Validation of a Novel Semi-Quantitative Immunoassay to Differentiate Bacterial vs Viral Infections from Serum Samples

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Introduction

Clinical manifestations of viral and bacterial infections can be overlapping. A rapid test that could aid in in the differentiation of bacterial from viral infections could improve patient management by reducing unnecessary antibiotic treatment. In this study we evaluated a validation panel for a novel immunoassay that measures three host proteins (TRAIL, IP-10, and CRP) that reports out a likelihood score for viral or bacterial infections.

Method

The MeMedBV assay is a novel immunoassay that provides semi-quantitative results for three host proteins known to be either up or downregulated during infections with bacterial or viral pathogens. To aid in validation of the assay, a reference panel was developed that allows for rapid testing of analytic sensitivity and specificity and analytic measurement range. Each sample was thawed prior to testing and 100 ul added to the test cartridge, which was loaded to the analyzer (called MeMed Key) and results recorded after a 15-minute run time. Accuracy was determined by testing 20 samples containing a range of the 3 proteins: 15-300pg/mL for TRAIL, 100-6000pg/mL for IP-10, and 1-250ug/mL for CRP. Precision was evaluated by testing 20 replicates of two samples, with high and low bacterial likelihood scores. Testing was duplicated on a second analyzer to measure inter-instrument reliability.

Accuracy

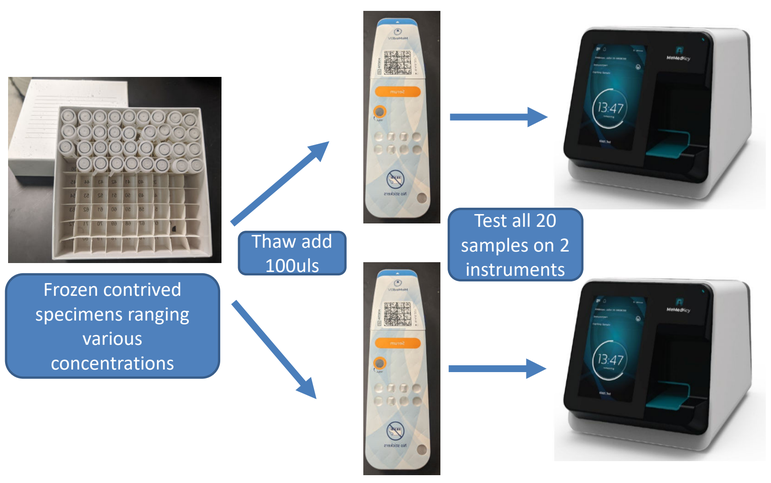


Figure 1. Workflow of Accuracy testing using contrived samples. Specimens were shipped from the manufacturer on dry ice. These came in as 20 specimens per instrument that range between the reportable range of each target and ranges in final score. Each specimen was thawed and tested on two instruments to determine linearity and R^2 value.

