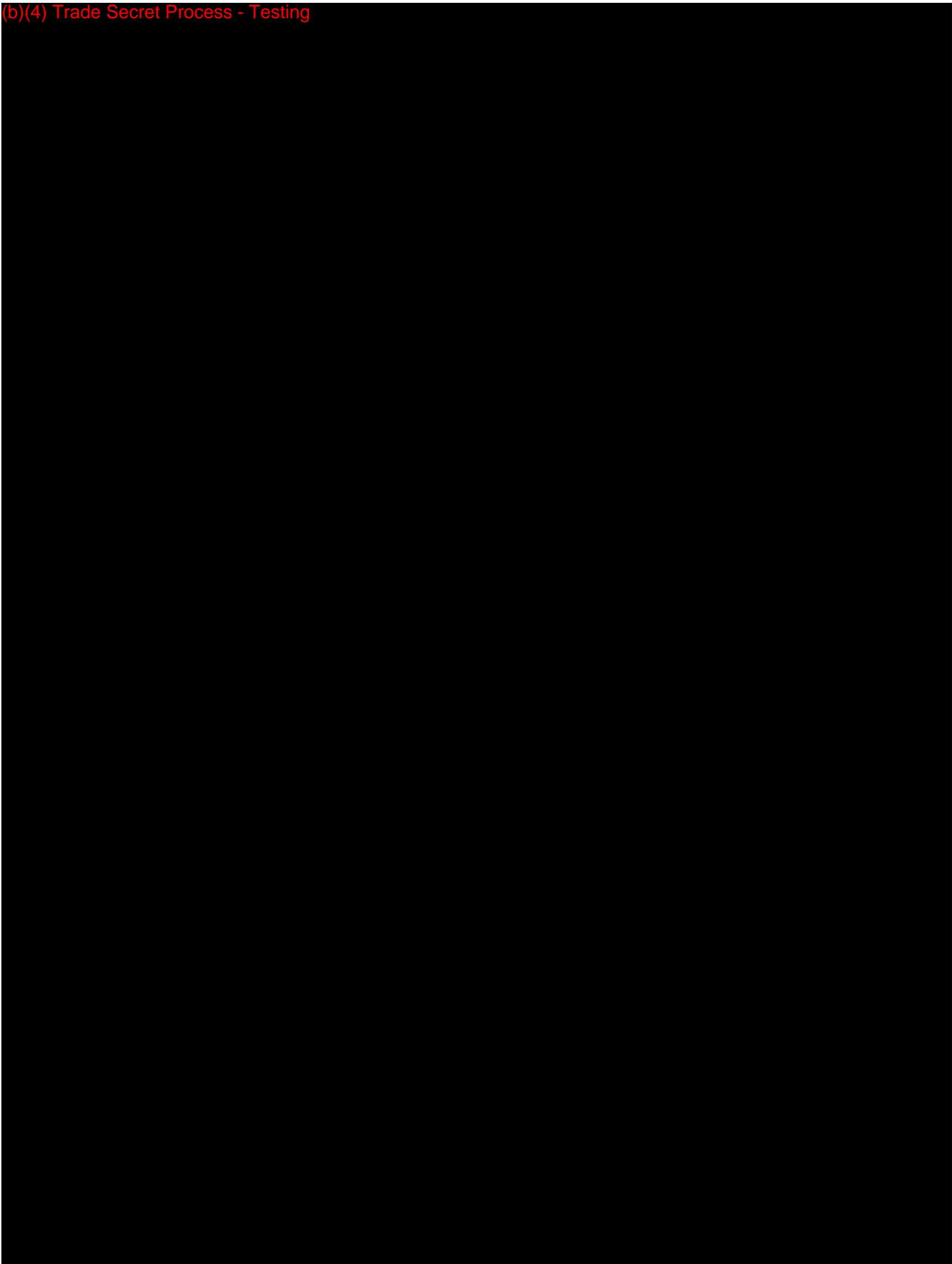
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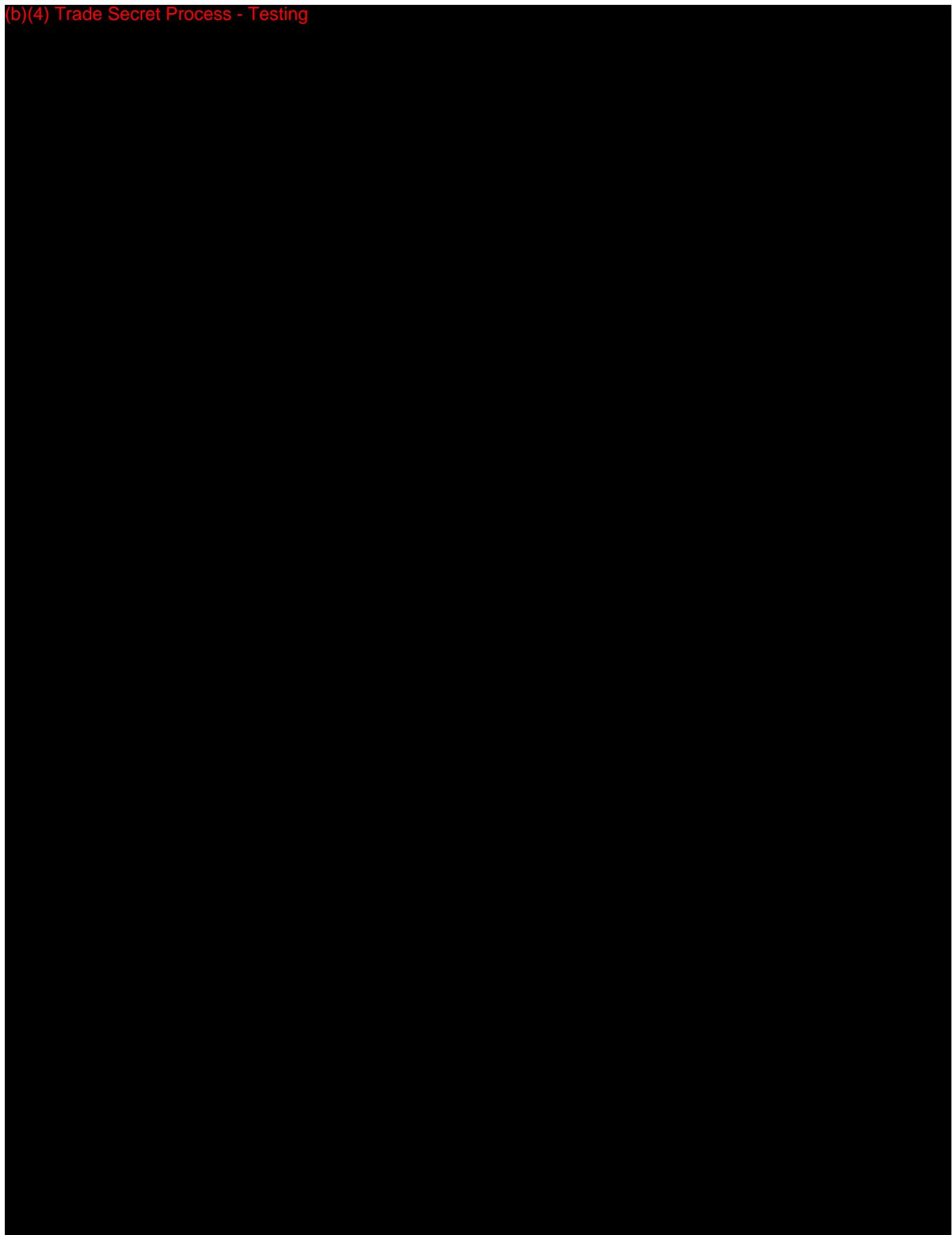
