Expert Opinion: What To Do When There Is *Coccidioides* Exposure in a Laboratory

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Inadvertent exposure to *Coccidioides* species by laboratory staff and others as a result of a mishap is not an uncommon cause of infection in clinical microbiology laboratories. These types of infection may occur in laboratories outside the endemic areas, because the etiologic agent is unexpected in the submitted specimens and because personnel may be unfamiliar with the hazards of dealing with *Coccidioides* species in the laboratory. Coccidioidal infections are often difficult to treat, and outcomes can be poor. Here, we emphasize prevention and an approach to a laboratory accident that minimizes the risk of exposure to laboratory staff and staff in adjacent areas. On the basis of an artificially large exposure to arthroconidia that may occur as a result of a laboratory accident, a conservative approach of close observation and early treatment of exposed staff is discussed.

Coccidioides is a fungus maintaining a saprophytic cycle in soil in geographic regions with hospitable climatic conditions. In soil, it grows as mycelia, eventually bearing arthroconidia, the infectious propagule. The conidia are inhaled, initiating a respiratory infection. In some people, particularly those of dark-skinned races, immunosuppressed patients, and pregnant women, the infection can disseminate and cause life-threatening conditions. Most persons with only a primary infection do not require treatment, but all with disseminated disease do [1, 2]. Of the deep mycoses, coccidioidomycosis is thought to be the least responsive to treatment [1, 2].

Accidental laboratory exposure to *Coccidioides* species is the major cause of clinical laboratory-acquired fungal infections [3]. With the increase in travel and tourism to endemic regions, exposure to patient specimens for culture becomes even more likely. We have received queries regarding clinical laboratory exposure to *Coccidioides* species. The consequences of past exposure have been described elsewhere [4], but to our knowledge, this subject has not been fully addressed for 26 years [4]. The Web site for the Centers for Disease Control and Prevention indicates that "there are currently no guidelines about *Coccidioides* exposure in the laboratory" [5]. Thus, we have formulated a recommended plan for dealing with such incidents. Useful background reading regarding the pathogenesis of coccidioidomycosis [1], laboratory safety in general [6], and laboratory safety with regard to fungal pathogens in particular [7, 8] is given in the references.

Prevention. The most important step is preventing such exposures. Most queries have come from laboratories outside the endemic area. A mold culture was opened, and later it was realized that the plate contained coccidioidal arthroconidia. The principle to be followed is as follows: no culture of an unknown mold should be opened outside a biological safety cabinet appropriate for containing *Coccidioides*. *Coccidioides* growth may be visible in 48 h as grey-white wisps on culture media, later as white and/or buff-colored colonies with aerial hyphae. The formation of alternating barrel-shaped arthroconidia begins as early as 4 days of culture [9]. Further information about the maturation of *Coccidioides* and the degree of risk is presented below. If physicians would regularly alert the laboratory that *Coccidioides* is suspected in a submitted specimen or that the patient has a history of travel to endemic areas, then this information would require laboratory staff to maintain appropriate precautions.

A technique used in some laboratories to avoid the problem of examining an unknown mold that might prove to be *Coccidioides* is to freeze a petri dish with an

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unknown mold and then transfer (with a spatula or tweezers) ice crystals of the frozen culture; if performed carefully, this technique can be done in lower grade biosafety cabinets (where permissible by regulations). This would allow transfer and subculture for later inspection in sealed agar culture containers or as stained preparations under sealed cover slips.

Assessing the risk. The different types of exposure to *Coccidioides* range over a continuum of risk. Momentarily lifting the lid of a petri dish of a young coccidioidal culture with a single colony of immature hyphae represents a vastly smaller risk to exposed personnel than does breaking a vessel that contains a confluent culture with mature arthroconidia. However, often the risk is difficult to assess acutely. In such instances, it may be prudent to take all the recommended precautions and to assess the situation from a safe locale.

