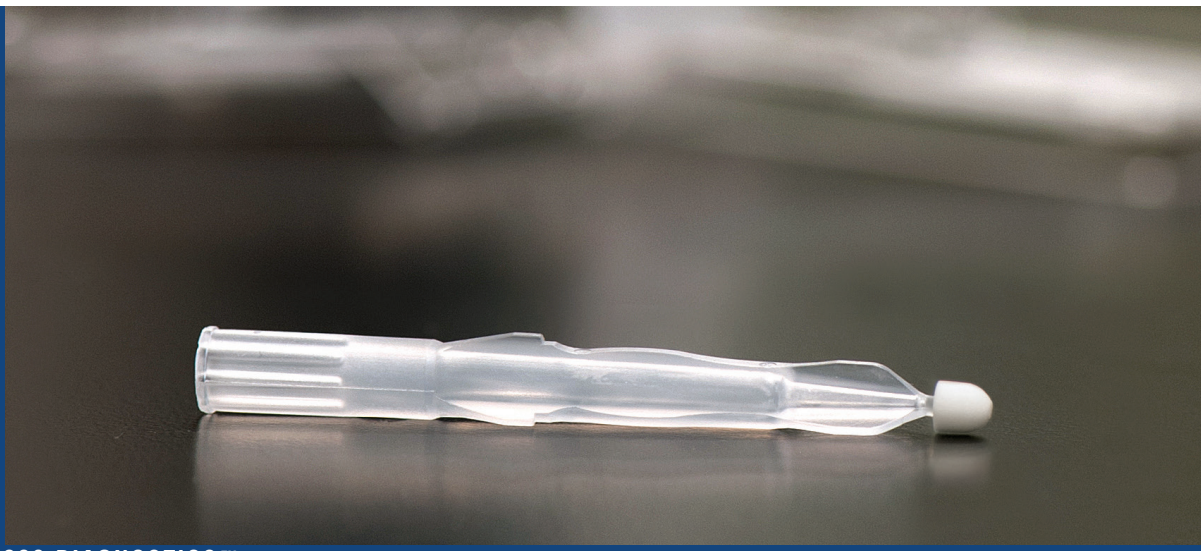
charles river

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 rodent serology.



360 DIAGNOSTICS™

Qualification of the Charles River HemaTIP™ Microsampler for Rodent Serology

For over 50 years, serologic immunoassays for specific antibodies have been used to monitor specific-pathogen-free (SPF) laboratory animals for adventitious infections with viruses and other pathogens capable of confounding research by causing disease and distorting experimental responses. Traditionally, separate "singleplex" immunoassays for antibodies to individual pathogens have been performed on serum collected from clotted blood. To assure that the volume of serum obtained would be adequate to perform a large panel of singleplex serologic assays as well as confirmatory repeat testing, small rodents such as mice and rats typically had to be euthanized for blood collection.[8]

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 now able to perform the most comprehensive serosurveillance panel in a single test well on as little as 2 μL of serum or the equivalent volume of blood eluted from a dried blood spot (DBS) card.[1,2,7] The small sample volume needed for MFIA® has facilitated survival blood collection, which is consistent with the 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.

In 2014, Neoteryx introduced the Mitra® microsampler based on volumetric absorptive microsampling (VAMS™) technology for the collection, transport and storage of biological fluids. The tip of the Mitra® device (shown above) consists of an inert, porous, hydrophilic material that rapidly wicks up a constant volume (e.g., 10 or 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, Mitra® tips are immersed in elution buffer for sample extraction and testing. Important advantages of the Mitra® microsampling system compared to DBS card systems like EZ-Spot® include more quantitative and simpler blood collection, and improved reproducibility, as the Mitra® microsamplers are designed for organization and automated extraction in a 96-well plate format.[3,4,9]

Materials and Methods

Samples

MFIA® were performed on matching serum 20 μL, HemaTIP™ and EZ-Spot® samples from naturally and intentionally infected adult mice and rats and from Charles River barrier-reared SPF rodents. The HemaTIP™ and EZ-Spot® samples were collected, stored and extracted according to the Mitra Microsampling Device User Manual (www.neoteryx.com) and the EZ-Spot® Instruction Sheet (www.criver.com/ezspot), respectively. Blood samples prepared from naïve rodents intentionally 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 rodents naturally infected with a variety of pathogens or by combining various monospecific antisera and mixing the resultant pool with an equal volume of packed rodent red blood cells.

