



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT**

I Background Information:

A 510(k) Number

K241676

B Applicant

Inflammatix, Inc.

C Proprietary and Established Names

TriVerity

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PRE	Class II	21 CFR 866.3215 - Device To Detect And Measure Non-Microbial Analyte(S) In Human Clinical Specimens To Aid In Assessment Of Patients With Suspected Sepsis	MI - Microbiology
OOI	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the TriVerity device

B Measurand:

Twenty-nine mRNA transcripts of host response genes and three housekeeping genes

C Type of Test:

Quantitative reverse transcription loop-mediated isothermal amplification (qRT-LAMP)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The TriVerity test is an automated, semi-quantitative *in vitro* diagnostic test that measures the relative expression levels of host response genes in RNA isolated from whole blood collected in the PAXgene Blood RNA tube using reverse transcription loop-mediated isothermal amplification (RT-LAMP) on the Myrna instrument.

The TriVerity test is indicated for use in conjunction with clinical assessments and other laboratory findings as an aid to differentiate bacterial infections, viral infections, and non-infectious illness, as well as to determine the likelihood of 7-day need for mechanical ventilation, vasopressors, and/or renal replacement therapy in adult patients with suspected acute infection or suspected sepsis presenting to the emergency department.

The test generates three scores that each fall within one of five discrete interpretation bands based on the increasing likelihood of

1) bacterial infection,

2) viral infection, and

3) severe illness, as defined by the need for mechanical ventilation, vasopressors, and/or renal replacement therapy (RRT) within seven days.

Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

C Special Instrument Requirements:

For use with the Myrna Instrument

IV Device/System Characteristics:

A Device Description:

The TriVerity test is an *in-vitro* diagnostic device that uses reverse transcription loop-mediated isothermal amplification (RT-LAMP) to measure the relative expression levels of 29 host response genes and 3 housekeeping transcripts (Table 1) from whole blood samples collected in PAXgene blood collection tubes (K042613). The assay includes single-use, disposable TriVerity Cartridges which contain all the reagents necessary to perform a single test. The TriVerity test is

processed on the Myrna instrument and includes built-in software integrated with the TriVerity computational algorithm to generate a result for each sample in approximately 30 minutes.

Table 1: Target Genes for the TriVerity Acute Infection and Sepsis Test

Host Response Genes		
<i>ANKRD22</i>	<i>ARG1</i>	<i>BATF</i>
<i>C3AR1</i>	<i>CD163</i>	<i>CEACAM1</i>
<i>CLEC5A</i>	<i>CTSL1</i>	<i>DEFA4</i>
<i>HERC5</i>	<i>HLA-DMB</i>	<i>IFI27</i>
<i>IFI44</i>	<i>IFI44L</i>	<i>IL18R1</i>
<i>IL1R2</i>	<i>ISG15</i>	<i>JUP</i>
<i>KCNJ2</i>	<i>LY86</i>	<i>OASL</i>
<i>OLFM4</i>	<i>PSMB9</i>	<i>RSAD2</i>
<i>S100A12</i>	<i>TDRD9</i>	<i>TGFBI</i>
<i>XAF1</i>	<i>ZDHHC19</i>	
Housekeeping Genes		
<i>KPNA6</i>	<i>RREB1</i>	<i>YWHAB</i>

B Principle of Operation:

The TriVerity test is composed of the TriVerity Cartridge that is run on the Myrna Instrument. The Myrna Instrument is a cartridge-based, molecular diagnostic instrument designed to measure relative gene expression. The single-use TriVerity Cartridge contains all the necessary reagents to perform RNA isolation from the sample and analysis. The PAXgene Blood RNA Tube is directly docked to the cartridge by the user before the cartridge is loaded into the instrument. Approximately 550 µL of blood and PAXgene solution is drawn into the cartridge from the tube. Nucleic acid extraction and purification are completed on-board using standard solid phase reversible immobilization (SPRI) chemistry facilitated by magnetic particles. Purified RNA is eluted and then mixed with master mix reagents, and then is channeled to separate reaction vessels. The Myrna Instrument's analyzer/detector is an incubator designed for isothermal amplification with single color fluorescence detection capability. The quantitative gene expression assay is based on real-time generation of fluorescence from an intercalating dye which increases its fluorescence signal due to the increasing abundance of double stranded DNA generated during amplification. Each singleplex reaction is performed in a 2 µL reaction volume.

The test simultaneously runs 64 isothermal amplification reactions. The test uses a multiplex array which contains primers to measure, in duplicate, 29 mRNA host response transcripts, 3 housekeeping transcripts, and 2 process controls. The housekeeping genes are used together with process controls to normalize input RNA, while the 'host response genes' are inputs to the two fixed classifiers that generate the assay results: IMX-BVN-4 (bacterial-viral-noninfected) and IMX-SEV-4 (illness severity). The IMX-BVN-4 classifier estimates of the likelihood of a bacterial or a viral infection and the IMX-SEV-4 classifier estimates the risk of severe illness as defined by the 7-day need for vasopressors, mechanical ventilation, and/or renal replacement therapy.

For each patient sample, the TriVerity test will generate three numerical results which indicate the likelihood of a bacterial infection, the likelihood of a viral infection, and the likelihood of severe illness. For each numerical result, there will also be an interpretation provided that is

related to the specific risk band within which the quantitative number falls (corresponding to Very High, High, Moderate, Low, and Very Low).

The TriVerity Cartridge is designed to dock the PAXgene venipuncture tube directly to avoid potential issues (including use error, contamination, and biohazards) in transferring samples from one vessel to another. The pre-analytical steps are designed to be simple and consist of tube inversion, insertion of the tube into the cartridge, and insertion of the cartridge into the device, with an estimated hands-on time of less than one minute.

C Instrument Description Information:

1. Instrument Name:

Myrna Instrument

2. Specimen Identification:

The instrument software reads a unique barcode on the assay cartridge to identify the cartridge type, the cartridge assay, the cartridge lot/expiration details, and lot-to-lot correction factors. This unique cartridge barcode is linked to the sample ID through scanning or manual entry. The cartridge barcode also includes traceability information including the cartridge manufacture date and expiration dates.

3. Specimen Sampling and Handling:

A medical professional is required for collecting the whole blood sample by venipuncture using the PAXgene blood collection tubes within the PAXgene Blood RNA System (K042613). The user then mixes the sample by inversion followed by loading the tube directly into the cartridge and then inserting the cartridge into the Myrna instrument. All waste and amplified material remain sealed within the TriVerity Cartridge after the test is performed.

4. Calibration:

All necessary configuration, qualification, and calibration (where applicable) is performed by Inflammatrix including calibration of the TriVerity cartridges. Further configuration and calibration procedures are not required by the operator.

5. Quality Control:

Each cartridge contains two internal process controls for monitoring amplification inhibition, assay reagents, and sample processing effectiveness. The controls are RNA spike in controls from the External RNA Controls Consortium. External controls are not provided with the assay, but may be used in accordance with local, state, and federal accrediting organizations, as applicable.

