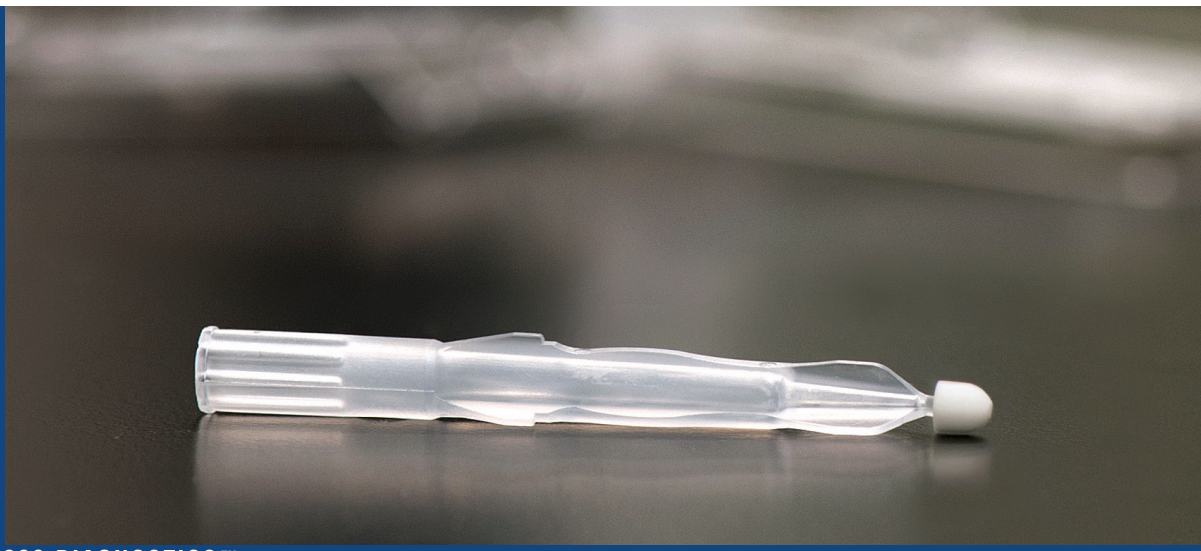
charles river

Summary

Hema-TIP™ 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 Hema-TIP™ microsampling for rabbit, guinea pig, and hamster serology.



360 DIAGNOSTICS™

Qualification of the Charles River HemaTIP™ Microsampler for Rabbit, Guinea Pig, and Hamster Serology

With the recent advent of multiplexed immunoassay platforms, such as the Luminex xMAP®-based multiplexed fluorometric immunoassay (MFIA®) developed by our laboratory[1,2], we are able to perform the most comprehensive serosurveillance panel on a drop of blood, which can be collected humanely from an unanesthetized laboratory animal. Survival sample collection is consistent with goals of the 3Rs to reduce and refine the use of animals in biomedical research, and has enabled direct sampling of rodent colony animals in quarantine 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® microsampling device by Neoteryx (www.neoteryx.com), which 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, refrigerated storage, and ambient temperature shipping. In the laboratory, tips are immersed in elution buffer for sample extraction and testing. Important advantages of the HemaTIP™ microsampling system compared to the dried blood spot (DBS) sample collection[3–5] (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.[6–8]

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

Materials and Methods

Samples

MFIA® was performed on matching serum and HemaTIP™ samples from seropositive and SPF rabbits, guinea pigs, and hamsters. 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 each 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

Rabbit, guinea pig, and hamster samples were tested by MFIA® panels comprising 10, 9, and 6 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.[9] 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, or LOD) and specificity. Then, to evaluate the diagnostic performance and repeatability (i.e., agreement between the results of replicate testing) of MFIA® with HemaTIP™ versus serum samples, 8 polyspecific and 8 SPF serum-HemaTIP™ sample pairs per animal species were tested in 3 separate runs by each of two analysts.

Results

Analytical Performance

The graphs in Figure 1 show MFIA® endpoint titration curves for monospecific serum-HemaTIP™ sample pairs prepared from standard antisera collected from intentionally or naturally infected rabbits, guinea pigs, and hamsters. 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 per animal species were tested by MFIA® in 3 separate runs carried out by each of the two analysts, for a total of 6 MFIA® runs per species. The results for known-positive and negative sample-assay combinations are summarized in Tables 1 and 2, respectively. As shown in Table 1, the diagnostic sensitivities for assay-positive serum and HemaTIP™ samples were 100.0% (with average Net MFI/1,000 of 18.5 and CV of 8.9%), and 99.4% (with average Net MFI/1,000 of 18.2 and CV of 13.0%), respectively. Aside from the few false-negative MFIA® results obtained by analyst 2 for guinea pig HemaTIP™ samples, the serum and HemaTIP results were virtually identical.

The linear regression analysis plots for the immune samples presented in Figures 2 and 3 demonstrate the strong correlation between the Net MFI for HemaTIP™ versus serum ($R^2 = 0.97$, slope $= 0.97$) and the Net MFI for analyst 1 versus analyst 2 for serum ($R^2 = 0.94$, slope $=$ 1.05) and HemaTIP™ ($R^2 = 0.94$, slope $= 0.97$).

As shown in Table 2, the diagnostic specificity of MFIA® with serum and HemaTIP™ samples were 100% and 99.7%, respectively. The average Net MFI/1,000 was 0.0 for serum and 0.1 for HemaTIP™.