MFIA® Testing

Mouse and rat samples were tested by Assessment Plus MFIA® panels comprising 29 and 23 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

Table 1 contains a summary of the study experiments. Briefly, serial dilutions of polyspecific serum, HemaTIP™ and EZ-Spot® samples and 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 polyspecific and SPF serum: HemaTIP™ sample pairs were tested in three separate runs by each of two analysts to evaluate the diagnostic performance and repeatability (i.e., agreement between the results of replicate testing) of MFIA® with HemaTIP™.

Results

Analytical Performance

The graphs in Figure 1 show MFIA® endpoint titration curves for: (1) polyspecific serum, HemaTIP™ and EZ-Spot® samples from naturally infected rodents; and (2) monospecific antiserum: HemaTip™ pairs prepared from standard antisera collected from intentionally infected mice and rats. Titration curves and LOD were the same irrespective of sample type.

Table 2 shows the average net MFI for the assay-positive, -negative and -equivocal samples (at the titration starting dilution). Table 3 shows the same data, but for the monospecific antiserum: HemaTIP™ pairs at the 1/200 titration dilution. For both sample sets, positive net MFI results for HemaTIP™ samples were comparable to those for sera; the average net MFI/1,000 for assay-negative samples was 0, thus demonstrating that the specificities of MFIA® with HemaTIP™ and serum samples were equivalent.

Diagnostic Performance and Repeatability

Table 4 summarizes the MFIA® results for triplicate test runs carried out by each of two analysts (for a total of six MFIA® runs) on serum and HemaTIP™ sample pairs. These samples were obtained from eight naturally infected and eight SPF animals per rodent species. For assay-positive samples, the percentages of positive results were 99.8% for serum and 99.2% for HemaTIP™, with comparable net MFI and average % CV below 10%. For assay-negative samples, the percentages of positive results for serum and HemaTIP™ samples were both 0%, with average net MFI/1,000 of 0.1± 0.0.

The linear regression analysis plots presented in Figures 3 and 4 demonstrate the strong correlation between the net MFI for HemaTIP™ versus serum ($R^2 = 0.99$) and analyst 1 versus 2 ($R^2 = 0.99$). Thus, the levels of repeatability (i.e., agreement between the results of replicate testing) and of diagnostic sensitivity and specificity achieved by MFIA® of HemaTIP™ samples were excellent and equivalent to those for paired serum samples.

Conclusion

This study was undertaken to qualify the HemaTIP™ microsampling system (using the 20 µL Mitra® microsampler from Neoteryx) for laboratory animal serology. HemaTIP™ (like DBS) microsampling 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™ microsampler as a suitable alternative to serum or DBS sample collection for rodent serology.

Table 1: Summary of Experiments to Qualify HemaTIP™ Microsampling

		Samples		Serum	Hema-TIP™	EZ-Spot®
Qualification	**Experiment**	**Source**	**Species (#)**			
Analytical performance	MFIA® titration of polyspecific samples	Naturally infected rodents	Mouse (8) Rat (8)	•	•	•
	MFIA® titration of monospecific samples	Standard antiserum pools	Mouse (12) Rat (9)	•	•	
Diagnostic performance and repeatability	Triplicate MFIA® runs performed by each of 2 analysts on polyspecific and SPF samples	Standard antiserum pools	Mouse (12) Rat (9)	•	•	
		Barrier-reared SPF rodents	Mouse (8) Rat (8)	•	•	

Table 2: Analytical Performance – MFIA® of Polyspecific Samples from Naturally Infected Rodents*

Sample			Samples	Mean Net MFI/1,000		
Species	#	Status	x Assays	Serum	HemaTIP™	EZ-Spot®
Mouse	8	Positive	60	13.9	10.8	9.0
		Negative	148	0.0	0.0	0.0
		Equivocal	24	3.4	2.6	1.4
Rat	8	Positive	73	13.3	12.5	10.8
		Negative	82	0.4	-0.3	-0.5
		Equivocal	29	2.9	2.7	2.0

* Blood from each rodent was used to prepare serum, HemaTIP™ and EZ-Spot® samples. As the described in the Materials and Methods, the mouse and rat samples were tested by Assessment Plus panels comprising 29 and 23 assays, respectively. The net MFI/1,000 are for the serum and HemaTIP™ samples diluted 40-fold and the EZ-Spot® samples diluted 80-fold. A sample was assigned an assay status based on the net MFI/1,000 for the serum diluted 40-fold, with values of < 1.5 and $>= 5$ classified as negative and positive, respectively.

