



**DEPARTMENT OF HEALTH & HUMAN SERVICES
FDA/CBER/OVRR/DVRPA**

Date: 27 June 2011

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Medical Officer, Clinical Review Branch 1

Through: Douglas Pratt, M.D, MPH
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Subject: Clinical Review of Biologics License Application for Spherusol Skin test antigen

To: BLA STN# 125354/0

BLA review committee:

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1 Title and General Information**1.1 Medical Officer's Review Identifiers and Dates****1.1.1 BLA/NDA #: 125354 / 0****1.1.2 Related IND/BLA #(s):**

- IND -(b)(4)-: (Submission 07 Dec 2001) *Coccidioides immitis* Spherule-derived Skin Test Antigen (-----(b)(4)-----) – Allermed
- IND -----(b)(4)-----

- PLA -----(b)(4)-----

1.1.3 Reviewer Name, Division and Mail Code (HFM number):

Ann T. Schwartz, M.D.
Division of Vaccines and Related Products Applications
HFM-485

1.1.4 Submission Received by FDA: 27 May 2009**1.1.5 Complete Response Review Completed: 05 March 2010****1.2 Product****1.2.1 Proper Name or Established Name:** *Coccidioides immitis*, Spherule-Derived Skin Test Antigen**1.2.2 Proposed Trade Name:** Spherusol

The Applicant submitted a proposal (10 February 2010) for use of the proprietary name, Spherusol (application for trademark submitted to the US Patent Office 02 Feb 2010). This proposal was reviewed by APLB and found to be acceptable. The proposed name of Spherusol will be used throughout this review to identify the study product.

1.2.3 Product Formulation(s) Including Adjuvants, Preservatives, etc.:

Spherule-derived antigen	
<i>C. immitis</i>	1.27 mcg /0.1 mL
NaCl	0.9%
Phenol	0.4%
Thimerosal	1:1,000,000 (residual)
Sodium borate	0.014%

1.2.4 Chemical Name, Structure: N/A

1.3 Applicant: Allermed Laboratories Inc.
7203 Convoy Court
San Diego, CA 92111

1.4 Pharmacologic Class or Category: Skin Test Antigen

- 1.5 Proposed Indication(s):** Spherusol is a skin test antigen indicated for the detection of delayed type hypersensitivity to *Coccidioides immitis* in individuals with a history of pulmonary coccidioidomycosis. Spherusol is approved for use in individuals 18-64 years of age.
- 1.6 Proposed Populations(s):** Not indicated by the Sponsor. Subjects enrolled in the clinical studies were 18 to 64 years of age.
- 1.7 Dosage Form(s) and Route(s) of Administration:**
Solution for intradermal injection, 1.27 mcg/0.1 mL

2 Table of Contents

1.0	GENERAL INFORMATION	2
1.1	CONTENT	
1.1.1	BLA#	
1.1.2	Date application submitted	
1.1.3	Date clinical review completed	
1.1.4	Reviewer Name, Division and Mail Code	
1.2	PRODUCT NAME	2
1.2.1	Proper name	
1.2.2	Trade name	
1.2.3	Product formulation	
1.3	APPLICANT	2
1.4	PHARMACOLOGIC CLASS	2
1.5	PROPOSED INDICATION	3
1.6	PROPOSED POPULATIONS	3
1.7	DOSING REGIMEN AND ROUTE OF ADMINISTRATION	3
2.0	TABLE OF CONTENTS	4
3.0	EXECUTIVE SUMMARY	5
4.0	SIGNIFICANT FINDINGS FROM OTHER REVIEW DISCIPLES	6
5.0	CLINICAL AND REGULATORY BACKGROUND	6
6.0	CLINICAL DATA SOURCES	8
6.2	TABLE(s) OF CLINICAL STUDIES	9
7.0	HUMAN PHARMACOLOGY	10
8.0	CLINICAL STUDIES IN SUPPORT OF LICENSURE	10
8.1	“A Dose-Response Study of ---(b)(4)-- Skin Test Antigen”	10
8.2	"Skin Test Sensitivity of 1.27 mcg per 0.1 mL Spherule-derived Coccidioidin in Adult Volunteers with a History of Pulmonary Coccidioidomycosis"	18
8.3	“Skin Test Specificity of 1.27 mcg per 0.1 mL Spherule-derived Coccidioidin in Adult Volunteers Without a History of Pulmonary Coccidioidomycosis”	27
8.4	“Skin Test Specificity of 1.27 mcg per 0.1 mL Spherule-derived Coccidioidin in Adult Volunteers With a History of Pulmonary Histoplasmosis”	33
9.0	OVERVIEW OF INDURATION RESPONSES ACROSS TRIALS	37
10	OVERVIEW OF SAFETY ACROSS TRIALS	38
11	ADDITIONAL CLINICAL ISSUES	39
12	CONCLUSIONS	39
13	RECOMMENDATIONS	39
14	LABELING	40

3 Executive Summary

This submission contains four studies evaluating the skin test antigen, Spherusol, that support the indication for the detection of delayed type hypersensitivity to *Coccidioides immitis* in healthy individuals with a history of pulmonary coccidioidomycosis. The indication for the product will be limited to use in individuals who have previously been diagnosed with pulmonary coccidioidomycosis by serology, culture or radiographs. Spherusol is not indicated for the diagnosis of acute infection with *C. immitis*. The utility in confirming past infections has not been demonstrated in a population in which information about positive serologies, cultures or radiographs were not available prior to testing with Spherusol. The four completed clinical studies enrolled subjects with a history of pulmonary coccidioidomycosis in an endemic area (S101A, S104-1), subjects without prior exposure to *C. immitis* in a non-endemic area (S104-2) and subjects who have a history of Histoplasmosis (S104-3) in an area non-endemic for *C. immitis*, to provide data on the sensitivity and specificity of Spherusol in each defined population. No positive or negative predictive value can be assessed for the product's use in the general population based upon these studies.

The initial study, S101A, was a dose response study to determine the dose to be used in the later clinical studies following the -----(b)(4)----- phenol as a preservative. Twenty subjects with a history of pulmonary coccidioidomycosis diagnosed by serologies, culture and/or radiographs received three to four different doses ranging from 0.4 – 2.4 mcg/0.1 mL on the volar surface of the forearms. Induration responses were read at 48 hours following placement. Two of six subjects who received the highest dose [2.4 mcg/0.1 mL] experienced accelerated, severe reactions. This dose was removed from further testing. A linear regression using the induration responses for the test doses supported the use of the 1.27 mcg/0.1 mL dose in the subsequent studies.

In study S104-1 a total of 56 subjects with a previous history of pulmonary coccidioidomycosis were enrolled at two sites (Bakersfield, CA and Tucson, AZ) to receive skin testing with five skin test antigens (Spherusol, Trichophyton, Candin, saline placebo and thimerosal negative control). Two subjects were excluded prior to skin testing for not meeting inclusion/exclusion criteria and one subject was later lost to follow-up when he did not return to have the skin test read. A total of fifty-four subjects had skin test antigens placed for evaluation at 48 hours and 53 subjects completed the study. Fifty-one subjects had valid skin test results. Two subjects, one from each site, reacted to one or both of the negative controls and were excluded from further analyses. Of the fifty-one subjects with evaluable skin test results, 50 subjects had a positive reaction to Spherusol. Thus, 98% (89.6, 1.00) of subjects with a previous history of pulmonary coccidioidomycosis reacted at 48 hours with induration responses measuring 5 millimeters or greater. No positive predictive value can be assessed for the use of Spherusol outside this specific population of subjects with a previous history of pulmonary coccidioidomycosis.

Study S104-2 provided data to demonstrate negative delayed type hypersensitivity reactions to Spherusol in a population of subjects (N=60) who had no previous history of a diagnosis of pulmonary coccidioidomycosis, negative serologies for antibodies to *C. immitis* and no history of travel to endemic areas. Five subjects (8%) did not show reactions to any of the skin test antigens or controls administered. These five subjects were evaluated for immune competence by lymphocytic profiles, which were all normal. To account for these findings the analyses included an evaluation in which these subjects were considered to have a positive response to Spherusol to illustrate the worst case scenario for the study product. This gave specificity for Spherusol in this population of 90% (79.5%, 96.2%). Again, no negative predictive value can be assessed for the use of Spherusol outside this specific population.

In Study S104-3, no induration reactions to the Spherusol were noted for the 12 subjects with a known diagnosis of histoplasmosis, and without history of travel to *C. immitis* endemic areas. All skin tests with Spherusol demonstrated a < 5mm induration and were considered to be negative. This finding is supportive for a lack of cross-reaction of Spherusol with *H. capsulatum*.

Safety data were not collected in a manner which allowed for the assessment of local adverse reactions caused by Spherusol alone. Three of the studies [S104-1, S104-2 and S104-3] administered three skin test antigens (Spherusol, Candin and Trichophyton) and two controls (Thimerosal control and Saline/phenol control) on the volar surfaces of the arms of each enrolled subject. Local adverse reactions were documented at 48 hours and through seven days, but not for the individual skin tests. Thus, all local adverse reactions have been ascribed to Spherusol.

The most common solicited adverse events reported were swelling and itching occurring in a range of 67-85% for subjects who received all five skin tests. The majority of reactions were mild to moderate in nature, but severe itching and swelling did occur in 2% of subjects in studies S104-1 and S104-2. There were no severe solicited local adverse reactions in study S104-3. Flu-like symptoms were the most common solicited systemic adverse event occurring in a range of 7-8%. One subject in S104-2 reported severe difficulty breathing requiring medical treatment in the 24 hours following administration of the test products.

There were serious adverse events or deaths in the clinical studies.

4 Significant Findings from Other Review Disciplines

4.1 Chemistry, Manufacturing and Controls (CMC)

Please see the reviews completed by the product reviewers.

4.2 Animal Pharmacology/Toxicology

Please see other discipline reviews.

5 Clinical and Regulatory Background

5.1 Disease or Health-Related Condition(s) Studied and Available Interventions

Coccidioides immitis is a dimorphic fungus endemic to the soil in the southwestern United States, northern Mexico and parts of Latin and South America. Hyperendemic areas located in the US include Kern County, California and Pima, Pinal and Maricopa counties in Arizona. Infections are acquired from the environment following the inhalation of arthroconidia (spores). Clinical manifestations of infection include non-specific complaints in immunocompetent individuals including fever, pleuritic substernal chest pain, cough, malaise anorexia or chills. In greater than 60 % of infected individuals the disease is asymptomatic. Pulmonary disease can present as pneumonia, hilar adenopathy associated with infiltrates, or pleural effusions. Extrapulmonary dissemination of disease can occur, often within a few months of the primary infection. Populations at higher risk for disseminated disease include pregnant women (particularly during the third trimester), Hispanics, African Americans, Filipinos and those with depressed cellular immunity (acquired or iatrogenic). Extrapulmonary disease can manifest as granulomas in the skin, coccidioidal meningitis (with or without abscess formation) or bone infection.

Serodiagnosis of coccidioidomycosis is the primary method of indirect diagnosis, with both qualitative and quantitative serologic tests being used in the diagnosis. Enzyme immunoassay, latex particle

agglutination, and immunodiffusion are used as qualitative techniques, which yield positive results early in the course of the infection. Enzyme immunoassays provide rapid qualitative assessment of both IgM and IgG coccidioidal antibodies. Acute primary coccidioidomycosis is associated with positive IgM primary tube precipitin (IDTP) tests. These highly specific titers usually correlate with the severity of disease with rising titers associated with progressive disease. False negative serologies do occur, primarily in those with HIV infection and immunosuppressed individuals. (Pappagianis & Zimmer. Clinical Microbiology Reviews, July 1990, p. 247-268; Yeo & Wong. Clinical Microbiology Reviews, July 2002, p. 465-484. Saubolle. Annals of the New York Academy of Science. 1111: 2007, p.301-304). The most specific method for diagnosis of disease is culture or histopathologic evaluation of affected tissue. Growth of the mycelial form will occur on most culture mediums within 7-10 days after inoculation. *C. immitis* is designated as a "select agent" for bioterrorism because of it is the most virulent fungal pathogen in humans and is the only fungal etiological agent designated as a Biosafety Level 3 organism (www.cdc.gov/od/sap, Dixon, J. Applied Microbiology 2001, p.602-605).