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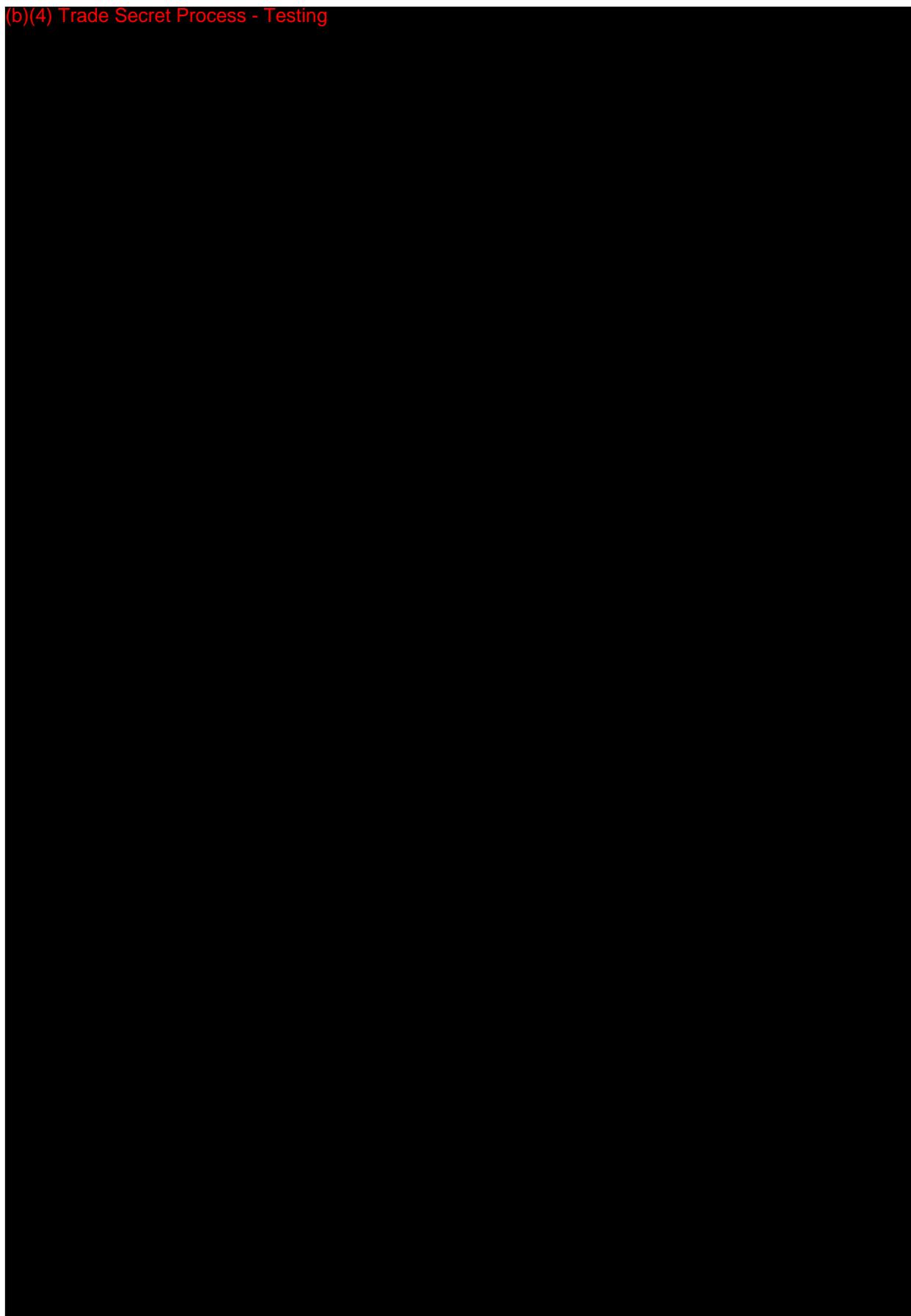
(b)(4) Trade Secret Process - Testing



