Identification of Endogenous *Coccidioides posadasii* Contamination of Commercial Primary Rhesus Monkey Kidney Cells

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Here we describe the identification of endogenous *Coccidioides posadasii* contamination in commercial rhesus monkey kidney (RhMK) cells and the subsequent nationwide alert that reduced the risk of laboratory exposure. This extraordinary event highlights the necessity for laboratories to remain vigilant in the use of appropriate biosafety procedures, particularly when working with unknown pathogens.

Bacteria, fungi, and viruses can contaminate cell cultures during production or postproduction when performing routine culture procedures (1–6). Inherent to the use of primary animal cells, including rhesus monkey kidney (RhMK) cells, is the risk that the tissue itself may harbor endogenous viruses, such as simian viruses, adenoviruses, herpes B virus, as well as parasites, fungi, and bacteria (1–7). Endogenous or exogenous contamination may be noticed only after cells are stimulated or stressed. Consequently, antibiotics, antifungals, and antisera are common additives to RhMK culture media and control tubes are included with testing (8, 9). We describe a recent event involving endogenous *Coccidioides posadasii* contamination in commercial RhMK cells. *C. posadasii* and *Coccidioides immitis* are highly infectious fungal pathogens that can cause coccidioidomycosis in healthy and immunocompromised individuals (10, 11).

**Identification of contamination.** On 14 December 2012, the Virology Laboratory at North Shore-LIJ Health System Laboratories (NS-LIJHSL), NY, received 700 RhMK culture tubes (lot A491206-T) from Diagnostic Hybrids (DHI; Quidel Corp., Athens, OH). On 19 December 2012, an unopened RhMK tube was noted to contain fungal contamination and examination of the remaining unopened lot identified another 31 contaminated tubes. DHI was immediately notified since the tubes were never opened, indicating possible contamination during production. The following day, the laboratory posted a warning regarding contaminated RhMK cells on the American Society for Microbiology (ASM) ClinMicroNet and Pan American Society for Clinical Virology (PASCV) Web servers and shipped 30 contaminated RhMK tubes to DHI for their internal investigation.

Initial wet mount examination at NS-LIJHSL identified hyphal elements without any distinguishing characteristics. Early cultures on Sabouraud’s agar (25°C) and 5% sheep blood agar (35°C) revealed white fuzzy colonies with sterile mycelia, but on 28 December 2012, the mold demonstrated hyaline hyphae with alternating barrel-shaped arthroconidia, classical of *Coccidioides* spp. The laboratory immediately notified the Wadsworth Center Virology Laboratory (WCVL), New York State Department of Health (NYSDOH). Additional postings on ClinMicroNet and PASCV, including a posting from the WCVL, warned of contaminated tubes observed in a second lot of RhMK cells (A-491216-B). The Wadsworth Center Mycology Laboratory (WCML) performed wet mounts on material from contaminated tubes in WCVL and noted thin hyaline hyphae without any discernible characteristics; this was not surprising, as fungi do not sporulate while submerged in liquid media (12).

The WCML performed a laboratory-developed genus-specific real-time PCR targeting the *prp2* gene for *Coccidioides* spp. on tubes from lot A-491216-B in the WCVL and on the culture that had been grown at NS-LIJHSL from lot-A491206-T. Samples were confirmed as *Coccidioides* spp. on 28 and 29 December 2012, respectively. Further molecular analysis in the WCML, including a species-specific real-time PCR assay targeting *prp2* and contig G45815, a modified size disparity PCR targeting contig G45815 (13), and a sequence analysis of the internal transcribed spacer (ITS) region, identified the mold from both cell lots as *C. posadasii*.

Distribution of *C. posadasii* contamination was low and erratic. However, the large batch of cells received at NS-LIJHSL and the 6% contamination rate facilitated the original observation and prompted the laboratory to identify the mold. Ninety unused tubes from the same lot received at WCVL never showed contamination, even after 3 weeks. Interestingly, inoculated RhMK tubes containing medium with amphotericin B, the drug of choice for the treatment of coccidioidomycosis, had breakthrough contamination, suggesting a high level of mold in some tubes. If contamination had only been noted postmanipulation, as it was in other laboratories, it could have been erroneously associated with laboratory or sample contamination.