5.2 Important Information from Pharmacologically Related Products, Including Marketed Products

Two products were previously licensed as skin test antigens to detect delayed type sensitivity to *C. immitis*. Coccidioidin and Spherulin™, were made from extracts of either the mycelial phase or spherule phase of *Coccidioides immitis*, respectively. Neither product is currently approved for use. Both products were used previously to detect delayed type hypersensitivity to *C. immitis*, the causal agent of coccidioidomycosis. Results from previously conducted research have disagreed on which skin test antigen is more sensitive (Am J Public Health 1985; 75: 863-865). The skin tests have been of limited usefulness in the diagnosis of acute or disseminated infection as anergy may develop during active and disseminated infection. The time from acute infection to the ability to respond with a delayed type hypersensitivity reaction is thought to be several weeks. A positive skin test will not differentiate a current infection from prior infection. A positive skin test with induration ≥ 5 mm has been accepted by clinicians and researchers as indicative of prior exposure to the fungus. These products have been used as an epidemiological tool to assess for prior exposure to the fungus in various populations and demographic regions.

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

The product for licensure is produced by dilution with --(b)(4)-- saline containing 0.4% phenol as preservative ----- (b)(4) ----- of *C. immitis* antigen contains 12.7 mcg/mL of antigen in the final product. ----- (b)(4) -----

5.3 Previous Human Experience with the Product, Including Foreign Experience

See above. No previous history of the safety and use of this product is provided by the Applicant.

5.4 Regulatory Background Information

Pre-BLA meeting: 27 August 2007

6 Clinical Data Sources

6.1 Material Reviewed

6.1.1 BLA/NDA Volume Numbers Which Serve as a Basis for the Clinical Review

IND --(b)(4)--, amendments as required
BLA STN 125354/0 (electronic submission in a modified eCTD)
Sections reviewed:
Section 2 - Labeling
Section 3 - Summary
Section 8 - Clinical
Section 11 – Case report tabulations
Section 12 – Case report forms
Amendments to the original submission

6.1.2 Literature

- Dodge et al. AJPH August 1985, Vol. 75, No. 8 (page 863-865)
- DiCaudo, D. J Am Acad Dermatol, December 2006, Volume 55, Number 6.
- Larwood, T. CID, March 2000; 30.
- Galgiani et al. CID April 2000; 30: 658-61.

- others as cited in review

6.1.3 Post-Marketing Experience

No data available for review.

6.2 Table(s) of Clinical Studies

Four clinical studies were performed under the IND and are submitted for review in support of licensure.

TABLE 1: Clinical Studies Submitted to BLA 125354*

Study number (site)	Title	Primary Objective	Number of subjects enrolled (age)
S101A (Bakersfield, CA)	A Dose-Response Study of - --(b)(4)-- Skin Test Antigen**	To compare the dose- response of the current product at varying concentrations	20 (>17 years)
S104-1 (Bakersfield, CA & Tucson, AZ)	Skin Test Sensitivity of 1.27mcg per 0.1mL Spherule-Derived Coccidioidin in Adult Volunteers With a History of Pulmonary Coccidioidomycosis	To evaluate the DTH skin test response to Spherusol in persons with a history of pulmonary coccidioidomycosis confirmed by laboratory findings	54 (18-65 years)
S104-2 (Spokane)	Skin Test Specificity of 1.27mcg per 0.1 mL Spherule-Derived Coccidioidin in Adult Volunteers without A History of Pulmonary Coccidioidomycosis	To evaluate the DTH response to Spherusol in persons without a history of coccidioidomycosis or exposure to the fungus.	60 (18-60 years)
S104-3 (Blair, NE)	Skin Test Specificity of 1.27mcg per 0.1mL Spherule-Derived Coccidioidin in Adult Volunteers With a History of Pulmonary Histoplasmosis	To evaluate the DTH skin test response to Spherusol in persons with a history of pulmonary Histoplasmosis.	12 (> 18 years)

* Studies S104-1, S104- 2 and S104-3 are listed as one study NCT00690092 at <http://clinicaltrials.gov>.

** In Study S101A, the product Spherusol is referred to as ---(b)(4)--.

6.3 Financial Disclosures

The applicant provided a statement which certifies that the physicians listed below and their sub-investigators and research associates who participated in the “Spherusol” studies submitted to the BLA had no financial interest in the outcome of these studies. Specifically, (a) the compensation paid to the investigator was not affected by the outcome of the clinical trial; (b) the investigator did not have an equity interest in Spherusol; (c) no significant monetary payments, goods or services were made to the investigator exclusive of the costs associated with conducting the trial.

Form FDA 3454 were submitted in the 125354/002 by the Applicant for each investigator:

- Steven Kernerman, D.O. Spokane, WA
- Royce H. Johnson, M.D., Bakersfield, CA
- Neil M. Ampel, M.D., Tucson, AZ
- Brad Sawtelle, M.D., Blair, NE

7 Human Pharmacology

See section 8.0 for review of clinical studies conducted to support licensure.

8 Clinical Studies in Support of Licensure

8.1 “A Dose-Response Study of ----(b)(4)---- Skin Test Antigen”

8.1.1 Protocol # and Protocol Title:

Study S101A (Bakersfield, CA): “A Dose-Response Study of --(b)(4)--- Skin Test Antigen”

8.1.1.1 Objective/Rationale:

To evaluate the cellular hypersensitivity response to four dose levels of -----(b)(4)-----
----- of 1.27 mcg/0.1mL -----(b)(4)-----
----- Re-evaluation of
the dose concentration was necessary because --(b)(4)-- is formulated with 0.4% phenol as a
preservative, -----(b)(4)-----
-----.

Please see statistical reviewer’s comments on the methodology for evaluation of dose determination.

8.1.1.2 Design Overview

This study was designed to evaluate the delayed-type hypersensitivity response (induration) at 48 hours after the administration of four strengths of the study product (referred to here as --(b)(4)--) formulated with 0.4% phenol and a placebo control which was the diluent used to formulate the highest dose of 2.4 mcg/0.1 mL in subjects who had previously been diagnosed with pulmonary coccidioidomycosis. In this dose-response study each subject was to receive 4 different doses of the study product and the placebo. The concentrations of the study product that were administered were:

1. Placebo Control*
2. --(b)(4)--- 0.4 mcg/0.1 mL with 0.4% phenol
3. --(b)(4)--- 0.8 mcg/0.1 mL with 0.4% phenol
4. --(b)(4)--- 1.6 mcg/0.1 mL with 0.4% phenol
5. --(b)(4)--- 2.4 mcg/0.1 mL with 0.4% phenol

* Diluent used to prepare --(b)(4)--- contains 0.4% phenol and thimerosal at the same concentration as the residual thimerosal in 2.4 µg/0.1 mL --(b)(4)---

Allermed chose these test dose concentrations -----(b)(4)-----
----- 1.27 mcg/ 0.1mL.

The diagnosis of pulmonary coccidioidomycosis was established using serologies (complement fixation, EIA, and IgG and IgM) and radiography. The skin test results were evaluated and measured at 48 hours

following placement of four doses of “---(b)(4)---” and the placebo control. Test materials were coded and double-blinded. Each subject was to be observed for thirty minutes after administration to assess for immediate adverse reactions.

Procedures

Visit 1

1. Subjects were asked to complete a study related questionnaire and consent form.
 2. Subjects were skin tested with the diluent control and three concentrations of --(b)(4)---*.
- The test materials were coded, double-blinded, and administered in a volume of 0.1 mL intradermally.
3. Subjects were required to wait in the physician's office for 30 minutes after the last skin test had been administered.

Visit 2 (48 hrs. later)

1. Subjects were asked to report any adverse reactions during the past 48 hours.
 2. Skin test sites were examined for induration. If present, was to be measured in two diameters (longest and orthogonal) and outlined with a black ballpoint pen. The tracing was recorded on the skin test record using transparent tape. This was done by pressing the tape firmly over the tracing, removing it, and placing the tape on the skin test record.
- * Initially four doses of --(b)(4)--- were to be used in the dose response study, with the highest dose being 2.4 mcg / 0.1 mL. The first two subjects to receive this dose had induration reactions > 70 mm. The study was halted and the decision was made not to continue with the highest dose product. Therefore, the remaining 14 subjects received three strengths of the study product.

Analysis of skin test

The induration responses for each dose and the placebo were supplied, along with the codes for unblinding the skin test placements.

The mean induration for each injection was assessed at 48 hours. Mean results were calculated by averaging the greatest diameter of induration and its orthogonal diameter. The mean induration values at each dose were used to calculate the linear regression.

8.1.1.3 Population

The study enrolled twenty subjects greater than 17 years of age with a history of pulmonary coccidioidomycosis diagnosed by laboratory and radiographic testing and confirmed by chart review.

Inclusion Criteria

- History of infection with *C. immitis* or positive delayed-type hypersensitivity response to Coccidioidin
- Male or non-pregnant female; women of child-bearing potential must have a negative urine or serum pregnancy test 48 hours prior to enrolment, or have evidence of surgical sterilization
- Greater than 17 years of age

Exclusion Criteria

- History of histoplasmosis
- History of an adverse reaction to antigens of *Coccidioides immitis*, i.e., strong local reaction to skin test with Spherulin or Coccidioidin ≥ 70 mm and/or systemic response
- HIV positive
- Presence of erythema nodosum

- Presence of eczema
- Presence of psoriasis
- Presence of cellulitis
- Treatment with immunosuppressive drugs
- History of immunodeficiency disease
- Breastfeeding

8.1.1.4 Products mandated by the protocol

Table 2: Summary Table of Products used in Study S101A, Bakersfield, CA

Product Name	Lot Number	Color Code
Spherule-derived Coccidioidin 0.4 µg/0.1mL	XSN11020101	Green
Spherule-derived Coccidioidin 0.8 µg/0.1mL	XSN11020102	Red
Spherule-derived Coccidioidin 1.6 µg/0.1mL	XSN11020103	Black
Spherule-derived Coccidioidin 2.4 µg/0.1mL	XSN11020104	Yellow
Placebo Control*	XDf10180101	Blue
Diluent (used to dilute active ingredient in the first 4 products listed above)	XDf10180102	

Source: STN 124345/005, Table Q10-1, page 29/66.

* Diluent used to prepare Spherosol contains 0.4% phenol and thimerosal at the same concentration as the residual thimerosal in 2.4 mcg/0.1 mL Spherosol

Table 3: Study S101A: Composition of Phenol Saline Control (Placebo) Lot -----(b)(4)-----

Ingredient	Lot #	Specification
Sodium Chloride, (b)(4)	-(b)(4)-----	-(b)(4)-
Sodium Borate, ----- (b)(4)-----	-(b)(4)-	-(b)(4)-
Thimerosal, (b)(4)	-(b)(4)---	-(b)(4)-----
Phenol, liquefied (b)(4)	-(b)(4)--	-(b)(4)-
Water for Injection	-(b)(4)-----	Q.S.

Source: STN 124345/005, Table Q10-2, page 29/66.