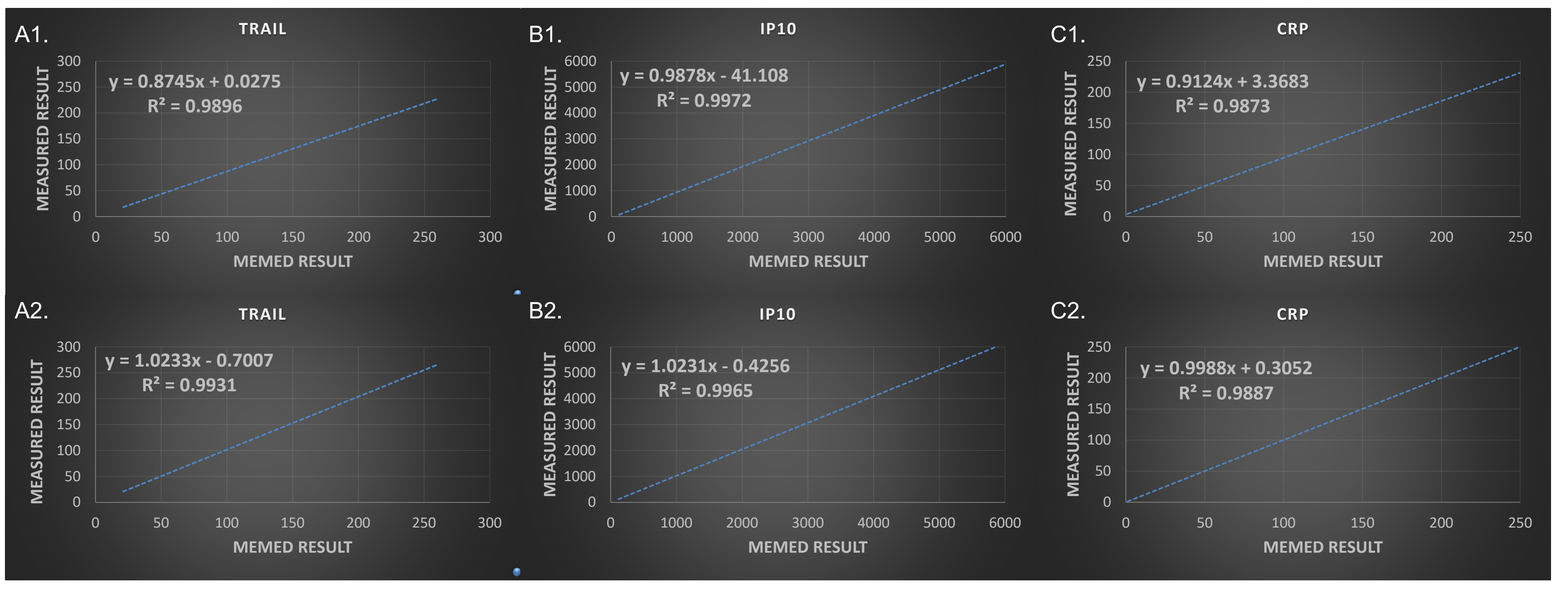
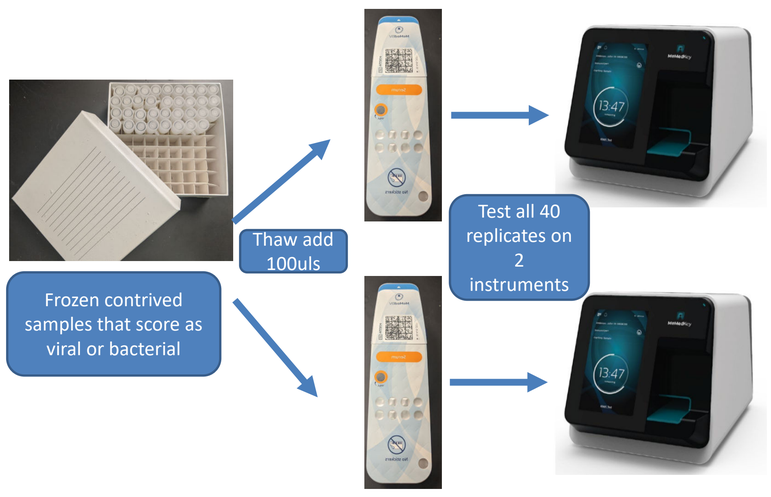


Figure 2. Accuracy performance of the MeMed BV assay between two instruments for each target over the 20 samples ranging from high to low concentration levels. TRAIL was tested from 15-30 pg/mL for instrument 1 (A1) and instrument 2 (A2), IP10 tested at 100-6000pg/mL for instrument 1 (B1) and instrument 2 (B2), and CRP tested with a range of 1-250 mg/L for instrument 1 (C1) and instrument 2 (C2). Linear regression analysis was performed to calculate R^2.

Reproducibility



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Instrument 1		Trail (pg/mL)	IP-10 (pg/mL)	CRP (mg/L)	Score
Precision Test – Level 19	Average	45	4250	190	97
	SD	3.3	204.4	12.5	1.1
	%CV	7%	5%	7%	
	2.5 x SD	8.25	511	31.25	2.75
	% within 2.5 SD	100%	100%	100%	100%
	Pass/Fail	Pass	Pass	Pass	Pass
Precision Test- Level 20	Average	151	362	16	2
	SD	11.0	62.6	1.7	0.8
	%CV	7%	17%	11%	
	2.5 x SD	27.5	156.5	4.25	2
	% within 2.5 SD	100%	95%	95%	100%
	Pass/Fail	Pass	Pass	Pass	Pass

Instrument 2		Trail (pg/mL)	IP-10 (pg/mL)	CRP (mg/L)	Score
Precision Test – Level 19	Average	54	4842	195	96
	SD	3.5	166.4	10.6	1.6
	%CV	7%	3%	5%	
	2.5 x SD	6	416	26.5	4
	% within 2.5 SD	100%	100%	100%	100%
	Pass/Fail	Pass	Pass	Pass	Pass
Precision Test- Level 20	Average	182	355	16	1
	SD	11.1	46.4	1.3	0.3
	%CV	6%	13%	8%	
	2.5 x SD	27.75	116	3.25	0.75
	% within 2.5 SD	100%	94.4%	100%	100%
	Pass/Fail	Pass	Pass	Pass	Pass

Table 1. Results of reproducibility study on two instruments. For each biomarker, two concentrations were tested: one that represents a physiological concentration of the biomarker in the presence of a bacterial infection and the other, of a viral infection. 20 replicates per sample were tested. Measurements were carried out by 3 operators, on 2 instruments, over 5 nonconsecutive days.

Conclusions

The MeMed BV assay is a highly accurate and sensitive test for the detection and quantitation of the three host proteins. The validation panel was easy to use and could help implementation of the assay in near patient settings. Future studies evaluating the assay with patient specimens is needed to evaluate the clinical utility of the assay.