Conclusion

This study was undertaken to qualify the HemaTIP™ microsampling system, employing the 20 µL Mitra microsampler from Neoteryx, for rabbit, guinea pig, and hamster serology. The results of a comparable study to qualify the HemaTIP™ for serology of mice and rats are available in a separate technical note (www.criver.com/hematip). HemaTIP™ microsampling, like DBS, facilitates antemortem blood sample collection, thereby reducing sentinel usage and enabling non-lethal, direct sampling of colony animals; 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 rabbit, guinea pig, and hamster serology.

Table 1. Diagnostic Sensitivity and Repeatability of MFIA® of 8 Immune Serum-HemaTIP™ Sample Pairs*

Sample-Assay Negative			Serum					HemaTIP™				
				Net/1,000					Net/1,000			
Species	#	Analyst	% Pos	Avg	SD	%CV	TC/1,000	% Pos	Avg	SD	%CV	TC/1,000
Rabbit	20	1	100.0%	18.9	2.6	15.2%	0.0	100.0%	19.7	1.3	6.9%	0.0
		2	100.0%	19.8	1.0	5.2%	0.0	100.0%	20.3	1.5	7.8%	0.0
			100.0%	19.4	2.1	11.7%	0.0	100.0%	20.0	1.6	8.1%	0.0
Guinea Pig	33	1	100.0%	17.1	1.2	8.1%	0.0	100.0%	16.0	2.1	14.4%	0.0
		2	100.0%	16.8	1.2	8.2%	0.0	97.5%	16.2	3.4	22.3%	0.0
			100.0%	17.0	1.3	9.0%	0.0	98.7%	16.1	2.9	19.2%	0.0
Hamster	16	1	100.0%	20.3	1.2	6.2%	0.0	100.0%	20.6	1.0	5.2%	0.0
		2	100.0%	20.6	0.7	3.8%	0.0	100.0%	20.3	1.2	6.3%	0.0
			100.0%	20.4	1.0	5.3%	0.0	100.0%	20.5	1.2	6.2%	0.0
All	**69**		**100.0%**	**18.5**	**1.5**	**8.9%**	**0.0**	**99.4%**	**18.2**	**2.1**	**13.0%**	**0.0**

* As the described in the Materials and Methods, 8 known immune and 8 SPF serum-HemaTIP™ sample pairs per animal species were tested in 3 separate runs by each of the two 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.

Table 2. Diagnostic Specificity of MFIA® of 8 Immune and 8 SPF Serum-HemaTIP™ Sample Pairs*

Sample-Assay Positive			Serum				HemaTIP™			
				Net/1,000				Net/1,000		
Species	#	Analyst	% Pos	Avg	SD	TC/1,000	% Pos	Avg	SD	TC/1,000
Rabbit	120	1	0.0%	0.1	0.0	0.0	0.1%	0.1	0.1	0.0
		2	0.0%	0.0	0.0	0.0	1.1%	0.2	0.1	0.0
Guinea Pig	43	1	0.0%	0.1	0.0	0.0	0.0%	0.1	0.1	0.0
		2	0.0%	0.0	0.0	0.0	0.0%	0.1	0.1	0.0
Hamster	80	1	0.0%	0.0	0.0	0.0	0.0%	0.0	0.0	0.0
		2	0.0%	0.0	0.0	0.0	0.0%	0.0	0.0	0.0
All	**243**		**0.0%**	**0.0**	**0.0**	**0.0**	**0.3%**	**0.1**	**0.1**	**0.0**

* See the Table 1 footnotes and Material and Methods.

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

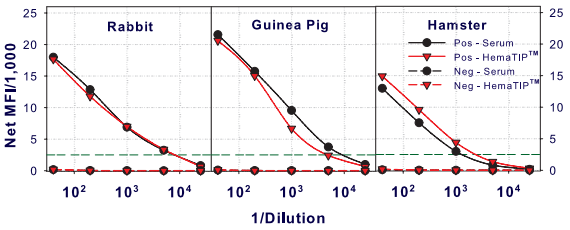


Figure 2. Linear Regression of HemaTIP™ Versus Serum Net MFI/1,000 for MFIA® of Immune Rabbit, Hamster, and Guinea Pig Samples: The data points represent the average serum and HemaTIP™ net MFI/1,000 for 6 runs per sample species. The linear regression analysis was done in SigmaPlot version 11 (Systat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

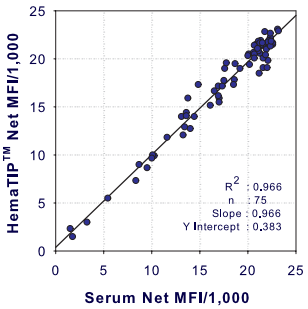
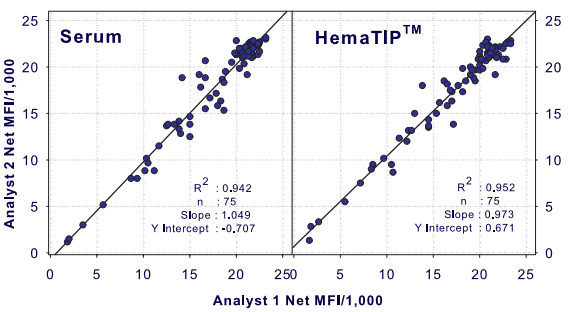


Figure 3. Linear Regression of Analyst 1 versus 2 Net MFI/1,000 for MFIA® of Immune Rabbit, Hamster, and Guinea Pig Samples: The data points denote the average serum and HemaTIP™ net MFI/1,000 for 3 separate runs per species performed by each analyst. The linear regression analysis was done in SigmaPlot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



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askcharlesriver@criver.com • www.criver.com