



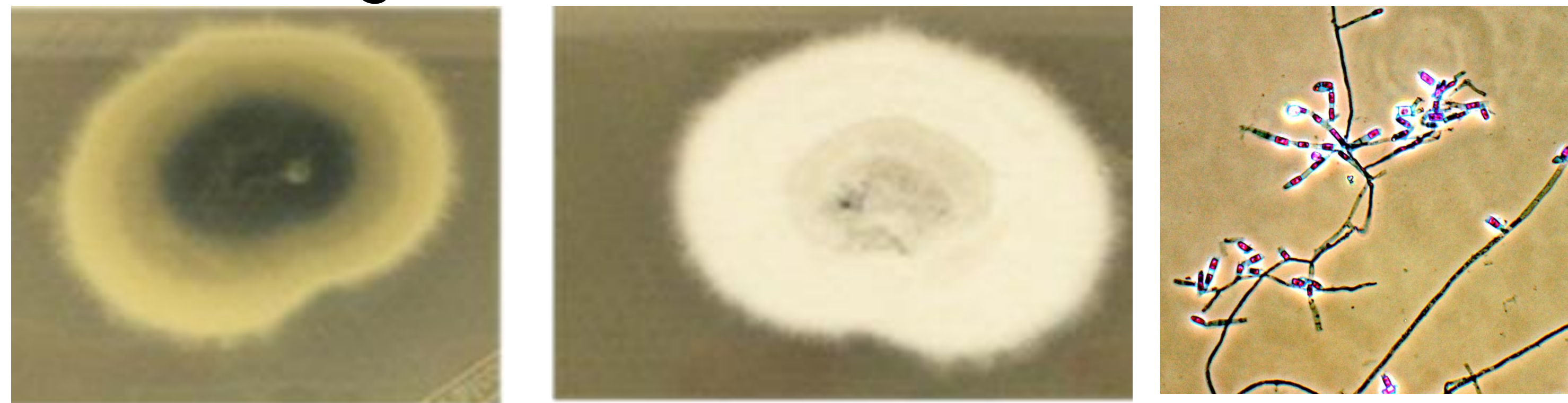
Introduction

Coccidioidomycosis, also known as valley fever, is an infection caused by inhalation of *Coccidioides spp.* spores. *Coccidioides spp.* are dimorphic, soil-dwelling fungi, known to cause a broad spectrum of disease ranging from a mild febrile illness to severe pulmonary manifestations or disseminated disease.

The genus *Coccidioides* is comprised of two genetically distinct species: *Coccidioides immitis* and *C. posadasii*. These two species cause similar clinical symptoms; however, they are present in different geographic regions. *C. immitis* is found in central and southern California, with the San Joaquin Valley being the region of greatest endemicity.

The most concentrated region for *C. posadasii* is in Arizona and sporadic sites in southern Utah and Nevada, Oregon, and El Paso, TX. Beyond the USA, *C. posadasii* is present in parts of Mexico and Central and South America. Members of our group has been working for the last year in identifying the epidemiologic and clinical/radiographical presentations of Coccidiomycosis in the Permian Basin.

This is of great concern to women of childbearing age that become infected prior to conception, as the risk of dissemination increases during pregnancy and requires increased vigilance.¹



From: Litvintseva et al, CID 2014 ²

OBJECTIVES

The goal of this study was to evaluate the soil microbiome, including those of *Coccidioides spp.* in soil from the counties served by Texas Tech University Health Sciences Center at the Permian Basin.

Methods and Materials

Six soil samples were collected at Loop 250 and Lamesa Rd, Midland, TX Midland County. Using an N-99 respirator and sterile techniques, approximately 10 grams of soil at a depth of 10 inches was collected from an established Prairie Dog colony. DNA was then isolated using Mo-Bio PowerLyzer Power Soil kit (Carlsbad, CA, U.S.A.).

DNA Extraction

- MoBio PowerLyzer Power soil
 - no heating and proteinase K treatment
 - glass beads for cleaner DNA
- Bead beating is the most critical step

From: Litvintseva et al, CID 2014 ²

Real- time Quantitation PCR was performed on the isolated DNA samples. The samples were quantified using a Standard Curve and detected utilizing FastStart Essential DNA Green Master (Roche, Indianapolis, Indiana, U.S.A.).