V Substantial Equivalence Information:

A Predicate Device Name(s):SeptiCyt[®]e RAPID**B Predicate 510(k) Number(s):**

K203748

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K241676</u>	<u>K203748</u>
Device Trade Name	TriVerity	SeptiCyt [®] e RAPID
General Device Characteristic Similarities		
Intended Use/Indications For Use	<p>The TriVerity test is an automated, semi-quantitative in vitro diagnostic test that measures the relative expression levels of host response genes in RNA isolated from whole blood collected in the PAXgene Blood RNA tube using reverse transcription loop-mediated isothermal amplification (RT-LAMP) on the Myrna instrument.</p> <p>The TriVerity test is indicated for use in conjunction with clinical assessments and other laboratory findings as an aid to differentiate bacterial infections, viral infections, and non-infectious illness, as well as to determine the likelihood of 7-day need for mechanical ventilation, vasopressors, and/or renal replacement therapy in adult patients with suspected acute infection or suspected sepsis presenting to the emergency department.</p> <p>The test generates three</p>	<p>The SeptiCyt[®]e RAPID test is a gene expression assay using reverse transcription polymerase chain reaction to quantify the relative expression levels of host response genes isolated from whole blood collected in the PAXgene Blood RNA Tube. The SeptiCyt[®]e RAPID test is used in conjunction with clinical assessments and other laboratory findings as an aid to differentiate infection-positive (sepsis) from infection- negative systemic inflammation in patients suspected of sepsis on their first day of ICU admission. The SeptiCyt[®]e RAPID test generates a score (SeptiScore) that falls within one of four discrete Interpretation Bands based on the increasing likelihood of infection positive systemic inflammation. SeptiCyt[®]e RAPID is intended for in vitro diagnostic use on the Biocartis Idylla System.</p>

	<p>scores that each fall within one of five discrete interpretation bands based on the increasing likelihood of</p> <ol style="list-style-type: none"> 1) bacterial infection, 2) viral infection, and 3) severe illness, as defined by the need for mechanical ventilation, vasopressors, and/or renal replacement therapy (RRT) within seven days. 	
Specimen Type	Same	Whole blood collected in PAXgene Blood RNA tube
Specimen Processing	Same	Automated extraction within the instrument platform
General Device Characteristic Differences		
Intended Use Population	Patients presenting with suspected acute infection or sepsis in the emergency department	Patients suspected of sepsis on their first day of Intensive Care Unit (ICU) admission
Assay Principle	Quantitative gene expression assay, based on real-time generation of fluorescence from intercalating fluorescent dye during RT-LAMP amplification of nucleic acid templates	Quantitative gene expression assay, based on real-time generation of fluorescence from hydrolysis of dye quencher hydrolysis probes during cycles of PCR amplification of nucleic acid templates
Detection Technology	RT-LAMP	RT-PCR
Analytes	<p>29 mRNA host response genes:</p> <p>ANKRD22, ARG1, BATF, C3AR1, CD163, CEACAM1, CLEC5A, CTSL1, DEFA4, HERC5, HLADMB, IFI27, IFI44, IFI44L, IL18R1, IL1R2, ISG15, JUP, KCNJ2, LY86, OASL, OLFM4, PSMB9, RSAD2,</p>	Two mRNA transcript immune biomarkers: PLA2G7, PLAC8

	S100A12, TDRD9, TGFB1, XAF1, ZDHHC19 3 housekeeping genes: KPNA6, RREB1, YWHAB	
Instrument Platform	Myrna Instrument	Biocartis Idylla System
Result Output	TriVerity measures the expression levels of 32 mRNA transcripts which are input into two fixed classifiers. The result is three separate scores, each with five discrete interpretation bands. The bands reflect monotonically increasing likelihood of bacterial infection, viral infection, and severe illness, as defined by the need for mechanical ventilation, vasopressors, and/or renal replacement therapy (RRT) within seven days.	SeptiScore, calculated from the expression levels of the two mRNA analytes PLA2G7, PLAC8. The SeptiScore is laced into four discrete bands that describe a monotonically increasing likelihood of sepsis vs. Systemic Inflammatory Response Syndrome (SIRS).
Controls	Two internal controls were developed, serving as within-cartridge positive controls. They serve as sample processing and qRT-LAMP controls. The controls are RNA spike in controls from the External RNA Controls Consortium. External controls are not provided with the assay and are not required for assay performance; however, labeling describes their setup and use.	MS2 bacteriophage particles, serving as sample processing control (SPC), i.e., as within-cartridge positive control for both the sample extraction step and the coupled RT-qPCR step. External controls not provided with the assay but are described in labeling with protocols available from sponsor.

VI Standards/Guidance Documents Referenced:

Standards

- CLSI EP05-A3 – Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition
- CLSI EP06 – Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline – Second Edition
- CLSI EP07 – Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition
- CLSI EP17-A2 – Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition
- CLSI EP25-A – Evaluation of Stability of In Vitro Diagnostic Reagents: Approved Guideline
- CLSI EP37 – Supplemental Tables for Interference Testing in Clinical Chemistry (1st Edition)
- ASTM D4169-23 – Standard Practice for Performance Testing of Shipping Containers and Systems
- ASTM F2825-18 – Standard Practice for Climatic Stressing of Packaging Systems for Single Parcel Delivery

Guidance Documents

- FDA Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

A multi-site reproducibility study was performed across three laboratories. The measurements were performed over five non-consecutive days. At each site, at least two operators conducted the tests on three different instruments using one cartridge lot/panel for a total of 90 runs per panel member.

The ‘infectious’ specimens were designed as contrived specimens. The samples were formulated, aliquoted (1.6 mL) then frozen at -80°C. Prior to initiation of the reproducibility study, the aliquots were sent on dry ice to the participating laboratories. Each aliquot was thawed at room temperature for up to 120 mins or until completely thawed. After thawing, PAXgene RNA Blood contrived samples were homogenized by inverting 10 times and immediately run on the Myrna instrument. The following panel members were utilized for the reproducibility study:

Table 2. Study Panel Members

Panel Member	Sample Type	Expected Risk Band Results
A	Contrived	Bacterial Result: High Viral Result: Moderate Severity Result: High

B	Contrived	Bacterial Result: Very high Viral Result: Very Low Severity Result: Very High
C	Contrived	Bacterial Result: Very Low Viral Result: Very high Severity Result: Very Low
F	Contrived	Bacterial Result: Very Low Viral Result: Very Low Severity Result: Very Low

Results from the reproducibility study are summarized in the table below including between-days, instruments, and sites. Results met the pre-specified acceptance criteria of the standard deviation (SD) for the evaluated interpretation band being less than 5.50 score units, which reflects a small probability of scores falling into nonadjacent bands. The TriVerity scores are on a logistic scale and therefore CV analysis was not considered for these parameters; however, SD and %CV for the reproducibility study were included in Table 4 for the 29 host response genes and two housekeeping genes.

Table 3. Reproducibility Study Results

Panel Member	Score Type	Mean Score	Standard Deviation			
			Between Days	Between Instruments	Between Sites	Reproducibility
A	Bacterial	38.2	0.0	1.2	1.8	4.9
	Viral	22.3	0.0	1.5	1.8	5.2
	Severity	37.5	1.3	1.6	0.0	3.6
B	Bacterial	47.4	0.0	0.1	0.0	0.7
	Viral	6.2	0.6	0.3	0.0	1.9
	Severity	48.2	0.0	0.2	0.0	0.8
C	Bacterial	1.1	0.0	0.2	0.1	0.5
	Viral	49.1	0.2	0.2	0.0	0.4
	Severity	6.9	0.0	0.6	0.0	1.6
F	Bacterial	3.5	0.2	0.2	0.2	0.8
	Viral	3.2	0.0	0.4	0.0	1.0
	Severity	8.9	0.0	0.0	0.0	2.5

Table 4. Reproducibility Study Results for the Individual Assay Markers

Target Genes	Reproducibility							
	Panel A		Panel B		Panel C		Panel F	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
ANKRD22	1.2	6.8	0.9	5.4	0.9	5.9	2.9	11.1
ARG1	1.8	8.6	0.9	6.0	1.2	6.6	1.1	4.7
BATF	3.3	12.4	1.9	8.2	2.6	10.3	2.2	7.3
C3AR1	1.7	8.6	1.0	5.9	1.3	7.4	1.5	6.4

