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Molecular detection of airborne *Coccidioides* in Tucson, Arizona

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Abstract

Environmental surveillance of the soil-dwelling fungus *Coccidioides* is essential for the prevention of Valley fever, a disease primarily caused by inhalation of the arthroconidia. Methods for collecting and detecting *Coccidioides* in soil samples are currently in use by several laboratories; however, a method utilizing current air sampling technologies has not been formally demonstrated for the capture of airborne arthroconidia. In this study, we collected air/dust samples at two sites (Site A and Site B) in the endemic region of Tucson, Arizona, and tested a variety of air samplers and membrane matrices. We then employed a single-tube nested qPCR assay for molecular detection. At both sites, numerous soil samples ($n = 10$ at Site A and $n = 24$ at Site B) were collected and *Coccidioides* was detected in two samples (20%) at Site A and in eight samples (33%) at Site B. Of the 25 air/dust samples collected at both sites using five different air sampling methods, we detected *Coccidioides* in three samples from site B. All three samples were collected using a high-volume sampler with glass-fiber filters. In this report, we describe these methods and propose the use of these air sampling and molecular detection strategies for environmental surveillance of *Coccidioides*.

Keywords

Coccidioides; Valley fever; atmospheric sampling; air/dust samples; molecular detection

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Supplementary material

Supplementary material is available at *Medical Mycology* online (http://www.mmy.oxfordjournals.org/).

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Introduction

Valley fever, also known as coccidioidomycosis, is a fungal disease that develops when aerosolized spores of *Coccidioides immitis* and *C. posadasii* are inhaled.[1] Approximately 150,000 new human infections occur annually in the United States;[2] however, this number is thought to be a gross underestimate due to underreporting and the lack of mandatory reporting for some endemic areas.[3] *Coccidioides* is endemic to the arid regions of southwestern United States, Mexico, Central America, and South America;[4] however, recent findings demonstrating its presence in Washington and Utah are challenging our current understanding of its geographic distribution.[5–7] Although the precise ecological niche of *Coccidioides* remains unknown, it is widely accepted that the fungus grows in soil as hyphae and produces infective arthroconidia (asexual spores of 3 to 5 μm in diameter) that become airborne upon soil disturbance.[8] An association with rodent habitats has also been suggested.[9–11] Once inside the host, *Coccidioides* produces spherules, specialized parasitic structures that reinfect host tissues and disseminate inside the host.[12] In most cases, infection is asymptomatic, and the majority of symptomatic cases present as a flu-like illness;[13,14] however, some cases can result in a chronic or disseminated life-threatening disease.[15]

Although instances of infection through organ transplantation and open wounds have been reported,[5,16] inhalation is by far the most common mode of transmission. Certain environmental conditions and human activities that involve soil disturbance are thought to increase aerosolization of arthroconidia and therefore risk of infection. The role of weather, particularly severe dust storms and earthquakes, has been reported to correlate with coccidioidomycosis outbreaks.[17–19] Uniquely, the outbreak of Valley fever in 1978 was reported to have been caused by a high-velocity dust storm that generated winds gusting up to 160 km/h in the endemic area of the San Joaquin Valley, California.[17] Importantly, smaller dust storms that are more frequent have not been formally demonstrated to increase infection rates, and the role of climate and seasonal weather changes on the number of cases in endemic regions has yet to be determined.[20,21] In addition, outbreaks of Valley fever have been associated with construction and archaeological digs, both activities that involve extreme soil disturbance.[22–24] Environmental surveillance using air sampling will help establish links between the effects of seasonal patterns, severe weather, earthquakes and certain human activities on the density of airborne arthroconidia.[25–27] Overall, data from environmental sampling coupled with public health initiatives can help inform individuals residing in endemic areas on how to make choices that lower their risk for infection.

