

The Return of Delayed-Type Hypersensitivity Skin Testing for Coccidioidomycosis

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A skin test that detects dermal hypersensitivity in persons with past infection with *Coccidioides* species is again available for clinical use. Nearly all of the clinical studies with similar materials were published prior to the 1990s, and as a result, many practicing physicians will be unfamiliar with how skin testing for coccidioidomycosis might be useful in patient management or as a research tool. We review clinical and epidemiological studies with past skin test antigens, the composition of past and current skin test preparations with particular attention to differences in the preservatives, and how the current preparation could be used today.

Keywords. coccidioidomycosis; delayed type hypersensitivity; skin test; cellular immunity; diagnostic test.

Coccidioidomycosis, also known as Valley fever, causes approximately 150 000 infections per year in the United States, resulting in 111 717 case reports to the Centers for Disease Control and Prevention between 1998 and 2011 [1]. The endemic regions for coccidioidomycosis lie in the San Joaquin Valley of California and the deserts of the southwestern United States and northwestern Mexico, with foci in areas of Central and South America [2]. Isolated locations have been found unexpectedly in northern Utah, and as far north as Washington State [3, 4]. Coccidioidomycosis is caused by soil-dwelling fungi now recognized as 2 species, *Coccidioides immitis* and *Coccidioides posadasii* [5]. They grow as mycelia in the soil and produce 3- to 5- μ M spores called arthroconidia [6], which, if inhaled by a human or other mammal, initiate infection. Once in the host, arthroconidia undergo a unique transformation, enlarging isotropically to 50–100 μ M in diameter

and developing internal septations. The resulting structures, called spherules, contain scores of viable progeny (endospores), each of which can propagate when the mature spherule ruptures. In most human infections, this process is arrested by a cellular immune response that results in lifelong immunity [7]. Infection is often subclinical, but can also cause a range of syndromes from a self-limited pneumonia [8, 9] to progressive infection in the lungs [10] to destructive lesions beyond the thoracic cavity (disseminated infection) [11]. Severe or disseminated disease is known to be associated with male sex, extremes of age, and Filipino and African descent.

Like tuberculosis, coccidioidomycosis is a granulomatous disease that stimulates a cellular immune response and generates a delayed-type hypersensitivity (DTH) reaction to the intradermal inoculation of antigens prepared from the organism. This DTH response is commonly called a skin test (ST) conversion. Since the 1940s, DTH to coccidioidal extracts have been used both to study the epidemiology of coccidioidomycosis and to assist in the clinical management of patients. However, since the late 1990s, there has not been a commercial source of these reagents in the United States and, as a result, clinicians who have trained in the past 20 years generally have no experience with skin testing for coccidioidomycosis. Recently, a newly formulated, spherule-derived antigen preparation (Spherusol,

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Nielsen Biosciences, San Diego California) has been approved by the Food and Drug Administration (FDA) as a test for cellular immunity in patients known to have had a coccidioidal infection. Because this will be an unfamiliar resource for many currently practicing clinicians, we provide here a review of experience with past skin testing products and discuss how Spherusol might be useful in practice now. A more detailed analysis of past skin testing products can be found in an excellent review by Drutz and Catanzaro [12].

EARLY WORK WITH COCCIDIOIDIN

A filtrate of coccidioidal mycelia grown in broth was developed and standardized by C. E. Smith and colleagues in the 1930s for ST purposes [13]. “Coccidioidin,” as this ST antigen was called, was derived from multiple isolates of *Coccidioides* species from patients in California, Arizona, Texas, and elsewhere in the endemic regions of the United States. Although the genetic differences between *C. immitis* and *C. posadasii* were not yet known, the diverse geographic sources of the isolates almost certainly included representatives of both species [13, 14]. Coccidioidin was eventually made commercially available in a 1:100 dilution as the standard dose and in a 1:10 dilution as the high test strength and administered intradermally (0.1 mL). Preparations of coccidioidin were available in the United States until the late 1990s.