Table 3: Analytical Performance – MFIA® of Monospecific Serum: HemaTIP™ Sample Pairs from Intentionally Infected Rodents*

Sample			Samples	Mean Net MFI/1,000	
Species	#	Status	x Assays	Serum	HemaTIP™
Mouse	12	Positive	18	16.4	17.2
		Negative	328	0.0	0.1
Rat	9	Positive	18	15.7	15.0
		Negative	187	0.1	0.1

* See the Table 2 footnote. As described in the Materials and Methods, the paired HemaTIP™ sample pairs were prepared by combining monospecific antisera and mixing the resultant polyspecific sample with an equal volume of packed mouse or rat red blood cells. The net MFI/1,000 are for samples diluted 200-fold.

Table 4: Diagnostic Performance – Two Analysts Each Perform Three MFIA® Runs of Polyspecific and SPF Rodent Serum: HemaTIP™ Sample Pairs*

				MFI/1,000			
				Net			
Sample-Assay Status (N)	Type	Analyst	% Pos	Avg	SD	% CV	TC
Positive (125)	**Serum**	**1**	99.7%	17.9	1.1	7.8%	0.3
		2	100.0%	17.8	0.8	5.7%	0.2
		Total	99.9%	17.8	1.1	7.8%	0.3
	HemaTIP™	**1**	99.7%	16.5	1.0	7.4%	0.2
		2	98.7%	16.7	1.1	8.9%	0.2
		Total	99.2%	16.6	1.1	8.6%	0.2
Negative (673)	**Serum**	**1**	0.0%	0.1	0.0		0.1
		2	0.0%	0.1	0.0		0.1
	HemaTIP™	**1**	0.0%	0.1	0.0		0.1
		2	0.0%	0.1	0.0		0.1

* For each species, 8 polyspecific and 8 nonimmune SPF serum: HemaTIP™ sample pairs were tested in 3 separate runs by each of 2 analysts. A sample was assigned an assay status based on the average net MFI/1,000 of the serum for all 6 runs, with values of < 1.5 and $>= 5$ classified as negative and positive, respectively. The coefficient of variation (%CV) = standard deviation (SD)/ average (Avg).

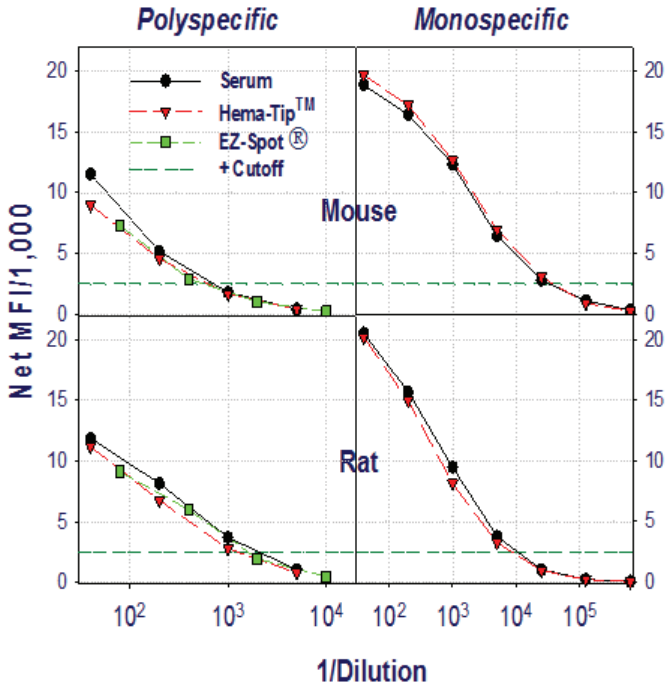


Figure 1. Analytical Performance – MFIA® LOD Titration of Polyspecific and Monospecific Samples: Samples were diluted 5-fold starting at 1/40 for serum and HemaTIP™, and 1/80 for EZ-Spot®. The titration data points represent the average for all serum samples with assay net MFI/1,000 ≥ -5 at the starting dilution.