In some instances of laboratory exposure to *Coccidioides,* the number of arthroconidia inhaled far exceeded that which would occur in most instances of natural exposure in endemic regions (although exposure to large numbers of arthroconidia has occurred among archeology students digging in contaminated soil) [10]. Attack rates for symptomatic illness from laboratory exposure exceed that seen from natural exposure [6]. The consequence of a massive type of exposure can sometimes be a multifocal pneumonia, exceeding the severity of the disease that would occur in an instance of natural exposure. Another point is that the dose of arthroconidia needed to establish a human coccidioidal infection appears to be very small, possibly even 1–10 arthroconidia; experimental studies suggest that 1 arthroconidium is the dose needed to cause a mouse to become infected [1, 6]. Incidents of nonlaboratory inadvertent exposure may be instructive: a window was sawed into the cast of a patient with coccidioidal arthritis, to view the wound. *Coccidioides* had leaked from the wound onto the inside of the cast, where it had found a hospitable temperature and sufficient nutrients in the traces of blood and pus, and had germinated. Several staff members who were merely present in the room, including nurses and physicians, became infected as a result of the saw-generated aerosol [11]. The period of exposure does not need to be long: a woman became infected as a result of changing planes (which lasted a few hours) in an airport (admittedly, in a dust-blown site) in an endemic region, her only known time in an endemic area.

The degree of risk can also be gauged by examination of culture about the time of the exposure. This examination may have occurred before the exposure, or if the exposure did not result in obliteration of the culture with wet toweling or disposal (eg, if the exposure resulted from inappropriate opening of a petri dish, which was then closed), then the examination may take place after the exposure. The development of mature arthroconidia, at risk of disarticulation from the parent hyphae, takes a few days (dependent on the culture temperature and conditions) in mycelial development, and if there are few such structures visible on immature hyphae, then the risk of exposure is considerably less. Large numbers of mature arthroconidia usually develop by 7–10 days of culture. At this stage, the arthroconidia are readily dispersed by air currents as gentle as those generated when removing the lid of a petri dish [12].

We present the following guidelines for situations in which a significant type of exposure may have occurred. It is hoped that, from our guidelines, a laboratory can develop a plan of action appropriate to its unique physical environment and equipment and can assess the different risks of exposure.

Initial steps after an exposure. If an exposure occurs, personnel should be evacuated (preferably holding their breath, if possible, until after exiting the affected area), and protective laboratory clothing removed just inside the affected room before exiting (clothing to be later added to biological hazardous waste). If the laboratory has ultraviolet lights (eg, above workstations or at ceiling height), then they can be clicked on while exiting.

The laboratory's designated biosafety officer must be notified, and that individual may also need to notify public health and governmental officials. Doors to the laboratory should be closed, and the exposed room set at negative pressure with respect to adjacent rooms or corridors, if that capability exists. If the air system for the laboratory results in positive pressure with respect to common hallways or other laboratory areas, then the system should be shut down immediately.

If windows were open, then they need to be closed. If there is a possibility of an air leak via windows, then their margins can be temporarily sealed with tape (preferably from the outside, if they are accessible). If any fans in the room were on, then they should be turned off. If the exposure occurred as a result of a spill, the dropping of a dish or container, or any breakage, then any liquids, broken glass, other solids, or open containers can be covered with towels and flooded with the laboratory's approved sporocidal detergent (see below), allowing at least 20 minutes of contact time. Afterward, the moist towels and/or solid materials should be lifted with a pair of forceps, placed in a bag clearly demarcated for biohazardous waste, and autoclaved. The preceding measures could be done during the initial evacuation of the scene; if they were not, then attending to the windows, fans, and mishaps could be the initial duties of those appointed to do the cleaning, using personal protective equipment (discussed further below). These measures are performed to allow most airborne arthroconidia to settle; it is best to be cautious and wait 1–2 h before proceeding with cleanup after the initial evacuation. If there is a possibility of an air leak via doors, then their margins can be temporarily sealed with tape from the outside. Skin surfaces of exposed personnel should be washed thoroughly with soap (germicidal soap if possible) and water.

Second step measures. Signs should be posted to alert personnel that this is a contaminated area that should not be entered. A list should be made of everyone present in the room at the time of exposure, for later follow-up [13, 14].