EPIDEMIOLOGIC STUDIES ON MILITARY PERSONNEL IN THE SAN JOAQUIN VALLEY, 1940–1941

Epidemiologic studies using coccidioidin were carried out during 1940–1941 on military personnel in the San Joaquin Valley in California to identify persons who had not been infected before entering the endemic area, to determine the extent of illness associated with ST conversion, and to evaluate the relationship of infection to the time of year, climatic conditions, and geography [15]. To summarize the conclusions of these studies, ST conversions were often asymptomatic, recruits with negative ST were susceptible to acquiring clinical coccidioidomycosis, and the development of a positive ST was associated with protection against illness from a subsequent exposure to *Coccidioides* species. Furthermore, symptomatic infections were rarely fatal. It was also noted that black male soldiers were 10-fold more likely to develop disseminated disease, and in these persons the ST often failed to convert to positive. With respect to incidence, there was a distinct seasonal variation in the infection rate, with highest rates in the summer when there was very little rainfall and conditions were dusty [16]. It was also noted that risk of infection was very uneven in areas of known risk, and the infection was not limited to the San Joaquin Valley [17].

SPHERULIN REPLACES COCCIDIOIDIN

In the 1950s, methods were developed to grow spherules in the laboratory on a synthetic medium [18–20]. Using this new technology, in vitro–grown spherules were used as a new source of a coccidioidal ST antigen [21–27]. A commercial ST preparation, spherulin (Berkeley Biologicals, Emeryville, California), was introduced in the late 1970s. This spherulin was made available in the usual strength (2.8 µg per dose), roughly equivalent to the 1:100 coccidioidin, and the high test strength (28 µg per dose), comparable to 1:10 coccidioidin. In studies of persons living in the San Joaquin Valley using standardized preparations of coccidioidin and spherulin, spherulin was reactive in approximately one-third more individuals than was coccidioidin, and rates of reactivity increased with length of time of endemic exposure [24]. Furthermore, subjects who reacted to coccidioidin always appeared to react to spherulin [26]. Both ST reagents were demonstrated to be nonreactive in patients from outside endemic areas and rarely reactive in patients with fungal infections other than coccidioidomycosis [25]. Whether this superior performance of spherulin was due to its production from the tissue phase of the fungus or simply the result of slightly larger doses of the antigens was never resolved [28]. In 1987 a new, more potent spherulin with the “usual strength” of 1.27 µg/0.1 mL, titrated to the 1:100 coccidioidin standard, replaced the earlier preparation. This was available to clinicians until sales were discontinued in 1999 (T. Carpenter, Nielsen Biosciences, personal communication).

THIMEROSAL AND DERMAL SENSITIZATION

Thimerosal is a mercury-containing compound used as a preservative for vaccines and other biological products. C. E. Smith and colleagues originally selected thimerosal in a concentration of 1:10 000 for the early coccidioidin preparation, and this practice continued as the ST product evolved. Although it is generally considered to be safe and effective, there is evidence associating its use in vaccines with dermal sensitization. Work with coccidioidin containing thimerosal and trials using thimerosal alone indicated that there is an increasing frequency of allergy to thimerosal, which appears to increase the likelihood of a positive ST on retesting [29, 30]. Further studies have shown that this tendency can be resolved by dilution [31].

THE REINTRODUCTION OF COCCIDIOIDAL SKIN TESTING

Since the discontinuation of spherulin in the late 1990s and until 2014, a commercial coccidioidal ST preparation has been unavailable. However, a large bulk supply of the concentrated antigen preparation used to make spherulin still existed,

