Table 2b. IgM Positive Percent Agreement for 48 Serum and Plasma Samples by 'Days from Symptom Onset'

Days from Symptom Onset	Number of Samples Tested	MidaSpot™ COVID-19 Antibody Combo Detection Kit Results		
		IgM Positive results	IgM PPA	95% CI
0-7 days	0	0	N/A	N/A
8-14 days	13	13	100%	77.2 – 100%
⩾15 days	35*	35	100%	90.1 – 100%

* Ten samples did not have an associated 'date from symptom onset', however these 10 samples were collected from patients with prior reported symptoms at least 16 days after the PCR positive results, and therefore are presumed to be ≥ 15 days post symptom onset. Two additional samples for which symptom onset was unknown and could not be presumed were not included in this analysis.

Table 2c. IgG Positive Percent Agreement for 12 Serum and Plasma Samples by 'Days from PCR Result'

(Performance data excludes 38 samples without "date of PCR positive" information)

Days from PCR Positive Result	Number of Samples Tested	MidaSpot™ COVID-19 Antibody Combo Detection Kit Results		
		IgG Positive Results	IgG PPA	95% CI
0-7 days	0	0	N/A	N/A
8-14 days	2	2	100%	34.2 – 100%
⩾15 days	10	10	100%	72.2 – 100%

Table 2d. IgM Positive Percent Agreement for 12 Serum and Plasma Samples by 'Days from PCR Result'

(Performance data excludes 38 samples without "date of PCR positive" information)

Days from PCR Positive Result	Number of Samples Tested	MidaSpot™ COVID-19 Antibody Combo Detection Kit Results		
		IgM Positive results	IgM PPA	95% CI
0-7 days	0	0	N/A	N/A
8-14 days	2	2	100%	34.2 – 100%
⩾15 days	10	10	100%	72.2 – 100%

Independent Clinical Agreement Evaluation

The MidaSpot™ COVID-19 Antibody Combo Detection Kit was tested on December 14, 2020 at the Frederick National Laboratory for Cancer Research (FNLCR) sponsored by the National Cancer Institute (NCI). The test was validated against a panel of previously frozen samples consisting of 30 SARS-CoV-2 antibody-positive serum samples and 80 antibody-negative serum and plasma (ACD) samples. Each of the 30 antibody-positive samples were confirmed with a nucleic acid amplification test (NAAT) and both IgM and IgG antibodies were confirmed to be present in all 30 samples. The presence of antibodies in the samples was confirmed by several orthogonal methods prior to testing with the MidaSpot™ COVID-19 Antibody Combo Detection Kit. The presence of IgM and IgG antibodies specifically was confirmed by one or more comparator methods. Antibody-positive samples were selected at different antibody titers.

All antibody-negative samples were collected prior to 2020 and include: i) Seventy (70) samples selected without regard to clinical status, "Negatives" and ii) Ten (10) samples selected from banked serum from HIV+ patients, "HIV+". Testing was performed by one operator using one lot of the MidaSpot™ COVID-19 Antibody Combo Detection Kit. Confidence intervals for sensitivity and specificity were calculated per a score method described in CLSI EP12-A2 (2008).

For evaluation of cross-reactivity with HIV+, it was evaluated whether an increased false positive rate among antibody-negative samples with HIV was statistically higher than the false positive rate among antibody-negative samples without HIV (for this, a confidence interval for the difference in false positive rates was calculated per a score method described by Altman). The results and data analysis are shown in Table A below.

Table A. FNLCR/NCI Independent Evaluation – Summary Results

	Comparator Method			Collected pre-2020		
	Antibody Positive			Antibody Negative		
MidaSpot COVID-19 Antibody Combo Detection Kit (NBPC-0007)	IgM+, IgG+	IgM+, IgG-	IgM-, IgG+	Negative	HIV+	Total
IgM+, IgG+	29					29
IgM+, IgG-	1			1		2
IgM-, IgG+				2		2
IgM-, IgG-				67	10	77
Total	30			70	10	110