Table 4: Study S101A: Composition of Diluent Lot -----(b)(4)---- used to prepare lots of Spherosol

Ingredient	Lot#	Specification
Sodium Chloride, (b)(4)	-(b)(4)-----	-(b)(4)-
Sodium Borate, ----- (b)(4)-----	-(b)(4)-	-(b)(4)-
Phenol, liquefied (b)(4)	-(b)(4)--	-(b)(4)-
Water for Injection	-(b)(4)-----	Q.S.

Source: STN 124345/005, Table Q10-3, page 30/66.

The products were monitored for stability during the course of the study.

8.1.1.5 Endpoints

This study was conducted to identify the appropriate dose of Spherosol to be used in subsequent studies. Spherosol was formulated by Allermid ------(b)(4)-----

----- 0.4% phenol ------(b)(4)----- as a preservative. The dose-response study reported in this submission was designed to evaluate the cellular hypersensitivity response

to four doses of -----(b)(4)-----

The endpoint of the dose-response study was the size of induration 48 hours after receiving intradermal injections of 0.1 mL of the four dose levels of study product and 0.1 mL of the placebo control. At 48 hours each area of induration was measured along two axes and the mean was reported as the final measurement.

The mean response data were then plotted against the dose concentration and analyzed by linear regression using the following equation:

$$E(\text{induration/concentration}) = \text{-----(b)(4)----- concentration}$$

Where “E”- Expectation is the mean of the conditional distribution of induration at any given concentration.

The Applicant also provided another equation for verification of the --- (b)(4) -- Dose-Response Study results as:

$$E(\text{induration/concentration}) = \text{-----(b)(4)-----}$$

Discrepancy to be addressed in the Statistical Reviewer’s comments.

Please see section 8.1.1.7 below.

Please statistical reviewer’s comments for full review.

8.1.1.6 Surveillance

Subjects were to be monitored for 30 minutes after the skin tests were administered per protocol.

Subjects were also seen in follow-up at 48 hours after skin test administered. Skin test sites were measured at that time and adverse events recorded.

The subject was to record all adverse events on a diary card. Adverse events were to be graded for time, nature, severity and outcome of the event using the grading scale below:

1+ (mild) Noticeable systemic response, transient, symptoms subside within 20 minutes and/or induration or swelling at test site 30 to 40 mm in diameter.

2+ (moderate) Systemic response which persists for more than 20 minutes but self-limiting and/or induration or swelling at test site > 40 mm in diameter.

3+ (severe) Strong response which persists for more than 20 minutes and which requires intervention with emergency procedures/medication for treatment and/or necrosis at test site.

Adverse events listed by the sponsor in the text of the protocol included: swollen, painful arm, difficulty breathing, faintness, flushing, dizziness, weakness, tachycardia, abdominal cramps, marked hypertension or hypotension or other systemic reactions. However, a review of the subject diary card shows that no adverse events were specifically solicited for in this study. Instead subjects were asked to report any events they considered “abnormal” following administration of the skin test products. The toxicity grading scale included in the protocol was not used to assess the reported adverse events.

8.1.1.7 Statistical considerations

The data were plotted on a dose response curve with the mean dose response plotted against the dose concentration. The data was analyzed by linear regression, using the formula below:

$$E(\text{induration} \mid \text{concentration}) = \text{-----}(b)(4)\text{----- concentration}$$

At CBER's request an analysis of the data was performed after removal of subjects that had test results that could not be read, had un-evaluable test results because of reaction to the placebo, or had a non-linear response to increasing doses of Spherusol. The estimated induration based upon results from 16 subjects for the proposed dose of 1.27 mcg/0.1 mL was 22.6 mm. A variance +/- 20% (18.9 to 28.3 mm) in the induration response was acceptable based on the statistical design. Since all of the regression results showed that the estimated induration values are well within the range, the dose concentration at the 1.27mcg/0.1mL level was found acceptable for further studies using Spherusol.

Please see the statistical review for further analysis.

8.1.2 Results

8.1.2.1 Populations enrolled/analyzed

Twenty adults ages 22 to 54 years age (M/F = 8/12), with a previous medical diagnosis of coccidioidomycosis evaluated by radiography, serology and culture (data not shown). Median age was 35 years. Eighty-five percent of the enrolled subjects were Hispanic. Caucasians (2/20, 10%) and Black (1/20, 5%) represented the remainder of the study population.

None of the subjects enrolled had previously been tested with a coccidioidin antigen (Amendment /005, Other, Letter from R. Johnson, MD)

Initial study start: 04 June 2002

Study stopped: 04 June 2002

Study recommenced: 16 July 2002 (using doses of 0.4 µg, 0.8 µg, 1.6 µg and placebo)

Study completed: 14 January 2003

8.1.2.2 Dose-Response endpoints/outcomes

The evaluation of the dose- response for Spherusol formulated with phenol using a linear regression of the induration responses to doses of 0.4 mcg/0.1 mL, 0.8 mcg/0.1 mL, 1.6 mcg/0.1 mL and 2.4 mcg/ 0.1 mL demonstrated that a dose of 1.27 mcg/0.1 mL was acceptable for further studies of Spherusol. Based upon the calculation of the linear regression, the estimated induration for a dose of 1.27 mcg/mL would be 22.6 mm, which falls within the +/- 20% variance accepted around the previous internal reference of 23.6 mm.

8.1.2.3 Safety outcomes

Although surveillance guidelines were presented in the protocol (see 8.1.1.6 above) the principal investigator did not follow the protocol prescribed monitoring of subjects and evaluation of adverse events.

No summary of safety data was submitted to the BLA which included immediate, solicited, unsolicited or serious adverse events. No adverse event data were submitted to the file except for the brief narrative on subjects --(b)(6)-- in section V of the study results.

In response to the “Complete Response” letter of 26 March 2010, the Applicant states that all AEs were unsolicited. Subjects documented the AEs on the “Daily Diary Form” which were then transcribed by the principal investigator under the “Physician’s Notes” in the Case Report Forms. Although a grading scale was presented in the original protocol for the study, it does not appear that it was used to assess the intensity of the adverse events.

Based on available information, ten of the 20 subjects enrolled reported AEs.

This reviewer’s interpretation of the raw data notes that 7/10 subjects who documented adverse reactions reported itching, with one subject (study number [SN] (b)(6)) applying an unknown cream at the site secondary to the AE. This same subject also reported swelling and vesiculation at a test site (dose and site not indicated) in the first 24-48 hours following skin test placement. One subject (SN (b)(6)) reported a headache of three day duration which began on the day of administration of the study product. Two subjects (SN ---(b)(6)--) noted a rash described as “red bumps” within 24-48 hours after administration of Spherusol dilutions.

Two subjects (SN ---(b)(6)-- respectively) had accelerated (defined as occurring within the first 24 hours after test placement) responses to the highest dose, 2.4 µg/0.1 mL “---(b)(4)--” within the first day of testing. One of the subjects (SN (b)(6)) was treated with topical hydrocortisone.

Review of the unblinded skin test measurements submitted for Subject (b)(6), who had the skin test antigen (2.4 µg/0.01mL) placed on 04 June 2002 with the result read on 06 June 2002, showed a reaction of 62 x 90 mm was recorded for R1, 72 x 90 mm for R2 and the reaction for R3 could not be read due to extension. No reading is present for L2 and L1 which are recorded as negative. (where R= the right arm and L= the left arm). Data for Subject (b)(6) are presented as a single measurement of 55 mm across for R1 and 44 mm across for R2, which were doses of 2.4 and 1.6 µg/mL, respectively.

Due to the accelerated response in two individuals the study was paused, and the decision was made to discontinue further testing at the 2.4 mcg dose. Five of the 20 subjects enrolled received the 2.4 µg dose of Spherusol before its use in the trial was discontinued.

Table 5 below shows the induration responses that were seen at 48 hours following administration of three or four different strengths of Spherusol and the diluent placebo. Induration responses were measured on two orthogonal axes and the mean of the measurements recorded.

Table 5. Study S101A: Induration responses following administration of different concentrations of Spherusol

Subject ID	0.4 mcg Spherusol (mm)	0.8 mcg Spherusol (mm)	1.6 mcg Spherusol (mm)	2.4* mcg Spherusol (mm)	Placebo diluent (mm)
^01	ND	ND	ND	ND	ND
02	10.0	13.0	13.0	21.5	0.0
03	13.0	19.0	17.5	28.0	0.0
04	25.0	25.5	44.0	55.0	0.0
05	12.0	17.5	21.5	36.0	0.0
06	8.5	12.0	16.0	22.0	0.0
07	10.5	17.0	39.5	NT	0.0
08	6.0	9.5	16.0	NT	0.0
09	16.0	29.5	45.5	NT	0.0
^10	4.5	11.0	19.5	NT	6.5
11	11.5	20.5	42.0	NT	0.0
12	10.5	26.0	32.0	NT	0.0
13	0.0	10.5	33.0	NT	0.0
^14	15.0	34.0	41.0	NT	10.0
^15	6.0	0.0	12.5	NT	0.0
16	8.0	15.5	21.0	NT	0.0
17	6.5	11.5	15.0	NT	0.0
18	9.0	15.0	28.5	NT	0.0
19	0.0	10.0	17.0	NT	0.0
20	23.5	24.0	25.0	NT	0.0
MEAN	10.6	17.3	26.6	-	-

Source: Table 3, S101A, STN 125345/0. (no pagination provided)

ND= size of induration could not be determined

NT=not tested

mm= millimeters

mcg= micrograms/0.1 mL

*After two subjects experienced large induration responses to the 2.4 mcg/0.1 mL dose, testing with this dose was suspended.

^ induration responses were unevaluable due to reaction to placebo, inability to read induration responses, or non-linear induration responses

Placebo diluent = Diluent used to prepare --(b)(4)--- contains 0.4% phenol and thimerosal at the same concentration as the residual thimerosal in 2.4 µg/0.1 mL --(b)(4)---.

Mean values do not include unevaluable subjects

All safety data reported by subjects was considered to be unsolicited. Data was collected from subject diary cards or by interview at the 48 hour visit or during the telephone follow-up at 48 hours (+/- 4 hours).

The applicant states in the response to the IR letter of 11 May 2011 that not all subjects returned the subject diary card during this study [page 2/17, STN 125345/0/10] in his response to requests for further

safety information. From the data available it appears that half of the 20 subjects enrolled in the study did not return diary cards. Intensity of reactions was not recorded for adverse events that were seen following administration of the differing concentrations of Spherusol.

Table 6 below provides the available safety information for study S101A.

Table 6. Study S101A, Bakersfield ,CA: Subjects for whom adverse events were reported

Subject No.	ID.	Adverse Events	Intervention
--(b)(6)--		Large DTH response	topical hydrocortisone
		itching	none
		Large DTH response	none
		itching	none
		Hungry, tired	none
		Itching, swelling, vesiculation	topical cream
		itching	none
		itching, red bumps	none
		headache, itching	none
		itching	none

Source: STN 125345/0/005, Table Q5-1, page 25/66.

8.1.3 Comments & Conclusions

The study results include minimal safety data and brief narratives of the “accelerated” responses that were seen in two subjects (Subject (b)(6) and Subject (b)(6)) who were administered the 2.4 µg dose of Spherusol. Due to the accelerated responses, after review by the IRB and discussion with the CBER it was decided that testing of the 2.4 µg dose would be discontinued. It appears from Table 3 in the BLA submission (no pagination provided) that six subjects received the 2.4 µg dose of the skin test antigen prior to this decision.

No analysis of the available safety data was provided by the Applicant. CBER’s review of the available data showed that itching was the most commonly reported AE from 7/10 (70%) subjects with available data (Table Q5-2, Response to question 5, 26 March, 2010 letter, page 26/66).