was kept in appropriate storage, and was very stable. A company (Allermed Laboratories, San Diego, California) obtained the rights to this supply and reformulated the preparation by substituting phenol for thimerosal as the preservative used during dilution of the stock, thereby reducing the thimerosal concentration to 0.0001% of that in spherulin. Allermed then opened an Investigational New Drug application with the FDA, and conducted the clinical trials necessary to support an FDA approval to market a new commercial product (Spherusol) as a test for prior coccidioidal infection [32]. In one study, individuals with no history of or likely exposure to coccidioidal infections in Spokane, Washington, were tested. Of 59 evaluable patients, only 1 individual had a positive response. In another study in Blair, Nebraska, an area endemic for histoplasmosis, 12 of 12 subjects with a past history of pulmonary histoplasmosis tested with Spherusol were negative. In a pair of phase 3 sensitivity studies undertaken in Bakersfield, California, and Tucson, Arizona, a total of 52 of 53 subjects known to have had primary pulmonary coccidioidomycosis reacted positively, and the adverse event rate was low. These 4 studies established that the reformulated spherule-based ST antigen was safe and demonstrated sensitivity and specificity of >98% [32]. Prior therapy with an azole antifungal did not appear to alter the sensitivity of the test in these patients. Specifically, 14 subjects with a recent diagnosis of primary pulmonary coccidioidomycosis, positive skin tests, and therapy with fluconazole were compared with 26 subjects with the same diagnosis and ST results but no fluconazole therapy. There was no difference in magnitude of ST reaction. These studies subsequently led to the approval of Spherusol by the FDA as an ST reagent specifically to test for cellular immunity in patients 18–64 years of age with an established history of pulmonary coccidioidomycosis on 29 July 2011. Because the results of prior studies with spherulin were not considered applicable for the FDA approval process of Spherusol, the approved use for Spherusol is restricted to this narrow indication. However, because the new Spherusol is prepared from the same antigen concentrate as was spherulin, it is reasonable for clinicians to extrapolate the published literature with the older spherulin to the now available Spherusol.

METHOD OF ADMINISTERING THE SKIN TEST AND ITS INTERPRETATION

Just as with tuberculin ST, the correct application of Spherusol is critical for an accurate result. The ST is administered intradermally (0.1 mL) using a short, beveled 24- to 26-gauge needle on the volar surface of the arm and read 48 hours after placement by measuring with a ruler the diameter of the visible and palpable induration at the injection site. Dermal redness or other discoloration is not used in the measurement. Induration of ≥ 5 mm is considered a positive test.

It has been recommended to avoid administration of a coccidioidal ST in patients with erythema nodosum due to coccidioidomycosis because of the possibility of local necrosis at the injection site. Smith and colleagues also noted that exacerbations of *E. nodosum* may occur after coccidioidin skin testing in patients with coccidioidomycosis [13]. In some patients, a skin reaction may develop between 15 minutes and 1 hour after ST placement. These are considered “immediate reactions,” usually consisting of a wheal that may persist up to 24 hours, and may be mistaken as a positive DTH response during that time period. However, these do not represent “positive reactions.” Although DTH may be detected as early as 6 hours after ST placement, it is usually maximal by 36 hours [13]. Hence, readings for DTH should be performed at 48 hours from the time of placement to avoid confusion with an immediate reaction.

SKIN TEST RESULTS AND CLINICAL DISEASE

Surveys have demonstrated that a positive ST usually develops within 3 days to 3 weeks after the onset of symptoms in primary pulmonary coccidioidomycosis. Within the first week, 83% of subjects have converted and 99% of healthy subjects have converted by the third week. ST does not adversely affect illness or serologic testing [33]. Persistent expression of DTH in response to coccidioidal ST suggests the development of a protective cellular immune response and a good clinical outcome. On the other hand, patients with severe coccidioidomycosis may never develop ST reactivity, or it may wane coincident with worsening of their infection and with increase of their complement-fixing antibody titer. Establishment or reestablishment of reactivity during treatment or convalescence may be a good prognostic sign [12, 34].

EPIDEMIOLOGIC USES OF COCCIDIOIDAL SKIN TESTING

Coccidioidal ST results can be used to estimate the prevalence of past infection. Knowing prevalence differences among various geographic regions can identify areas with endemic exposure risk so that public health agencies can target education and prevention messages both to the public and healthcare providers. Additionally, ST data would be useful for determining prevalence in a geographic location where coccidioidomycosis had not been previously identified to determine if the area is actually endemic. Recently, 3 laboratory-confirmed cases were identified and soil samples were found to contain *C. immitis* in Washington State, which lies outside of the known endemic area for the disease [4, 35]. Testing of residents in those geographic locations where environmental samples tested positive would help determine the degree of risk that these new findings have identified.