The difficulty of isolating this infectious agent from soils has been well documented,[11,28–31] and although it is well known that this pathogen is transmitted through the atmosphere, only a few studies from the 1950s have captured arthroconidia in air/dust samples.[32] To date, capture of airborne arthroconidia and molecular detection of *Coccidioides* from air/dust samples using current technologies that are suitable for environmental surveillance have not been formally demonstrated. In this study, we implemented methods used to collect atmospheric samples in desert environments and during dust storms for determining microbial presence.[33] One of the more efficient methods, due to the resistant nature of fungi to desiccation via airflow, is the use of high-volume membrane filtration. The benefit of

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utilizing high-volume filtration is the ability to quickly sample large volumes of air when the concentration of the target organism may be low. Recently, a few manufacturers have released kits designed for extraction of cells and nucleic acids from the membrane matrix of these filter units.[34,35] In addition, thin flat-surface and hydrophobic membranes have been developed for enhanced extraction of particulates from their surfaces. Several other newer means of efficient collection include high-volume liquid impingers and electrostatic precipitation.[36,37] To date, successful atmospheric collection of *Coccidioides* by these methods has not been described in the scientific literature.

Proper environmental surveillance of airborne *Coccidioides* heavily relies not just on effective capture but also the detection of *Coccidioides* DNA in environmental samples. Various methods, both culture dependent and independent, are employed to detect *Coccidioides* DNA. Recently, a TaqMan-based qPCR assay developed by the Translational Genomics Research Institute has significantly improved detection of *Coccidioides*[5] in soil samples. The assay targets a transposable element identified as a copia-like retrotransposon family protein found in both *C. immitis* and *C. posadasii*.[5,38]

In this study, we use DNA extraction and *Coccidioides* detection methods optimized for environmental samples to test various air sampling techniques and establish a novel approach to environmental surveillance of airborne arthroconidia. We show that sampling methods using a high-volume sampler with glass-fiber (GFF) filters are able to capture arthroconidia and that our extraction and molecular identification methods are adoptable for environmental surveillance of airborne *Coccidioides*.

Materials and Methods

Sample sites

Air and soil samples were collected on the 18th and 19th of November 2014. Samples obtained at Site A, just north of Florence, AZ, on November 18th, were collected within a military installation (the corner of a fenced compound) and next to a dry stream bed (~200 m west of the air sample location). Samples obtained at Site B, near the intersection of Banyan Wash and Pinal Pioneer Parkway (Highway 79) were collected on November 19th.

Particulate matter collection

Five different types of techniques were used to collect air samples. Table 1 contains sample type and collection data. Four of the techniques utilized low-volume airflows via the use of Cole Parmer Air Cadet vacuum pumps (Fisher Scientific; Pittsburgh, PA, USA). Two of these techniques utilized individual sample-dedicated Advantec MFS Inc, polypropylene 47-mm filter holders (Fisher Scientific) that contained either EMD Millipore Fluoropore FTFE 47-mm membrane filters (Fisher Scientific) or Sartorius Polyethersulfone 47-mm membrane filters (Fisher Scientific) at a flow rate of 0.23 ft^3 min^{-1}. A third membrane technique utilized the same flow rate and Whatman HEPA-CAP 36 (Fisher Scientific) bidirectional flow filters. A Multi-stage liquid impinger (Burkard Manufacturing Co. Ltd.; London, England, UK) that collected three size fractions (>10 μm, 10–4 μm, and <4 μm) each in 6 ml of 1× phosphate buffered saline (PBS) was also utilized at a flow rate of 0.23 ft^3 min^{-1}.

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Each fraction was transferred to sterile 15-ml tubes for storage, and the volume was reduced by evaporation and remaining volumes ranged from 5 to 5.5 ml. High-volume air samples were collected using TFAGF1 10.16-cm GFF filters (Staplex Co.) and a TFIA High Volume Air Sampler (Staplex Co.; Brooklyn, NY, USA) at a flow rate of 15 ft^3 min^{-1}. All low- and high-volume membrane filters and the HEPA cartridges were stored in sterile Whirl-Pak bags in a cooler with cool packs and refrigerated at 4 °C for transport and storage.

Artificial dust storm

Sample collection techniques were utilized during normal atmospheric conditions and were also challenged by artificially generated clouds of dust. Dust clouds were generated using an electric leaf blower. Table 1 identifies which samples were challenged. Samples collected on November 18th at 1155 h were normal-condition samples and no dust was artificially generated while they were collected. Dust clouds were generated from surface soils around the sample units for the remaining samples collected on November 18th and samples on November 19th with start times of 1020, 1130, and 1235 h. For the November 19th samples with start times of 1345 and 1445 h, subsurface soils collected from nearby locations were sprinkled around the samplers using several standard size bucket loads prior to generating the clouds of dust.

