Clinical Validation of the IntelliSep® Host-Response Sepsis Test in the Absence of a Reference Standard

Alyssa Hutto, MHA[1]; Dhruvangkumar Modi, MD, MPH[1]; Karla A. Guier, MSN, RN, CNL[1]; Benjamin Fragano, MSN, RN[1]; An Nguyen, MD[1]; Timothy Harris, MD[1]; Kelly Brown, MSN, RN, NE-BC[1]; Roya Sheybani, PhD[2]; Andrew Masica, MD, MSCI[1]; & Ferdinand Velasco, MD[1]

[1]Texas Health Resources Presbyterian Hospital, Denton, TX, USA [2] Cytovale Inc., S. San Francisco, CA, USA

Introduction

- Diagnostic evaluation relies on comparison to a reference standard (i.e., a "gold-standard")
- When such reference standards are unavailable, laboratories must develop alternative methods
- Sepsis is a dysregulated host immune response to infection leading to organ dysfunction[1] for which no diagnostic reference standard currently exists
- IntelliSep is an FDA-cleared rapid diagnostic that assesses the state of immune activation
- It generates the IntelliSep Index (ISI), reported as three interpretation bands indicating low (Band 1) to high (Band 3) probability of sepsis with organ dysfunction manifesting within three days after testing

This study describes a practical validation strategy for assessing sepsis diagnostic accuracy when no reference standard exists.

Methods

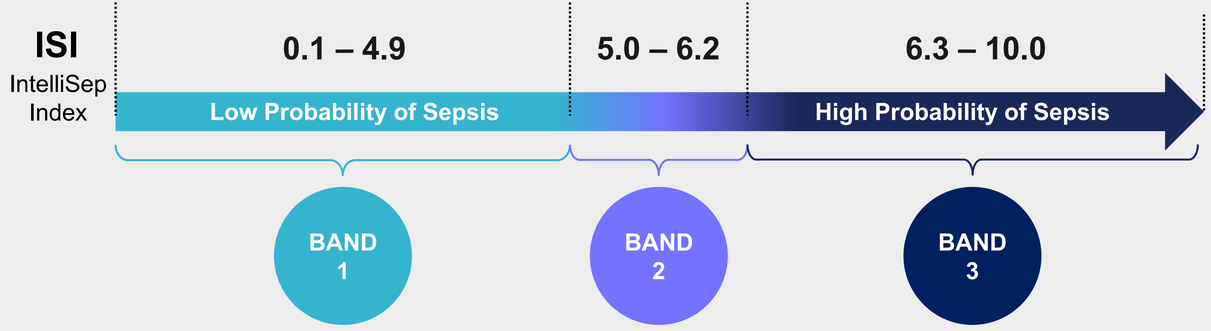
- The IntelliSep test was performed on 100 µL of K_2EDTA anticoagulated whole blood using remnant CBC samples from patients meeting pre-defined criteria
- Patients were selected based on clinical characteristics and treatment consistent with a low- or high-likelihood of the patient having or developing sepsis
- These criteria were intended to enrich the study population for Band 1 and 3 results and were not intended to establish or define sepsis

Sepsis Likelihood Criteria	
LOW likelihood sepsis criteria (More likely to yield a Band 1 result)	**HIGH likelihood sepsis criteria** (More likely to yield a Band 3 result)
• ≥ 18-year-old • CBC ordered, sample ≤ 4 hours of collection • Pending ED Discharge Order	• ≥ 18-year-old • CBC ordered, sample ≤ 4 hours of collection • ≥ 2 SIRS criteria, with ≥ 1 temperature or WBC • Order placed for blood cultures • Prescribed antibiotics or antivirals • Pending admission to hospital or admission order placed

- IntelliSep results inconsistent with the assigned likelihood category (e.g., a low-likelihood patient resulting in a Band 3) were labelled as discordant
- All discordant results and a subset of concordant results (n=6) were assessed by blinded (to the IntelliSep results) retrospective physician case review of the patient's medical record.

The Cytovale IntelliSep Test:

The IntelliSep test is an FDA cleared, semi-quantitative blood test that assesses the state of immune activation at the cellular level and is intended to aid in the early detection of sepsis with organ dysfunction manifesting within the first 3 days after testing for adults with signs and symptoms of infection presenting to the ED.



All results should be interpreted in the context of the other clinical observations and laboratory test results for the patient.

The test results in the IntelliSep Index (ISI), a single score between 0.1-10.0, in <10 minutes. Results are grouped into three interpretation bands reflecting increasing likelihood of sepsis, Band 1 (low) to Band 3 (high).

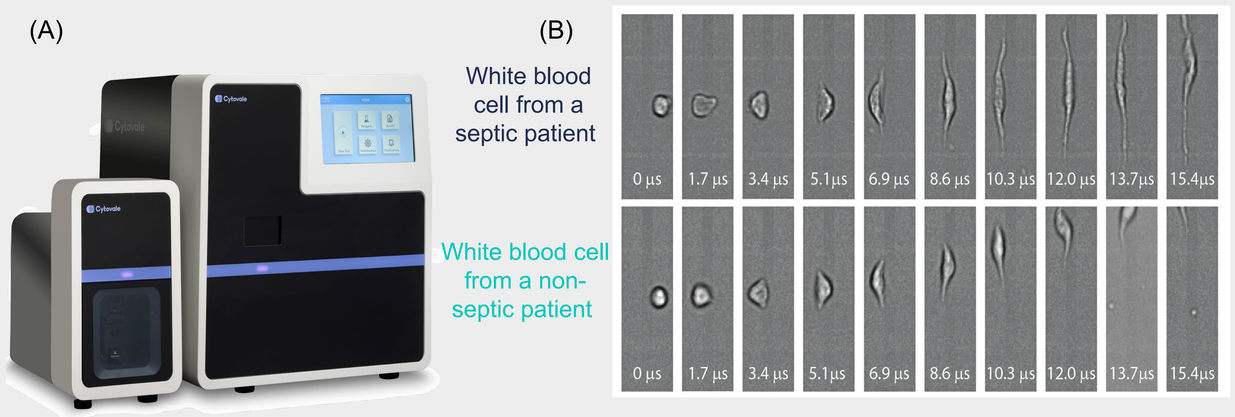


Figure 1: (A) Photograph of the Cytovale system, a benchtop instrument on which the IntelliSep test is performed; (B) Time series of cell deformation for a representative leukocyte of a septic Band 3 patient (top) and a non-septic Band 1 patient (bottom).