Table 2: Summary Statistics

Measure	Estimate	Confidence Interval
IgM Sensitivity	100% (30/30)	(88.7%; 100%)
IgM Specificity	98.8% (79/80)	(93.3%; 99.8%)
IgG Sensitivity	96.7% (29/30)	(83.3%; 99.4%)
IgG Specificity	97.5% (78/80)	(91.3%; 99.3%)
Combined Sensitivity	100% (30/30)	(88.7%; 100%)
Combined Specificity	96.2% (77/80)	(89.5%; 98.7%)
Combined PPV for prevalence = 5.0%	58.4%	(30.9%; 80.4%)
Combined NPV for prevalence = 5.0%	100%	(99.3%; 100%)
Cross-reactivity with HIV+	0.0% (0/10), not detected	

Limitations of the FNLCR/NCI Independent Evaluation:

- Samples were not randomly selected, and sensitivity and specificity estimates may not be indicative of the real-world performance of the device.
- These results are based on serum and plasma samples only and may not be indicative of performance with other sample types, such as whole blood, including finger stick blood.
- The number of samples in the panel is a minimally viable sample size that still provides reasonable estimates and confidence intervals for test performance, and the samples used may not be representative of the antibody profile observed in patient populations.

Class Specificity

Using 5 samples (testing with 2 replicates each), DTT removal experiments were conducted to assess IgM removal from samples positive for both IgG and IgM. After DTT treatment, the IgM results were negative, and IgG results remained positive. This was in 100% agreement with expected outcomes. Study summary data is illustrated in Table 3.

Table 3. Class Specificity Results.

Sample ID	Replicate	Result NO DTT Treatment (IgM/IgG)	Result DTT Treatment (IgM/IgG)	Expected Result with DTT Treatment (IgM/IgG)	Result Agreement
FL 205	1	+/+	-/+	-/+	yes
FL 205	2	+/+	-/+	-/+	yes
FL 217	1	+/+	-/+	-/+	yes
FL 217	2	+/+	-/+	-/+	yes
FL 221	1	+/+	-/+	-/+	yes
FL 221	2	+/+	-/+	-/+	yes
FL 448	1	+/+	-/+	-/+	yes
FL 448	2	+/+	-/+	-/+	yes
FL 452	1	+/+	-/+	-/+	yes
FL 452	2	+/+	-/+	-/+	yes

Matrix Equivalency

Studies were performed with venous serum, and venous plasma (lithium heparin and dipotassium EDTA) using contrived negative, low positive, and moderate/high positive samples. Five complete sets of matrices were used in the study, and each assay was performed twice. The agreement was 100% between both plasma matrices compared to serum. Study summary data are illustrated in Tables 4a. and 4b.

Table 4a. Dipotassium EDTA plasma compared to serum.

Plasma (Dipotassium EDTA) compared to Serum			
IgG		IgM	
True Positives	False Positives	True Positives	False Positives
20	0	20	0
False Negatives	True Negatives	False Negatives	True Negatives
0	10	0	10
PPA	NPA	PPA	NPA
100%	100%	100%	100%

Table 4b. Lithium Heparin plasma compared to serum.

Plasma (Li Heparin) compared to Serum			
IgG		IgM	
True Positives	False Positives	True Positives	False Positives
20	0	20	0
False Negatives	True Negatives	False Negatives	True Negatives
0	10	0	10
PPA	NPA	PPA	NPA
100%	100%	100%	100%

Point of Care (POC) use

Clinical Performance Studies

Nirmidas conducted a prospective clinical study using natural fingerstick whole blood samples at two sites to assess the clinical agreement of the MidaSpot™ COVID-19 Antibody Combo Detection Kit results versus clinical status determined by PCR. To assess positive percent agreement, samples from PCR confirmed SARS-2-CoV positive participants were tested. To assess negative percent agreement, samples from both presumed healthy donors and PCR confirmed SARS-CoV-2 negative participants were tested. Study site 1 was a hospital in Maryland. Samples from a total of 38 PCR positive, and 11 PCR negative participants were tested. Five (5) operators performed the fingerstick testing. Study site 2 was in California. Samples from a total of 44 healthy participants were tested. Five (5) operators performed the fingerstick testing. The performance summary data are illustrated in Tables 5a – 5d.