Together with recognition of proven cases and ecologic studies, ST data are used to estimate the distribution of *Coccidioides* species in nature and to potentially track changes in distribution over time. For instance, in a prevalence of coccidioidomycosis ST study of the ambulatory population in Maricopa County in 1983, the overall prevalence rate for all ages was 21.5% [36]. The rate was directly related to length of residence in an endemic area, with a marked increase when residence was ≥ 5 years. Repeating this prevalence study in Maricopa County with Spherusol would provide valuable information for public health, clinicians, and researchers to interpret the ≥ 5 -fold rising rates of reported coccidioidomycosis in Maricopa County and Arizona over the last decade [1].

Additionally, there has been much discussion regarding high-risk occupations for coccidioidomycosis exposure, with specific interest in the risk associated with construction [37–39]. ST could be used to evaluate the risk of certain occupational exposures such as construction by identifying susceptible individuals prior to a large construction project or other potential exposure, with repeat testing performed after project completion to determine the rate of conversion. This study design would not rule out the possibility of an exposure outside the occupational setting. ST could also be used to collect baseline incidence estimates of coccidioidomycosis in endemic areas. These data could be used as a comparison or control for epidemiologic studies of individuals who have participated in high-risk activities, such as construction, to estimate the additional risk of the occupational activity. Last, ST might be used to identify nonreactive recipients for a coccidioidal vaccine, should one become available.

CLINICAL USES OF COCCIDIOIDAL SKIN TESTING

A patient's ST status can aid in diagnosis and prognosis during several stages of infection. Even without clinical illness, documented conversion from negative to positive should indicate that a patient underwent a coccidioidal infection sometime during that interval. Because in most persons a coccidioidal infection confers lifelong protection, a patient when healthy who has a positive ST can be advised that future coccidioidal illness is very unlikely in the absence of subsequent major immunosuppression. Although not yet commonly practiced, indexing healthy patients as to their ST status and having such information entered into and retrievable from their electronic health record may become useful information in managing subsequent illness. In patients presenting for care with community-acquired pneumonia in the endemic area, as many as 29% are thought to have primary pulmonary coccidioidomycosis [8]. Presumably, persons elsewhere with recent travel to an endemic region would have a similar likelihood. In an otherwise healthy patient,

ST results, available in 48 hours, will add to the limited diagnostic armamentarium available, along with serology, sputum, and chest radiography. Because an ST is often positive early in coccidioidal infections, a negative ST would provide further evidence that the current illness is not due to a coccidioidal infection. Development of a positive ST in a patient with known pulmonary coccidioidomycosis is a good prognostic sign, and the patient can be reassured. Alternatively, failure to develop a positive ST in a patient with known pulmonary coccidioidomycosis may presage a worse outcome and denote a patient who warrants closer follow-up. Results of serial STs performed during and after completion of therapy for a variety of types of coccidioidal infection were available for 33 patients. Seven of 14 patients with serially negative STs relapsed, whereas 2 of 19 with serially positive STs relapsed. Having serial positive STs after therapy for all types of coccidioidal infection appears to be a strong predictor that patients will not relapse [34]. In those with complicated pulmonary and disseminated coccidioidomycosis, ST results, as a marker for the patient's DTH, could be used as one factor in determining the follow-up and length of therapy. The exact role of ST in the evaluation of patients with solitary pulmonary nodules is under investigation and, currently, recommendations have not been established. Last, testing also might be useful in anticipation of relocation into an endemic area for certain populations such as military personnel, farmworkers, firefighters, archeologists, and prisoners. Awareness of one's ST status might reassure or heighten awareness for those living in the endemic area.

Notes

Disclaimer. Use of trade names and commercial sources is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention, the Public Health Service, or the US Department of Health and Human Services.

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