Methods (continued)

Physicians blinded to the IntelliSep results, assigned "sepsis" or "not sepsis" determinations per the Sepsis-3 consensus definition[1] following a structured adjudication scheme described in the IntelliSep Instructions for Use (IFU), consisting of 3 questions:

1) Was there infection at time of testing?
2) Was there organ dysfunction manifesting within 3 days of testing?
3) Was the organ dysfunction caused by a dysregulated immune response to the infection?

Diagnostic accuracy was evaluated by comparing the observed proportion of adjudicated septic cases within Bands 1 and 3 to the performance characteristics reported in the test IFU.

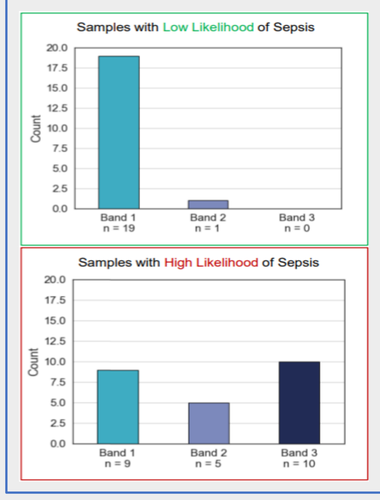
Workflow Summary

Sample Identification in the Emergency Department	Run IntelliSep Test	Assess discordant results: Adjudication
Select 30–50 patients that have remnant CBC samples. Choose patients meeting low and high likelihood sepsis criteria to capture **Band 1** and **Band 3** results. • Criteria increase chance but do not guarantee targeted band results • Additional samples may be needed to achieve the desired band distribution	Run samples on instrument(s). Samples must be run within 5 hours of collection and have not been refrigerated, frozen or spun down. Review results once the desired band distribution is reached: • Low-likelihood samples → more likely to yield Band 1 • High-likelihood samples → more likely to yield Band 3	Physician case review used to assess accuracy—especially for discordant cases: • Patients with low-likelihood clinical criteria with a **Band 3** result • Patients with high-likelihood clinical criteria with a **Band 1** result

Results & Discussion

- 44 patients were evaluated (20 low-likelihood and 24 high-likelihood)
- 28 patients were Band 1, 6 patients Band 2, and 10 patients were Band 3
  - Zero low-likelihood patients produced a Band 3 result
  - 9 high-likelihood patients produced a Band 1 result
- After blinded adjudication, 1 Band 1 result was determined to be septic and two Band 3 results not septic.
- Rate of sepsis was consistent with the IFU (3.6% in Band 1 and 80% in Band 3)

Initial Results



Adjudication

	Case #	Sample Drawn Date/Time	Enrollment Category	Adjudicated Sepsis Status	IntelliSep Test Results: Index	IntelliSep Test Results: Interpretation Band
Discordant Cases	1	2/4/25	high risk criteria	not sepsis	3.4	1
	36	2/12/25	high risk criteria	not sepsis	3.5	1
	44	2/13/25	high risk criteria	not sepsis	3.5	1
	31	2/6/25	high risk criteria	not sepsis	4.2	1
	34	2/12/25	high risk criteria	sepsis	4.2	1
	28	2/6/25	high risk criteria	not sepsis	4.4	1
	25	2/5/25	high risk criteria	not sepsis	4.5	1
	17	2/5/25	high risk criteria	not sepsis	4.7	1
	29	2/6/25	high risk criteria	not sepsis	4.7	1
Concordant Cases	4	2/4/25	low risk criteria	not sepsis	2.1	1
	16	2/5/25	low risk criteria	not sepsis	3.4	1
	8	2/4/25	low risk criteria	not sepsis	5.6	2
	41	2/13/25	high risk criteria	sepsis	7.5	3
	42	2/13/25	high risk criteria	not sepsis	8	3
	40	2/13/25	high risk criteria	not sepsis	9	3

Final Read Out

	Total # in Band	# discordant	Final Read Out (# discordant/total samples)	Status
% Sepsis: Band 1	28	1	3.6%	Pass
% Sepsis: Band 3	10	2	80.0%	Pass

Conclusions

Diagnostic accuracy of a novel sepsis test was verified in the absence of a reference standard using predefined clinical selection criteria and a physician adjudication framework. **These findings demonstrate that a targeted patient selection strategy combined with blinded physician adjudication provides a practical approach for validating host-response diagnostics in the absence of a diagnostic reference standard.**

Acknowledgements

We would like to thank the providers and nursing staff of the Texas Health Resources Denton Hospital Emergency Department for their tireless efforts in adopting new process and technology to improve the care of our patients who present with signs or suspicion of infection. We also thank the clinical laboratory staff who have worked with us over the last year to implement this test. Finally, we thank the THR Denton Quality and Safety team that works to ensure we are providing the best possible care to our patients.

References

[1] Singer, *JAMA*, 2016