Table 5a. POC Study – Site 1 – Negative Percent Agreement for 11 PCR Confirmed Negative Participants Using Fingerstick Whole Blood

Number of Samples Tested	MidaSpot™ COVID-19 Antibody Combo Detection Kit Results	
	IgG Negative Results	IgG NPA (95% CI)
11	11	100% (74.1 – 100%)
	IgM Negative Results	IgM NPA (95% CI)
11	11	100% (74.1 – 100%)

Table 5b. POC Study – Site 2 – Negative Percent Agreement for 44 Presumed Healthy Participants Using Fingerstick Whole Blood

Number of Samples Tested	MidaSpot™ COVID-19 Antibody Combo Detection Kit Results	
	IgG Negative Results	IgG NPA (95% CI)
44	44	100% (92.0 – 100%)
	IgM Negative Results	IgM NPA (95% CI)
44	43	97.7% (91.8 – 100%)

Table 5c. POC Study – IgG Positive Percent Agreement for 38 PCR Positive Participants Using Fingerstick Whole Blood by Days from Symptom Onset

Days from Symptom Onset	Number of Samples Tested	MidaSpot™ COVID-19 Antibody Combo Detection Kit Results		
		IgG Positive results	IgG PPA	95% CI
0-7 days	0	0	NA	NA
8-14 days	8	6	75%	40.9 – 92.7%
⩾15 days	30	30	100%	88.6 – 100%

Table 5d. POC Study – IgM Positive Percent Agreement for 38 PCR positive participants Using Fingerstick Whole Blood.

Days from Symptom Onset	Number of Samples Tested	MidaSpot™ COVID-19 Antibody Combo Detection Kit Results		
		IgM Positive Results	IgM PPA	95% CI
0-7 days	0	0	N/A	N/A
8-14 days	8	8	100.0%	63.1% - 100.0%
≥15 days	30	30	100.0%	88.4% - 100.0%

Robustness

Studies were performed to test the robust use of this test in a point of care setting. Varying amounts of sample, varying amounts of Sample Diluent, temperature, humidity, and lighting conditions were assessed. The results from this testing indicate that the test will perform as expected across environmental and use variations that may occur in POC settings. Testing in elevated environmental temperature combined with high humidity (40°C + 95% Relative Humidity) resulted in 2 false negative IgG results out of 5 samples tested.

14. CONTACT INFORMATION

For technical and product related questions, please contact
Nirmidas Biotech, Inc.
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Tel: +1 (669) 207-9813
Email: support@nirmidas.com

Created December 2020

NIRMIDAS BIOTECH

MidaSpot™ COVID-19 IgM IgG Positive Control and Negative Control Kit

(NBPC-0010)

Instructions for Use

For use under an Emergency Use Authorization Only.

For prescription use only.

For in vitro diagnostic use only.

This product has not been FDA cleared or approved, but has been authorized by FDA under an EUA for use by authorized laboratories certified under CLIA, that meet requirements to perform waived, moderate or high complexity tests.

This product has been authorized only for detecting the presence of IgM and IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens

The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

1. Intended Use

The MidaSpot™ COVID-19 IgM IgG Positive Control and Negative Control Kit is intended for use as an assayed quality control to monitor the performance of the MidaSpot™ COVID-19 Antibody Combo Detection Kit (controls sold separately). The performance characteristics of the MidaSpot™ COVID-19 IgM IgG Positive Control and Negative Control Kit have not been established for any other assays or instrument platforms.

2. Materials Provided

Positive Control Solution (20 x 50 µL)	Twenty (20), each sufficient for 4 tests. A combination of human plasma reactive for IgG, purified IgM antibody in a matrix of fetal bovine serum, and sodium azide preservative. Single Use Only. Ready to use.
Negative Control Solution (20 x 50 µL)	Twenty (20) vials, each sufficient for 4 tests. A combination of human serum that is non-reactive for SARS-CoV-2 IgM and IgG antibodies diluted in fetal bovine serum, and sodium azide preservative. Single Use Only. Ready to use.

3. Storage Conditions

The MidaSpot™ COVID-19 IgM IgG Positive Control and Negative Control Kit is shipped at 4°C. Upon

receipt, without delay, store the kit components at -20°C or lower until the expiration date on the label. Once each vial is thawed, use within 24 hours; store at 4°C when not in use.