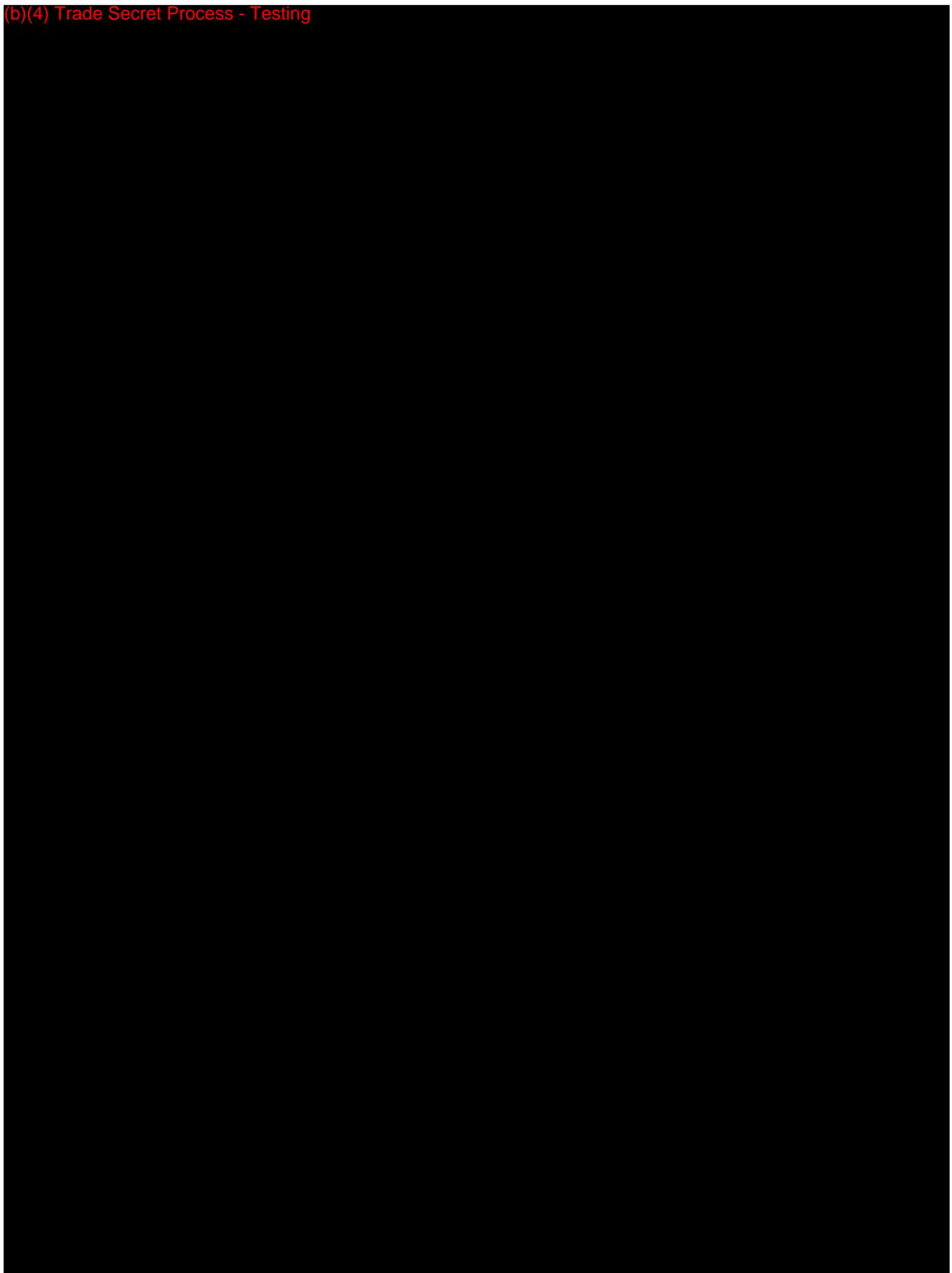
(b)(4) Trade Secret Process - Testing



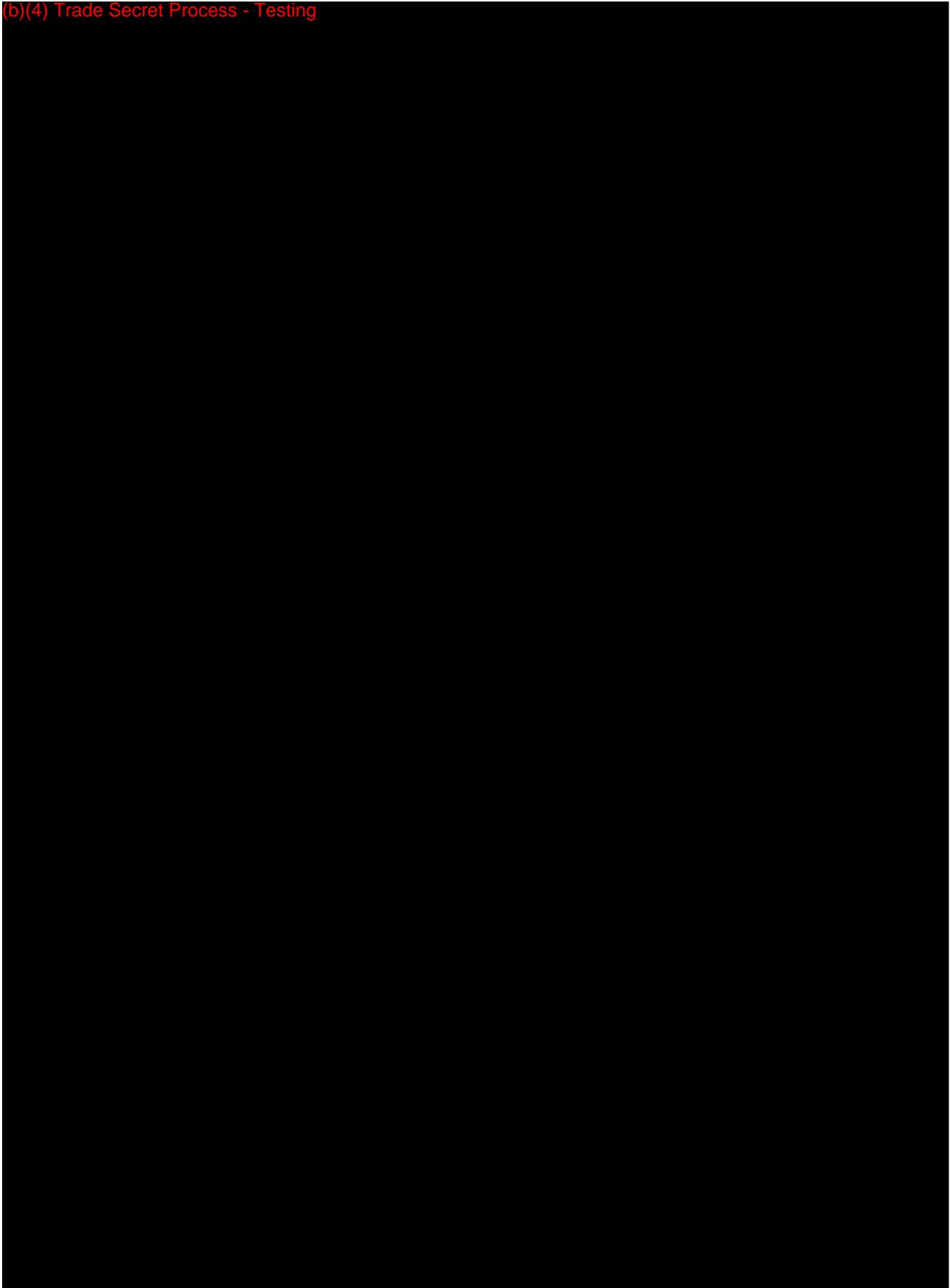
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