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Application Brief

Comparison of CSA: Simian TB with a Competitor's ELISA for Detection of NHPs Infected with *M. tuberculosis*.

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Introduction

Nonhuman primates (NHPs) in captivity are regularly screened for M. tuberculosis infection, both as part of transport associated quarantine and for routine colony health maintenance programs. The tuberculin skin test (TST) is the required screening method to identify animals with active or latent M. tuberculosis infection. However, the TST screening method has been insufficient to identify infected animals found with M. tuberculosis particularly in cynomolgus macaques (*Macaca fasicularis*), a commonly used species in biomedical research(1-3). Because of the low sensitivity of the TST, additional testing methods should be incorporated into the pre and post-quarantine routine to reduce the risk of TB in colonies.

Previous studies have shown that commercially available ELISA tests have not performed well to identify NHPs with *M. tuberculosis* infection. In the study by Choi et al. in 2016, several animals negative by semi-annual TST screening were imported for a study and underwent TB antibody test by ELISA and were also reported negative prior to immunosuppressive drug administration and allo-kidney transplantation. Multiple animals were found to have latent TB during necropsy, and of those with TB associated pathology only 50% of those were PCR positive (2). Despite the data demonstrating poor performance of ELISA-based tests they are still on the market. However, there is a clear need for supplemental testing alongside the TST to better identify asymptomatic or latent TB infected animals before study initiation.

These examples highlight the risk in using screening methods with low sensitivity, like ELISA-based methods. Intuitive Biosciences has developed the most sensitive screening test for TB in NHPs as part of the Colony Surveillance Assay (CSA) kits and services. The CSA: Simian TB serology screening test is built on the Array Intuitive Multiplex (AIM) platform, a multiplex immunoassay method that has been demonstrated to provide high diagnostic sensitivity and specificity when compared to other serological methods (4). To better compare the high accuracy of the CSA: Simian TB test with other available kits a set of test samples were evaluated by a commercially available ELISA and by the CSA: Simian TB test. Our results demonstrate the superior performance of the CSA: Simian TB kits and highlight the need for using well-validated and sensitive methods for supplemental TB testing to better identify NHP with active or latent TB infection.

Materials and Methods

Subjects. A validation set of samples was assembled from specific pathogen-free research macaques. A total of 183 Negative samples were selected, 104 cynomolgus macaques and 79 rhesus macaques. Negative samples were considered "negative" by a combination of history of negative tuberculin skin tests (TST), low risk of transmission, and history of healthy veterinary examination. A total of 54 Positive samples were selected, including 16 cynomolgus macaques and 38 rhesus macaques. The "positive" criteria included experimentally infected and those naturally exposed. For naturally occurring infections, the criteria include positive TST reaction with TB associated pathology, TB pathology at necropsy, *M. tuberculosis* culture or PCR positive results, or TB-positive histology by acid-fast stain and/or immunofluorescent microscopy.

ELISA Protocol. Samples were run according to the manufacturer's protocol on commercially available "Monkey Mycobacterium Tuberculosis ELISA IgG Kits for 96 tests." Briefly, samples were diluted and added to assay plates for 60 minutes incubation at 25°C. Plates were washed, then incubated for 30 minutes with anti-monkey HRP at 25°C. Following incubation,plates were washed and HRP substrate was added to wells in incubated for 20 minutes in the dark. Reaction was quenched with Stop Solution, and absorbance was read at 450 nm on a Tecan GENios instrument (Männedorf, Switzerland). The cut-off value was determined by taking the mean of triplicate measurement of the included Cut-off Control sample.

CSA: Simian TB Protocol. Samples were run according to the manufacturer's protocol (Intuitive Biosciences, Madison WI, USA). Briefly, samples were diluted and incubated for 60 minutes at 25°C in the assay plates. Plates were washed and incubated for 60 minutes with the anti-simian IgG gold conjugate at 25°C. Plates were washed and developed with SilverQuant detection reagents for 3 minutes, and Relative Intensity Units (RIU) determined by scanning and analysis using the AQ 1000 (Intuitive