All of the surfaces of the room (ie, floor, ceiling, and walls) and all of the surfaces of objects in the room (eg, equipment, culture plates, and vessels) that are needed for continued use should be thoroughly cleaned with the sporocidal detergent. Some of the following cleaning agents have been used: bleach at a 1:10 dilution of the undiluted commercial products in water (not 1:100 as for most other degerming procedures), hydrogen peroxide (≥6%) [15], and formaldehyde (8%), which is controversial because it is a potential carcinogen; phenolics (3%) are a lesser-endorsed alternative. Note that a 1 :10 dilution of bleach can be corrosive to some materials [16]. As with all disinfectants, wetting the affected areas and the time of exposure to the active cleaning agent are critical to achieve optimal killing; ≥20 minutes is recommended, although disinfecting can be extended to sterilization and the killing of spores if the time of exposure to the cleaning agent is extended to 6–10 h. The cleaning staff should wear respirators, such as individually fitted N95 or N99 respirators, that block the entry of microbes, or a hooded air-filtered personal protective unit. Personnel assigned to perform the cleaning activities should also wear other appropriate personal protective equipment, including gloves, moisture-resistant gowns, shoe covers, and hair covers. Because of the severity of coccidioidomycosis in individuals who are immunocompromised or pregnant, such individuals should not be a part of the cleaning staff. The ideal cleaning person would be someone in good health with a history of either a successfully resolved prior coccidioidal infection or a positive coccidioidal skin test result. Culture materials and other items present in the laboratory at the time of the exposure and no longer needed, as well as disposable cleaning materials, can be wetted with the sporicide, placed in a bag clearly demarcated for biohazardous waste or covered bucket, autoclaved, and discarded. Objects (eg, laboratory materials and mop heads) that can be autoclaved and reused should be placed in a bag clearly demarcated for biohazardous waste or covered bucket and autoclaved. If an accident occurred in (or near) a biological safety cabinet, then it should be set at negative pressure to the room, and the interior decontaminated using paraformaldehyde fumes, preferably by trained certified personnel [17].

Extensive exposures potentially involving the entire laboratory. If the exposure incident resulted in a major release of arthroconidia in the laboratory outside of a biological safety cabinet, full decontamination of the (sealed) laboratory may be required using paraformaldehyde or hydrogen peroxide fumes; the advantage of using hydrogen peroxide is that, after the fumes dissipate, the residual end product (ie, water) is nontoxic. Heating paraformaldehyde (optimally at 60%–80% humidity) results in gaseous formaldehyde; the humidity has been increased in cabinets or rooms by boiling water in open vessels on hot plates during the decontamination procedure. Decontamination is best done by certified professionals [18]. A research laboratory that was growing *Coccidioides* had a malfunction, presumably resulting in a greater dispersion of the organisms than would occur in a diagnostic laboratory exposure, and had to have one of its rooms sealed for 24 h, and a 37% commercial formaldehyde solution was distilled into the room air using an electric hot plate. The volume of formaldehyde to be used would depend on the size of the room. With paraformaldehyde, 0.3 g/ft^3 of room air is recommended. After the formaldehyde or paraformaldehyde used to ventilate a room has been vaporized, the room should remain sealed for at least 3 h, and then it should be ventilated by opening windows (if there are windows that can be opened), and possibly doors (depending on the area into which the doors open), for 24 h before the staff returns. Chlorine dioxide gas is available for use in some medical centers; it is sporicidal, as is chlorine, but does not form toxic chloramines. It is generated from a column, in the room, but is sensitive to light.

We emphasize that coccidioidal arthroconidia are quite hardy and can survive on inanimate surfaces for a long time. Infections have been recorded in nonendemic areas from fomites such as cotton, packing materials, or fruit exported from endemic regions or cacti purchased in endemic areas. Moreover, *Coccidioides* has few nutritional requirements and can briefly grow on materials with only traces of carbon and nitrogen, although establishing a new environmental locus for sustained growth requires soil and temperature conditions to which *Coccidioides* is adapted.

Interviews with occupational health or building maintenance personnel should be pursued to consider the possibility of *Coccidioides* exposure to persons not present in the room where the exposure occurred, via airflow patterns that may not exhaust directly to the exterior of the building. For example, this would apply if laboratory air is recirculated to the building supply or if a laboratory room is at positive pressure to common hallways or other areas of the building. During smallpox outbreaks in a UK research center and in a German hospital, persons were infected on floors other than the floor where the laboratory exposure occurred, owing to building airflow patterns [19].

After *Coccidioides* exposure occurs, it is desirable to perform a review of the events and to review laboratory safety training. A periodic drill to review what each person is required to do in case of *Coccidioides* exposure is important, just as in a fire drill.

Exposed personnel. We recommend that baseline serum samples be obtained promptly from persons exposed to *Coccidioides* and that these samples be stored

for eventual testing for coccidioidal immunoglobulin G and M antibodies by a laboratory experienced in coccidioidal testing. These tests will determine whether there was any prior exposure to *Coccidioides,* and those persons who did indeed have a prior exposure will be at lesser risk of infection, although, in one case, an individual was suspected of being infected a second time as a result of massive exposure to *Coccidioides.* The prior medical and travel history of a person exposed to *Coccidioides* could also be helpful in determining prior exposure.