RESULTS

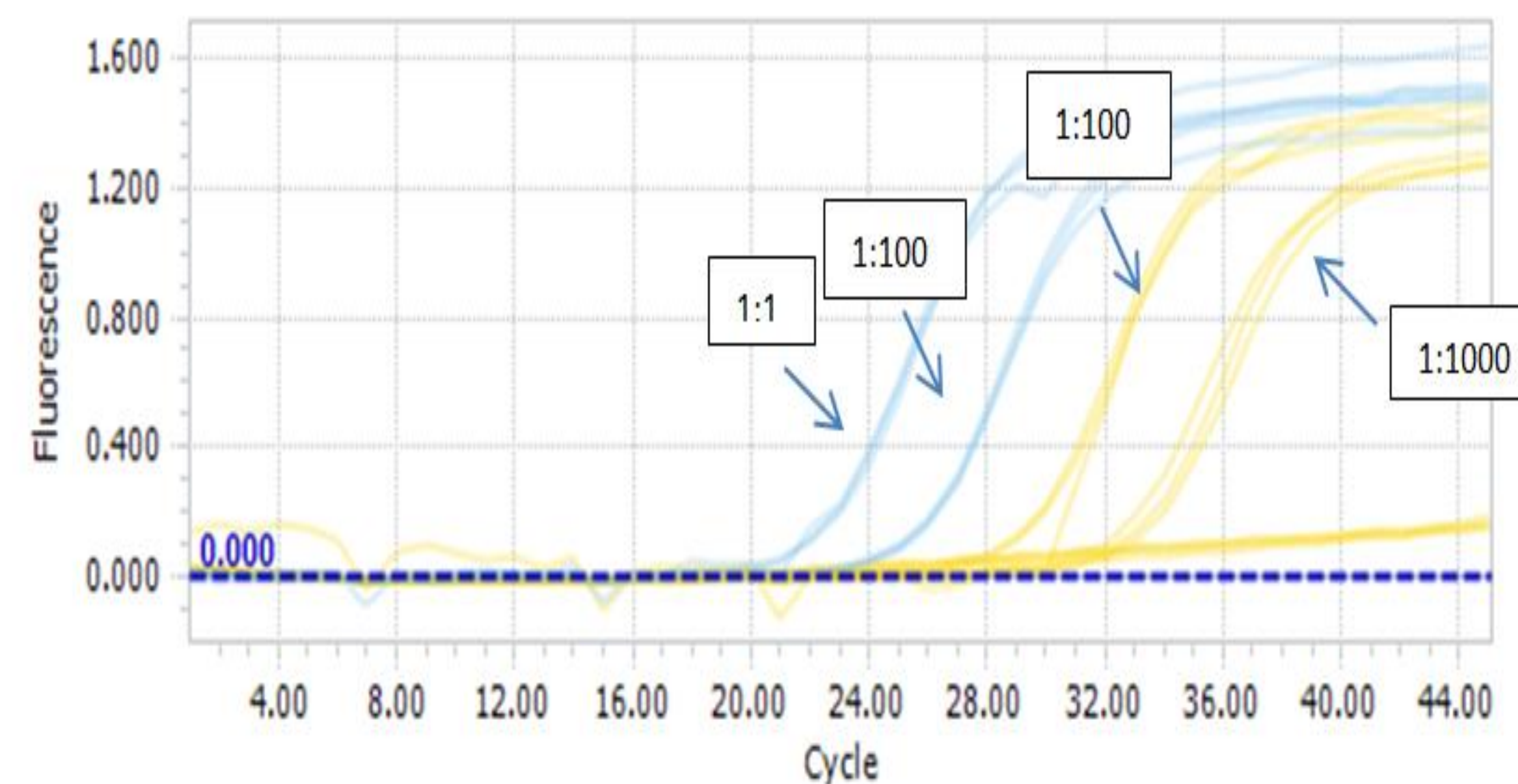


Figure 1 shows the Standard Curve 10- Fold Change of the *Coccidioides immitis* control (Vircell, Granada, Spain) . The dilutions were 1:1 (60,000 copies/ rxn), 1:10 (6,000 copies/ rxn), 1:100 (600 copies/ rxn), and 1:1000 (6 copies/ rxn).

Results (cont).