Particulate matter concentration

High-volume samples: Following collection, filters were removed from the bags (most bags contained particulate matter that had fallen off the filter) and cut in half using sterile scissors. One half was stored in a ziplock bag for culture-based analyses. The remaining half was placed into a ziplock with 9 ml of 1× PBS. To the original storage bag, 5ml of 1× PBS were added. Both bags were then shaken (bag with filter face down) at 450 rpm using a tabletop shaker for 30 min. Volumes from both bags were then combined in a 15-ml tube via aspiration with a 10-ml pipette and pipette bulb. Samples were then centrifuged at 5,900 × g for 30 min and supernatants were discarded. Pellets were suspended to 1 ml using 1× PBS and split into two 0.25 ml aliquots in microcentrifuge tubes.

Low-volume 47-mm membrane samples: Following collection, all samples were back-flushed three times (using 10-cc syringes) with a total of 15 ml of 1× PBS (first with 6 ml, second with 6 ml, and finally with 3 ml) into 15-ml tubes. Samples were centrifuged at 5,900 × g for 30 min. The supernatant from these samples was discarded and the remaining pellets were suspended to 0.5 ml in 1× PBS. This volume was then split into two 0.25 ml aliquots and stored in microcentrifuge tubes.

Low-volume HEPA-CAP samples: All cartridges were reverse-loaded with 60 ml of 1× PBS by capping the intake port with Parafilm and using a 60-cc syringe. Samples were shaken by hand for 1 min and back-flushed into a 50-ml tube. These steps were repeated using 50 ml of 1× PBS. Both 50-ml volumes were then centrifuged at 5,900 × g for 30 min. Supernatants were discarded and the pellets combined through subsequent suspension in 10 ml of 1× PBS. Samples were centrifuged at 5,900 × g for 30 min, and the supernatant was discarded. The pellet was suspended to 0.5 ml and transferred to a microcentrifuge tube.

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Liquid-impinger samples: All fraction sizes of sample numbers 1 and 2 were combined in respective 15-ml tubes. Samples number 1, 2, and the remaining sample size fractions (for samples number 3, 4, 5, 6, and 7) were centrifuged at 5,900 × g for 30 min. The supernatant from these samples was discarded, and the remaining pellets were suspended to 0.5 ml in 1× PBS. This volume was then split into two 0.25 ml aliquots and stored in microcentrifuge tubes.

Soil collection

Soil samples were collected from Site A and Site B at the time of air/dust sampling. Samples were collected inside or within a 5-cm radius of active and dormant rodent holes at varying depths (surface, 10 cm, and 20 cm). Samples were transported at room temperature (RT) and stored at 4 °C prior to DNA extraction.

DNA extraction

Genomic DNA was extracted using the PowerLyzer Power-Soil DNA Isolation Kit (MO BIO Laboratories, Inc.; Carlsbad, CA, USA) and following the manufacturer's instructions. For air/dust samples, 125–250 μl of particulate matter were loaded into a bead tube. For soil samples, ~0.75 g of soil were loaded into a bead tube. The FastPrep-24 5G (MP Biomedicals, LLC.; Santa Ana, CA, USA) high-speed benchtop homogenizer was used for optimal homogenization and cell lysis. Samples were homogenized for seven 1-min cycles at 6 m/s with 5-min rest breaks between each cycle. Upon addition of elution buffer to the spin filter, samples sat at RT for 5 min before centrifugation.

Single-tube (ST) nested qPCR

Coccidioides was detected by amplifying a 249-bp region of a transposable element[5] using a TaqMan-based single-tube (ST) nested qPCR assay. Each sample contained 240 nM each of outer primers [Outer Forward (OF) 1 and Outer Reverse (OR) 1–4], inner primers [Inner Forward (IF) 1–3 and Inner Reverse (IR) 1], TaqMan probe (Supplementary Table S1; probe synthesized by Applied Biosystems [Grand Island, NY, USA]), BSA (2 ng/μl; BSA), and TaqMan Universal PCR Master Mix (Applied Biosystems). Reactions were performed using a Rotor-Gene 6000 thermocycler (Qiagen; Valencia, CA, USA) with a thermal program consisting of two amplification phases distinguished by their annealing temperature. Specifically, there was an initial denaturation of 95 °C for 10 min; 25 cycles of denaturation at 95 °C for 10 s, annealing at 65 °C for 30 s, polymerization at 72 °C for 15 s; and 45 cycles of denaturation at 95 °C for 10 s, annealing at 52°C for 30 s, and polymerization at 72 °C for 15 s. C_t values were calculated using a manual threshold setting at a fluorescence value of $10^{-1.0}$. Positive and non-template controls were included in each qPCR run.