All persons deemed to have been exposed to *Coccidioides* should then be given a therapeutic dose of either itraconazole or fluconazole orally (400 mg daily, for adults) for 6 weeks, as prophylaxis. Treatment of natural primary infections in endemic regions is controversial, and the consensus is not to do so unless the patient has comorbidities (such as immunosuppression), is in a high-risk group for dissemination (such as those of Filipino ancestry), or has other conditions that will be adversely affected by primary coccidioidal pneumonia (such as chronic obstructive lung disease), because only a small percent of those infected via a natural exposure to *Coccidioides* will develop either disseminated disease or a progressive primary infection [2]. Our recommendation to administer prophylaxis as a result of laboratory exposure relates to the risks of inhaling, at a short distance from the point source, a possibly artificially large inoculum. The benefits of such a prophylactic approach have not been proven, although early treatment of experimental coccidioidal infection in animal models clearly disposes to a favorable outcome [1]. The risks of a short course of azoles are minimal, and the expenses are not great.

The pregnant female. Azoles are deemed teratogenic, so that if a pregnant female were exposed, then the problem of infection becomes more complicated, and the development of a coccidioidal infection will have greater consequences. If it is uncertain whether a pregnant female is really infected (eg, she might have been located in a distant part of the room at the moment of exposure and was evacuated promptly), then one option might be to observe her more closely and at first suggestion of a possible coccidioidal infection to initiate intravenous amphotericin B therapy (which is safe during pregnancy). If it is fairly certain that a pregnant female has been exposed to *Coccidioides* (eg, a culture plate was opened in front of her), then the only prophylactic option would be an intravenous amphotericin preparation, although in a prophylactic rather than therapeutic mode; a once-weekly administration of amphotericin would be reasonable until either the pregnancy was concluded (then azoles could be given instead), the 6-week period concluded without any signs of coccidioidal disease (then prophylaxis could be discontinued), or coccidioidal infection was suspected (then weekly administration of amphotericin could be switched to a daily administration).

The postexposure period. During the 6 weeks of prophylaxis (which includes the incubation period for coccidioidal infection), and probably for some months thereafter, if the person who was given prophylaxis developed a fever or cough, then they should present to a clinician, and the clinician should be made aware of the possible exposure to *Coccidioides,* so that coccidioidomycosis is considered in the differential diagnosis, and the appropriate diagnostic testing with culture, serum samples, and imaging is performed. This is when the concurrent testing of the stored baseline serum samples, obtained during the presentation in which coccidioidal infection was suspected, can be especially useful. Development of coccidioidal seropositivity can be delayed for a time after the appearance of symptoms of a primary infection, so retesting serum samples 3–12 weeks later may be needed. If a person receiving azole prophylaxis is diagnosed with a primary infection (with therapeutic blood and tissue levels provided even before infection had been established), then at least several months of treatment for that primary infection should be considered, probably with an alternative antifungal agent. We would then use an intravenous amphotericin B preparation, although some clinicians might be tempted to try another oral azole, avoiding intravenous therapy and the toxicities of polyenes. We note that no other azoles have the extensive favorable clinical experience as do itraconazole and fluconazole in treating coccidioidal infection [2]. In this setting of breakthrough infection as a result of prophylaxis, in vitro susceptibility testing of an isolate could be useful in choosing a therapeutic option. Any patients with disseminated coccidioidomycosis would require longer-term therapy and follow-up.

At the end of the 6-week period of prophylaxis after *Coccidioides* exposure, if there is no clinical suspicion of coccidioidomycosis, then testing for coccidioidal immunoglobulin G and M antibodies should again be performed, concurrently with the testing of some of the baseline serum sample, for comparative purposes. If there is no seroconversion, then prophylaxis can be stopped. If there is serological evidence of a subclinical infection, then prophylaxis can probably also be stopped, although continuing treatment, for another few months, of the subclinical primary infection might be contemplated for those with comorbidities and risk factors mentioned above. From natural exposure to *Coccidioides* resulting in undiagnosed primary infections, disseminated disease can later arise, so it is advisable to observe persons with laboratory exposure and infection, subclinical or not, for a follow-up period of 1 year, for the possibility of developing disseminated disease. This means that if the patient develops a fever, respiratory symptoms, skin lesions, arthritis, bone or abdominal pain, and/or headaches, then coccidioidomycosis should be considered in the differential diagnosis, and an appropriate diag-

nostic workup should be pursued for that possibility.

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