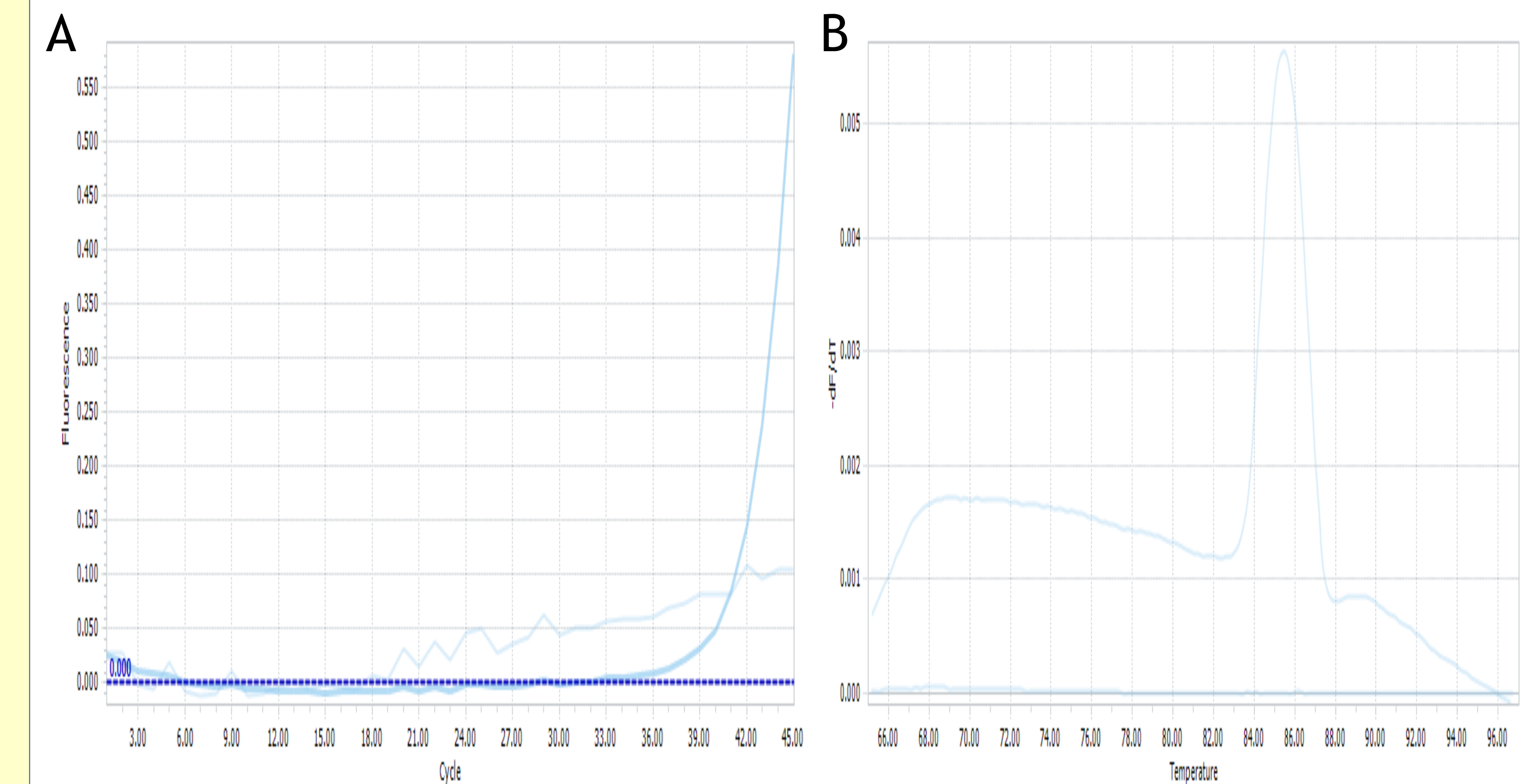


Figure 2a) This is the amplification curve for sample 1. The negative sample is a bit noisy but does not pass the minimal EPF to be considered positive. The amplification is late in the cycle indicating how little of coccidioides's species gene is in the sample. Figure 2b) This is the melting peak for sample 1. The expected melting temperature the product is about 85°C. The sample has a melting temperature of about 85°C showing this is our expected product.

CONCLUSIONS

The DNA extraction from the soil presents a more challenging methodology compared to human tissue DNA extraction.

The soil pH and matrix should be taken in consideration during extraction process, which may require additional processing.

While the DNA controls worked for the Q-RT PCR, all soil samples were negative for the gene used as a known control.

REFERENCES

- 1 Arch Pathol Lab Med. 2007; 131, :652-655. Disseminated Coccidioidomycosis in Pregnancy. Hooper, JE, Lu, Q, Pepkowitz, S.
- 2 Litvintseva et al. 2015. Investigating the expanding geographic range of coccidioides [PowerPoint Slides].

ACKNOWLEDGEMENT

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