CD163	1.5	8.8	0.8	5.2	1.2	6.8	1.0	4.8
CEACAM1	3.2	12.4	2.0	8.8	2.1	9.9	2.0	6.8
CLEC5A	1.9	10.0	1.1	6.3	1.1	7.0	3.1	11.0
CTSL1	1.7	7.5	1.2	6.0	1.3	6.9	2.1	7.4
DEFA4	1.8	9.5	1.0	5.7	1.8	8.5	1.3	5.6
HERC5	2.3	9.9	1.6	7.6	1.2	7.5	1.3	5.4
HLA-DMB	2.8	8.5	2.3	8.1	1.9	7.9	1.4	5.2
IFI27	2.5	10.2	1.7	7.8	1.0	6.3	1.1	4.8
IFI44	1.2	6.6	1.3	6.9	0.8	6.3	0.9	4.1
IFI44L	2.4	8.8	1.9	6.7	1.5	7.8	1.5	4.9
IL18R1	1.3	7.7	0.9	5.9	0.9	6.3	1.2	5.3
IL1R2	2.7	12.6	1.2	7.0	2.2	10.2	2.6	9.8
ISG15	1.8	7.8	0.9	4.4	0.8	5.1	0.7	3.2
JUP	2.4	9.4	2.5	9.3	1.3	6.3	2.3	7.8
KCNJ2	0.8	5.1	0.7	4.4	0.8	5.0	0.6	3.1
LY86	1.1	6.5	1.3	6.9	1.0	6.2	0.6	3.6
OASL	1.9	10.5	1.8	8.8	1.3	8.5	1.6	7.4
OLFM4	2.3	10.3	1.9	7.7	2.2	9.4	1.7	5.2
PSMB9	2.0	9.7	1.4	6.3	1.4	7.7	1.2	5.0
RSAD2	2.1	10.0	1.9	8.7	1.4	8.0	1.4	5.8
S100A12	3.0	11.2	1.0	6.2	1.6	7.6	1.0	4.1
TDRD9	1.2	6.6	1.1	6.3	3.3	15.6	3.4	10.9
TGFBI	3.0	12.1	2.1	8.6	1.8	8.1	1.1	4.5
XAF1	1.3	7.4	1.6	7.2	1.3	7.2	2.2	7.5
ZDHHC19	1.6	8.7	1.5	6.7	2.3	6.6	1.5	4.4
KPNA6	1.6	8.8	1.0	5.7	1.3	7.2	0.8	4.5
RREB1	2.5	11.8	1.8	8.9	1.9	9.1	1.0	5.2
YWHAB	2.1	8.7	1.8	7.6	1.6	7.3	1.2	5.0

A separate repeatability study was conducted by one operator using one instrument per panel member. Each panel was tested in duplicate runs for 12 non-consecutive days with one cartridge lot/panel member. Samples were evaluated twice per day for a total of 48 test results per panel member. Repeatability results met the acceptance criteria of SD <5.50 score units.

Table 5. Repeatability Study Results

Panel Member	Score Type	Mean Score	Repeatability SD
A	Bacterial	24.9	5.0
	Viral	32.0	4.0
	Severity	36.6	4.7
B	Bacterial	45.9	1.1
	Viral	10.4	3.2
	Severity	47.9	0.9

C	Bacterial	0.5	0.5
	Viral	49.8	0.4
	Severity	7.2	1.2
F	Bacterial	3.2	0.4
	Viral	3.3	0.9
	Severity	8.1	1.8

2. Lot-to-Lot Reproducibility:

To evaluate variance of the TriVerity scores between cartridge lots a lot-to-lot reproducibility study was conducted using the four panel members. Samples were tested by one operator at one site on four separate instruments using three cartridges lots. For each run, cartridge lots were alternated resulting in a total of six replicates per lot for each panel member for a total of 18 samples per panel member. Results from the lot variability study, included in Table 6 below, were acceptable and met the pre-defined acceptance criteria.

Table 6. Lot-to-Lot Variability Study Results

Panel Member	Score Type	Mean Score	Standard Deviation	
			Between Lots	Within Lab
A	Bacterial	33.6	0.0	4.7
	Viral	27.3	0.0	3.5
	Severity	37.5	1.1	2.7
B	Bacterial	47.6	0.0	0.8
	Viral	6.3	0.0	1.8
	Severity	47.9	0.0	0.6
C	Bacterial	1.0	0.0	0.0
	Viral	49.2	0.0	0.4
	Severity	7.3	0.4	1.2
F	Bacterial	3.3	0.0	0.5
	Viral	3.9	0.0	0.7
	Severity	9.3	1.0	2.5

An additional study was performed to assess the TriVerity device measurements across the score ranges not fully evaluated with contrived samples during the reproducibility study. Clinical samples that covered all score types were evaluated with at least 20 results in every score band. Each sample was tested in duplicate with the same instrument and cartridge lot. The study was performed over five days using a total of three different cartridge lots and 12 different instruments. The SD measured in each band score type was less than 5.50 score units, meeting the pre-defined acceptance criteria for repeatability.

3. Linearity:

Linearity is not applicable to the TriVerity test score. To ensure that the markers that are used to generate the score are being detected quantitatively within the linear range a linearity study

was performed for the gene expression of the individual markers. Using serial dilutions prepared with pure *in vitro* transcribed RNA sequences which contained each of the 32 markers measured by the TriVerity test, six dilutions, ranging from 5×10^9 copies/mL to 5×10^5 copies/mL, were prepared in an RNA stabilizing solution and measured in duplicate. The study was performed in a single laboratory, on a single day using one instrument. The data showed linearity, for the analytical measuring range of 1×10^9 copies/mL to 1×10^6 copies/mL, and device results met the acceptance criteria of a 5% allowable deviation from linearity for each of the 32 assay markers.

4. Analytical Specificity/Interference:

Analytical Specificity/Cross-reactivity

Non-specific amplification from genomic DNA (20 ng/ μ L of gDNA added to RNA stabilization buffer) as well as from a no-template control (NTC) was evaluated. Both the NTC and the human genomic DNA showed no amplification signal. Separately, cross-reactivity was assessed by spiking 10 ng/ μ L of gDNA into the two contrived panel members B and C then comparing the results to un-spiked samples. Results demonstrated no significant bias or imprecision when compared with control conditions at the tested concentrations. The mean drift between the control and test samples was <5.50 score units and the difference in standard deviation for each results band was also <5.50 score units.

Interference

An interference study was performed to evaluate the impact of select endogenous and exogenous interferents on the ability of the assay to detect the 32 genes in the TriVerity test, specifically the impact of those substances on score results. Interferents were appropriately prepared, then spiked into each of the two panel members B (Very High Bacterial Score/Very Low Viral Score/Very High Severity Score) and C (Very Low Bacterial Score/Very High Viral Score/Very Low Severity Score), at concentrations higher than the normal or expected reference ranges. Four replicates per panel were tested at each concentration of potential interferent using two cartridge lots. Interference was assessed by estimating the bias for each specimen when compared to an un-spiked control (containing no interferent and the appropriate solvent, when applicable). The substances tested, concentration of each, and results are listed below in Table 7.

Table 7. Interference Study Results

Interferent	Concentration	Panel Member	Delta Score Relative to Control		
			Bacterial	Viral	Severity
Unconjugated Bilirubin	400 mg/L	B	0.2	-1.0	0.5
		C	0.0	0.0	0.2
Hemoglobin	10 g/L	B	0.5	-1.5	0.5
		C	-0.2	0.0	-1.0
Rheumatoid Factor	45 U/mL	B	0.7	-1.5	0.0
		C	0.0	-0.2	-0.5

Triglycerides	2000 mg/dl	B	0.5	-1.5	1.2
		C	0.2	-0.2	0.2
Albumin	50 g/L	B	0.5	0.0	-0.2
		C	0.0	0.0	0.5
Heparin	10 U/mL	B	0.7	-1.7	-0.7
		C	0.5	-0.2	1.7
Imipenem/Cilastatin	100 mg/L	B	1.0	-2.5	-0.5
		C	1.0	-0.5	0.7
Vancomycin	100 mg/L	B	0.7	-1.5	-0.2
		C	-0.2	0.0	-0.5
Cefotaxime	400 mg/L	B	1.5	-2.7	1.2
		C	0.0	-0.2	-0.5
Dopamine	500 mg/dL	B	0.7	-1.7	0.5
		C	0.5	0.0	1.0
CRP (C-reactive protein)	60 mg/L	B	0.5	-0.5	0.5
		C	0.5	-0.2	-0.2
Norepinephrine	670 µmol/L	B	0.2	-1.0	1.0
		C	0.0	0.0	-1.2
Dobutamine	11.2 mg/L	B	1.0	-2.5	0.7
		C	-0.2	0.0	0.0
Furosemide	59.9 mg/L	B	0.5	-0.2	-0.2
		C	0.0	-0.2	-0.2
IL-6 (Interleukin-6)	2000 pg/mL	B	2.0	-4.0	2.0
		C	-0.2	0.0	0.0
sCD14	5 µg/mL	B	0.5	-1.0	0.2
		C	0.0	-0.2	-0.7
LPS (Lipopolysaccharides)	5 ng/mL	B	0.0	0.0	0.7
		C	0.2	-0.2	1.7
Control NaOH	N/A	B	0.5	-2.0	1.2
		C	-0.2	-0.2	0.0
Control Serum	N/A	B	0.0	1.0	0.0
		C	0.0	0.0	0.5
Control Water	N/A	B	-0.2	0.4	0.5
		C	0.0	-0.1	-0.4

5. Assay Reportable Range:

See linearity section above.

6. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

PAXgene Sample Collection/Handling

A specimen stability study was conducted to support the recommended handling conditions from blood collection with PAXgene sample tubes to testing on the TriVerity Cartridge. Blood samples were collected from 15 healthy donors with the first sample processed within one hour of collection, then tested in duplicate after a 2-hour, 12-hour, and 14-hour incubation at room temperature (15°C and 25°C). Results from the stability study were not statistically significant and demonstrated a standard deviation less than 5.50 score units in the TriVerity Severity score when evaluated after 12-hour or 14-hour storage compared to time 0. This acceptance criteria ensures that less than 2.5% of the observed scores fell into a non-adjacent reporting bin, which would change the clinical interpretation. These data support the recommended handling claims that samples can be stored at room temperature up to 12 hours after collection.

Freeze-thaw Stability Study

To support the use of banked frozen samples in the clinical validation studies an additional study was performed to demonstrate equivalence between fresh and frozen specimens. A total of 60 whole blood specimens collected in PAXgene sample tubes were tested. Following incubation at room temperature, specimens were stored for 24 hrs. at -80°C then underwent one freeze thaw cycle. TriVerity results were compared to results from the same sample that was tested within one hour of collection. Across all evaluated samples, the mean Viral and Severity TriVerity score shifts were not statistically significant. The mean shift for the Bacterial score was significant; however, the shift was less than 5.50 score units. Cumulatively, these results demonstrate that a single freeze-thaw cycle did not significantly affect the TriVerity reported results.

Real-time Stability

Real-time reagent stability studies are ongoing to support product claims of room temperature storage for 12 months. Testing will be performed on three cartridge lots when stored at 15-30°C for up to 18 months. Stability testing for one month demonstrated no significant change in performance for any of the three TriVerity scores.

Shipping Stability

Shipping studies were conducted for both the TriVerity cartridges and the Myrna instrument. For the Myrna instrument, control samples were tested prior to ship testing. The instrument in its packaging was then subjected to distribution cycle 13 at assurance level II as defined in ASTM D4169:23. A second set of samples were tested and compared to the control samples. Standard deviation in Bacterial, Viral, and Severity scores of post ship test runs were < 5.50 score units.

Sixty (60) cartridges underwent ship testing and more than 30 cartridges were tested after conditioning and ship testing. The cartridges were pre-conditioned per ASTM F2825-18 then subjected to simulated ship testing per ASTM D4169:23, distribution cycle 13 with assurance level II. The cartridges and packaging passed all post ship test inspections. A contrived sample panel was tested. Standard deviation in Bacterial, Viral, and Severity scores of post ship test runs were < 5.50 score units. There was no impact on cartridge or instrument performance after testing.

7. Detection Limit:

Limit of Blank

Leukoreduced blood was used to determine the Limit of Blank. Ten replicates of the leukocyte reduced blood did not generate a valid TriVerity test score for any score type.

Limit of Detection

To determine the Limit of Detection, an RNA stabilizing solution was spiked with serial dilutions of the transcribed RNA IVT mix. From testing 20 replicates of concentrations ranging from 1×10^6 copies/mL to 1×10^5 copies/mL, the analytical sensitivity was determined to be 1×10^6 copies/mL, which was the lowest concentration at which 95% (19/20) of the replicates for each individual marker tested demonstrated measurable amplification.

Limit of Quantitation

Two clinical pools were prepared: a Viral pool (Low Bacterial/High Viral/ Low Severity) and a Bacterial/Severe pool (High Bacterial/Low Viral/ High Severity). Samples were then serially diluted in leukoreduced blood. The Limit of Quantitation (LOQ) for each pool was defined as the lowest WBC concentration at which 95% (19/20) of the replicates provided a score with a standard deviation less than 5.50 score units for all 3 score types. The overall LOQ was defined as the higher of the LOQs calculated for each sample pool. Samples were tested with two cartridge lots across multiple days, instruments, and sample types, for a total of 220 runs. Results indicated that the higher LoQ was approximately 500 WBC cells/ μ L for both sample pools.

8. Assay Cut-Off:

There are three score types (Bacterial, Viral, and Severity) with five interpretation bands (Very High, High, Moderate, Low, Very Low). Cut-offs were defined ahead of the clinical validation study, and the assay performance was evaluated in the clinical study using those pre-defined cutoffs as compared to adjudicated results.

9. Accuracy (Instrument):

Not applicable.

10. Carry-Over:

A study was performed to evaluate the risk of carry-over or amplicon contamination between runs tested on the Myrna instrument. Carry-over was assessed by alternating testing between panel members B (Very High Bacterial Score/Very Low Viral Score/Very High Severity Score), C (Very Low Bacterial Score/Very High Viral Score/Very Low Severity Score), and F (Very Low Bacterial Score/Very Low Viral Score/Very Low Severity Score). A total of 60 samples were tested over two days using three instruments, with 10 runs per instrument per day, as shown below:

Day	Run	Panel Member
1	1	B
	2	C
	3	F
	4	B
	5	B
	6	C
	7	C
	8	B
	9	F
	10	F
2	1	B
	2	C
	3	F
	4	B
	5	B
	6	C
	7	C
	8	B
	9	F
	10	F

Within-run standard deviation was <5.50 for all tested samples, panel members B, C and F for each sample score result (Bacterial, Viral, and Severity). Overall standard deviation for all runs and instruments was also <5.50. Results, presented in Table 8 below, indicate that the carry-over results are acceptable.

Table 8. Carry-over Results

Panel Member	Bacterial		Viral		Severity	
	Mean	SD	Mean	SD	Mean	SD
B	47.9	0.6	5.4	1.5	47.9	0.9
C	1.0	0.0	49.2	0.4	8.3	2.5
F	3.4	0.5	3.5	0.7	10.1	3.3

Instrument	Panel Member	Bacterial		Viral		Severity	
		Mean	SD	Mean	SD	Mean	SD
1	B	48.0	0.5	4.9	1.4	47.6	1.1
	C	1.0	0.0	49.0	0.0	7.3	2.5
	F	3.7	0.5	3.3	0.5	9.3	1.6
2	B	47.9	0.8	5.4	1.9	47.7	0.9
	C	1.0	0.0	49.0	0.0	9.5	2.8
	F	3.3	0.5	3.2	0.7	10.5	2.1
3	B	47.9	0.6	5.9	1.1	48.2	0.7

	C	1.0	0.0	49.5	0.5	8.2	1.9
	F	3.3	0.5	4.0	0.6	10.5	5.3

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable.

2. Matrix Comparison:

Not applicable.

C Clinical Studies:

1. Clinical Sensitivity:

The clinical performance of the TriVerity test was evaluated through a multi-center, non-interventional study (SEPSIS-SHIELD, Clinicaltrials.gov identifier: NCT04094818) conducted across 22 sites comprised of emergency departments (EDs) and academic hospitals in the United States and Europe. Patients were enrolled and samples collected during two sequential phases: a frozen phase where samples were banked for testing and a fresh phase where samples were collected and tested prospectively. A fresh versus frozen study was conducted and demonstrated comparable results, allowing for data to be pooled during analysis. There were two primary objectives, the first was to establish the diagnostic performance for differentiating bacterial from viral infection in adult patients with suspected acute infection (infection and at least one abnormal vital sign) or suspected sepsis (defined by a blood culture order and at least two abnormal vital signs). The second primary objective was to establish the prognostic performance for predicting severe illness in patients with suspected acute infection or sepsis. Illness severity was defined by clinical outcomes (i.e., whether a patient received mechanical ventilation, vasopressors, and/or renal replacement therapy [RRT] within 7 days of enrollment).