Although the intensity of the adverse events were not assessed by the clinical investigators, the large injection site reactions which accompanied administration of the 2.4 µg dose in two subjects can be considered severe or Grade 3 reactions. Another subject (SN (b)(6)) who received doses of 0.4, 0.8 and 1.6 mcg/0.1 mL reported swelling and vesiculation at the skin test sites and was treated with a topical cream, which could also be considered a severe or Grade 3 reaction. Safety data for each specific dose was not available per the Applicant.

No serious adverse events occurred during the study.

The safety and dose response data support the use of a dose of 1.27 mcg/0.1 mL in the subsequent clinical studies of Spherusol.

8.2 Skin Test Sensitivity of 1.27 µg per 0.1 mL Spherule-derived Coccidioidin in Adult Volunteers with a History of Pulmonary Coccidioidomycosis

8.2.1 Applicant's Protocol # and Protocol Title

Protocol S104-1 (Bakersfield, CA) (Tucson, AZ)

“Skin Test Sensitivity of 1.27 mcg per 0.1 mL Spherule-derived Coccidioidin in Adult Volunteers with a History of Pulmonary Coccidioidomycosis”

8.2.2 Objective/Rationale

From the Study Synopsis: To determine if Spherusol elicits a positive DTH skin test in persons with a history of pulmonary coccidioidomycosis

From Study Report: To evaluate the delayed-type hypersensitivity skin test response to Spherusol in persons with a history of pulmonary coccidioidomycosis confirmed by laboratory findings.

8.2.3 Design Overview

Subjects were screened prior to enrollment with a 1) a participant questionnaire, 2) signed informed consent, 3) pregnancy test (female), and 4) review of medical records to confirm previous pulmonary coccidioidomycosis.

On Visit #1, enrolled subjects were skin tested in a double-blind manner with the five test reagents injected intradermally in a pattern chosen according to a randomized procedure, and they were asked to complete a diary for the next 48 hours.

The results of skin tests were read after 48 hours (\pm 4 hours) on Visit #2 of the study.

Subjects were asked to continue to keep their Daily Diary to monitor possible adverse events until they return to the physician's office for Visit #3 on the 7th day after Visit #2 (Day 10 of the study). During this visit the Diary was to be reviewed and a Post Procedure Evaluation Record and Study Completion Record completed by the study physician.

Vital signs were to be measured during each visit (#1, 2, and 3). Adverse events were monitored and, if necessary, treated until resolution. The Applicant states that subjects with invalid skin tests would be replaced with alternate participants until the desired number of volunteers was achieved; however, an invalid test is not defined in the protocol.

8.2.4 Population

Up to 38 volunteers age 18-60 years, with a history of non-disseminated, non-cavitary pulmonary coccidioidomycosis confirmed by radiography and serologic or mycological findings could be enrolled at each of two sites; Tucson, AZ and Bakersfield, CA. The protocol for the Tucson, AZ site was later modified to allow enrollment of subjects to an upper age of 65 years. If volunteers were taking anti-fungal medications they should be in overt good health with evidence of convalescence (e.g., declining serologic titer). The first thirty-five subjects were to be used for the analysis of data.

* The Applicant did not follow the submitted protocol. Forty-two subjects were enrolled at the Tucson, AZ site and all subjects were included in the analyses. No revision to the protocol was made under the IND.

8.2.4.1 Inclusion Criteria

- 18 – 65 years of age
- Overt good health (absence of Active Medical Disease*)
- History of pulmonary coccidioidomycosis of at least 45 days duration confirmed by serologic, histologic or mycologic findings

8.2.4.2 Exclusion Criteria

- Active Medical Disease*
- Alcohol abuse or illicit drug use
- History of histoplasmosis or blastomycosis
- Influenza-like illness within the past 4 weeks
- Immunizations within the last 4 weeks
- Current atopic or contact dermatitis, psoriasis, erythema nodosum, urticaria
- Current treatment with corticosteroids, cytotoxic or immunosuppressive drugs
- Immunodeficiency disease
- HIV infection
- Previous skin test with coccidioidin
- Pregnant or lactating **
- Adverse reaction to thimerosal
- Adverse reaction to Candida or Trichophyton skin test antigen
- Current cavitory or disseminated coccidioidomycosis

* **Active Medical Disease:** Any active physical or psychiatric condition that may increase the risks associated with participation in the study or interferes with the interpretation of study results. Included chronic medical illnesses are cardiovascular disease, renal insufficiency, chronic respiratory illness, cirrhosis, chronic hepatitis, chronic pancreatitis, chronic diarrhea, malnutrition, malignancy, autoimmune disease, and asthma.

** **Pregnancy Prevention:** Females were instructed to abstain from sexual relations or practice a medically acceptable form of birth control during the study. They were also instructed that, except for surgical removal of the uterus, birth control methods such as the use of condoms, a diaphragm or a cervical cap, birth control pills, IUD, or sperm killing products are not totally effective in preventing pregnancy. Urine pregnancy tests were done on women of childbearing age prior to skin testing.

Subjects may have been treated with anti-fungal drugs prior to enrollment or were receiving anti-fungal drugs during the study treatments.

Diagnosis of Pulmonary Coccidioidomycosis

The diagnosis of previous pulmonary coccidioidomycosis could include radiographic and serologic or mycological findings from medical record review (Documentation of diagnostic criteria was not included in the BLA submission).

Radiographic findings to include:

1. soft, fuzzy hilar-thickenings
2. pneumonic-like infiltrate
3. parenchymatous well-circumscribed nodular lesions
4. mediastinal and hilar adenopathy
5. small pleural effusions

Positive Serology to *C.immitis* demonstrated by at least one of the following assays (no other information provided on laboratory assays and validation):

1. precipitation
2. complement-fixation
3. ELISA competition

Mycological diagnosis supported by:

1. Presence of spherules in sputum/biopsied tissue
2. Presence of mycelium with arthrospores on laboratory media, such as -----(b)(4)-----.

Withdrawal and Removal Criteria

Participant Initiated: Volunteers will be allowed to withdraw from the study at any time without prejudice or loss of benefits to which they are entitled.

Investigator Initiated: Volunteers could be removed from the study at any time by the Principal Investigator or Sponsor should their continued participation be injurious to their health and well being.

The IRB was to be notified whenever a subject is removed from the study for health related issues.

Volunteers removed from the study subsequent to receiving the skin test, were to have a follow up visit at 1-2 weeks.

Alternate Participants: Based on the data reported to the sponsor, subjects with invalid tests (not defined) will be removed from the study (in terms of data analysis) and replaced with alternate participants.

8.2.5 Products mandated by the protocol

A dose of product was to have contained 1.27 micrograms of --(b)(4)--/0.1 mL. The product was diluted in 0.9% sodium chloride, 0.4% phenol, and 0.014% sodium borate. The product contains 1:1,000,000 residual thimerosal preservative (NB: -----(b)(4)-----).

The Spherusol formulation and control skin tests reagents are listed in Table 7, below.

Table 7. Study S-104-1: Identification of products used in study

Reagent	Study Color Code	Code	Description
Spherusol (1.27µg/0.1mL) Lot # XSN04220301	Green	4101	Water extractables of <i>C.immitis</i> spherules in --(b)(4)-- saline containing 0.4% phenol as a preservative and 1:1,000,000 residual thimerosal preservative. Contains 1.27 mcg/0.1mL of dry spherule material as the active ingredient.
Thimerosal Control Lot # ----- (b)(4) ----	Red	6849	--(b)(4)-- saline without the active ingredient. The solution contains thimerosal 1:1,000,000. 0.4% phenol added as a preservative.
Placebo(Saline) Control Lot # XDf06020301	Black	3546	--(b)(4)-- saline without the active ingredient and without thimerosal. 0.4% phenol is added as a preservative.
Candin Lot # CA033	Blue	1287	Extract of <i>Candida albicans</i> preserved with 0.4% phenol
Trichophyton Extract Lot # XMm11080401	Yellow	5461	Extract of <i>T.rubrum</i> and <i>T.mentagrophytes</i> preserved with 0.4% phenol

Source: Study S-104-1, IND --(b)(4)--, Table: "Skin Reagents", section 8.4.2, page 13 of 37.

The five skin test reagents that were used in the study were color coded. Each reagent was assigned a clinical code to ensure the identity of the article. This code was different from the lot number. Candin and Trichophyton extracts are licensed for use and distribution by the FDA.

8.2.6 Endpoints

Endpoints assessed were:

- Proportion of subjects with an induration response (≥ 5 mm) at 48 hours (+/- 4 hours) for each antigen. (NB: The use of 5 mm induration indicating a positive response at 48 hours was used for the previously licensed product(s) SpherulinTM and Coccidioidin)
- Safety was assessed by collection of information on local and systemic adverse events occurring from time of placement until 48 hours after placement and then at one week following 48 hour evaluation.

8.2.7 Surveillance

Immediate Reactions

Subjects were to be monitored for 60 minutes following the last injection

Local Reactions

- Reactions considered mild include (Category 1)
 - Itching
 - Tenderness not compromising limb function
 - Erythema and/or edema 50-80mm in any dimension
- Reactions considered moderate (Category 2)
 - Tenderness compromising limb function
 - Induration 80mm in any dimension
- Reactions considered severe (Category 3)
 - Skin breakdown with ulceration

Generalized/Systemic Reactions

- Reactions considered mild (Category 1)
 - Weakness, faintness, dizziness, nausea, cough, rhinorrhea lasting less than 1 hour, not requiring intervention
- Reactions considered moderate (Category 2)
 - Category 1 symptoms that persist for more than 1 hour, not requiring intervention
 - Flu-like symptoms
- Reaction considered severe – requiring intervention (Category 3)
 - Category 1 and 2 events requiring intervention
 - Progressive signs of an anaphylactic reaction
 - Hypertension or hypotension
 - Feeling of intense anxiety or panic
 - Flushing and sweating
 - Onset of vomiting, cramps or diarrhea
 - Onset of generalized pruritus in skin or mucous membranes
 - Onset of urticaria or angioedema
 - Acute onset of wheezing or dyspnea

8.2.8 Statistical considerations

The skin tests were read at 48 hours +/- 4 hours post-placement by measuring the area of induration (independent of erythema). Readings were confirmed by a second independent reader.

Subjects who reacted to the saline control or the placebo control with induration ≥ 5 mm were to be excluded from analysis of induration response but not the analyses of safety.

Induration responses of ≥ 5 mm at 48 hours for Spherusol were considered a positive response to the skin test antigen.

8.2.9 Results

8.2.9.1 Populations enrolled/analyzed

A total of 56 subjects with a previous history of pulmonary coccidioidomycosis were recruited at two sites (Bakersfield, CA and Tucson, AZ)*. Each skin test was administered in a blinded fashion on the volar aspect of the arms. Each skin test dose was formulated in 0.1 mL volume. Two subjects were excluded prior to skin testing for not meeting inclusion/exclusion criteria and one subject was lost to follow-up after placement of skin test antigens.

* Of note, the Applicant did not follow the submitted protocol. Forty-two subjects were enrolled at the Tucson, AZ site and all subjects were included in the analyses. No revision to the protocol was made under the IND.

A total of fifty-four subjects had skin test antigens placed for evaluation at 48 hours and 53 subjects completed the study. Fifty-one subjects had valid skin test results. Two subjects, one from each site, reacted to one or both of the negative controls.

Fifty-three subjects completed the study [38 (72%) males, 15 (28%) females]. The age range was 23-64 years of age. Racial and ethnic breakdown showed: thirty-seven (70%) subjects were Caucasian, six (11%) Hispanic, six (11%) African-American, one (2%) Asian, one (2%) Native American and two (4%) subjects did not specify ethnicity.