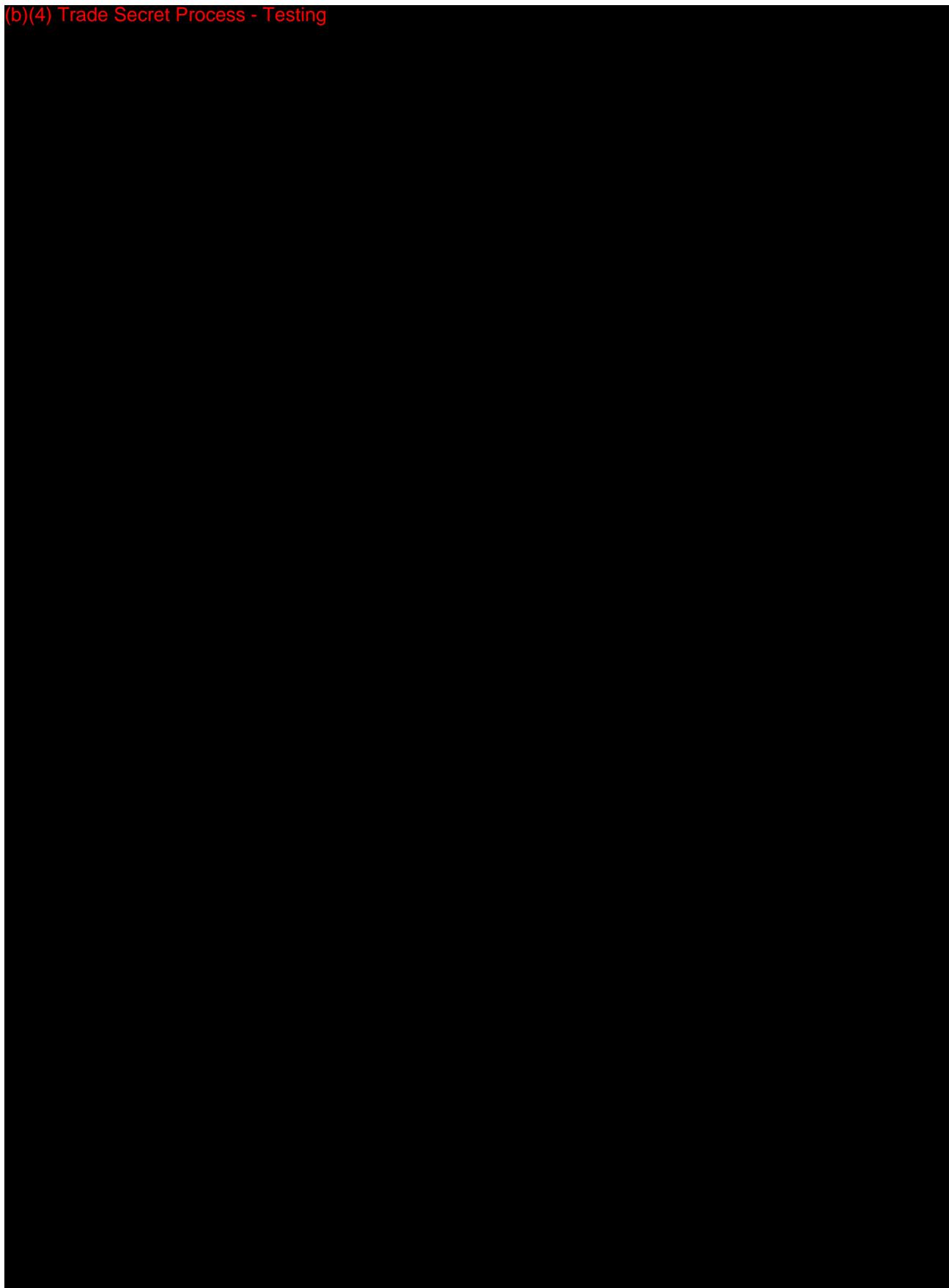
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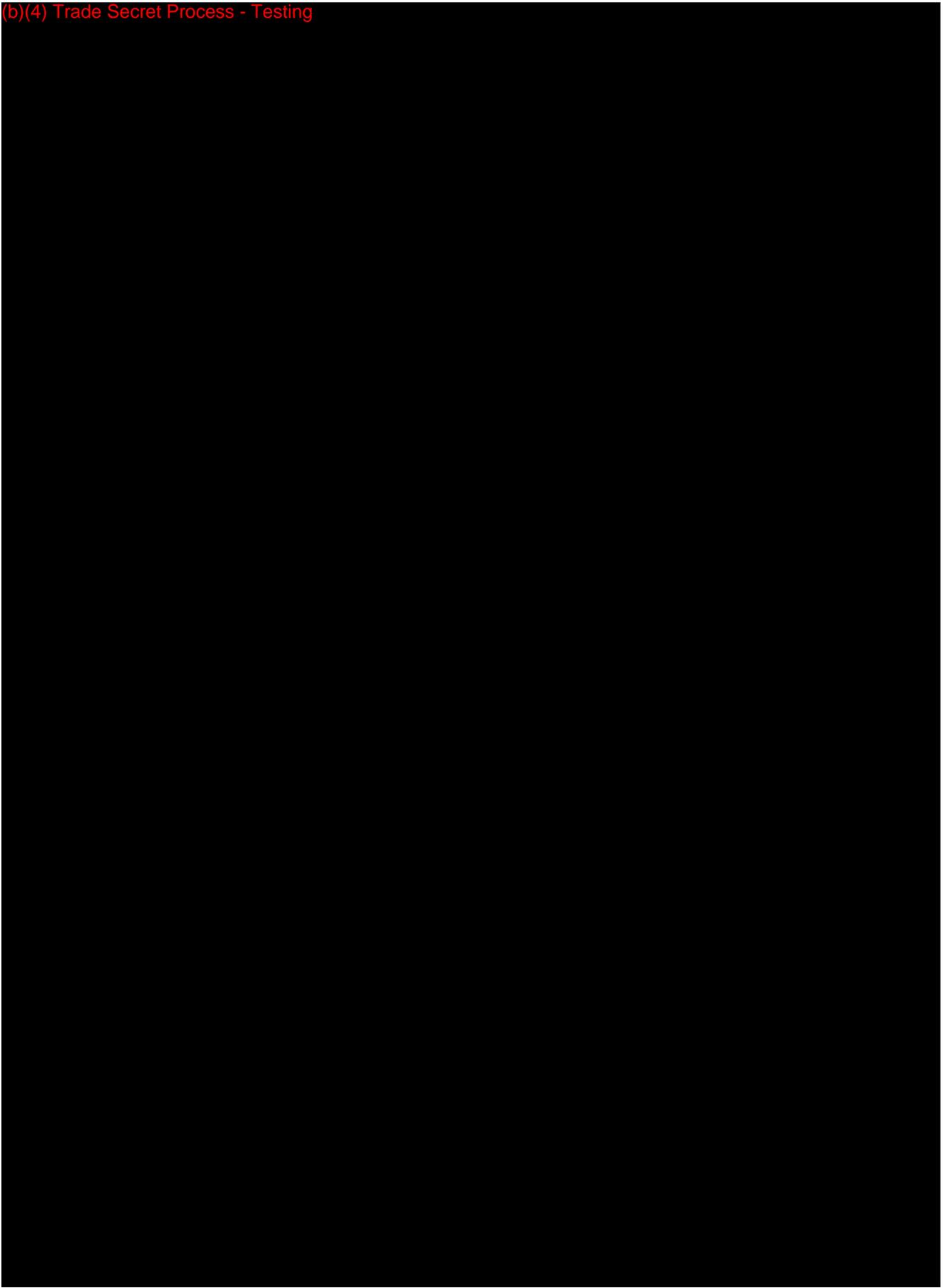
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