Generation of droplets and droplet digital PCR (ddPCR)

ddPCR provides absolute quantification of PCR targets by partitioning input DNA into ~20,000 droplets where each droplet functions as a separate PCR reaction. By assuming a Poisson distribution for how template DNA molecules are partitioned into droplets, the concentration of the input DNA can be calculated and expressed as copies/μl. Two genes were amplified: the target gene used for *Coccidioides* qPCR detection and a single-copy

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gene that encodes the enzyme chitin synthase.[39] We normalized the absolute copy number concentration of the qPCR gene target by the absolute copy number concentration of the single-copy gene, and hypothesized that this ratio is an estimation of the copy number/ genome of the transposable element targeted during *Coccidioides* qPCR detection.

Droplets were generated using the Bio-Rad QX100 Droplet Generator with Droplet Generation Oil for Probes (Bio-Rad;Hercules, CA, USA) and PCR reagents. Each PCR reaction was carried out in 37 μl volume containing ddPCR Supermix for Probes (Bio-Rad) and using the C1000 Touch Thermal Cycler (Bio-Rad). Droplets were detected using the Bio-Rad QX100 Droplet Reader. One hundred picograms of input DNA were used for each reaction and each isolate was tested in replicates of three. For the transposable element, primers/probes were used from the original qPCR assay developed by the Translational Genomics Research Institute.[5] For the single-copy gene, a 99-bp region was targeted with primers and a probe that were designed using IDT PrimerQuest and synthesized at Fisher Scientific. The amplification protocol was 95 °C for 10 min; 40 cycles of denaturation at 95 °C for 15 s, annealing at 54 °C for 30 s, and polymerization at 72 °C for 15 s.

Results

Development of single-tube (ST) nested qPCR assay for Coccidioides detection

In an effort to increase the sensitivity while limiting risk of laboratory contamination during *Coccidioides* qPCR detection for environmental samples, we adapted the “CocciDxQ” qPCR assay[5] developed by the Translational Genomics Research Institute into a single-tube (ST) nested qPCR reaction. We extended the amplicon region 133 bp upstream and 11 bp downstream. Outer primers (OF 1 and OR 1–4; see Supplementary Table S1) were used to amplify a 249-bp fragment and inner primers (IF 1–3 and IR 1) were used to amplify a 79-bp fragment within the first amplification product (Figure 1). The first and second rounds of amplification were controlled by employing different annealing temperatures; specifically, we designed primers so that the difference between annealing temperatures of the outer and inner primers was 13 °C. Products of the first and second rounds of amplification were verified using gel electrophoresis (data not shown).

A standard curve of reactions ranging from 6.8×10^5 to 6.8 copies/tube was generated using a plasmid containing the 249-bp amplicon region (Supplementary Figures S1A and 1B). Reactions containing 6.8 copies were detected at an average C_t value of 31.9 (five replicates ranging from 31.71 to 32.19). The assay has an efficiency (E) of 97.2% and a correlation coefficient (R^2) of 0.999, both within an acceptable range.

In order to further assess the sensitivity of the qPCR assay regarding the number of arthroconidia, we estimated the copy number of the transposable element that is targeted during *Coccidioides* qPCR detection by using droplet digital PCR (ddPCR). For four different *Coccidioides* isolates, two identified as *C. immitis* and two identified as *C. posadasii*, we found that the copy number/genome ranged from 57.0 to 85.7 (Table 2). In Supplementary Figure S2, we show ddPCR plots of the *Coccidioides* isolates and the non-template control (NTC). These results suggest that ~70 copies of this transposable element are found in a single *Coccidioides* genome or arthroconidium.

