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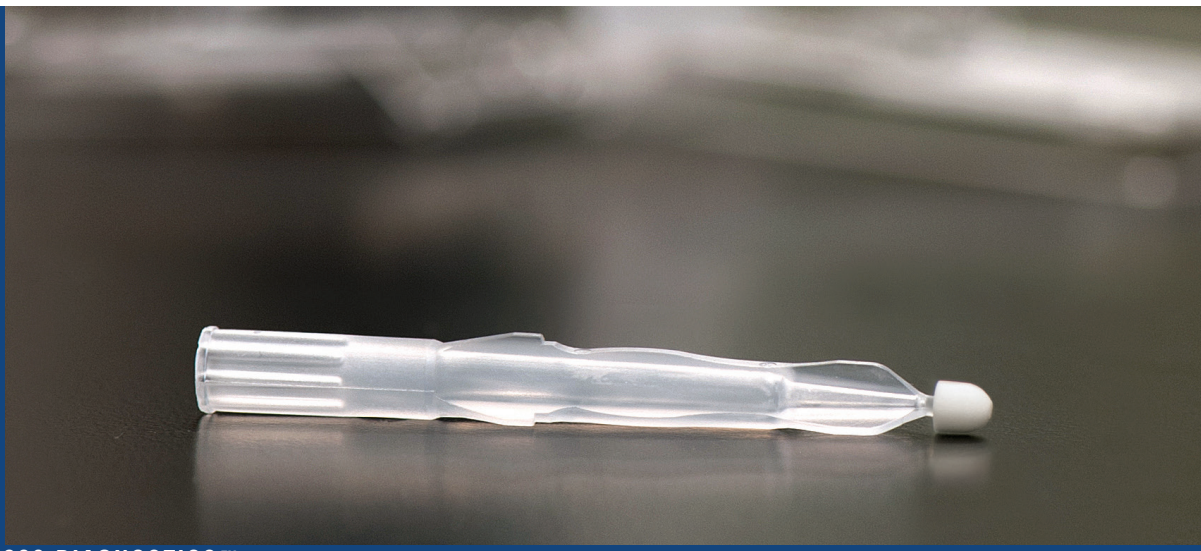
Summary

HemaTIP™ is the 360 Diagnostics™ offering of Mitra® VAMS™ technology. The introduction of this technology was preceded by the comprehensive qualification study presented here to demonstrate the suitability of HemaTIP™ microsampling for nonhuman primate serology.



360 DIAGNOSTICS™

Qualification of the Charles River HemaTIP™ Microsampler for Nonhuman Primate Serology

With the recent advent of multiplexed immunoassay platforms, such as the Luminex xMAP®-based multiplexed fluorometric immunoassay (MFIA®) developed by our laboratory[5,6], we are able to perform the most comprehensive serosurveillance panel on a single drop of blood, which can be collected humanely from an unanesthetized laboratory animal. Survival sample collection is consistent with the goals of the 3Rs, to reduce and refine the use of animals in biomedical research. Similar to other species, microsampling has enabled sampling of nonhuman primate (NHP) colony animals with minimal discomfort in quarantine, during routine serosurveillance or following an outbreak.

Charles River has introduced the HemaTIP™ microsampling system for the collection, transport, and storage of research animal blood specimens for serologic testing. HemaTIP™ employs the Mitra® microsampler device by Neoteryx (www.neoteryx.com) that is based on volumetric absorptive microsampling (VAMS™). The tip of the Mitra® device (shown above) consists of an inert, porous, hydrophilic material that rapidly wicks up a constant volume of 20 µL from a drop of whole blood. Microsamplers are then placed in a clamshell holder for drying, ambient temperature shipping, and refrigerated storage. In the laboratory, the HemaTIP™ is immersed in elution buffer for sample extraction followed by MFIA® testing. Important advantages of the HemaTIP™ microsampling system compared to the dried blood spot (DBS) sample collection[1,2,7,11] (such as our EZ-Spot®) include more quantitative and simpler blood collection, and improved reproducibility, as the microsamplers are designed for organization and automated extraction in a 96-well plate format[3,4,9].

The results of the HemaTIP™ qualification study presented here demonstrate that the sensitivity, specificity, and reproducibility of MFIA® for antibodies to NHP pathogens with HemaTIP™ and serum samples are equivalent. Data presented separately show the equivalence of HemaTIP™ to serum samples for MFIA® of mouse, rat, guinea pig, rabbit, and hamster samples (www.criver.com/hematip).