No subjects withdrew secondary to adverse events. One subject, enrolled to the Bakersfield site, had skin test reagents placed but did not return for skin test readings. The subject stated he had forgotten to return at 48 hours after placement of the study products.

Confirmation of coccidioidomycosis was had previously been made by positive serological findings for the majority of subjects enrolled in the study. At the Bakersfield site, the complement fixation (CF) assay was the most frequently used assay. At the Tucson site, the precipitation test was the most frequently used assay for diagnosis. Confirmational culture and observation of spherules in sputum or fine needle aspirates was done in 12 of 42 subjects enrolled at the Tucson, AZ site, but for none of the 11 subjects from the Bakersfield, CA site.

For subjects on antifungal therapy, remission was considered to have occurred at the time therapy was stopped. For subjects not on antifungal therapy, remission was considered to have occurred when significant clinical improvement was observed.

8.2.9.2 Delayed Type Hypersensitivity Reactions

The Applicant presented results for the Intent-to-Treat population (ITT) which contained 53 subjects. Two of these subjects reacted to the thimerosal and/or the saline placebo control and were excluded from the According to Protocol (ATP) population for the induration response at 48 hours.

Table 8. Study S104-1: Induration \geq 5mm at 48 hours for all skin test antigens, read at 48 hours (+/- 4 hours) N=53

Negative Controls		Positive Controls		Product
Saline Control	Thimerosal Control	Candin	Trichophyton	Spherusol
2 (4%)	1 (2%)	45 (85%)	46 (87%)	52 (98%)

Source: Study S104-1. Section 10.4.1, page 16 of 37.

Two subjects demonstrated a positive response to the negative controls, which were saline and the thimerosal placebo [4% phenol and thimerosal (1:1,000,000)]. Subject (---(b)(6)--) at the Bakersfield site had an 8.0 mm response to the Saline Control and a 10.5mm response to the Thimerosal Control. Subject (---(b)(6)--) at Tucson had a 7 mm induration response to the Saline Control. These two subjects were excluded in the analysis of induration response to Spherusol.

One subject (---(b)(6)--) did not react to the positive or negative controls, but had an 8.0 mm induration at the Spherusol administration site and was included in the analysis of induration response.

A summary of the number of subjects exhibiting induration responses at 48 hours following administration of the skin test antigens and controls is presented in Table 9 below.

Table 9. Study S104-1: Induration response \geq 5mm for Skin Test Reagents at 48 hours Post Placement by Study Site (N=53)

Product	Bakersfield	Tucson	Combined Results
Spherusol	11/11*	41/42	52/53
Positive Controls:			
Candin	10/11	31/42	41/53
Trichophyton	11/11	31/42	42/53
Negative Controls			
Thimerosal	1/11^	0/42	1/42
Saline placebo	1/11^	1/42	2/42

Source: STN 125345/0, Table page 7/37 unlabeled table- Synopsis

*numerator = number of subjects with a positive induration response; denominator = number of subjects tested

^same subject

Excluding the two invalid tests mentioned above (one at each study site) a total of 50 of 51 subjects with a past history of pulmonary coccidiomycosis demonstrated induration responses of \geq 5mm at the Spherusol injection site. Positive reactions to Spherusol were demonstrated by 98% (CI 0.896, 1.00) of subjects in this population.

Positive skin tests for Candin and Trichophyton were observed in approximately 85% of subjects.

A comparison is shown below of the size of the delayed type hypersensitivity (DTH) responses for subjects with a responses \geq 5mm to the positive controls and to the study product.

Table 10. Study S104-1: Mean Induration Responses and Range of Response at 48 hours Following Administration to Positive Controls and Spherusol *

	Candin n=43	Trichophyton n=44	Spherusol n=50
Range	6-32 mm	5-90 mm	5 – 39.5 mm
Mean	13.2 mm	18.9 mm	17.0 mm

Source: STN 125354/005, Response to FDA letter dated March 26, 2010, response to question 13, page 34/66.

*Subjects with induration responses at each of the particular antigen placements sites measuring < 5 mm were excluded.

Four subjects exhibited induration responses of > 40 mm (42.0, 51.0, 55.0, 71.0 mm) for Trichophyton.

The mean induration response for Spherusol was 17 mm in the 50 subjects with evaluable results.

Due to enrollment difficulties, the protocol was amended to allow enrollment of subjects who had received or were receiving antifungal drug treatment for coccidioidomycosis

Below is a summary of the mean induration response 48 hours after administration of Spherusol in subjects with evaluable responses who had never received anti-fungal medications, had previously been treated with anti-fungal medications for pulmonary coccidioidomycosis and subjects who were receiving anti-fungal medications during the study.

Table 11. Study S104-1. Induration Responses at 48 hours (+/- 4 hours) for Subjects with a Previous History of Pulmonary Coccidioidomycosis without a Reaction to Placebo and/or Thimerosal Who Had Never Received Antifungal Therapy (N=51)

Subjects who Never Received Antifungal Therapy	Mean Induration Response (mm)	Range of Responses (mm)
26/51	14.2	5 -35

Source: STN 125345/0/10, Table 2-1, page 3/17.

Table 12. Study S104-1. Induration Responses at 48 hours (+/- 4 hours) for Subjects with a Previous History of Pulmonary Coccidioidomycosis without a Reaction to Placebo and/or Thimerosal Who Had Received Antifungal Therapy *(N=51)

Subjects Who Previously Received Antifungal Therapy	Mean Induration Response (mm)	Range of Responses (mm)
25/51	20	4 -39.5

Source: STN 125345/0/10, Table 2-2, page 4/17.

*Includes subjects who are currently on anti-fungal therapy

Table 13. Study S104-1. Induration Responses at 48 hours (+/- 4 hours) for the Subset of Subjects with a Previous History of Pulmonary Coccidioidomycosis without a Reaction to Placebo and/or Thimerosal Who Were Receiving Antifungal Therapy at Time of Testing (N=51)

Subjects Receiving Antifungal Medication at the time of skin testing	Mean Induration Response (mm)	Range of Responses (mm)
7/51	21	6 -34

Source: STN 125345/0/10, Table 2-3, page 4/17.

Of the subjects who had received antifungal treatment previously; 13 subjects had been treated before January 2006 and 12 subjects had received anti-fungal treatment after January 2006.

The mean responses for these groups were 17.4 mm and 21.9 mm, respectively [STN 125345/0/0, Table 12.3.3, page 37/37]. Although the cohorts are small, it appears that subjects with a more recent diagnosis of pulmonary coccidioimycosis may have a larger delayed type hypersensitivity induration response to Spherusol than those diagnosed at an earlier time point. No studies have been done to evaluate the persistence of the delayed type hypersensitivity reaction following a remote (diagnosed greater than 10 years earlier) history of pulmonary coccidioimycosis.

8.2.9.3 Safety outcomes

The Applicant did not collect local reactogenicity data for reactions occurring at each skin test site individually. Reported local reactogenicity is therefore presumed to be associated with the study product. Events were recorded at 48 (+/- 4 hours) following administration.

A summary of adverse events is presented in Table 14 which corresponds to the cumulative adverse reactions seen in subjects who received Spherusol, Candin and Trichophyton as well as the positive and negative controls. Solicited local reactions were not collected for each individual reagent placed. Local adverse events are presented for the overall occurrences following injection of all skin test reagents and controls. Severe local reactions were seen in two subjects and included itching, swelling and ulceration.

Table 14. Study S104-1. Solicited Local and Systemic reactions within 7 days following administration of Spherusol (1.27 µg/0.1 mL), Candin, Trichophyton, Thimerosal control, Saline Placebo to Subjects with a History of Pulmonary Coccidioidomycosis (N=53)

Symptom	Intensity of adverse event n (%)			
	Any	Mild	Moderate	Severe
Itching;*	45 (85)	19 (36)	25 (47)	1 (2)
Swelling*	42 (79)	19 (36)	22 (41)	1 (2) [±]
Pain*	9 (17)	7 (13)	2 (4)	0
Necrosis/Ulceration*	2 (4)	1 (2)	0	1 (2) [±]
Increased heart rate	2 (4)	1 (2)	1 (2)	0
Weakness	3 (6)	1 (2)	2 (4)	0
Faintness	0	0	0	0
Dizziness	1 (2)	1 (2)	0	0
Nausea/cramps	1 (2)	1 (2)	0	0
Flu-like symptoms	4 (7)	1 (2)	3 (6)	0
Difficulty breathing/shortness of breath	0	0	0	0

Source: STN 125345/0/10, Table 3-1, page 6/18.

Mild= Barely noticeable, not bothersome; Moderate = Distinctly noticeable discomfort; Severe = Needs medical attention

Any = Subjects experiencing adverse event of any intensity

Thimerosal Control contains --(b)(4)-- saline, thimerosal 1: 1,000,000 and 0.4% phenol. The thimerosal concentration is the same as in Spherusol.

N=Number of subjects % = percentage of subjects

*Number and percentages include local reactions occurring at any injection site.

± Same subject --(b)(6)--: treated with oral prednisone

Of the 53 subjects evaluated following administration of the five skin test reagents, 48 (91%) reported solicited adverse events during the 7 days following injection. The most commonly reported solicited local reactions were itching and swelling occurring in 85% and 79% of subjects respectively. Flu-like symptoms were the most commonly occurring systemic reactions, reported by 7% of subjects.

Severe Solicited and Unsolicited Adverse Events

Two subjects reported three severe adverse events.

Subject -(b)(6)-, a 62 year old male, reported 3+ (severe) swelling and oozing 48 hours following administration of Trichophyton. Induration measuring 9.0cm was noted with a 2.0cm central area of vesiculation oozing serous fluid. Oral prednisone 30mg daily was prescribed and after 24 hours marked improvement was observed. The prednisone was decreased to 10mg daily for five days and the subject continued to improve, with resolution four weeks after administration.

Subject -(b)(6)-, a 53 year old female, reported 3+ (severe) itching by diary card. Itching, swelling and ulceration were reported in the CRF. No record was made that identified the reported adverse event with a specific test article.

Unsolicited Adverse Events

One subject reported joint pain, fatigue and cough during the seven days following skin test administration. This finding was reported in the investigator's notes, but was not graded for intensity.

8.2.10 Comments & Conclusions for Study S104-1

A total of 56 subjects with a previous history of pulmonary coccidioidomycosis were enrolled at two sites (Bakersfield, CA and Tucson, AZ) to receive skin testing with five skin test antigens (Spherusol, Trichophyton, Candin, saline placebo and thimerosal negative control). Two subjects were excluded prior to skin testing for not meeting inclusion/exclusion criteria and one subject was later lost to follow-up. A total of fifty-four subjects had skin test antigens placed for evaluation at 48 hours and 53 subjects completed the study. Fifty-one subjects had valid skin test results. Two subjects, one from each site, reacted to one or both of the negative controls and were excluded from further analyses.

Of the fifty-one subjects with evaluable skin test results, 50 subjects had a positive reaction to Spherusol. Thus, 98% (89.6. 1.00) of subjects with a documented history of pulmonary coccidioidomycosis reacted at 48 hours with induration responses measuring 5 millimeters or greater. Based on results of this study, no positive predictive value can be assessed for the use of Spherusol outside this specific population of subjects with a previous history of pulmonary coccidioimycosis.

Of the 53 subjects evaluated following administration of the five skin test reagents, 48 (91%) reported solicited adverse events during the 7 days following injection. The most commonly reported solicited local reactions were itching and swelling occurring in 85% and 79% of subjects respectively. Flu-like symptoms was the most commonly occurring systemic reacting, reported by 7% of subjects. Three severe adverse events (swelling and itching) occurred in two subjects. No serious adverse events occurred during the study.