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Detection of Coccidioides in soil locations neighboring air sampling site

We first extracted DNA from soil samples collected at both sites and then tested for the presence of *Coccidioides* using the ST-nested qPCR assay. At Site A, we detected *Coccidioides* in one location represented by two samples, 18–9 and 18-10. The location was near a Creosote bush (*Larrea tridentata*) and contained many rodent holes; 18–9 was collected at a depth of 10 cm and 18-10 was a composite sample. At Site B, we detected *Coccidioides* in four locations represented by eight samples. Samples 19-2A, 19-2B, and 19-2C were from varying depths (surface, 10 cm, and 20 cm, respectively) at a location characterized by a rodent hole and brittlebush (*Encelia farinosa*). Sample 19-6C was collected at a depth of 15 cm at a location near a rodent hole. Samples 19-8B and 19-8C were collected inside a rodent hole at a depth of 10 cm and 20 cm, respectively. Samples 19-9B and 19-C were collected at a depth of 10 cm and 20 cm, respectively, and at a location characterized by a Creosote bush and many rodent holes. These results demonstrate that both Site A and Site B contained soil positive for *Coccidioides*, thereby qualifying them as suitable locations for air sampling. In addition, we observed the commonly described ‘patchy’ distribution of *Coccidioides*[40,41] in that several locations negative for *Coccidioides* were less than 1.5 m away from a location positive for *Coccidioides*.

Detection of Coccidioides in air/dust samples

We next tested all air/dust samples for the presence of *Coccidioides* using the ST-nested qPCR assay and detected *Coccidioides* in samples High 5, High 6, and High 7 with C_t values of 35.9, 27.0, and 27.1, respectively (Figure 2). To verify that we were indeed detecting *Coccidioides*, we performed Sanger sequencing on the qPCR products for samples High 5, High 6, and High 7, and detected a 66-bp region in samples High 5 and High 6 that is recognized by the probe, inner reverse, and outer reverse primers (Supplementary Figure S3). All three samples were collected from Site B toward the end of the day using the Staplex TFIA High Volume Sampler with GFF filters. Sample High 5 was collected by generating dust clouds but prior to amendment of topsoils with subsurface soils (suspected of being positive for *Coccidioides*). Conversely, samples High 6 and 7 were collected by generating dust clouds after topsoils were amended with subsurface soils thereby explaining the large difference in C_t values for High 5 and High 6/7. These results show that a high-volume air sampler with GFF filters is capable of capturing airborne *Coccidioides* and that the PowerLyzer PowerSoil DNA Isolation Kit coupled with our ST-nested qPCR assay is capable of extracting and detecting *Coccidioides* genomic DNA from air/dust samples.

For both air and soil samples, we were unable to recover *Coccidioides* using culturing methods recently employed by our laboratory on soil samples.[5] Even with our recent success, culturing *Coccidioides* from environmental samples is still challenging, and the benefits of increased sensitivity from molecular based methods as compared to that of culture based methods are essential for feasible environmental surveillance.

Discussion

Each year, thousands of people develop Valley fever by inhaling infectious propagules from the environment. However, there is little knowledge about the prevalence and seasonal

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variation of *Coccidioides* in the environment because many fundamental questions remain unanswered. For example, 'What is the distribution of *Coccidioides* in a given atmospheric location?', 'How long do arthroconidia remain in the air after soil disturbance?', and 'How do weather changes and certain human activities affect the density of airborne arthroconidia?' Development of air sampling and detection methods will provide a first step for addressing these questions, which are important for understanding disease ecology and determining risk factors for occupational coccidioidomycosis. Our results provide proof of concept of a novel approach to environmental surveillance of airborne arthroconidia. We show that a high-volume air sampling method is capable of capturing arthroconidia and that DNA extraction and detection methods used for soil samples are also suitable for analyzing the particulate matter derived from air/dust samples.

Although both Site A and Site B contained at least one soil location positive for *Coccidioides*, we detected positive air/dust samples only from Site B suggesting that the distribution of *Coccidioides* in the air is 'patchy' and/or that the sensitivity of this method can be improved. We also observed a difference in average C_t values between sample High 5 (35.9) and samples High 6/7 (27.0/27.1), suggesting that samples High 6/7 contained significantly more arthroconidia in the air filters than that of sample High 5, which was not surprising since samples High 6/7 were collected after generating dust clouds with topsoils amended with subsurface soils. The ability to detect *Coccidioides* from artificial dust clouds is important for developing methods that measure exposure to this pathogen during activities that cause soil disturbance (i.e., construction and farming).