Materials and Methods

Samples

MFIA® testing was performed on matching serum and HemaTIP™ samples from seropositive and specific pathogen-free (SPF) nonhuman primates (NHPs) (rhesus and/or cynomolgus macaques). The HemaTIP™ samples were collected, stored, and extracted according to the Mitra® Microsampling Device User Manual (www.neoteryx.com); note that instructions are also available on the Charles River website (www.criver.com/hematip). Blood samples prepared from animals infected with an individual agent are referred to as monospecific to indicate that they contain antibodies to a single pathogen. Polyspecific antisera with antibodies to multiple pathogens were prepared using blood from conventionally housed animals naturally infected with a variety of pathogens or by combining various monospecific antisera and mixing the resultant pool with an equal volume of packed red blood cells.

MFIA® Testing

NHP samples were tested by MFIA® assessment panel plus Chagas and lymphocryptovirus (LCV) beads comprising 17 assays, respectively. The panel assays can be found on the Charles River website (www.criver.com/serology). The MFIA® procedure was performed as described elsewhere[10]. For each assay, the net median fluorescence intensity signal (MFI) was calculated by subtracting the tissue control (TC) from the antigen (AG) MFI. In the following tables and graphs, the results are presented as Net MFI/1,000 (or Net MFI in thousands). In this study, values of <1.5 and ≥ 5 were classified as negative and positive, respectively; net signals between these cutoffs were called equivocal.

Experiment Summary

Serial dilutions of monospecific serum: HemaTIP™ sample pairs were tested by MFIA® to assess the effect of sample type on analytical sensitivity (i.e., limit of detection [LOD]) and specificity. Then, to evaluate the diagnostic performance of MFIA® repeatability (i.e., agreement between the results of replicate testing) and ruggedness of MFIA® with HemaTIP™ versus serum, 8 polyspecific and 8 SPF serum: HemaTIP™ NHP (rhesus and/or cynomolgus macaques) sample pairs were tested in 3 separate runs by each of 2 analysts.

Results

Analytical Performance

The graphs in Figure 1 show MFIA® endpoint titration curves for monospecific serum-HemaTIP™ pairs prepared from standard antisera collected from intentionally or naturally infected NHPs (rhesus and/or cynomolgus macaques). Titration curves and LOD for the serum and corresponding HemaTIP™ samples were essentially identical.

Diagnostic Performance and Repeatability

As described above, 8 polyspecific immune and 8 SPF serum-HemaTIP™ sample pairs for NHPs were tested by MFIA® in 3 separate runs carried out by each of 2 analysts, for a total of 6 MFIA® runs. The results for known-positive and negative sample-assay combinations are summarized in Table 1. The diagnostic sensitivities for assay-positive serum and HemaTIP™ samples were 99.8% (with average Net MFI/1,000 of 15.0 and CV of 14.3%), and 99.6% (with average Net MFI/1,000 of 13.4 and CV of 13.4%), respectively. Aside from the few false-negative MFIA® results obtained by analyst 2 for serum and HemaTIP™ samples (99.5% and 99.3%, respectively), the results between the 2 analysts were virtually identical.

As shown in Table 1, the diagnostic specificity of MFIA® with serum and HemaTIP™ samples were 99.6% (0.4% false positives) and 98.6% (1.4% false positives), respectively. The average Net MFI/1,000 was 0.1 for both serum and HemaTIP™.

The linear regression analysis plots for the immune samples presented in Figures 2 and 3 demonstrate the strong correlation between the Net MFI for of HemaTIP™ versus serum ($R^2 = 0.902$, slope $= 0.897$) and the Net MFI for analyst 1 versus analyst 2 for serum ($R^2 = 0.962$, slope $=$ 1.009) and HemaTIP™ ($R^2 = 0.964$, slope $= 0.977$).