Company statements from DHI indicated that investigations had revealed the donor animal organ as the most likely source of contamination. A portion of the cells harvested from the donor...
kidney had been frozen and used to supplement the production of subsequent RhMK lots, hence the contamination of multiple lots. RhMK stocks prepared from that organ were exhausted by the time of the investigation, and future cell lots would therefore not contain material from that stock. No contamination was reported in other RhMK lots, including others that were received on the same days at WCVL. Coccidioides infections have been noted in a variety of animals, including domesticated, captive, exotic, or wild mammals and some reptiles (14–17). In regions of endemicity, clinical cases are relatively common in non-native zoo animals, dogs, cats, horses, llamas, and nonhuman primates, and rare infections are reported in numerous other animals. Similar to human infections, animal infections can be symptomatic or asymptomatic (16), and while primary infection is pulmonary, dissemination to multiple tissues or organs, including the kidney, occurs.

**Reporting.** Following initial discussions with NS-LIJHSL regarding the Coccidioides-like mold, on 28 December 2012, Wadsworth Center contacted the NYS Bureau of Communicable Disease Control epidemiologists, who contacted the Centers for Disease Control and Prevention (CDC) Mycology Division (Atlanta, GA). Following extensive communications between the four parties and DHI, and PCR confirmation of Coccidioides spp. in two RhMK cell lots, on the evening of 29 December 2012, the NYSDOH issued a health alert via its Integrated Health Alerting and Notification System (IHANS) and through Epi-X, the CDC’s national and international Web-based communications portal. The health alert notifications were also posted on ClinMicroNet, PASCV, and Division C (ASM) listservs and the Association of Public Health Laboratories directors and microbiology discussion lists.

**Exposure assessment.** Prior to noticing contamination, NS-LIJHSL inoculated 162 RhMK tubes using contaminated lots and WCVL inoculated 10 tubes from the same lot (A-491206-T). NS-LIJHSL had performed immunofluorescence antibody confirmations on some tubes and subpassages on others to RhMK tubes without visible contamination. WCVL had performed medium changes only, on the 10 inoculated tubes from the lot. Once contamination was noted, no further RhMK tubes from suspect lots were used for specimen inoculation at either facility. Virus culture inoculations and postinoculation manipulations had been performed under appropriate biosafety level 2 (BSL2) conditions by well-trained technologists adhering to strict cleaning procedures. Maintenance and service records indicated appropriate functioning of the BSL2 cabinets at both facilities (18). Potentially contaminated material in RhMK tubes was kept in liquid media, and tubes were maintained in incubators, except for brief periods when tubes were examined microscopically or manipulated in BSL2 cabinets, conditions not favorable for the formation of infectious arthroconidia. Visibly contaminated RhMK tubes were only opened in mycology laboratories by technologists trained in handling pathogenic fungi. Once Coccidioides spp. were tentatively identified, tubes were sequestered, cultures were terminated, and tubes were autoclaved regardless of the presence of visible contamination.

Although virology laboratories frequently deal with endogenous viral contamination, fungal contamination is rare. The assumption that the mold was an environmental nonpathogenic contaminant could have led to laboratory exposure due to inhalation of infectious arthroconidia (12, 18, 19). This incident highlights the need for vigilance in dealing with unknown organisms. Both facilities determined that due to appropriate precautions, the exposure risk to staff was extremely low. However, given the large number of tubes that had been handled at NS-LIJHSL, the culture identification of Coccidioides, and the well-documented history of lab-acquired infections with Coccidioides (9), baseline Coccidioides serology was performed for selected NS-LIJHSL staff, and two staff members received prophylaxis: one due to self-concern and the other due to medical history.

This investigation emphasizes the importance of communication within the microbiology community and with manufacturers when contamination is noted. Further, it highlights the importance of clinical laboratories working in close concert with public health laboratories, as well as state laboratories and their communicable disease bureaus. These highly cooperative relationships allowed for the rapid exchange and widespread dissemination of critical information, thus reducing the risk of C. posadasii exposure to laboratorians across the United States.

**Nucleotide sequence accession numbers.** Nucleotide sequences of the ITS gene were submitted to GenBank under the accession numbers KC469982 to KC469984.

**ACKNOWLEDGMENTS**

We thank the Mycology Division at the CDC for helpful discussions and assistance during this event and the Diagnostic Hybrids Company of Quidel for their willingness to supply all available information openly and promptly. We also sincerely thank Matthew Shudt in the Applied Genomics Technology Core at the Wadsworth Center for performing sequencing. We especially thank the technical staff in their respective departments for their dedicated work in identifying the contaminant and Nina Ahmad, EIS Officer for NYSDOH, who assisted with the prompt and widespread dissemination of the information.

**REFERENCES**