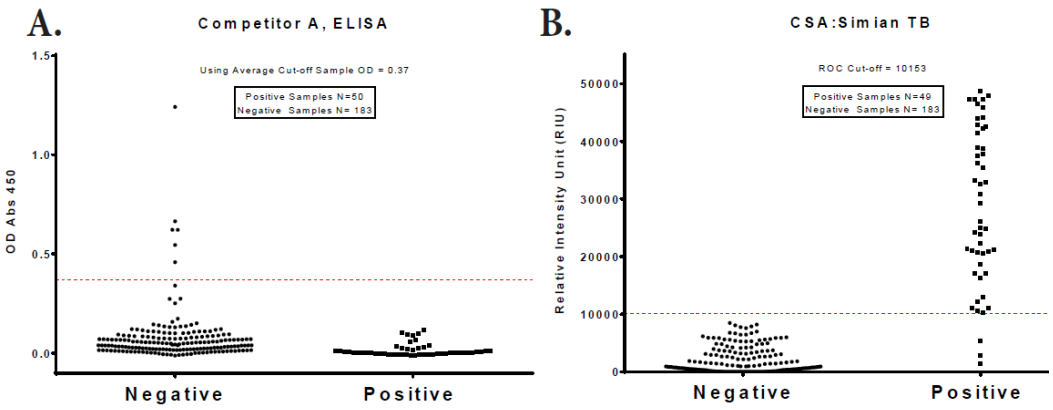

Figure 1. Validation sample set on two commercial kits. **A.** Results graphed as O.D. at abs 450m, for the negative (N=183) and Positive (N=50) samples sets when assay by Competitor A ELISA. **B.** Results graphed as Relative Intensity Units (RIU) for the Negative and Positive samples sets assayed on the CSA: Simian TB kit. Cut-off values are shown by the dotted red line.

Biosciences), using the established cut-off values in the analysis software to determine Positive and Negative calls.

Results

The results from "Competitor A" ELISA kits were surprising in the lack of signal in all positive samples. In each plate the included assay controls produced the expected signal. As shown the Table below, the Calibrator control samples all produced the expected signal with an average Calibrator B Cut-off value of OD 0.37. Calibrator C and D both had signal greater than the Cut-off, and Calibrator A was below. This demonstrates that the ELISA assay was performed according to the protocol and that the assay was functioning as intended. However, using the CSA: Simian TB controls from confirmed TB positive sera no signal was observed above the cut-off value. This suggests that the ELISA kit has a very low sensitivity for true positive samples from macaques.

Included Control:	Abs 450 (OD)	Diagnostic Call
Calibrator A, Negative	-0.02	Negative
Calibrator B, Cut-off	0.37	-
Calibrator C, Low Positive	0.57	Positive
Calibrator D, Positive	1.21	Positive
CSA: Simian TB Positive Control	0.04	Negative

When running the complete validation set of samples using the ELISA assay, not a single "Positive" sample was detected above the cut-off value of the ELISA kit. However, of the 183 "Negative" samples 6 produced OD values above the cut-off of the ELISA and were false positive results. The individual values for each sample is graphed in Figure 2a, grouped into "Negative" and "Positive" sample sets. This results in an overall Diagnostic Sensitivity of 0%, and an overall Accuracy of all samples at 76.0%. This same sample set when run on the CSA: Simian TB kits, shown in Figure 2b, has an overall accuracy of 98.7%. The CSA: Simian TB kit correctly identified all the "Negative" samples, but missed 3 in the "Positive" sample set. The cut-off value for the CSA: Simian TB was determined by an extensive validation with hundreds of macaque samples, and uses an internal positive control to normalize results between assays. These results are consistent with the reported validation data, where the CSA: Simian TB had a calculated Diagnostic Sensitivity of 96% and Specificity of 99%.

Conclusions

Overall, the CSA: Simian TB vastly outperformed the ELISA based method, with an overall accuracy of 98.7% compared to the ELISA with 76.0% accuracy using the same sample set. The diagnostic sensitivity of the CSA: Simian TB test was demonstrated with correctly identifying 94% of the TB positive samples, compared to 0% identified by the ELISA. Surprisingly, the ELISA assay did not correctly identify any of the Positive samples. Given the limited information on validation of this ELISA kit, it appears that there is very low sensitivity for detecting TB positive macaques. While other manufacturers have adapted human TB kits for NHPs, the CSA: Simian TB was developed using samples from naturally and experimentally infected macaques and determined the ideal epitopes for NHP (US Patent No. 9,404,923). Moreover, the CSA: Simian TB kits continually monitor NHPs throughout the world to stay current with circulating strain of M. tuberculosis. The higher performance and accuracy of the CSA: Simian TB kits is demonstrated here in a side-by-side comparison of identical samples, and highlight the sensitivity of this NHP-specific test for TB.

References:
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