The adverse event profile was similar to what was seen in the dose-response study done in a similar population with a history of pulmonary coccidioidomycosis. In the dose-response study most subjects were Hispanic (80%), while the majority of subjects in this study were Caucasian (70%). It can not be ascertained from the limited data obtained from these two studies whether populations that experience

more severe disease (e.g., Blacks, Pilipino and Asians) may exhibit more adverse events in association with the use the Spherusol skin test.

8.3 Skin Test Specificity in Adult Volunteers Without a History of Pulmonary Coccidioidomycosis

8.3.1 Applicant's Protocol # and Protocol Title

S104-2 (Spokane, WA) "Skin Test Specificity of 1.27 mcg per 0.1 mL Spherule- derived Coccidioidin in Adult Volunteers Without a History of Pulmonary Coccidioidomycosis"

8.3.2 Objective/Rationale

To evaluate the DTH skin test response to Spherusol in persons without a history of pulmonary coccidioidomycosis or known exposure to the fungus by prior residence or travel in endemic areas for *C.immitis*.

8.3.3 Design Overview

Subjects were screened prior to enrollment with a 1) a medical history questionnaire, 2) signed informed consent, 3) pregnancy test (female), 4) serological evaluation for *C. immitis* (ELISA, immunodiffusion and complement fixation and 5) a residential and travel history to rule out exposure to *C. immitis* or history of coccidioidomycosis.

Enrolled subjects were skin tested with five blinded, randomized reagents on Visit #1 of the study and asked to complete a diary for the next 48 hours.

The results of skin tests were read after 48 hours (\pm 4 hours) on Visit #2 of the study.

Subjects were asked to continue to keep their Daily Diary to monitor possible adverse events until they return to the physician's office for Visit #3 on the 7th day after Visit #2 (Day 10 of the study). Vital signs were to be measured during each visit (#1, 2, and 3).

If subjects were found to be negative to all positive controls a lymphocytic profile was to be done to assess for immunosuppression.

8.3.4 Population

8.3.4.1 Inclusion Criteria

- 18 - 60 years of age
- Overt good health (absence of Active Medical Disease*)
- Lifetime residence in the states of WA, OR, ID, or MT
- Never employed as an agricultural worker
- Serology negative for *C.immitis* antibodies

8.3.4.2 Exclusion Criteria

- Active Medical Disease*
- Alcohol abuse or illicit drug use
- History of coccidioidomycosis, histoplasmosis, blastomycosis
- Influenza-like illness within the past 4 weeks
- Immunizations within the last 4 weeks
- Current atopic or contact dermatitis, psoriasis, erythema nodosum, urticaria
- Current treatment with corticosteroids, cytotoxic or immunosuppressive drugs, systemic antifungal medications
- Immunodeficiency disease

- HIV infection
- Previous skin test with coccidioidin or *Spherulin*
- Pregnant or lactating **
- Adverse reaction to thimerosal
- Adverse reaction to Candida or Trichophyton skin test antigen
- Travel for more than 30 days in designated areas of CA, AZ, NV, UT, NM, TX and Mexico, Central and South America. Travel for more than 7 days in restricted areas of CA, AZ, and TX

* **Active Medical Disease:** Any active physical or psychiatric condition that may increase the risks associated with participation in the study or interferes with the interpretation of study results. Included chronic medical illnesses are cardiovascular disease, renal insufficiency, chronic respiratory illness, cirrhosis, chronic hepatitis, chronic pancreatitis, chronic diarrhea, malnutrition, malignancy, autoimmune disease, and asthma.

** **Pregnancy Prevention:** Females were instructed to abstain from sexual relations or practice a medically acceptable form of birth control during the study. They were also instructed that, except for surgical removal of the uterus, birth control methods such as the use of condoms, a diaphragm or a cervical cap, birth control pills, IUD, or sperm killing products are not totally effective in preventing pregnancy.

To meet study entry criteria subjects were seronegative for *C. immitis* antibodies tested by EIA, immunodiffusion or complement fixation assays. Interpretation of serological results is shown in Table 15.

Table 15. Study S104-2: *Coccidioides immitis* Serologic Tests and Interpretation

EIA (IgM) ¹	Immunodiffusion ²		Complement Fixation ³	Interpretation
	IgG	IgM		
-	-	-	-	negative
i	-	-	-	negative
i	Any other positive test			positive
+	+ or -	+ or -	+ or -	positive
+ or -	+	+ or -	+ or -	positive
+ or -	+ or -	+	+ or -	positive
+ or -	+ or -	+ or -	> 1:2	positive

Source: Laboratory reference values for serologic tests used to determine positive or negative antibodies to *C. immitis*. [Source Supplement C, IND -(b)(4)-, Protocol S104-2, pagination not provided]

- = negative test

+ = positive test

I = indeterminate (inconclusive)

+ or - = positive or negative

Reported Laboratory Results

- | | | |
|---|---------------|---------------|
| 1. EIA (IgM) | O.D. -(b)(4)- | Negative |
| | O.D. -(b)(4)- | Indeterminate |
| | OD -(b)(4)- | Positive |
| 2. Immunodiffusion (precipitin band) | Absent | Negative |
| | Present | Positive |
| 3. Complement Fixation (IgG) – serum dilution | | |

----- (b)(4) ----- Positive

*For EIA (IgM) and Immunodiffusion laboratory results are based on values established by ----- (b)(4) ----- with reagents and references supplied by serologic test manufacturer (----- (b)(4) -----). For Complement fixation (igG) laboratory results, reference values were established by --- (b)(4) --- based on internal controls using reagents prepared by --- (b)(4) --- and commercial vendors. [Source Supplement C, IND - (b)(4) -, Protocol S104-2, pagination not provided]

8.3.5 Products mandated by the protocol

Each subject was skin tested with an intradermal injection (0.1 mL) of the study product, two positive controls, Candin and Trichophyton and two negative controls (Saline and thimerosal control).

Table 16. Study S104-2: Skin Test Reagents for use in Study S104-2

Reagent	Color	Code	Purpose
Spherusol (1.27µg/0.1mL) Lot # XSN04220301	Green	4101	Evaluate DTH response in subjects without a history of pulmonary coccidioidomycosis or known exposure to <i>C.immitis</i> from prior residence or travel in endemic areas
Thimerosal Control Lot # ----- (b)(4) -----	Red	6849	Evaluate DTH response to residual thimerosal(1:1,000,000) in Spherusol from ----- (b)(4) ----- with diluent containing 1: 1,000,000 concentration of thimerosal
Placebo Control Lot # XDf06020301	Black	3546	Evaluate DTH responses to ingredients in the -- (b)(4) -- saline solution used to prepare Spherusol
Candin Lot #CA033	Blue	1287	Evaluate subject's ability to elicit a positive DTH response
Trichophyton Extract Lot # XMm11080401	Yellow	5461	Evaluate subject's ability to elicit a positive DTH response

Source: Table: Skin test Reagents, page 12/31, section 8.4.2.

8.3.6 Endpoints

Primary endpoint was induration response for each antigen at 48 hours.

The delayed type hypersensitivity response of each skin test agent was measured by recording induration at the skin test site 48 hours after injection. The induration response was outlined with a black ballpoint pen and a permanent record was made by overlaying the tracing with transparent tape and placing the tape on the skin test record. The longest and orthogonal diameters of the tracing were measured in mm. Reactions ≥ 5 mm were considered to be a positive skin test. Induration less than 5mm at 48 hours demonstrated the absence of sensitivity to the test article and induration ≥ 5 mm at 48 hours demonstrated sensitivity to the test article in the population studied.

Safety was measured by reporting local and systemic reactions that occurred for 7 days after the skin tests were administered. A diary of adverse events was completed by each subject for the duration of the study.

Solicited local reactions included swelling, itching, pain, and necrosis. It does not appear that reactions were assessed by reagent received but as occurrence of any of the solicited reactions at any of the skin test

placement sites. Systemic responses that were solicited included flu-like symptoms, increased heart rate, nausea/cramps, fatigue, weakness, faintness, difficulty breathing.

8.3.7 Safety Surveillance

The study report does not provide documentation of specific monitoring at each skin test site. Systemic responses that were monitored included flu-like symptoms, increased heart rate, nausea/cramps, fatigue, weakness, faintness, difficulty breathing. The Sponsor had stated that a diary card was to be used to capture adverse events, but no toxicity grading scale for events or diary card examples are provided.

8.3.8 Statistical considerations

The Applicant- defined specificity was the proportion of persons in the study population without previous exposure to *C.immitis* who demonstrated a negative DTH test after placement of Spherusol. Subjects were to be excluded from the specificity analysis if they reacted to the negative controls, the saline placebo and/or the thimerosal control.

8.3.9 Results

8.3.9.1 Populations enrolled/analyzed

Sixty-one (61) subjects signed informed consent documents. One subject failed to meet all inclusion criteria. Sixty (60) subjects were enrolled and completed the trial as outlined in the protocol. By the Applicant's report, serological testing for *C. immitis* antibodies performed prior to administration of the skin test antigens and controls was negative for all enrolled subjects.

Of the 60 subjects enrolled, 38 (65%) were women. Fifty-eight (96%) subjects were Caucasian. One (1) subject (2%) was Hispanic and one (2%) subject was Asian. The median age of study participants was 31 (range 18-56).

Study participants had never lived in endemic areas for *C.immitis* including CA, AZ, NV, UT, NM, TX or Mexico and South and Central America. Enrollment was limited to subjects whose travel history was limited to 7 days in highly endemic areas and 30 days in other endemic locales.

8.3.9.2 Delayed Type Hypersensitivity Reactions

A total of 60 subjects had skin test antigens placed per the protocol. One subject (-(b)(6)-) demonstrated a 5 mm reaction to thimerosal diluent control and a negative reaction to Spherusol. This subject was not excluded from further analyses. The Placebo Control (saline) elicited induration responses of 2.0mm (-(b)(6)-) and 4.0mm (-(b)(6)-). The Thimerosal Control elicited induration responses of 3.5mm (-(b)(6)-), 4.0mm (------(b)(6)-----), and 5.0mm (-(b)(6)-), none of these subjects exhibited a positive induration response to Spherusol.

Spherusol did not elicit a positive DTH skin test in 54 of 55 subjects with skin test results. One subject (-(b)(6)-) had a 5mm induration response to Spherusol which was reported as a positive skin test to the antigen. This subject (-(b)(6)-) had traveled on a limited basis in AZ and CA. A second subject (-(b)(6)-) showed an induration response of 4.5 mm which was reported as negative. Five subjects had a negative response to all skin test antigens and controls. Assessment of the lymphocyte subsets was performed to ensure that skin test negative subjects were not immunocompromised and incapable of mounting an immune response. Blood samples were taken from five subjects (------(b)(6)-----) for lymphocyte analysis. In all five cases, the lymphocyte profile was normal. It is unclear why these five subjects did not react to any skin test antigen. Placement technique may have been a factor since this finding of anergy to all test articles was not seen at other clinical sites.

The five subjects with unevaluable results (negative to all skin test antigens and controls) in the analysis of induration reactions were evaluated as if these responses had represented positive reactions to Spherusol. In this analysis, a negative induration response was seen in 54 of 60 (90% with 95% CI [79.5%, 96.2%]) subjects who had no history of coccidioimycosis or prolonged travel to an area endemic for *C. immitis*.