4. Procedure

- The positive and negative controls are to be treated as a serum/plasma sample and tested and interpreted following the Instruction for Use provided for the MidaSpot™ COVID-19 Antibody Combo Detection Kit.
- Good laboratory practices include the use of external controls on a regular basis. State and local regulations should be followed.
- Each vial is for single use only, but may be used for up to four (4) times within 24 hours, once thawed.

5. Warnings and Precautions

- Controls are not specific to lots of the MidaSpot™ COVID-19 Antibody Combo Detection Assay Kit and may be safely used with multiple Assay Kit lots.
- Do not use kit components beyond the expiration date given on the label.
- All specimens of human origin should be considered potentially infectious and handled with care.
- Observe the normal precautions required for handling all laboratory reagents.
- Waste must be handled with care and disposed of in compliance with laboratory guidelines and the statutory provisions enforced in each country.

Safety Precautions

- Do not eat, drink, smoke or apply cosmetics in the assay laboratory. Do not pipette by mouth.
- Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles, and disposable gloves. Wash hands thoroughly at the end of each assay.
- Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with appropriate disinfectants and the means used must be treated as infected waste.

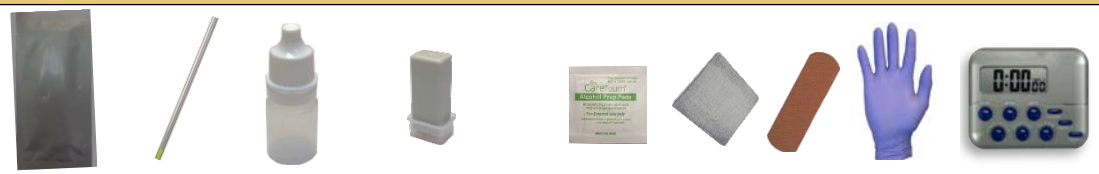
Nirmidas Biotech, Inc.
2458 Embarcadero Way, Palo Alto, CA 94303 USA
Tel: +1 (669) 207-9813
Email: support@nirmidas.com

Created December 2020

For in vitro diagnostic use. Rx only, For use under Emergency Use Authorization (EUA) only.
This test has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use with fingerstick whole blood specimens by laboratories certified under CLIA that meet the requirements to perform high, moderate or waived complexity tests. Testing of fingerstick whole blood specimens is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.
This test has been authorized only for detecting the presence of IgM and IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens; and The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Food, Drug and Cosmetics Act , 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

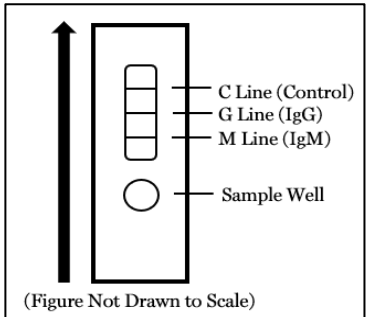
MidaSpot™ COVID-19 Antibody Combo Detection Kit
Quick Reference Guide

Materials



Provided: Test Card in pouch, Non-Sterile Capillary Tube, Dropper Bottle of Sample Diluent, Lancet

Not Provided: Alcohol Pad, Gauze, Bandage, Gloves, Timer

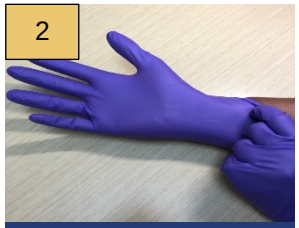


Test Card Overview

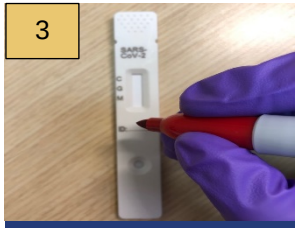
IMPORTANT NOTE: Each test is for SINGLE USE. Do NOT reuse the test device.
Bring Test Card and Sample Diluent to room temperature before testing.