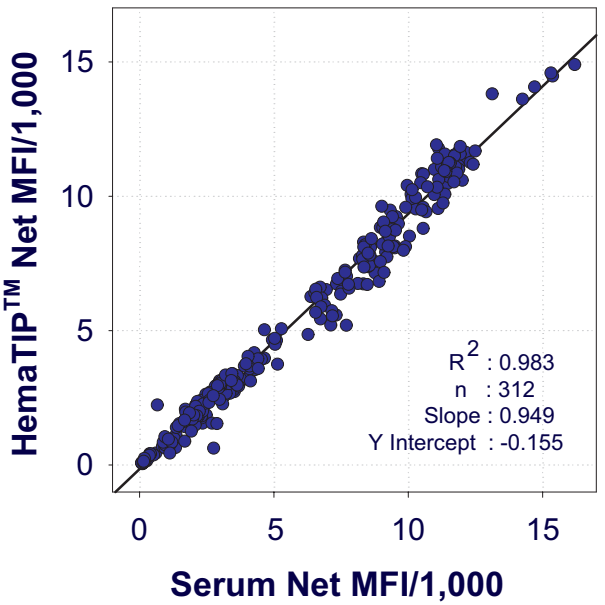


Figure 2. Linear Regression of HemaTIP™ versus Serum Net MFI/1,000 for Six MFIA® Runs: Samples diluted 40-fold were tested in three separate runs performed by each of two analysts. The data points represent individual Serum: HemaTIP™ net MFI/1,000 value pairs by assay. The linear regression analysis was done in SigmaPlot version 11.

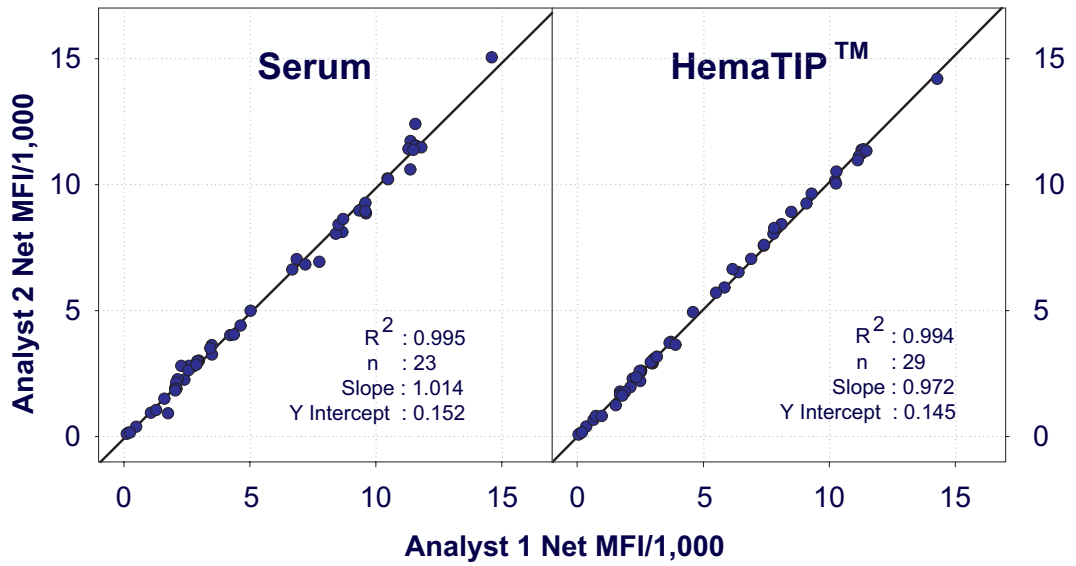


Figure 3. Linear Regression of Sample – Assay Average Net MFI/1,000 for Analyst One versus Analyst Two: Samples diluted 40-fold were tested in 3 separate runs performed by each analyst. The linear regression analysis was done in SigmaPlot version 11.

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