8.3.9.3 Safety outcomes

For the sixty subjects enrolled in S104-2 adverse events were reported by 53 (83%) subjects following the administration of the five skin test reagents. The most frequently reported adverse events were itching, swelling and pain. Swelling was reported by 42 (70%) subjects, itching was reported by 40 (67%) subjects, and pain was reported by 15 (25%) subjects over the 7 day study period. During the first 48 hours after skin tests were administered 15 (25%) subjects reported itching, 7 (12%) subjects reported swelling and 7 (12%) subjects reported pain. During the 48-72 hour time period, 5 (8%) subjects reported itching, 4 (7%) subjects reported swelling and 1 (2%) subject reported pain. Beyond 72 hours, itching was reported by 18 (30%) subjects, swelling by 31 (52%) subjects and pain by 7 (12%) subjects.

Solicited Local and Systemic Adverse Events

Local adverse events included mild to moderate itching, swelling, and pain attributed to the sites of positive skin tests. According to the Applicant, these events were observed as reactions to the Candin and/or Trichophyton Extract and not to the study product, Spherusol. However, no data were submitted to support this claim. Since subjects were not evaluated for reactions at each specific site, all local reactions were considered as attributable to Spherusol.

Table 17. Study S104-2: Solicited Local and Systemic Reactions within 7 Days following Administration of Spherusol (1.27 µg/0.1 mL), Candin, Trichophyton, Thimerosal control, Saline Placebo to Subjects Without a History of Pulmonary Coccidioidomycosis or Travel to Endemic Areas (N=60)

Symptom	Intensity of adverse event n (%)			
	Any	Mild	Moderate	Severe
Itching*	41 (68)	25 (42)	15 (25)	1± (~2)
Swelling*	44 (73)	29 (48)	14 (23)	1± (~2)
Pain*	16 (27)	11 (18)	5 (8)	0
Necrosis/Ulceration*	3 (5)	1 (~2)	1 (~2)	1 (~2)**
				0
Increased heart rate	2 (3)	2 (3)	0	0
Weakness	3 (5)	3 (5)	0	0
Faintness	2 (3)	2 (3)	0	0
Dizziness	1 (2)	1 (~2)	0	0
Nausea/cramps	5 (8)	3 (5)	2 (3)	0
Flu-like symptoms	4 (7)	2 (3)	2 (3)	0
Difficulty breathing/shortness of breath	2 (3)	1 (~2)	1 (2)	0

Source: STN 125345/0/10, Table 3-2, page 6/17.

Mild= Barely noticeable, not bothersome

Severe = Needs medical attention

Moderate = Distinctly noticeable discomfort

Any = Subjects experiencing adverse event of any intensity

Thimerosal Control contains --(b)(4)-- saline, thimerosal 1: 1,000,000 and 0.4% phenol. The thimerosal concentration is the same as in Spherusol.

N=Number of subjects %= percentage of subjects

*Number and percentages include local reactions occurring at any injection site

± Same subject, -(b)(6)- reported grade 3 swelling and itching that did not require medical treatment.

** Subject -(b)(6)- reported the ulceration as mild, but was treated by the attending physician with Elocon (Mometasone Topical), thus meeting the criteria for a severe reaction

Severe Adverse Events

Three subjects (------(b)(6)-----) reported ulceration at a skin test site. Each of these subjects had an induration response at the skin test site for Candin only. The induration response at the Candin site was measured at 7, 11, and 13 mm respectively. Of note, two of the cases of reported ulceration were felt to be “dermatitis” when evaluated by the study physicians.

Subject -(b)(6)- self-treated the area of induration/ulceration with Neosporin for 6 days. Subject -(b)(6)- was treated with Elocon (Mometasone Topical) for two weeks by the attending physician. Subject -(b)(6)- reported ulceration within the first 48 hours after administration and was graded mild in severity.

Subject -(b)(6)- reported severe itching and swelling via daily diary card. No treatment was sought and no medications used to treat these reactions. The event resolved without sequelae.

The systemic adverse events shown above were reported by five subjects in their daily diaries. Several events were reported by the same subjects (------(b)(6)-----). All of the events resolved without intervention.

Unsolicited Adverse Events

Unsolicited adverse events of a mild or moderate intensity were reported by twelve subjects and included: headache, diarrhea, fatigue, dermatitis, upper respiratory symptoms, muscle tightness and fever. All events occurred within 7 days of the administration of the skin tests and resolved without sequelae.

Serious Adverse Events

No serious adverse events were reported during the study.

8.3.10 Comments & Conclusions for Study S104-2

The Applicant has provided data to demonstrate negative delayed type hypersensitivity reactions to Spherusol in a population of subjects who had no previous history of a diagnosis of pulmonary coccidioidomycosis, negative serologies for antibodies to *C. immitis* and no history of prolonged travel to endemic areas. Five subjects (8%) did not show reactions to any of the skin test antigens or controls administered. Analyses of the rate of negative reactions to account for this finding led to including the subjects as if they had had a positive response to Spherusol to illustrate the worst case scenario for the study product. This gave specificity for Spherusol in this population of 90% (79.5%, 96.2%).

For the sixty subjects enrolled in S104-2 adverse events were reported by 53 (83%) subjects following the administration of the five skin test reagents. The most frequently reported adverse events were itching, swelling and pain. Swelling was reported by 42 (70%) subjects, itching was reported by 40 (67%) subjects, and pain was reported by 15 (25%) subjects over the 7 day study period.

8.4 Skin Test Specificity in Adult Volunteers with a History of Pulmonary Histoplasmosis

8.4.1 Applicant's Protocol # and Protocol Title

S104-3: "Skin Test Specificity of 1.27 mcg per 0.1 mL Spherule-derived Coccidioidin in Adult Volunteers With a History of Pulmonary Histoplasmosis" (Blair, NE)

8.4.2 Objective/Rationale

- To determine if Spherusol elicits a positive DTH skin test in persons with a history of pulmonary Histoplasmosis.

It was unknown if the spherule-derived *C. immitis* skin test antigen would cross-react with antibodies to *H. capsulatum* to give a delayed type hypersensitivity reaction in individuals who had previously been diagnosed with Histoplasmosis. To determine if there would be cross reaction in individuals who were previously infected with *H. capsulatum*, a study was designed to enroll subjects in a known *H. capsulatum* endemic area with a history of pulmonary Histoplasmosis who had not travelled to regions endemic for *C. immitis*.

Clinical definition of Histoplasmosis included fever and at least one additional symptom (headache, cough, chest pains and/or shortness of breath). In the individuals with the above symptom complex, serology was performed with confirming evidence being a complement fixation (CF) titer $\geq 1:32$ and/or the presence of an "H" or "M" band by immunodiffusion. From a MMWR report [November 5, 2004 / 53(43); 1020-1022], of the 724 potentially exposed persons, 108 had symptoms that were consistent with the case definition. Twenty-five (25) of these individuals had a positive Histoplasma serology.

8.4.3 Design Overview

The study was a double blinded, non-randomized observational study.

Eligible study participants were identified by -----(b)(4)----- . Of the twenty-five identified individuals, 13 volunteers consented to participate in the study. All volunteers were screened prior to enrollment with a participant questionnaire, informed consent documents, pregnancy test (female), serologic evaluation for *C.immitis* and *H. capsulatum* infection and a residential and travel history. Eligible participants were skin tested on Visit # 1 and asked to complete a diary for the next 48 hours. The results of skin tests were read after 48 hours (\pm 4 hours) on Visit # 2. Subjects were asked to continue to keep a diary to monitor possible adverse events until they returned to the physician's office one week later. Vital signs were measured during each visit and subjects with a positive serology to *H. Capsulatum* were given a final physical exam to assess the status of their health.

No subjects received anti-fungal medications/treatments during the course of the study.

8.4.4 Population

Subjects with previous history of pulmonary histoplasmosis confirmed by laboratory testing with no travel or residence in areas endemic for *C. immitis*.

No age limits or gender specifications are noted in the study.

Inclusion Criteria

- 18 years of age or older
- Overt good health (absence of Active Medical Disease*)
- History of pulmonary histoplasmosis confirmed by serology

Exclusion Criteria

- Active Medical Disease*
- Alcohol abuse or illicit drug use
- History of coccidioidomycosis
- Influenza-like illness within the past 4 weeks
- Immunizations within the last 4 weeks
- Current atopic or contact dermatitis, psoriasis, erythema nodosum, urticaria
- Current treatment with corticosteroids, cytotoxic or immunosuppressive drugs
- Immunodeficiency disease
- HIV infection
- Previous skin test with coccidioidin
- Pregnant or lactating**
- Adverse reaction to thimerosal
- Adverse reaction to Candida or Trichophyton skin test antigen

***Active Medical Disease:** Any active physical or psychiatric condition that may increase the risks associated with participation in the study or interferes with the interpretation of study results. Included chronic medical illnesses are cardiovascular disease, renal insufficiency, chronic respiratory illness, cirrhosis, chronic hepatitis, chronic pancreatitis, chronic diarrhea, malnutrition, malignancy, autoimmune disease, and asthma.

** **Pregnancy Prevention:** Females were instructed to abstain from sexual relations or practice a medically acceptable form of birth control during the study. They were also instructed that, except for surgical removal of the uterus, birth control methods such as the use of condoms, a diaphragm or a cervical cap, birth control pills, IUD, or sperm killing products are not totally effective in preventing pregnancy.

Removal of Subjects from the Study

Participant Initiated: Volunteers were allowed to withdraw from the study at any time without prejudice or loss of benefits to which they were entitled.

Investigator Initiated: Volunteers could be removed from the study at any time by the principal investigator if they failed to meet all inclusion criteria or if their continued participation was judged to be injurious to their health and well being.

8.4.5 Products mandated by the protocol

Each subject received an intradermal injection of the study product, Spherusol, Trichophyton skin test antigen, Candin skin test antigen and the negative controls of placebo (--(b)(4)-- saline) and 1:1,000,000 thimerosal.

Table 18. Study S104-3: Skin Test Reagents (Blair, NE)

Reagent	Color	Code	Purpose
Spherusol (1.27µg/0.1mL) Lot # XSN04220301	Green	4101	Evaluate DTH response in subjects with a history of pulmonary histoplasmosis
Thimerosal Control Lot # ----(b)(4)-----	Red	6849	Evaluate DTH response to residual thimerosal(1:1,000,000) in Spherusol from Thimerosal Positive Control
Placebo Control Lot # XDF06020301	Black	3546	Evaluate DTH responses to ingredients in the --(b)(4)-- saline solution used to prepare Spherusol
Candin Lot # CA033	Blue	1287	Evaluate subject's ability to elicit a positive DTH response
Trichophyton Extract Lot # XMm11080401	Yellow	5461	Evaluate subject's ability to elicit a positive DTH response

Source: Study 104-3, section 8.4.2, page 11/21.

8.4.6 Endpoints

The endpoints of the study are not clearly elucidated in the study report. The outcome monitored was the induration response for each skin test agent at 48 hours following placement. Safety (local and systemic adverse events) was monitored by diary card. No information is provided on the length of monitoring, the parameters for assessment, or grading scale of adverse events.

8.4.7 Surveillance

“Efficacy”: measured by recording induration at the skin test site after 48 hours. The induration response was outlined with a black ballpoint pen and a permanent record was made by overlaying the tracing with transparent tape and placing the tape on the skin test record. The longest and orthogonal diameters of the tracing were measured in mm. Reactions ≥ 5 mm were considered to be a positive skin test.

“Safety”: measured by reporting local and systemic reactions that occurred after skin tests were administered. A diary of adverse events was completed for the duration of the study.

8.4.8 Statistical considerations

The Fisher's Exact Test was used to calculate the 95% two-sided confidence limits for the specificity of Spherusol.

8.4.9 Results for Study S104-3

8.4.9.1 Populations enrolled/analyzed

From a population of 25 adults previously diagnosed with Histoplasmosis in 2004, 13 volunteers consented to participate in the study. Twelve subjects qualified for enrollment.