Assay Procedure

1 Check Test Card. Do not use if expired or if pouch is open.

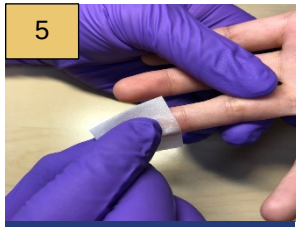


2 Put on gloves. Use new gloves for each patient.

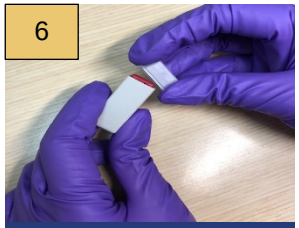


3 Open Test Card pouch and label with patient ID.

4 Remove cap from Sample Diluent and set aside.

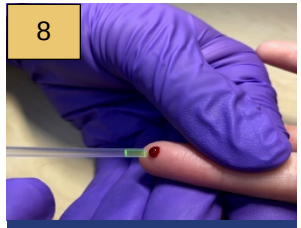


5 Clean patient s fingertip with alcohol pad; let dry.

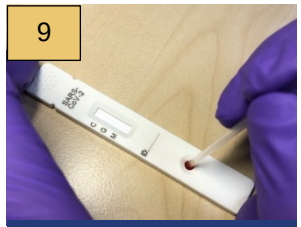


6 Remove cap on lancet.

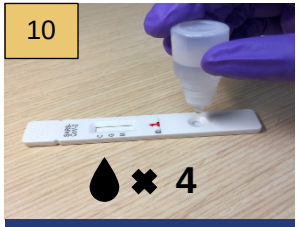
7 Squeeze finger; push lancet firmly into side of finger pad.



8 Touch colored end of capillary to blood; fill yellowish region.

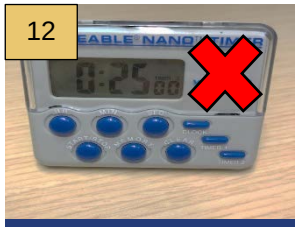


9 Gently touch capillary to Sample Well to dispense.



10 Add 4 drops of Sample Diluent to Sample Well.

11 Start timer. Read results in 20 ± 2 minutes. Do not read the result before 18 minutes.

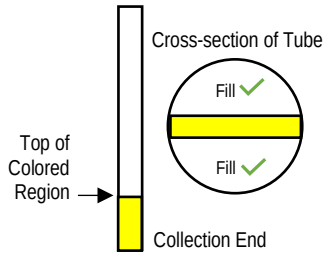


12 Do not read results after 25 minutes.

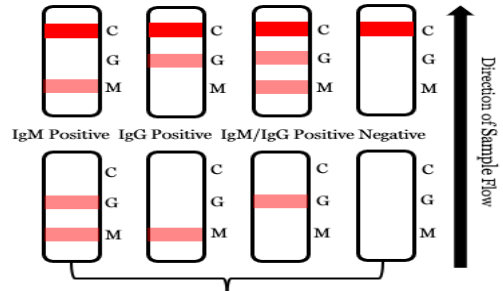
Dropper Bottle Usage

- Squeeze continuously until 4 drops are dispensed.
- Hold far enough away from Test Card so that complete, free-falling drops form.
- Hold vertically to dispense, avoid bubbles in tip.
- Wipe away the first drop if bubbles are forming.

Capillary Diagram



Test Results



Faint lines in test region M or G are considered positive.

Results from antibody testing should not be used to diagnose or exclude SARS-CoV-2 infection or to inform infection status.

Always wear gloves when performing this procedure and treat all specimens and used devices as potentially infectious. Dispose of contaminated materials according to local regulations.

For technical and product related questions, please contact Nirmidas Biotech, Inc.
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Email: support@nirmidas.com

NIRMIDAS BIOTECH

Quick Reference Guide MidaSpot EUA
Created December 2020

MidaSpot™COVID-19 IgM IgG Positive Control and Negative Control Quick Reference Guide

The MidaSpot COVID-19 IgM IgG Positive Control and Negative Control are sold separately and should be purchased from Nirmidas Biotech, Inc. It is recommended that controls be run under the following circumstances: a new operator uses the test kits for the first time, a new shipment of test kits is received, device storage falls out of the 4-25°C range, to verify a higher or lower than expected frequency of positive or negative results, to investigate the cause of repeated invalid results, or a new test environment is used.

Assay Procedure

IMPORTANT NOTE: Each test is for SINGLE USE. Do NOT reuse the test device.
Bring Test Card and Sample Diluent to room temperature before testing.

