AUTOMATED ELISA MEASUREMENTS OF HOST-IMMUNE BIOMARKERS FOR DISTINGUISHING BETWEEN ACUTE BACTERIAL AND VIRAL INFECTIONS

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Background

ImmunoXpert™ is a novel assay that distinguishes between bacterial and viral infections based on the patient's immune response. It measures the serum levels of viral- and bacterial-induced host-proteins (TNF-related apoptosis-inducing ligand [TRAIL], Interferon gamma-induced protein-10 [IP-10], and C-reactive protein [CRP]) and computes a bacterial likelihood score. TRAIL and IP-10 are measured using manual enzyme-linked immunosorbent assays (ELISA). As other manual assays, it requires precious technician hands-on time, and is prone to human errors. Automated workstations offer several advantages compared to manual ELISA such as reduced hands-on time, improved analytical performance, and less human errors. Here we present a new protocol for simultaneous measurement of these biomarkers using an automated ELISA workstation.

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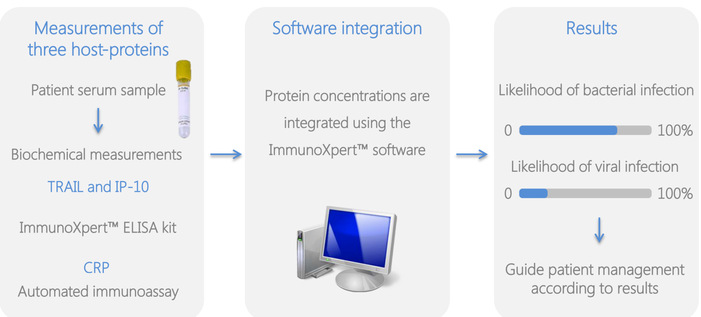

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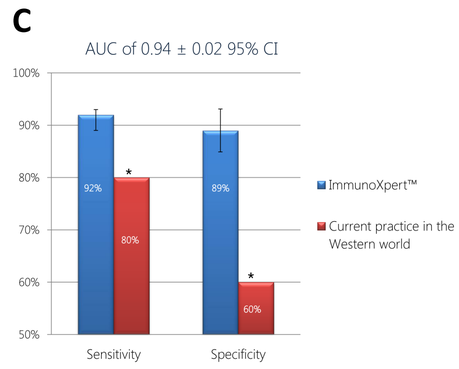

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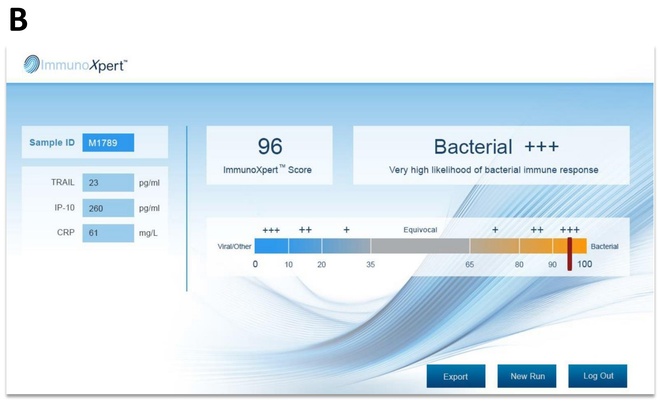


The ImmunoXpert™ assay

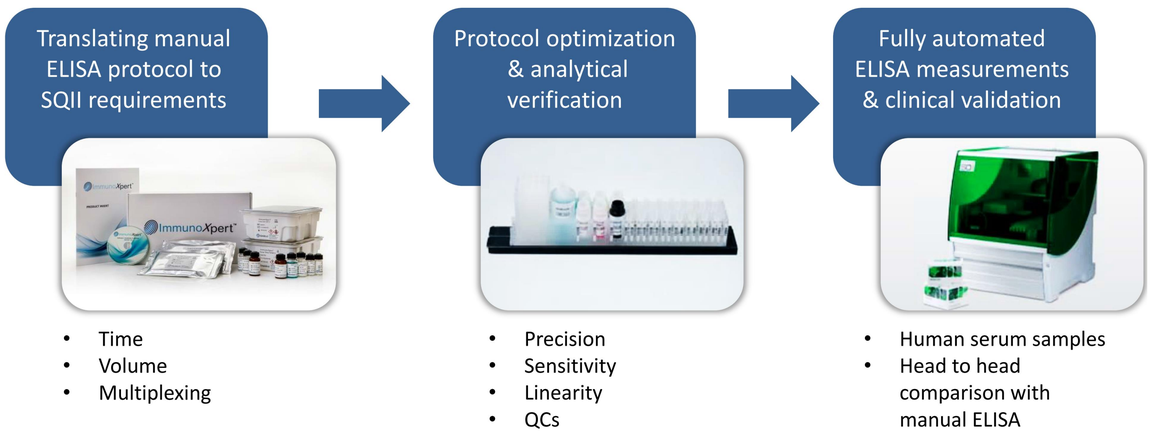
(A) Assay principle

(B) Snapshot of the ImmunoXpert™ results display

(C) Accuracy measures of ImmunoXpert™ for diagnosing bacterial vs viral infections. Performance estimates and corresponding 95% confidence intervals were obtained using a leave-10%-out cross-validation on patients enrolled in an observational, prospective clinical study (NCT01917461)[1]. Accuracy estimates of current practice in the Western world are based on previous studies[2-6].