For the first primary objective, diagnostic performance of the TriVerity test for identifying bacterial or viral infection was compared to physician adjudication. Medical experts independently reviewed all available clinical data collected during standard of care as well as data collected as part of the study protocol, such as C-reactive protein (CRP) and procalcitonin (PCT) values, while remaining blinded to the TriVerity test results. Two independent analyses were conducted based on the clinical adjudication results:

1. Forced Evaluation – To define the ground truth, forced adjudication was used as the comparator method where physicians were forced to make a bacterial or viral diagnosis (certain [Yes or No] or uncertain [Probable or Unlikely] infection status). The primary diagnostic performance evaluation of the device was based on the Forced Endpoint.
2. Consensus Evaluation – Consensus expert adjudication was used as a comparator method in which uncertain (Probable or Unlikely) cases were removed. Although data from these

analyses are presented, the consensus adjudication should be considered supplementary information.

For the second primary objective, all enrolled participants followed through the first seven days were eligible for inclusion if information was available on the presence of severe illness. For a patient to be evaluated the use of mechanical ventilation, vasopressor use, and/or RRT had to be associated with the acute episode of care in the ED but not chronic exposure (e.g., continuation of home care or short-term use around surgical or other procedures).

Patient demographics and medical history for the study population was comparable across all analyzed subgroups (forced evaluation, consensus evaluation, and severity prognosis). The forced evaluation endpoint population was comprised of 1,222 patients (289 prospectively recruited and 933 banked frozen samples). The mean age was 50.9 years with 49.5% female. The race and ethnicity representation were as follows: Hispanic/Latino 11.0%, Asian 0.9%, Black or African American 29.5%, and White 64.3%. Out of the 1,222 patients enrolled in the forced adjudication cohort, a total of 1,120 individuals were also included in the severity prognostic endpoint population (262 prospectively recruited and 858 banked frozen samples). The mean age for the severity prognostic cohort was 51.3 years with 48.6% of enrolled individuals identified as female. Race and ethnicity showed similar levels of distribution as the diagnostic population (Hispanic/Latino 11.4%, Asian 0.7%, Black or African American 28.7%, and White 65.5%). Metabolic/endocrinological, respiratory tract, and cardiovascular illnesses were the most frequently represented disorders across all study populations. Malignancies were the most frequent type of immunosuppression, reported in approximately 10% of all study participants, followed by solid organ transplantation, steroid treatment, and HIV/AIDS. A total of 219 (17.9%) participants in the diagnostic population were immunosuppressed; among these, 30 fell into more than one of the immunosuppression categories. A total of 206 (18.4%) participants in the severity prognosis population were immunosuppressed. Among these, 30 participants fell into more than one immunosuppression category. Vital signs at time of enrollment showed that most patients had heart beats > 90 beats/minute while altered mental status was observed in 4.7 – 5.1% of patients. A summary of the demographic data and other clinical characteristics are provided in Table 9 below.

Table 9. Demographics and Clinical Variables Stratified by Study Population

Variable	Forced Evaluation (n=1,222)	Consensus Evaluation (n=729)	Severity Prognosis Population (n=1,120)
Gender			
Female	605 (49.5%)	345 (47.3%)	544 (48.6%)
Male	617 (50.5%)	384 (52.7%)	576 (51.4%)
Race¹			
American Indian/Native	2 (0.2%)	0 (0.0%)	1 (0.1%)
Asian	11 (0.9%)	6 (0.8%)	8 (0.7%)
Black or African American	360 (29.5%)	223 (30.6%)	321 (28.7%)
Native Hawaiian or Other Pacific Islander	2 (0.2%)	2 (0.3%)	2 (0.2%)

White	786 (64.3%)	461 (63.2%)	734 (65.5%)
Other	63 (5.2%)	37 (5.1%)	56 (5.0%)
Ethnicity			
Hispanic or Latino	135 (11.0%)	97 (13.3%)	128 (11.4%)
Not Hispanic or Latino	1064 (87.1%)	623 (85.5%)	972 (86.8%)
Unknown	23 (1.9%)	9 (1.2%)	20 (1.8%)
Medical History²			
Blood Disorders	86 (7.0%)	51 (7.0%)	80 (7.1%)
Cardiovascular Diseases	519 (42.5%)	303 (41.6%)	485 (43.3%)
Kidney Diseases	224 (18.3%)	140 (19.2%)	213 (19.0%)
Gastrointestinal Diseases	284 (23.2%)	167 (22.9%)	265 (23.7%)
Metabolic/Endocrinological Diseases	524 (42.9%)	315 (43.2%)	490 (43.8%)
Musculoskeletal Diseases	177 (14.5%)	106 (14.5%)	167 (14.9%)
Neuropsychiatric Diseases	282 (23.1%)	162 (22.2%)	264 (23.6%)
Respiratory Tract Diseases	380 (31.1%)	229 (31.4%)	350 (31.3%)
Skin and Soft Tissue Diseases	61 (5.0%)	36 (4.9%)	58 (5.2%)
Urogenital Diseases	110 (9.0%)	68 (9.3%)	106 (9.5%)
Other	200 (16.4%)	110 (15.1%)	186 (16.6%)
Type of Immunosuppression³			
Bone Marrow, Stem Cell, Cell Transplant	3 (0.2%)	2 (0.3%)	3 (0.3%)
Malignancies	125 (10.2%)	73 (10.0%)	121 (10.8%)
HIV/AIDS	26 (2.1%)	16 (2.2%)	22 (2.0%)
Solid Organ Transplant	35 (2.9%)	27 (3.7%)	34 (3.0%)
Steroid Treatment	34 (2.8%)	17 (2.3%)	30 (2.7%)
Other	30 (2.5%)	17 (2.3%)	29 (2.6%)
Abnormal Vital Signs			
Heart rate: >90 beats/minute	1062 (86.9%)	624 (85.6%)	970 (86.6%)
Temperature: >38°C or <36°C	331 (27.1%)	213 (29.2%)	301 (26.9%)
Respiratory rate: >20 breaths/minute or PaO2 <60 mmHg or SpO2 <90%	413 (33.8%)	250 (34.3%)	379 (33.8%)
Systolic blood pressure <100 mmHg	186 (15.2%)	128 (17.6%)	174 (15.5%)
Altered mental status	58 (4.7%)	34 (4.7%)	57 (5.1%)

¹Two patients gave their race as biracial (Asian, White), added to forced and prognostic population.

²Determined using provider questionnaire information at time of enrollment.

³Patients may have been included in more than one immunosuppression type.

Out of the 1,120 patients evaluated for the severity prognostic endpoint, a total of 122 (10.9%) needed mechanical ventilation, vasopressors, and/or RRT within 7 days, with vasopressor use and mechanical ventilation the most frequent clinical outcome as shown in Table 10.

Table 10. Clinical Outcomes for Prognostic Objective Population

Clinical Outcomes	Severity Prognosis Population (n=1,120)
Need for Mechanical Ventilation, Vasopressor Use, and/or RRT within 7 days	122 (10.9%)
Mechanical Ventilation	63/122 (51.6%)
Vasopressor Use	99/122 (81.1%)
RRT	23/122 (18.8%)

The disease prevalence (pre-test probability), predictive values (post-test probability), likelihood ratios (with 80% confidence intervals calculated by bootstrap analysis), and the frequency of results for each score type and interpretation band generated by the of the TriVerity test are presented below for the diagnosis of bacterial and viral infections in addition to prognosis of illness severity. The likelihood ratios (LR) were calculated using the definition where LR equals the probability that an individual with disease has the test result divided by the probability that an individual without disease has the test result. This formula was applied to each interpretation band separately. Predictive values depend on the likelihood ratios and the prevalence of disease.

Clinical Performance Evaluation for Primary Diagnostic Endpoint – Bacterial Score

Results for the performance of the TriVerity device for diagnosing the likelihood of bacterial infection showed a monotonic increase with no overlapping of the 80% CIs between non-adjacent interpretation bands. This demonstrates a relationship between the TriVerity Bacterial score and the increasing likelihood of bacterial infection across each interpretation band. For the forced adjudicated population, 80.4% of the results fell into the clinically actionable Very High, High, Low or Very Low interpretation bands (40.8% Very High and High, 39.6% Very Low and Low interpretation bands); only 19.6% of results were found in the moderate interpretation band which had a LR of 0.9. At a prevalence of 60.6% for bacterial infections, the positive predictive values of the outer Very High and Very Low interpretation bands were 89.0% and 20.0%, respectively. Forced and consensus diagnostic results were similar; however, consensus adjudicated results performed better due to the removal of uncertain bacterial infection status. Results from the forced adjudication are included in Table 11 and results from the consensus adjudication are included in Table 12.