We acknowledge several limitations in our study. First, molecular methods of detection do not address the question of viability and infectivity of *Coccidioides* propagules and some of the detected arthroconidiamay be nonviable and/or noninfectious. Second, the infectious dose of *Coccidioides* is not well defined; although it is generally accepted that inhaling a single arthroconidium can result in infection, the actual number of arthroconidia needed to cause disease is unknown and likely depends on the strain of *Coccidioides* and ethnicity/ health condition of the host. Finally, high-volume sampling methods do not accurately reflect human breathing, and therefore, it may be difficult to correlate the amount of arthroconidia detected by this method with the amount of arthroconidia that are inhaled by humans. Further research is needed to address these limitations and further improve the methodology of capturing and detecting *Coccidioides* from air/dust samples.

Our results also highlighted the importance and challenges associated with the DNA extraction for molecular detection of *Coccidioides* in environmental samples and especially the critical role of sample homogenization. We tested various homogenizers and found the FastPrep-24 5 G using the specified protocol (see Materials and Methods) yielded the lowest and most consistent C_t values during qPCR analysis. This observation suggests that effective lysis of arthroconidia may be the limiting factor once arthroconidia are captured.

Detection of *Coccidioides* was performed using a ST-nested qPCR assay. Potential benefits to nested PCR assays include increased specificity of DNA amplification and increased yield or sensitivity of the assay. However, nested PCR increases the chance for laboratory contamination since the product of the first amplification is transferred to the tube in which

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the second amplification will be performed. Thus, combining both amplifications into a single reaction or tube limits the risk for laboratory contamination while increasing the chance for true detection. Several studies have employed the use of a ST-nested PCR assay for pathogen detection.[42,43]

Based on our findings, we recommend the use of a high-volume sampler paired with GFF filters for capturing arthroconidia. We tested one high-volume and four low-volume collection techniques and only detected arthroconidia using the high-volume collection and elution assays described. Arthroconidia may have been present in the low-volume samples but detection may have been limited due to assay thresholds. Alternative collection techniques that utilize high-volume liquid impingers or electrostatic precipitators may ultimately prove useful. Our results provide the proof of principal that detection of *Coccidioides* in the air/dust is technically feasible; however, additional research is needed to improve this methodology. In the future, this methodology can be used to address questions about ecology and public health aspects of this disease and has the potential to lead to the development of a *Coccidioides* monitoring system for air quality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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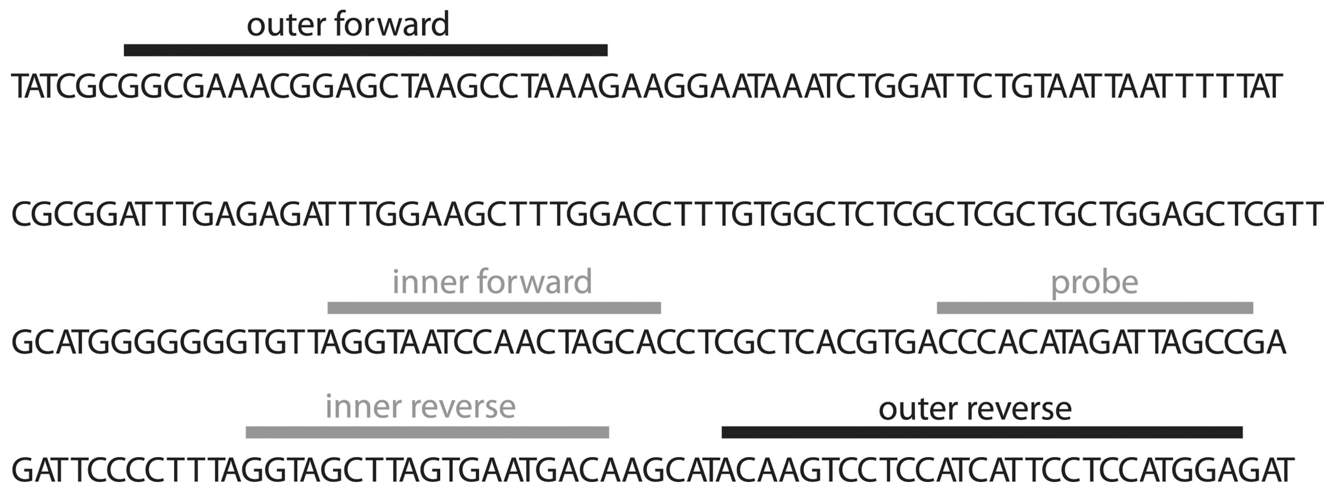

Figure 1.
Schematic of ST nested qPCR assay used for molecular detection of *Coccidioides* from particulate matter collected from air/dust samples. Bars highlight regions used for primer/probe design.