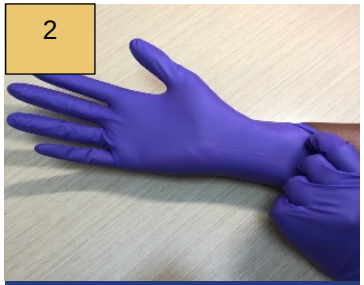
Before You Begin

Thaw the desired number of Positive Control vials or Negative Control vials and allow them to reach room temperature. Gently mix each vial before use.

1

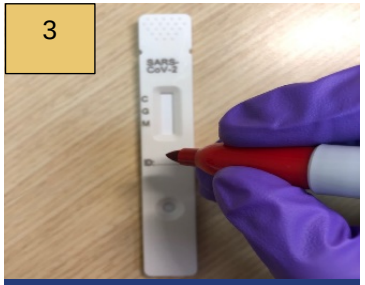
Check Test Card. Do not use if expired or if pouch is open.

2



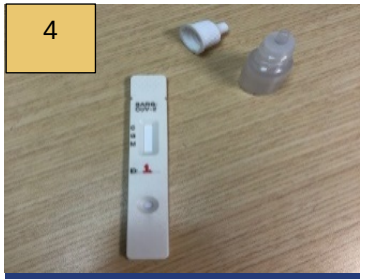
Put on gloves.

3



Open Test Card pouch and label with Control ID.

4

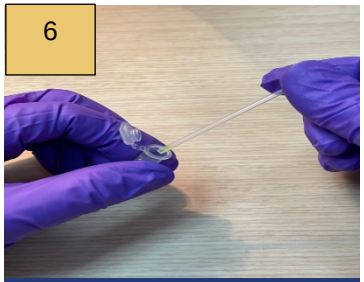


Remove cap from Sample Diluent and set aside.

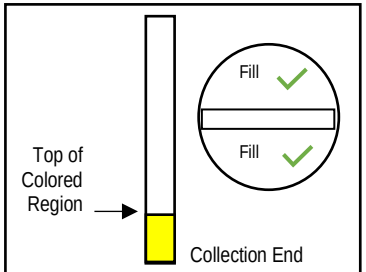
5

Open Control vial.

6



Dip colored end of capillary into vial; fill yellowish region.

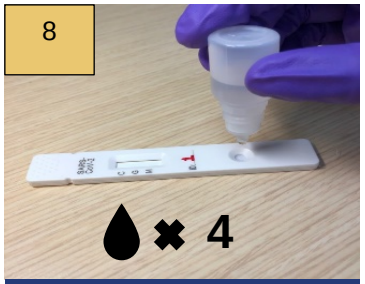


Side view and cross section diagram of the capillary tube.

7

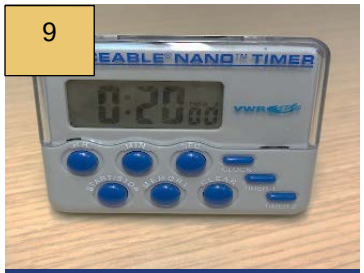
Gently touch capillary to Sample Well to dispense.

8



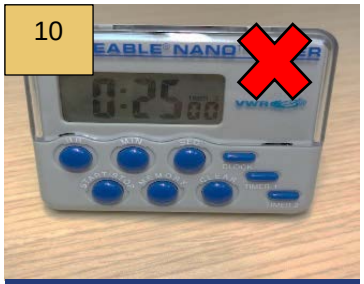
Add 4 drops of Sample Diluent to Sample Well.

9



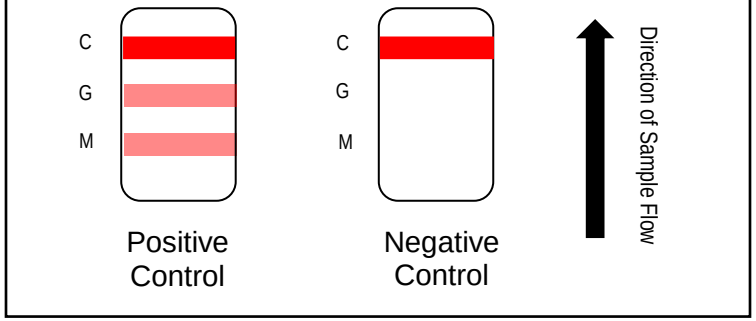
Start timer. Read results in 20 ± 2 minutes. Do not read the result before 18 minutes.

10



Do not read results after 25 minutes.

Test Results



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