SPECIFIC AIMS

Coccidioidomycosis (Valley Fever, VF) is a respiratory disease caused by *Coccidioides immitis* and *Coccidioides posadasii*. These fungi can cause disease in both human and non-human mammals when infectious spores are inhaled. Although ~60% of those infected do not seek medical attention, 40% experience delayed diagnosis and prolonged illness. Flu-like symptoms range from uncomplicated pneumonia with extreme fatigue and drenching night sweats to disseminated CNS disease that can be fatal. In endemic regions, VF has been reported to be the cause of almost one-third of community-acquired pneumonia (CAP) cases, often being misdiagnosed as a bacterial infection.[7] The median time from visiting a healthcare provider to diagnosis for VF in endemic regions is about 23 days, while in low- and non-endemic regions has been reported as 38 days.[31–33] Improving time to diagnosis by increasing the speed and availability of antibody detection is critical to limiting inappropriate use of antibiotics which are ineffective for fungal infections.[2] A major reason for the delay in diagnosis is that testing is often performed at clinical reference laboratories, and the time from drawing blood to test result may be 2 days at best to 2 weeks. Cactus Bio has developed and seeks to commercialize a rapid 10-minute antibody test for VF that can be performed near or at a point-of-care setting. This rapid test will provide a new tool to aid healthcare providers when considering diagnostic and treatment decisions.

The Cactus Bio team has advanced the speed of anti-coccidioidal antibody detection from days to just 10 minutes using an undiluted specimen directly in a lateral flow assay (LFA) format. One innovation of Cactus Bio's test is that while antibody detection methods traditionally rely on species-specific secondary antibodies, our LFA captures antigen-antibody complexes at the test line allowing the rapid test to be used with virtually any mammal that generates antibodies–which is helpful because *Coccidioides* is known to infect a wide variety of mammals. Our LFA utilizes an innovative double-antigen bridge assay format which has shown improved sensitivity compared to indirect antibody detection methods.[34,35] These features represent a substantial improvement in versatility, simplicity, and speed over current antibody detection methods such as the complement fixation (CF) test in which reagent and technical failures are common, immunodiffusion (ID) testing which has subjective interpretation and an incubation time of 48 hours (realistic turnaround time of 3-8 days), and enzyme immunoassays (EIAs) in which false positive IgM results occur.[36,37] Cactus Bio has licensed this LFA and demonstrated proof of principle in Phase I. In Phase II, we will build on our successes, pursuing commercialization of the LFA for qualitative use in Aim 1 [*note: qualitative Coccidioides serology (Product Code: MIY, Regulation Number 866.3135) is exempt from FDA 510(k) pre-market notification*] and pursuing additional uses of the test such that an antibody titer may be approximated using a lateral flow reader (Aim 2), and evaluating its use with additional biofluids such as finger-stick blood and cerebrospinal fluid (Aim 3).

Note: For the remainder of this proposal, Cactus Bio's antibody LFA test will be referred to as **Velox**.

Aim 1A: Perform equivalency study of Velox compared to current VF serologic antibody tests [i) EIA, ii) Immunodiffusion and iii) Complement Fixation] for qualitative (pos/neg) detection of anti-coccidioidal antibodies.

Aim 1B: Evaluate the utility of Velox rapid test in a clinical setting. Compare qualitative LFA results with EIA testing for every serum sample sent to the clinical laboratory from patients with pneumonia.

Aim 2: Evaluate the use of a lateral flow reader to approximate antibody titers compared to a standard of care complement fixation test. Analytical validation of Velox per FDA requirements will be completed with the following milestones:

Milestone 1. Evaluate the analytical performance of the Velox test when used with a Detekt lateral flow reader (dynamic range, analytical sensitivity, inter- and intra-test reproducibility).
Milestone 2. Determine performance of Velox as an aid to diagnose VF and provide an antibody titer for the patient serum samples tested with the standard of care assay at 2 different hospitals (same samples as in Aim 1B).
Milestone 3. Produce Velox kits for clinical validation (regulatory) studies and commercialization.
Milestone 4. Conduct inter-laboratory assay reproducibility.
Milestone 5. Conduct stability testing of Velox test kits.

Aim 3: Assess performance of the Velox LFA with cerebrospinal fluid (CSF) and fingerstick whole blood. Use of the test with CSF and fingerstick whole blood will allow for expansion of applications to central nervous system involvement and point-of-care testing, respectively. Analytical validation with these specimen types will be performed per FDA requirements, with similar milestones to those outlined in Aim 2.