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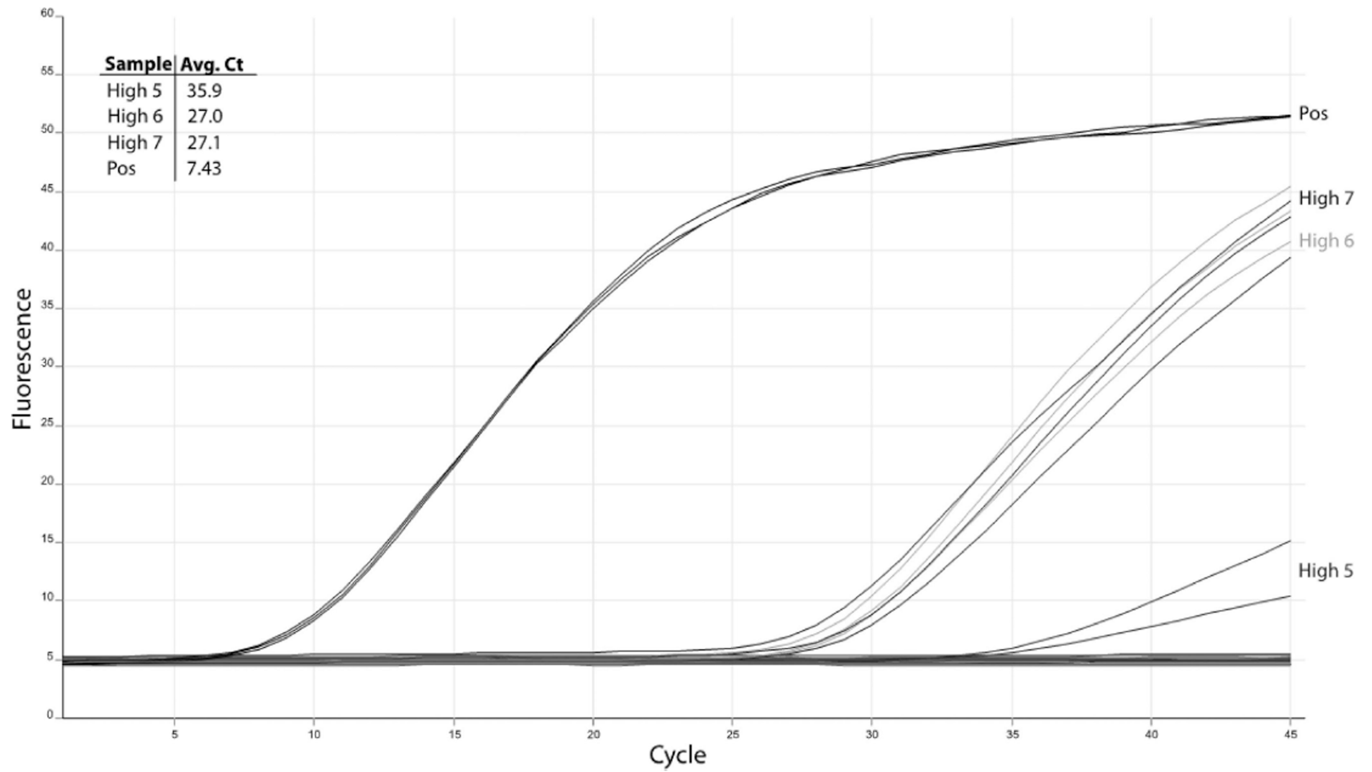

Figure 2.

Detection of *Coccidioides* in air/dust samples. *Coccidioides* was detected in samples High 5, High 6, and High 7 by the ST nested qPCR assay. One nanogram of input *Coccidioides* isolate DNA was used as a positive control (black). Non-template control (NTC) was undetected. Average Ct values (duplicates) are shown in top-left corner.

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Table 1

Air/dust samples collected at Site A and Site B.