Conclusion

This study was undertaken to qualify the HemaTIP™ microsampling system, employing the 20 µL Mitra® microsampler from Neoteryx, for NHP serology. The results of comparable studies qualifying the HemaTIP™ for serology of mice, rats, guinea pigs, rabbits, and hamsters are available in a separate technical note (www.criver.com/hematip). HemaTIP™ microsampling facilitates easy blood sample collection (point and collect), thereby reducing stress in NHPs during blood collection; it also eliminates the steps, reagents, materials, and equipment needed to prepare and ship serum samples. Important advantages of HemaTIP™ versus DBS microsampling include more quantitative and simpler blood collection, and improved reproducibility, as the HemaTIP™ microsamplers are designed for organization and automated extraction in a 96-well plate format. By comprehensively and conclusively demonstrating that the analytical and diagnostic performance of MFIA® with serum and HemaTIP™ samples were equivalent, the results of this study qualify the 20 µL HemaTIP™ microsampling system as a suitable alternative to serum or DBS sample collection for NHP serology.

Table 1. Diagnostic Sensitivity and Repeatability of MFIA® for Antibodies to NHP Pathogens Using HemaTIP™ Versus Serum*

			Serum					HemaTIP™				
Sample-Assay				Net/1,000					Net/1,000			
Status	#	Analyst	% Pos	Avg	SD	%CV	TC/1,000	% Pos	Avg	SD	%CV	TC/1,000
Positive	71	1	100.0%	15.6	1.0	8.0%	0.0	100.0%	14.1	0.9	7.8%	0.0
		2	99.5%	14.2	1.4	14.2%	0.0	99.3%	12.6	1.1	9.8%	0.0
			99.8%	15.0	1.6	14.3%	0.0	99.6%	13.4	1.5	13.4%	0.0
Negative	144	1	0.1%	0.0	0.0		0.0	1.5%	0.1	0.0		0.0
		2	0.7%	0.1	0.2		0.0	1.4%	0.1	0.0		0.0
			0.4%	0.1	0.2		0.0	1.4%	0.1	0.0		0.0

* As described in the Materials and Methods, 8 known immune and 8 SPF serum-HemaTIP™ NHP sample pairs were tested in 3 separate runs by each of 2 analysts. Sample Net MF1/1,000 of <1.5 and ≥ 5 were classified as negative and positive, respectively. The coefficient of variation (%CV) = Standard Deviation (SD)/ Average (Avg). TC = "Tissue" Control assay to detect nonspecific reactivity.

Figure 1. MFIA® LOD Titration of Monospecific Serum-HemaTIP™ NHP Sample Pairs: Samples were diluted 5-fold starting at 1/100. The titration data points represent the average for all serum samples with assay net MFI/1,000 ≥ 5 at the starting 40-fold dilution.

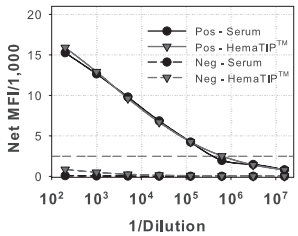


Figure 2. Linear Regression of HemaTIP™ versus Serum Net MFI/1,000 for MFIA® of Immune NHP Samples: The data points represent the average Serum and HemaTIP™ net MFI/1,000 for 6 runs. The linear regression analysis was done in SigmaPlot version 11 (Systat Software).

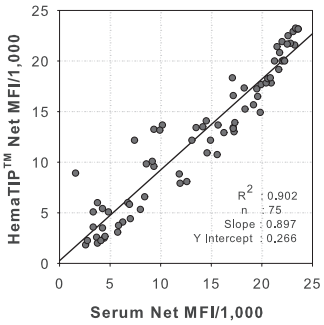
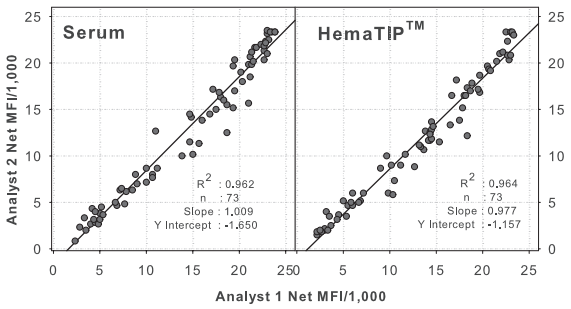


Figure 3. Linear Regression of Analyst 1 versus 2 Net MFI/1,000 for MFIA® of Immune NHP Samples: The data points denote the average serum and HemaTIP™ net MFI/1,000 for 3 separate runs performed by each analyst. The linear regression analysis was done in SigmaPlot version 11 (Systat Software).



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