All 12 subjects were Caucasian adults, 33 to 60 years of age [42%female]. The median age was 46 (range 33-60) years.

8.4.9.2 Delayed-type Hypersensitivity Reactions

Skin test responses were measured for induration at 48 hours after administration.

Table 19. Study S104-3: Induration \geq 5mm at 48 hours Following Administration for All Skin Test Reagents (N=12)

Negatives Controls		Positive Controls		Study product
Saline Placebo	Thimerosal	Candin	Trichophyton	Spherusol
0/12	0/12	11/12	6/12	0/12

Source: Table titled "Induration \geq mm at 48 hours to Skin Test Reagents", section 10.4.1, page 14/21.

All subjects enrolled were negative (defined as induration $<$ 5 mm) to the Spherusol skin test. All subjects had a positive induration response to either or both of the positive controls, Candin and Trichophyton. There were no induration responses to the negative controls, thimerosal diluent and --(b)(4)-- saline, thus all results were valid.

From these results it appears that a previous infection with *H. Capsulatum* does not induce conversion to a positive skin test response with Spherusol.

8.4.9.3 Safety outcomes

Adverse events were reported by each of the 12 subjects (100%) enrolled in this study. Itching and swelling at the skin tests sites were the most frequently documented adverse events. Itching was reported by 8 (67%) subjects and swelling was reported by 9 (75%) subjects. Other solicited adverse events included pain, nausea and cramps, flu-like symptoms and difficulty breathing or shortness of breath which were each reported by 8% of the study population. The duration of specific adverse events was as follows: Itching was reported during the 0 - 24 hour period by 3 (25%) subjects, swelling by 1 (8%) subject and nausea by 1 (8%) subject. Between 24 – 48 hours 5 (42%) subjects reported itching and swelling, 1 (8%) subject reported pain, 1 (8%) subject reported flu-like symptoms and 1 (8%) subject reported difficulty breathing. After 48 hours, 2 (16%) subjects reported swelling. All adverse events were mild to moderate in severity and resolved within 7 days after skin testing.

Table 20. Study S104-3: Solicited Local and Systemic Reactions within 7 Days following Administration of Spherusol (1.27 μ g/0.1 mL), Candin, Trichophyton, Thimerosal control, Saline Placebo to Subjects With a History of Pulmonary Histoplasmosis (N=12)

Symptom	Intensity of adverse event n(%)			
	Any	Mild	Moderate	Severe
Itching*	8 (67)	6 (50)	2 (17)	0
Swelling*	9 (75)	7 (58)	2 (17)	0
Pain"	1 (8)	1 (8)	0	0
Necrosis/Ulceration*	0	0	0	0
Increased heart rate	0	0	0	0
Weakness	0	0	0	0
Faintness -	0	0	0	0
Dizziness	0	0	0	0
Nausea/cramps	1 (8)	1 (8)	0	0
Flu-like symptoms	1 (8)	0	1 (8)	0
Difficulty breathing/shortness of breath	1 (8)	0	0	1(8)

Source: STN 125345/0/10, Table 3-3, page 6/17.

Mild= Barely noticeable, but not bothersome

Moderate= definitely noticeable, discomfort

Severe=needs medical attention

Any= Subjects experiencing adverse event of any intensity

Thimerosal Control contains --(b)(4)-- saline, thimerosal 1: 1,000,000 and 0.4% phenol. The thimerosal concentration is the same as in Spherusol.

N=Number of subjects experiencing intensity of adverse events

%= percentage of subjects experiencing intensity of adverse events

"Number and percentages include local reactions occurring at any injection site

Severe and Unsolicited Adverse Reactions

One severe reaction was reported during the course of the study. Subject -(b)(6)- experienced a single episode of chest tightness and wheezing lasting approximately 15 minutes within 24 hours of skin test placement. (Review of subject's entry criteria did not show any previous history of allergy or asthma). These symptoms were resolved with a single dose of Albuterol. This reaction was originally graded as a mild reaction by the Applicant, but because of the need for prescription medication the intensity of the reaction was revised to Grade 3/severe. This subject had a 30 x 30 mm DTH response to Candin; all other skin tests were negative. Information in the description of the adverse event and review of the case report forms does not clarify if this subject had a history of asthma or other respiratory conditions which might require the use of a beta-agonist inhaler.

Subject -(b)(6)- experienced nausea without vomiting, diarrhea, fever or chills the evening following skin testing. This person had positive DTH skin tests to Candin; all other skin tests were negative.

Serious Adverse Events

There were no serious adverse events reported during the course of the study.

8.4.10 Comments & Conclusions for Study S104-3

No induration reactions to Spherusol were noted in this study of 12 subjects with a known diagnosis of histoplasmosis, without history of prolonged travel to *C. immitis* endemic areas. All skin tests with Spherusol demonstrated a < 5 mm induration and were considered to be negative. This finding is supportive for an overall lack of cross-reaction of *C. immitis* and *H. capsulatum* induced cellular immune responses. It is likely that people with exposure to *H. capsulatum*, but with no disease manifestations, would be non-reactive to Spherusol.

The majority of local adverse reactions were itching and swelling at unidentified skin test sites. No severe reactions were seen. Two subjects reported systemic adverse events. One subject reported mild difficulty breathing, and required treatment with a prescription inhaled β agonist within 24 hours of antigen placements. Another subject reported mild flu-like symptoms which included nausea and vomiting within 24-48 hours after skin test placements. No information is provided on the resolution or follow-up to this event.

9 Overview of Positive and Negative Induration Responses across Trials

9.1 General Discussion of Endpoints

9.1.1 Study Design

The four studies submitted in the application included one dose finding study, one study to assess sensitivity in a specific population and two studies to assess specificity of Spherusol in different

populations. Three of the study populations [S101A, S104-1 and S104-2] were defined by past exposure and disease caused by *C. immitis*. The fourth study population [S104-3] represented individuals with previous diagnosis of exposure and disease caused by *H. capsulatum*.

Subjects in studies S104-1, S104-2 and S104-3 were evaluated at 48 hours after skin test placement for induration responses to the Spherusol and the positive and negative controls. Subjects were followed for solicited local and systemic reactions for 7 days following administration of the skin tests and controls. Local reactions other than induration were not assessed by findings at the individual skin test sites by the Applicant, so all local reactions were attributed to Spherusol in the evaluation of adverse events. Systemic adverse events were also attributed to Spherusol.

9.1.2 Findings

A positive induration response (defined as ≥ 5 mm of induration) to Spherusol was seen in 98% (95%CI: 89.6%, 100%) of subjects with a previous history of coccidioidomycosis at 48 hours following intra-dermal administration 0.1 μ g of Spherusol in Study S104-1.

A negative induration response (defined as <5 mm of induration) to Spherusol was seen in 90 % (95%CI: 79.5%, 96.2%) of subjects who had no history of disease caused by *C. immitis*, negative serologies for antibodies to *C. immitis* or prolonged travel to an area endemic for *C. immitis*. Five subjects enrolled in this study did not react to any skin test antigen or control. These subjects were analyzed as having a positive induration response to Spherusol to illustrate the worst case scenario for the study product.

A positive induration response to Spherusol was seen in 0% (1-sided 97.5% CI: 0%, 26.5%) of subjects (N=12) who had a previous history of disease caused by *H. capsulatum* and no history of travel to areas endemic for *C. immitis*. This supports the lack of cross-reaction between the cellular immune responses induced by the two fungus species.

9.2 Conclusions

In subjects with a known history of infection with *C. immitis* resulting in pulmonary coccidioimycosis, a positive induration reaction was seen 48 hours following administration of Spherusol in 98% (95%CI: 89.6%,100%) of individuals. In individuals with a no history of disease caused by *C. immitis* a negative induration response was seen in 90% (95%CI: 79.5%, 96.2%) of those skin tested with Spherusol. No induration was seen following administration of Spherusol in a small group of subjects (N=12) with antibodies to *H. capsulatum* and no infection known infection with *C. immitis*, supporting a lack of cross-reaction with the Spherusol skin test.

Although the sensitivity and specificity of the product can be assessed for each specific population enrolled in the corresponding studies, it is not possible to ascertain a predictive value for Spherusol in a general population. Caution should be used when interpreting results, either negative or positive induration, outside of the defined study populations. The available data do not support use of Spherusol as a diagnostic test of active infection with *C. immitis*.

10 Overview of Safety Across Trials

10.1 Safety Database - Number of Subjects, Types of Subjects and Extent of Exposure

Four trials enrolling 146 subjects who received at least one dose of Spherusol were submitted in support of licensure. Twenty subjects enrolled in Study S101A simultaneously received three to four doses of varying strengths of Spherusol as part of the dose response study. Please see Table 1 of this review for a brief description of the studies and the populations enrolled.

10.2 Potentially Significant Events

10.2.1 Deaths

No deaths were reported during the clinical studies.

10.2.2 Human Reproduction and Pregnancy Data

No pre-clinical or clinical studies have been conducted on the use of Spherusol and its effect on fertility and reproduction or during pregnancy.

11 Additional Clinical Issues

11.1 Directions for Use

Spherusol is supplied as a clear, colorless solution in a 1 mL multi-dose vial. Spherusol is injected intradermally with a 26-27 gauge needle and tuberculin syringe. The needle point should be inserted into the skin at a 15-20 degree angle. The angular distance of penetration should be approximately 1.5 mm to pass through the epidermis of the volar surface of the forearm.

11.2 Dose Regimens and Administration

The dose of Spherusol used in three clinical studies was 1.27 µg/0.1 mL. This dose will be specified in the Dosage and Administration section of the prescribing information. The route of administration will be as an intradermal injection on the volar surface of the forearm. The skin test is read at 48 hours following placement to assess for induration response. A mean of the orthogonal diameters of the indurated area around the injection site is used to determine a positive or negative response to Spherusol.

There have been no studies on re-administration of Spherusol.

11.3 Special Populations

No studies have been conducted in children, pregnant or nursing women or the elderly (≥ 65 years of age).

11.4 Pediatrics

Spherusol was designated as an Orphan Drug and qualifies for Orphan Drug exception status under section 736 (a) (1)(E) of the federal Food, Drug and Cosmetic act (21 USC 36bb). Therefore, per 21 USC 355c [505.b, section k], PREA regulations are not applicable. The Applicant has not proposed or conducted studies in pediatric populations. The prescribing information will be limited to use in adults.

12 Conclusions – Overall

The safety and induration response data support the approval of Spherusol for use as a skin test antigen for the detection of delayed type hypersensitivity in healthy individuals age 18-64 years with a past history of pulmonary coccidioimycosis.

13 Recommendations

13.1 Approval, Non-approval, Conditions

Spherusol is recommended for approval as a skin test antigen indicated for the detection of delayed type hypersensitivity to *Coccidioides immitis* in individuals with a history of pulmonary coccidioidomycosis. Spherusol is approved for use in individuals 18-64 years of age.

It is recommended that the limitations of use for Spherusol include:

- The use of Spherusol to detect delayed type hypersensitivity response in individuals with unknown exposure to *C. immitis* has not been evaluated.
- Persons with acute or disseminated coccidioidomycosis may not develop a delayed type hypersensitivity response to Spherusol.
- Persons with immunodeficiency and a history of coccidioidomycosis may not develop a delayed type hypersensitivity response to Spherusol."

13.2 Recommendation on Postmarketing Actions

There will be no post-market commitments or requirements for this application.

14 Labeling

The original package insert submitted in the BLA was in the format required by FDA's Final Rule titled "Requirements on Content and Format of Labeling for Human Prescription Drug and Biological Products" published in January 2006. Revisions to the prescribing information proposed by the applicant were made after CBER review of the BLA and communications with the applicant.