Sample ID	Time of sampling		Air sampler	Flow rate	Filter/medium
	Start	End			
Site A					
November 18, 2014					
High 2	1435	1635	Staplex TFIA High Volume	15 ft^3/min	GFF
C 1	1155	1435	Cole Parmer Air Cadet vacuum pumps	0.23 ft^3/min	HEPA
C 2	1435	1635	Cole Parmer Air Cadet vacuum pumps	0.23 ft^3/min	HEPA
Low 1	1155	1255	Cole Parmer Air Cadet vacuum pumps	0.23 ft^3/min	FGLP
Low 2	1255	1435	Cole Parmer Air Cadet vacuum pumps	0.23 ft^3/min	PESU
Low 3	1435	1635	Cole Parmer Air Cadet vacuum pumps	0.23 ft^3/min	FGLP
Liquid 1	1155	1435	Burkard mutli-stage liquid impinger	0.46 ft^3/min	PBS
Liquid 2	1435	1635	Burkard mutli-stage liquid impinger	0.46 ft^3/min	PBS
Site B					
November 19, 2014					
High 3	1020	1120	Staplex TFIA High Volume	15 ft^3/min	GFF
High 4	1130	1230	Staplex TFIA High Volume	15 ft^3/min	GFF
High 5	1235	1335	Staplex TFIA High Volume	15 ft^3/min	GFF
High 6	1345	1445	Staplex TFIA High Volume	15 ft^3/min	GFF
High 7	1455	1545	Staplex TFIA High Volume	15 ft^3/min	GFF
C 3	1020	1335	Cole Parmer Air Cadet vacuum pumps	0.23 ft^3/min	HEPA
C 4	1345	1545	Cole Parmer Air Cadet vacuum pumps	0.23 ft^3/min	HEPA
Low 4	1020	1120	Cole Parmer Air Cadet vacuum pumps	0.23 ft^3/min	PESU
Low 5	1130	1230	Cole Parmer Air Cadet vacuum pumps	0.23 ft^3/min	FGLP
Low 6	1235	1335	Cole Parmer Air Cadet vacuum pumps	0.23 ft^3/min	FGLP
Low 7	1345	1445	Cole Parmer Air Cadet vacuum pumps	0.23 ft^3/min	PESU
Low 8	1455	1545	Cole Parmer Air Cadet vacuum pumps	0.23 ft^3/min	FGLP
Liquid 3*	1020	1125	Burkard multi-stage liquid impinger	0.46 ft^3/min	PBS

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Sample ID	Time of sampling		Air sampler	Flow rate	Filter/medium
	Start	End			
Liquid 4 *	1130	1230	Burkard multi-stage liquid impinger	0.46 ft^3/min	PBS
Liquid 5 *	1235	1335	Burkard multi-stage liquid impinger	0.46 ft^3/min	PBS
Liquid 6 *	1345	1445	Burkard multi-stage liquid impinger	0.46 ft^3/min	PBS
Liquid 7 *	1455	1545	Burkard multi-stage liquid impinger	0.46 ft^3/min	PBS

Note: Sample type and collection data are shown for air/dust samples taken from Site A and Site B. High indicates high-volume filtration. C indicates low-volume HEPA filtration. Low indicates 47-mm membrane low-volume filtration. Liquid indicates low-volume liquid impingement. GFF indicates Staplex TFAGF1 10.16-cm glass fiber filters. HEPA indicates HEPA-CAP 36 filters. FGLP indicates hydrophobic 47-mm filters. PESU indicates 47-mm polyethersulfone filters.

* indicates samples that were collected in three size fractions (>10 μm, 10–4 μm, and <4 μm).

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Table 2

Estimation of copy number/genome for *Coccidioides* detection target gene.

Copy number concentration (copies/ul) **Isolate**	**Chitin synthase**	**Copia-like retrotranspososon**	**Ratio**
Coccidioides immitis 1	30.7	2630	**85.7**
Coccidioides immitis 2	65.2	3720	**57.0**
Coccidioides posadasii 1	31.1	1830	**58.8**
Coccidioides posadasii 2	60.6	3650	**60.2**
Non-template control (NTC)	No call	No call	–

Note: Estimation of the copy number/genome for the target gene used for *Coccidioides* detection. Copy number concentration of the single-copy gene and target gene for *Coccidioides* detection are shown for all four *Coccidioides* isolates. The ratio (copy number concentration of target gene/ copy number concentration of single-copy gene) is also shown. Copy number concentration is expressed as copies/μl.