Table 11. Diagnostic Performance for Bacterial Score in the Forced Adjudication Population

TriVerity Bacterial Score Band	N	Forced Adjudicated Bacterial Infection		Positive Predictive Value	Frequency of Result	LR (80% CI)
		Yes (N)	No (N)			
Very High	254	226	28	89.0%	20.8%	5.2 (4.2 - 6.8)
High	245	184	61	75.1%	20.0%	2.0 (1.6 - 2.4)
Moderate	239	142	97	59.4%	19.6%	0.9 (0.8 - 1.1)
Low	324	157	167	48.5%	26.5%	0.6 (0.5 - 0.7)

Very Low	160	32	128	20.0%	13.1%	0.2 (0.1 - 0.2)
Total	1222	741	481	Prevalence = 60.6%		

Table 12. Diagnostic Performance for Bacterial Score in the Consensus Adjudication Population

TriVerity Bacterial Score Band	N	Consensus Adjudicated Bacterial Infection		Positive Predictive Value	Frequency of Result	LR (80% CI)
		Yes (N)	No (N)			
Very High	177	165	12	93.2%	24.3%	8.0 (5.7 - 12.4)
High	132	107	25	81.1%	18.1%	2.5 (2.0 - 3.3)
Moderate	136	90	46	66.2%	18.7%	1.1 (0.9 - 1.4)
Low	177	85	92	48.0%	24.3%	0.5 (0.4 - 0.6)
Very Low	107	13	94	12.1%	14.7%	0.1 (0.0 - 0.1)
Total	729	460	269	Prevalence = 63.1%		

Clinical Performance Evaluation for Primary Diagnostic Endpoint – Viral Score

Results for the TriVerity test performance for diagnosis of the likelihood of viral infection demonstrated a monotonic increase between bands with no overlapping of the 80% CIs between non-adjacent interpretation bands. For the forced adjudicated population, 84.9% of the results fell into the clinically actionable Very High, High, Low or Very Low interpretation bands (20.0% Very High and High, 65.0% Very Low and Low interpretation bands); 15.1% of results were found in the moderate interpretation band which had a LR of 1.0. At a prevalence of 25.5% for viral infections, the positive predictive values of the outer Very High and Very Low interpretation bands were 85.3% and 6.8%, respectively. The consensus diagnostic results showed much higher LR for the Very High interpretation band (LR: 40.9) and much lower LR for the Very Low interpretation band (LR: 0.1). Results from the forced and consensus adjudication are included in Table 13 and Table 14, respectively.

Table 13. Diagnostic Performance for Viral Score in the Forced Adjudication Population

TriVerity Viral Score Band	N	Forced Adjudicated Viral Infection		Positive Predictive Value	Frequency of Result	LR (80% CI)
		Yes (N)	No (N)			
Very High	150	128	22	85.3%	12.3%	20.0 (15.4 - 27.3)
High	94	35	59	37.2%	7.7%	2.0 (1.6 - 2.6)
Moderate	184	40	144	21.7%	15.1%	1.0

						(0.8 - 1.2)
Low	336	41	295	12.2%	27.5%	0.5 (0.4 - 0.6)
Very Low	458	31	427	6.8%	37.5%	0.2 (0.2 - 0.3)
Total	1222	275	947	Prevalence = 22.5%		

Table 14. Diagnostic Performance for Viral Score in the Consensus Adjudication Population

TriVerity Viral Score Band	N	Consensus Adjudicated Viral Infection		Positive Predictive Value	Frequency of Result	LR (80% CI)
		Yes (N)	No (N)			
Very High	113	105	8	92.9%	15.5%	40.9 (27.7 - 72.2)
High	58	25	33	43.1%	8.0%	2.4 (1.7 - 3.2)
Moderate	101	22	79	21.8%	13.9%	0.9 (0.6 - 1.1)
Low	183	17	166	9.3%	25.1%	0.3 (0.2 - 0.4)
Very Low	274	8	266	2.9%	37.6%	0.1 (0.0 - 0.1)
Total	729	177	552	Prevalence = 24.3%		

Clinical Performance Evaluation for Prognostic Endpoint – Illness Severity Score

Table 15 shows the performance of the TriVerity test for the prediction of illness severity as defined by the need for mechanical ventilation, vasopressors, and/or RRT within 7 days. The Severity score demonstrated a monotonic increase between bands with no overlapping of the 80% CIs between non-adjacent interpretation bands. A total of 79.6% of results were observed in the clinically actionable Very High, High, Very Low and Low interpretation bands; 18.8% of these in the Very High and High bands, 60.7% in the Low and Very Low bands. At a prevalence of 10.9%, the probabilities of having severe illness were 58.1% and 2.7% for the Very High and Very Low interpretation bands. The data demonstrated a relationship between the TriVerity test results and the likelihood of needing mechanical ventilation, vasopressors, and/or RRT within 7 days.

Table 15. Prognostic Performance for Illness Severity as Defined by the Need for Mechanical Ventilation, Vasopressor Use, and/or RRT within 7 Days

TriVerity Severity Score Band	N	Need for Mechanical, Ventilation, Vasopressors, and/or RRT Within 7 Days		Positive Predictive Value	Frequency of Result	LR (80% CI)
		Yes (N)	No (N)			
Very High	31	18	13	58.1%	2.8%	11.3 (7.1 - 17.7)

High	180	41	139	22.8%	16.1%	2.4 (2.0 - 2.9)
Moderate	229	38	191	16.6%	20.4%	1.6 (1.3 - 2.0)
Low	203	15	288	5.0%	27.1%	0.4 (0.3 - 0.6)
Very Low	377	10	367	2.7%	33.7%	0.2 (0.1 - 0.3)
Total	1120	122	998	Prevalence = 10.9%		

2. Clinical Specificity:

See Clinical Sensitivity above.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Subgroup Analyses

Subgroup analyses were performed based on individual patient demographic data and medical history, including race/ethnicity, immunocompromised status, SARS-CoV-2 infection, as well as by enrollment criteria (suspected acute infection versus suspected sepsis).

For subgroups with a sufficient number of patients for statistical analysis, an increase in TriVerity scores appeared to correspond to an increase in likelihood for diagnosing bacterial and viral infections.

Performance by Race and Ethnicity

Substantial differences were not observed in the diagnostic performance of the TriVerity test between Black/African Americans and other races or between ethnicities (Hispanic or Latino versus Not Hispanic or Latino) using forced adjudication. AUROCs for the accuracy of the bacterial results of the TriVerity test among White, Black/African American vs. participants of other races were 0.76, 0.76 and 0.84, respectively. AUROCs for the viral results were 0.81, 0.83 and 0.90, respectively.

For the illness severity prognosis endpoint, race did not impact the performance of Severity result readout of the TriVerity test. Across a diverse cohort of participants enrolled, LRs for the five interpretation bands in the TriVerity test did not differ markedly between Black/African Americans and White patients or between Hispanic or Latino and Non-Hispanic or Latino patients. The number of Asian, American Indian or Alaskan Native, and Native Hawaiian or Other Pacific Islander patients was not sufficient to evaluate potential differences individually. AUROCs for the TriVerity illness severity results were 0.79 for Black/African Americans versus 0.78 for other races.