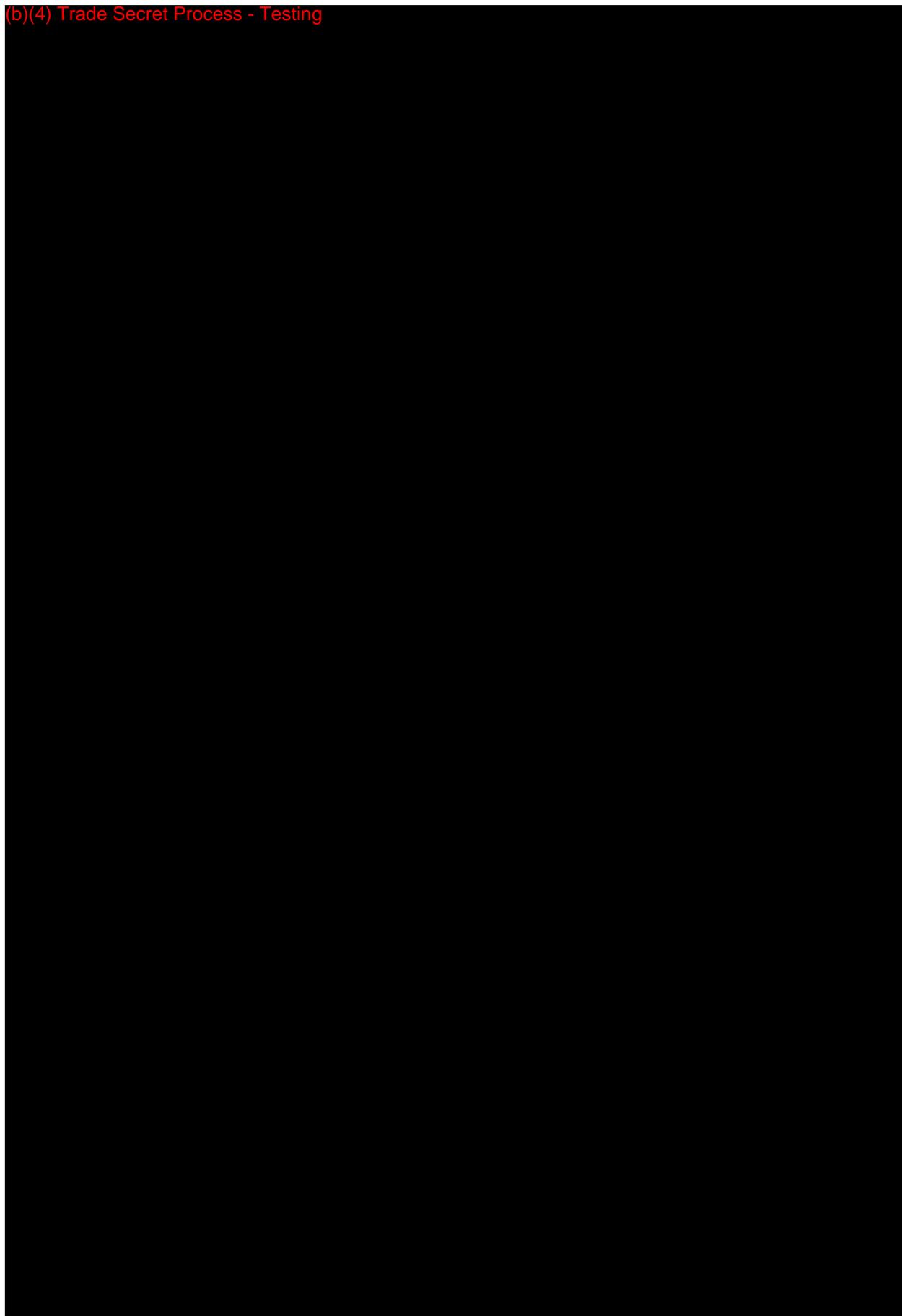
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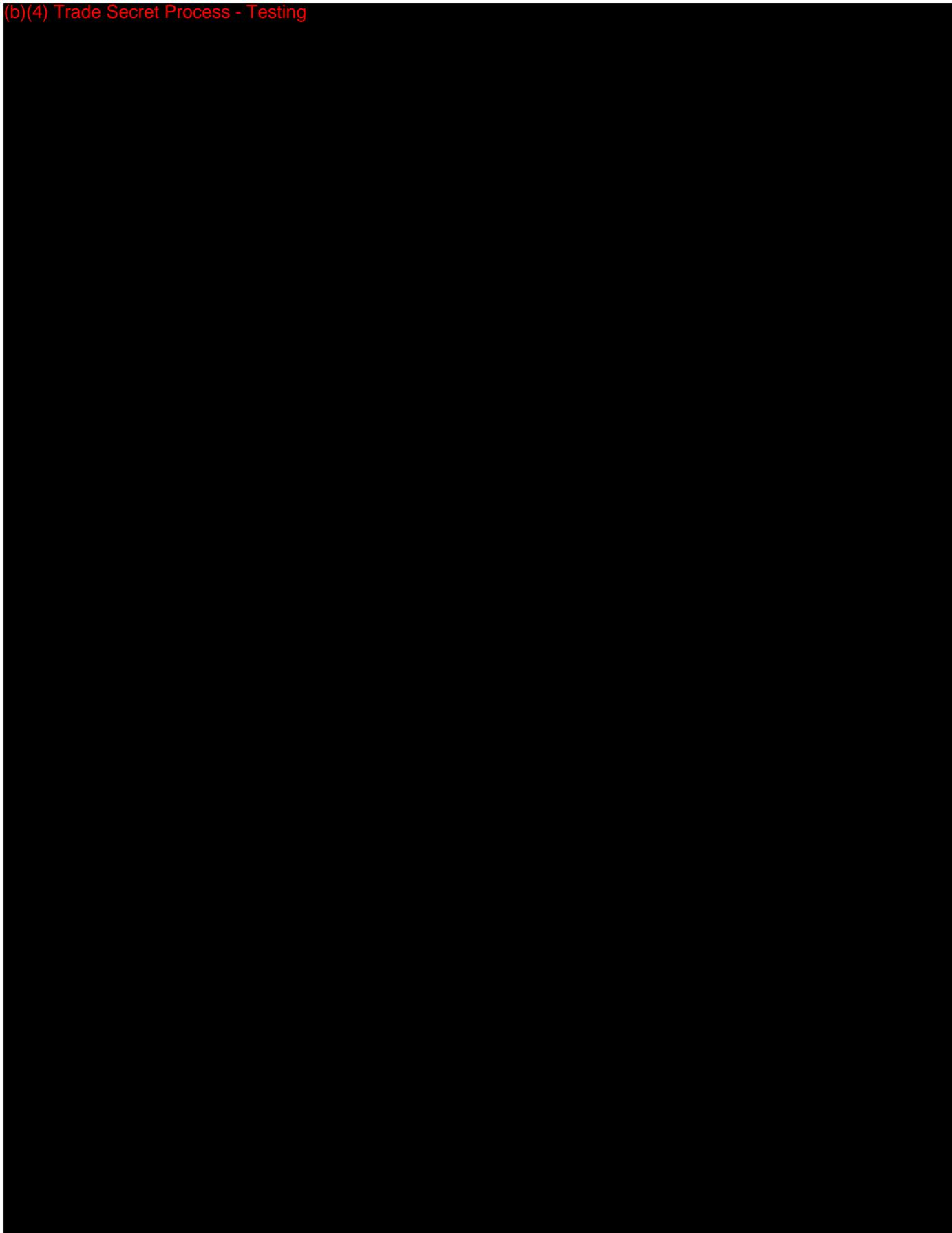
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