Methods

ImmunoXpert™ manual ELISA (MeMed Diagnostics, IL) was adapted to the SQII automated ELISA workstation (AESKU, DE). TRAIL and IP-10 protocols were synchronized to enable simultaneous measurement of the two analytes while minimizing the total assay time. Assay verification was performed to evaluate precision, sensitivity, and linearity of the automated protocol using IP-10 and TRAIL recombinant proteins and human serum samples.



Automated workstation protocol

TRAIL

- Prepare and load into the automated ELISA workstation all reagents, standards, and samples as instructed.
- Pipet 50µl Assay diluent MM1S to each well.
- Pipet 50µl standard, control, or sample to each well. **Incubate 30 min with continuous shaking**, at RT.
- Aspirate and wash 4 times.
- Pipet 200µl Conjugate to each well. **Incubate 45 min with continuous shaking**, at RT.
- Aspirate and wash 4 times.
- Pipet 100µl TMB substrate solution to each well. **Incubate 30 min** at RT.
- Pipet 50µl stop solution to each well.
- Read at 450nm.

IP-10

- Prepare and load into the automated ELISA workstation all reagents, standards, and samples as instructed.
- Pipet 50µl Assay diluent MM56 to each well.
- Pipet 50µl standard, control, or sample to each well. **Incubate 30 min with continuous shaking**, at RT.
- Aspirate and wash 4 times.
- Pipet 200µl Conjugate to each well. **Incubate 45 min with continuous shaking**, at RT.
- Aspirate and wash 4 times.
- Pipet 100µl TMB substrate solution to each well. **Incubate 10 min** at RT.
- Pipet 50µl stop solution to each well.
- Read at 450nm.

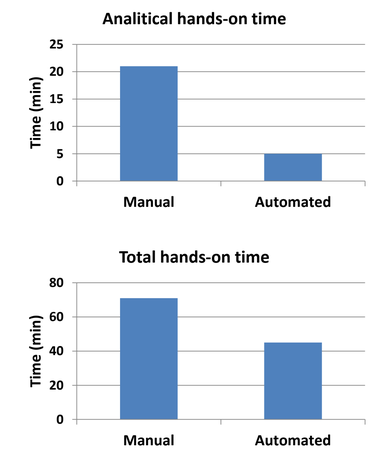
Results

1. Automated workstation facilitated a significant reduction in hands-on technician time

A

	Step	Time (min) Manual	Time (min) Automated
	Pre-processing	30	30
Analytical time	Samples load	4	5
	Wash cycle #1	4	-
	Conjugate	2	-
	Wash cycle #2	4	-
	TMB substrate	2	-
	Stop solution	2	-
	Read (450 nm)	3	-
	Analysis	10	-
	Clean work station	10	10

B



Measured hands-on technician time in automated and manual protocols

(A) A detailed description of manual and automated protocols and their time estimates (for handling 8 clinical samples). (B) The automated workstation facilitated a 16 min (76%) reduction in the analytical hands-on technician time, and a 26 min (37%) reduction in the total hands-on technician time, which also includes pre- and post analytical processing.

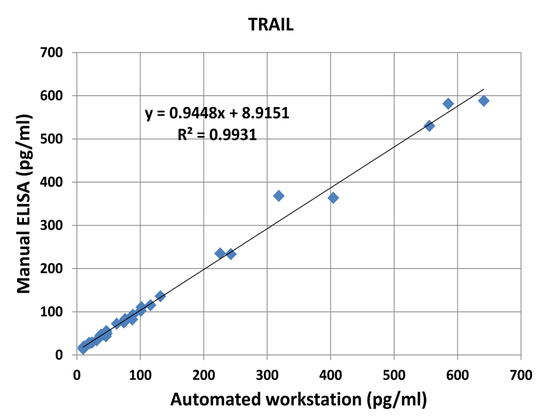
2. Automated protocol maintains high analytical performance

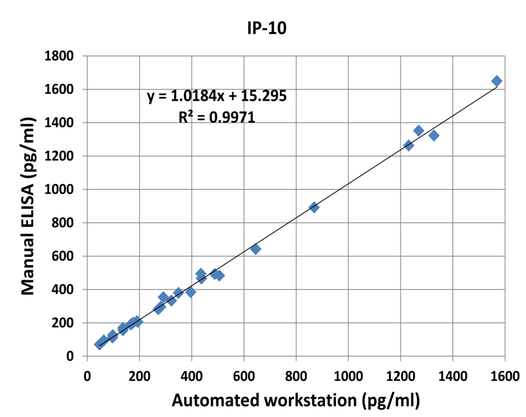
Criteria	Value (TRAIL)	Value (IP-10)
Precision		
Inter- Assay CV%	3%	4%
Intra –Assay CV%	3%	3%
Sensitivity		
Limitation of Blank (LOB)	1.4 pg/ml	10 pg/ml
Linearity		
Linear regression (R^2)	0.996	0.999

Assay performance measures for TRAIL and IP-10

Precision and sensitivity were evaluated using recombinant proteins (n= 5 and 10 respectively). Linearity was evaluated using clinical serum samples (n=3).

3. Automated and manual protocols demonstrate high correlation when measuring TRAIL and IP-10 from human serum samples





Manual and automated protocol are highly correlated

TRAIL and IP-10 were simultaneously measured using manual and automated protocols using clinical serum samples (n= 31 and 26 respectively).

Conclusions

We implemented a manual ELISA assay onto an automated workstation for a novel assay, the ImmunoXpert™. We were able to reduce technician hands-on time while maintaining high analytical performance. The automation process can potentially reduce the overall burden on the lab while facilitating a timely diagnosis of infectious disease patients, thus promoting antibiotic stewardship and improved patient management.

References

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