Table 24. Analysis for Race and Ethnicity, Bacterial Score – Black Patients

Black or African American Population				
TriVerity Bacterial Score Band	N	Forced Physician Adjudication	Frequency of Result	LR (80% CI)

		Yes (N)	No (N)		
Very High	49	40	9	13.6%	3.8 (2.5 - 6.4)
High	52	41	11	14.4%	3.1 (2.2 - 5.0)
Moderate	67	42	25	18.6%	1.4 (1.1 - 1.9)
Low	118	57	61	32.8%	0.8 (0.6 - 1.0)
Very Low	74	15	59	20.6%	0.2 (0.1 - 0.3)
Total	360	195	165		

Table 25. Analysis for Race and Ethnicity, Bacterial Score – White Patients

White Population					
TriVerity Bacterial Score Band	N	Forced Physician Adjudication		Frequency of Result	LR (80% CI)
		Yes (N)	No (N)		
Very High	193	174	19	24.6%	5.2 (4.1 - 7.3)
High	179	131	48	22.8%	1.6 (1.3 - 1.9)
Moderate	161	92	69	20.5%	0.8 (0.6 - 0.9)
Low	181	88	93	23.0%	0.5 (0.5 - 0.6)
Very Low	72	16	56	9.2%	0.2 (0.1 - 0.2)
Total	786	501	285		

Table 26. Analysis for Race and Ethnicity, Bacterial Score – Hispanic

Hispanic or Latino Population					
TriVerity Bacterial Score Band	N	Forced Physician Adjudication		Frequency of Result	LR (80% CI)
		Yes (N)	No (N)		
Very High	28	25	3	20.7%	6.9 (3.7 - 21.9)
High	28	21	7	20.7%	2.5 (1.5 - 4.7)
Moderate	21	16	5	15.6%	2.6 (1.7 - 6.0)
Low	33	11	22	24.4%	0.4 (0.2 - 0.6)
Very Low	25	1	24	18.5%	0.0 (0.0 - 0.1)
Total	135	74	61		

Table 27. Analysis for Race and Ethnicity, Bacterial Score – Not Hispanic

Non-Hispanic or Latino Population					
TriVerity Bacterial Score Band	N	Forced Physician Adjudication		Frequency of Result	LR (80% CI)
		Yes (N)	No (N)		
Very High	225	200	25	21.1%	5.0 (4.0 - 6.6)
High	211	158	53	19.8%	1.9 (1.5 - 2.2)
Moderate	218	126	92	20.5%	0.8 (0.7 - 1.0)
Low	279	140	139	26.2%	0.6 (0.6 - 0.7)
Very Low	131	31	100	12.3%	0.2 (0.1 - 0.2)
Total	1064	655	409		

Table 28. Analysis for Race and Ethnicity, Viral Score – Black Patients

Black or African American Population					
TriVerity Viral Score Band	N	Forced Physician Adjudication		Frequency of Result	LR (80% CI)
		Yes (N)	No (N)		
Very High	59	51	8	16.4%	16.6 (11.1 - 29.5)
High	39	14	25	10.8%	1.5 (0.9 - 2.1)
Moderate	59	14	45	16.4%	0.8 (0.5 - 1.1)
Low	86	14	72	23.9%	0.5 (0.3 - 0.7)
Very Low	117	7	110	32.5%	0.2 (0.1 - 0.2)
Total	360	100	260		

Table 29. Analysis for Race and Ethnicity, Viral Score – White Patients

White Population					
TriVerity Viral Score Band	N	Forced Physician Adjudication		Frequency of Result	LR (80% CI)
		Yes (N)	No (N)		
Very High	78	65	13	9.9%	20.2 (14.4 - 30.8)
High	49	18	31	6.2%	2.3 (1.6 - 3.4)
Moderate	115	24	91	14.6%	1.1 (0.8 - 1.4)
Low	235	26	209	29.9%	0.5

					(0.4 - 0.6)
Very Low	309	23	286	39.3%	0.3 (0.2 - 0.4)
Total	786	156	630		

Table 30. Analysis for Race and Ethnicity, Viral Score – Hispanic

Hispanic or Latino Population					
TriVerity Viral Score Band	N	Forced Physician Adjudication		Frequency of Result	LR (80% CI)
		Yes (N)	No (N)		
Very High	33	31	2	24.4%	25.5 (13.5 - ∞)
High	9	6	3	6.7%	3.3 (1.1 - 8.9)
Moderate	18	6	12	13.3%	0.8 (0.4 - 1.4)
Low	29	4	25	21.5%	0.3 (0.1 - 0.4)
Very Low	46	4	42	34.1%	0.2 (0.0 - 0.3)
Total	135	51	84		

Table 31. Analysis for Race and Ethnicity, Viral Score – Not Hispanic

Non-Hispanic or Latino Population					
TriVerity Viral Score Band	N	Forced Physician Adjudication		Frequency of Result	LR (80% CI)
		Yes (N)	No (N)		
Very High	114	94	20	10.7%	18.2 (13.9 - 24.9)
High	84	29	55	7.9%	2.0 (1.5 - 2.6)
Moderate	159	31	128	14.9%	0.9 (0.7 - 1.1)
Low	304	37	267	28.6%	0.5 (0.4 - 0.6)
Very Low	403	27	376	37.9%	0.3 (0.2 - 0.3)
Total	1064	218	846		

Table 32. Analysis for Race and Ethnicity, Illness Severity Score – Black Patients

Black or African American Population					
TriVerity Severity Score Band	N	Need for Mechanical Ventilation, Vasopressor, and/or RRT within 7 Days		Frequency of Result	LR (80% CI)
		Yes (N)	No (N)		
Very High	8	5	3	2.5%	14.5

					(5.7 - 50.3)
High	33	6	27	10.3%	1.9 (1.0 - 3.2)
Moderate	41	10	31	12.8%	2.8 (1.2 - 4.0)
Low	82	7	75	25.5%	0.8 (0.4 - 1.2)
Very Low	157	5	152	48.9%	0.3 (0.1 - 0.4)
Total	321	33	288		

Table 33. Analysis for Race and Ethnicity, Illness Severity Score – White Patients

White Population					
TriVerity Severity Score Band	N	Need for Mechanical Ventilation, Vasopressor, and/or RRT within 7 Days		Frequency of Result	LR (80% CI)
		Yes (N)	No (N)		
Very High	23	13	10	3.1%	9.8 (5.8 – 17.5)
High	137	33	104	18.7%	2.4 (1.9 – 2.9)
Moderate	178	28	150	24.3%	1.4 (1.1 - 1.7)
Low	206	7	199	28.1%	0.3 (0.1 - 0.4)
Very Low	190	5	185	25.9%	0.2 (0.1 - 0.3)
Total	734	86	648		

Table 34. Analysis for Race and Ethnicity, Illness Severity Score – Hispanic

Hispanic or Latino Population					
TriVerity Severity Score Band	N	Need for Mechanical Ventilation, Vasopressor, and/or RRT within 7 Days		Frequency of Result	LR (80% CI)
		Yes (N)	No (N)		
Very High	4	2	2	3.1%	15.0 (0 - ∞)
High	18	2	16	14.1%	1.9 (0.0 - 3.8)
Moderate	30	2	28	23.4%	1.1 (0.4 - 2.0)
Low	32	2	30	25.0%	1.0 (0.3 - 1.8)
Very Low	44	0	44	34.4%	0.0 (0.0 - 0.0)
Total	128	8	120		

Table 35. Analysis for Race and Ethnicity, Illness Severity Score – Not Hispanic

Non-Hispanic or Latino Population					
TriVerity Severity Score Band	N	Need for Mechanical Ventilation, Vasopressor, and/or RRT within 7 Days		Frequency of Result	LR (80% CI)
		Yes (N)	No (N)		
Very High	27	16	11	2.8%	11.1 (7.2 - 19.0)
High	160	39	121	16.5%	2.4 (1.9 - 2.9)
Moderate	195	35	160	20.1%	1.7 (1.3 - 1.9)
Low	268	13	255	27.6%	0.4 (0.3 - 0.5)
Very Low	322	10	312	33.1%	0.2 (0.1 - 0.4)
Total	972	113	859		

Performance by Immune Competency Status

Additional analyses were conducted to determine whether the presence of immunosuppression in study participants affected the performance of the TriVerity test. Likelihood ratios and AUROCs for the Bacterial and Viral TriVerity results in the forced adjudicated population did not differ markedly based on participants with (N = 219) or without immunosuppression (N = 1,003). Results are presented below in Tables 36 – 38. Likelihood ratios ranged from 0.1 to 20.3 in immunosuppressed participants and ranged from 0.2 to 20.0 in immunocompetent participants across all three score types.