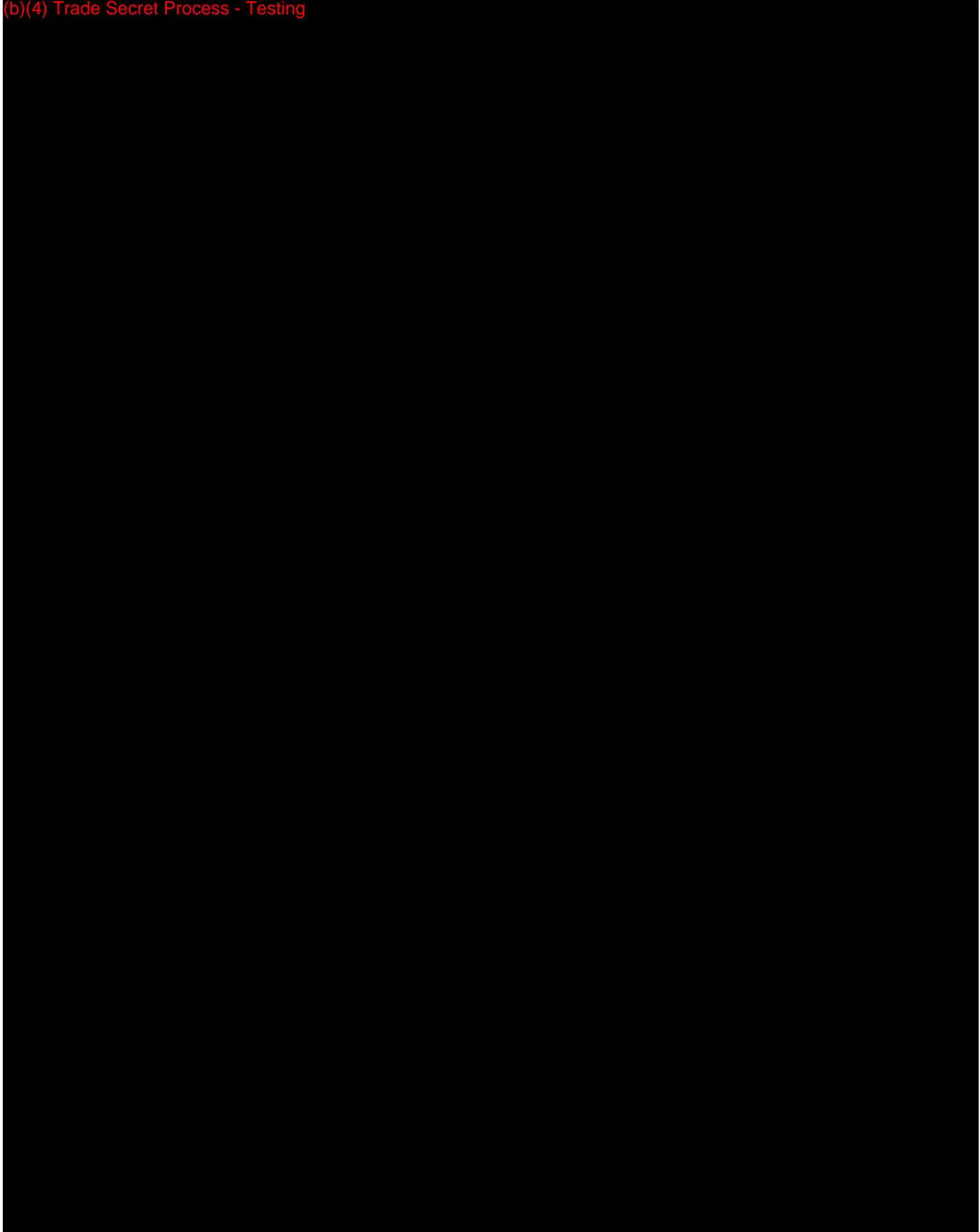
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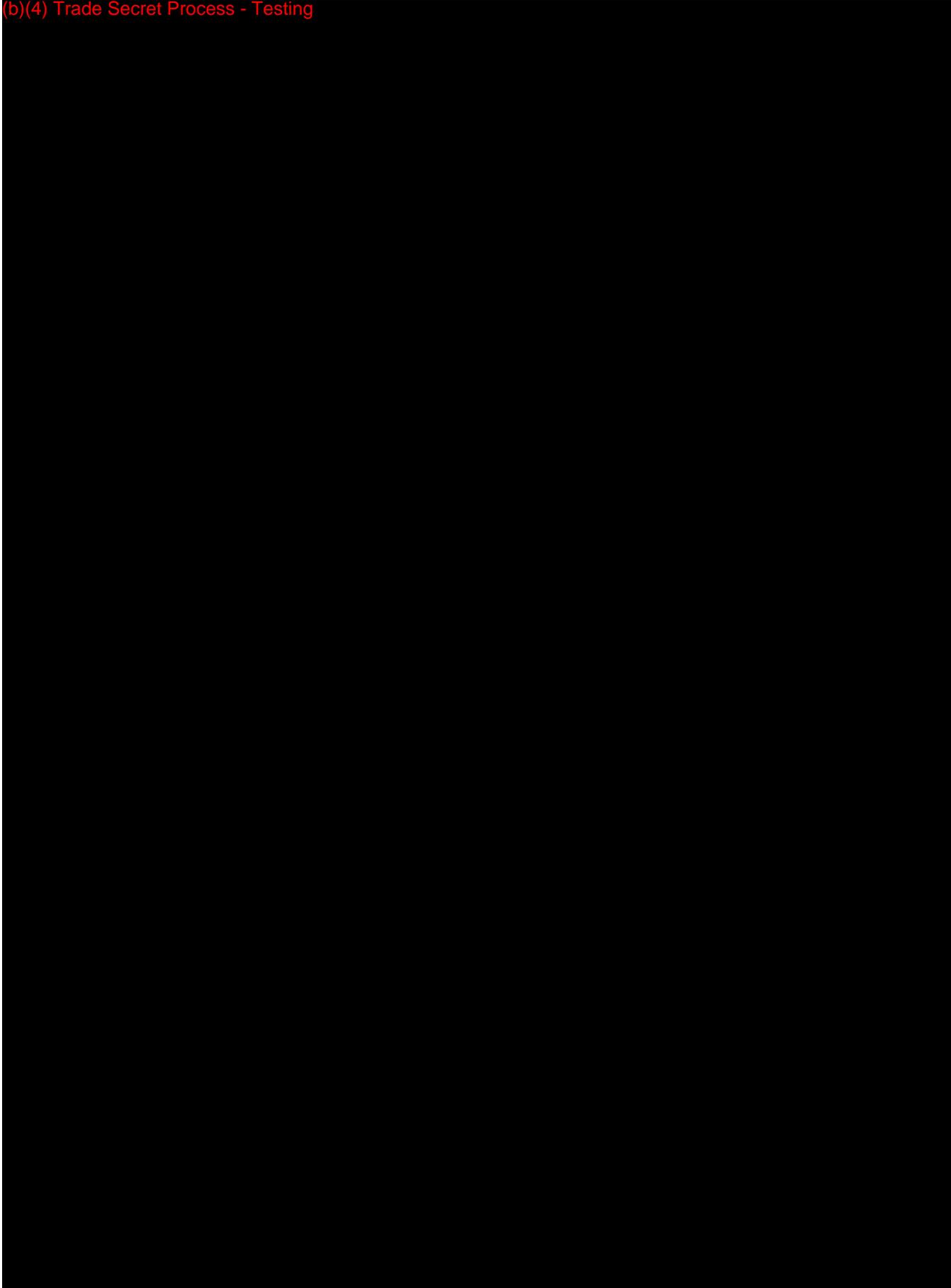
(b)(4) Trade Secret Process - Testing



(b)(4) Trade Secret Process - Testing



(b)(4) Trade Secret Process - Testing



(b)(4) Trade Secret Process - Testing

The above data was analyzed according to CLSI EP12 A2 and presented in a 2x2 contingency tables (Table 13, 15, and 17). The sensitivity, specificity, and 95% confidence intervals (CI), are also presented (Tables 14, 16 and 18).

(b)(4) Trade Secret Process - Testing

Table 17. CSF 2x2 Contingency Table: Culture/India Ink

		Culture/India Ink	
		Positive	Negative
CrAg LFA Assay	Positive	65	0
	Negative	0	99

Table 18. CSF Statistical Analysis: Culture/India Ink

	Calculated	95% CI
Sensitivity	100%	94.4-100.0%
Specificity	100%	96.3-100%

2. Comparison Conclusions:

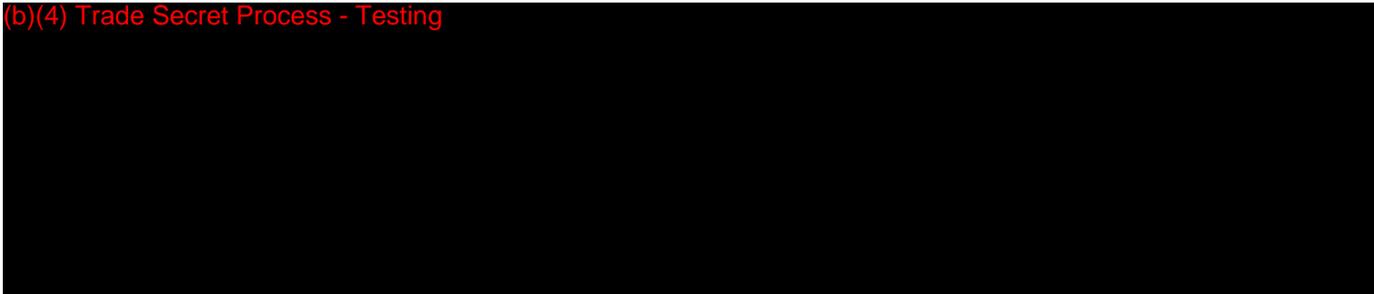
When the CrAg Lateral Flow Assay is compared to the gold standard in cryptococcosis diagnosis (culture or India Ink) using serum specimens, the new device is proven to be equivalent.

H. Other Method Comparisons – Semi-Quantitative Latex Agglutination

Test Objective:	To establish substantial equivalence to the latex agglutination (LA)
Test Articles Used:	Human serum specimens positive for cryptococcal antigen, collected retrospectively and stored at 80°C, CrAg Lateral Flow Assay (New Device), and Latex <i>Cryptococcus</i> Antigen Detection System (Predicate).
Test Methods:	See below.
Study Endpoint:	Latex versus CrAg Lateral Flow Assay Titer Regression Analysis
Acceptance Criteria:	r^2 value greater than or equal to 0.8.

1. Semi-Quantitative Method Comparison

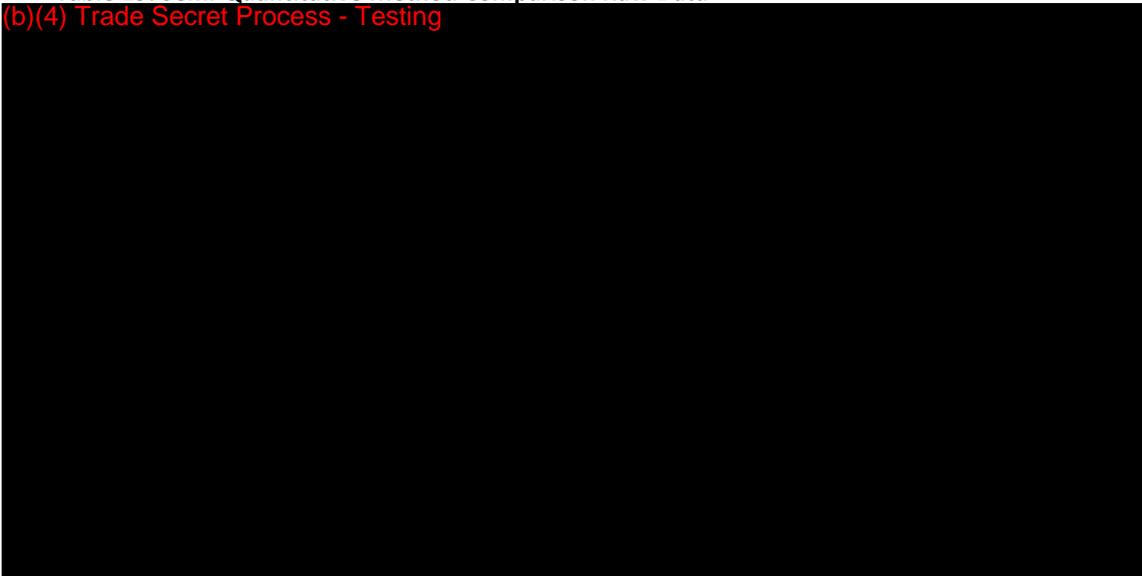
(b)(4) Trade Secret Process - Testing



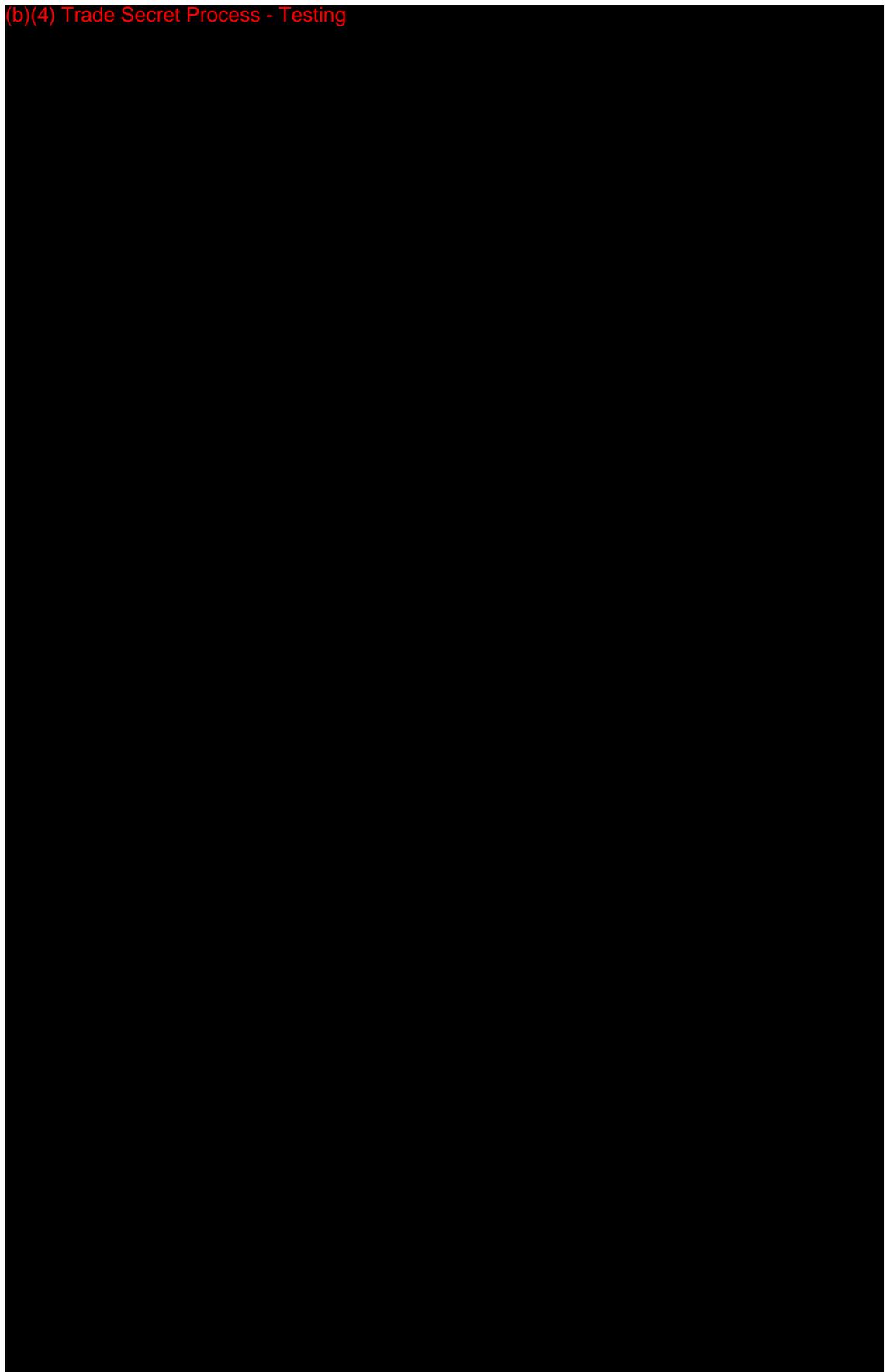
2. Semi-Quantitative Results:

Table 19. Semi-Quantitative Method Comparison Raw Data

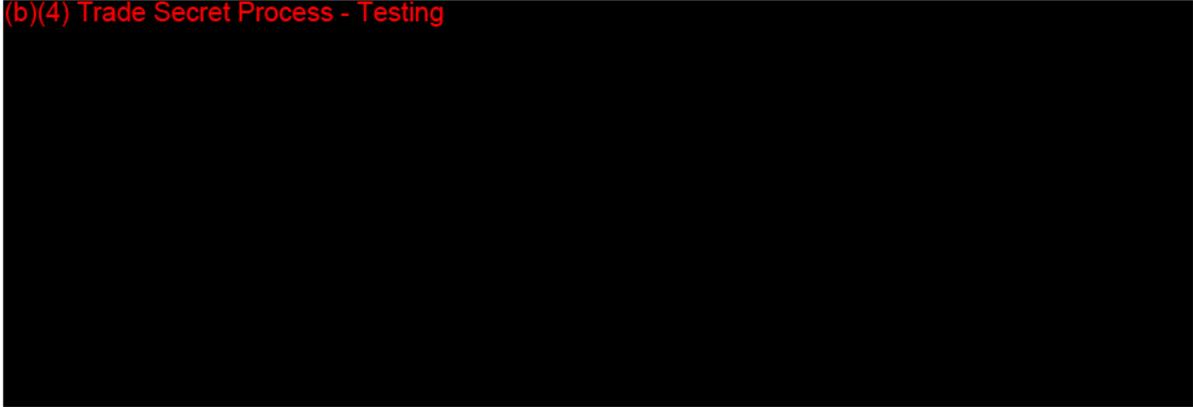
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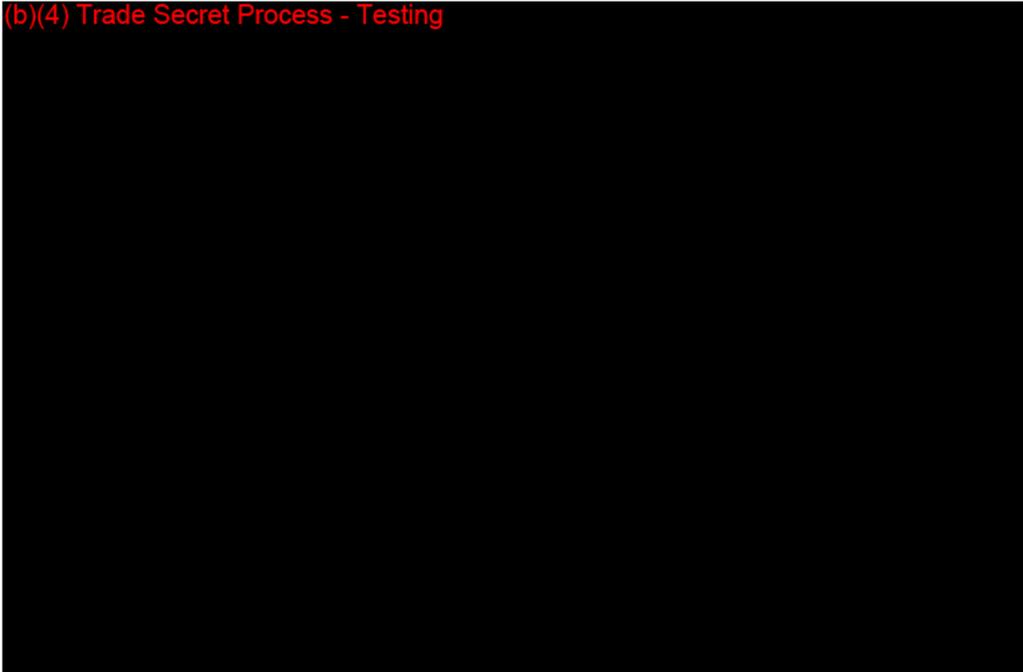
(b)(4) Trade Secret Process - Testing

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3. Semi-Quantitative Method Comparison Analysis:

Cryptococcus Antigen Latex titer (LA) versus CrAg Lateral Flow Assay titer (LFA) (1:n) was plotted and regression analysis was performed (Figure 2).

(b)(4) Trade Secret Process - Testing

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4. Semi-Quantitative Method Comparison Conclusions:

A strong correlation ($r^2 = 0.905$) exists when titers are determined on positive specimens using the CrAg Lateral Flow Assay and compared to the titers determined on the Latex *Cryptococcus* Antigen Detection System.

Final Conclusions:

The information submitted in this premarket notification is complete and supports a substantial equivalence decision.



Section 19 Performance Testing: Animal

N/A



Section 20 Performance Testing: Clinical

N/A