Table 36. Analysis for Immunosuppressed Population, Bacterial Score

Population	TriVerity Bacterial Score Band	N	Forced Physician Adjudication		LR (80% CI)
			Yes (N)	No (N)	
Immunosuppressed Population	Very High	64	57	7	4.3 (2.8 – 8.1)
	High	43	28	15	1.0 (0.7 - 1.5)
	Moderate	44	30	14	1.1 (0.8 - 1.7)
	Low	43	22	21	0.6 (0.4 - 0.8)
	Very Low	25	6	19	0.2 (0.1 - 0.3)
Immunocompetent Population	Very High	190	169	21	5.4 (4.2 - 7.4)
	High	202	156	46	2.3 (1.9 - 2.8)
	Moderate	195	112	83	0.9 (0.8 - 1.1)

	Low	281	135	146	0.6 (0.5 - 0.7)
	Very Low	135	26	109	0.2 (0.1 - 0.2)

Table 37. Analysis for Immunosuppressed Population, Viral Score

Population	TriVerity Viral Score Band	N	Forced Physician Adjudication		LR (80% CI)
			Yes (N)	No (N)	
Immunosuppressed Population	Very High	25	21	4	20.3 (11.6 - 51.2)
	High	22	7	16	1.7 (0.9 - 2.8)
	Moderate	33	4	29	0.5 (0.2 - 0.9)
	Low	55	6	49	0.5 (0.2 - 0.7)
	Very Low	83	7	76	0.4 (0.2 - 0.5)
Immunocompetent Population	Very High	125	107	18	20.0 (15.3 - 28.8)
	High	71	28	43	2.2 (1.6 - 2.9)
	Moderate	151	36	115	1.0 (0.8 - 1.3)
	Low	281	35	246	0.5 (0.4 - 0.6)
	Very Low	375	24	351	0.2 (0.2 - 0.3)

Table 38. Analysis for Immunosuppressed Population, Illness Severity Score

Population	TriVerity Severity Score Band	N	Need for Mechanical Ventilation, Vasopressor, and/or RRT within 7 Days		LR (80% CI)
			Yes (N)	No (N)	
Immunosuppressed Population	Very High	10	5	5	6.6 (2.7 - 15.9)
	High	44	10	34	1.9 (1.3 - 2.8)
	Moderate	44	7	37	1.2 (0.7 - 1.9)
	Low	57	4	53	0.5 (0.2 - 0.8)
	Very Low	51	1	50	0.1 (0.0 - 0.3)
Immunocompetent Population	Very High	21	13	8	14.0 (7.9 - 26.5)
	High	136	31	105	2.5

					(2.0 - 3.2)
	Moderate	185	31	154	1.7 (1.4 - 2.1)
	Low	246	11	235	0.4 (0.3 - 0.6)
	Very Low	326	9	317	0.2 (0.1 - 0.3)

Performance by enrollment criteria (suspected of acute infection versus suspected sepsis)

Additional analyses were performed to identify whether performance of the TriVerity test differed among different enrollment cohorts (i.e., individuals with suspected acute infection and at least 1 abnormal vital sign vs. individuals with suspected sepsis with at least 2 abnormal vital signs). Analysis of TriVerity Bacterial Score results (Table 39) and Viral Score (Table 39) test results are summarized below. Among the forced adjudication cohort of patients, LRs ranged from 0.2 in the Very Low to 5.4 in the Very High TriVerity Bacterial bands for participants enrolled with suspected acute infection with ≥ 1 vital sign change. Among participants with suspected sepsis with ≥ 2 vital sign changes, the likelihood ratios ranged from 0.1 to 4.9. LRs for the Viral TriVerity interpretation bands also did not differ markedly between participants enrolled under either of the two inclusion criteria. AUROCs for the Bacterial TriVerity results were 0.81 and 0.72 in participants with suspected acute infection with ≥ 1 vital sign change vs. suspected sepsis with ≥ 2 vital sign change. Similarly, AUROCs for the viral TriVerity results were 0.83 and 0.83 for participants with a suspected infection plus ≥ 1 vital sign change vs. suspected sepsis with ≥ 2 vital sign. These results demonstrate that the inclusion criteria used at the time of enrollment did not impact the performance of TriVerity Bacterial and Viral results. Table 41 outlines the prognostic severity results. AUROC for TriVerity severity results in the population with suspected acute Infection with ≥ 1 vital sign change was 0.84 and was 0.72 for the suspected sepsis with ≥ 2 vital signs change population.

Table 39. Analysis by Enrollment Criteria (Suspected Acute Infection vs. Suspected Sepsis), Bacterial Score

Population	TriVerity Bacterial Score Band	N	Forced Physician Adjudication		LR (80% CI)
			Yes (N)	No (N)	
Suspected Acute Infection with >1 Vital Sign Changes	Very High	200	177	23	5.4 (4.3 - 7.4)
	High	209	153	56	1.9 (1.6 - 2.4)
	Moderate	212	124	88	1.0 (0.8 - 1.2)
	Low	301	143	158	0.6 (0.6 - 0.7)
	Very Low	151	31	120	0.2 (0.1 - 0.2)
	Very High	128	120	8	4.9 (3.3 - 8.4)

Suspected Sepsis with >2 Vital Sign Changes	High	93	75	18	1.4 (1.0 - 1.9)
	Moderate	66	40	26	0.5 (0.4 - 0.7)
	Low	51	30	21	0.5 (0.3 - 0.7)
	Very Low	18	3	15	0.1 (0.0 - 0.1)

Table 40. Analysis by Enrollment Criteria (Suspected Acute Infection vs. Suspected Sepsis), Viral Score

Population	TriVerity Viral Score Band	N	Forced Physician Adjudication		LR (80% CI)
			Yes (N)	No (N)	
Suspected Acute Infection with >1 Vital Sign Changes	Very High	138	118	20	19.6 (14.9 - 26.5)
	High	79	32	47	2.3 (1.7 - 3.0)
	Moderate	161	35	126	0.9 (0.7 - 1.1)
	Low	292	37	255	0.5 (0.4 - 0.6)
	Very Low	403	26	377	0.2 (0.2 - 0.3)
Suspected Sepsis with >2 Vital Sign Changes	Very High	21	18	3	29.0 (14.9 - 101.8)
	High	30	8	22	1.8 (1.0 - 2.8)
	Moderate	60	13	47	1.3 (0.9 - 1.8)
	Low	105	12	93	0.6 (0.4 - 0.9)
	Very Low	140	10	130	0.4 (0.2 - 0.5)

Table 41. Analysis by Enrollment Criteria (Suspected Acute Infection vs. Suspected Sepsis), Illness Severity Score

Population	TriVerity Severity Score Band	N	Need for Mechanical Ventilation, Vasopressor, and/or RRT within 7 Days		LR (80% CI)
			Yes (N)	No (N)	
Suspected Acute Infection with >1 Vital Sign Changes	Very High	20	10	10	11.4 (6.5 - 21.0)
	High	138	22	116	2.2 (1.6 - 2.8)
	Moderate	193	29	164	2.0 (1.6 - 2.5)
	Low	278	13	265	0.6 (0.4 - 0.7)

	Very Low	353	5	348	0.2 (0.1 - 0.2)
Suspected Sepsis with >2 Vital Sign Changes	Very High	23	15	8	5.6 (3.3 - 10.5)
	High	84	32	52	1.8 (1.5 - 2.4)
	Moderate	95	22	73	0.9 (0.7 - 1.2)
	Low	76	10	66	0.4 (0.3 - 0.7)
	Very Low	58	5	53	0.3 (0.1 - 0.5)

Performance by Viral Respiratory Infection

To assess whether the TriVerity Viral score could adequately identify SARS-COV-2 infections, additional subgroup analysis was performed in enrolled individuals who tested positive for SARS-CoV-2 (COVID-19). Due to the lack of a control group (patients tested and negative for SARS-CoV-2) performance for this population could not be independently assess based on the LR. However, most patients were assigned to either the Very High, High, or Moderate likelihood interpretation bands. A limitation statement has been included in the labeling that this test is not meant to diagnose SARS-CoV-2 infections.

Table 42. Analysis for SARS-CoV-2 Performance, Viral Score

Population	TriVerity Viral Score Band	N	Forced Physician Adjudication		LR (80% CI)
			YES	NO	
Positive SARS-CoV-2 Test (COVID-19)	Very High	77	77	0	n/a
	High	17	16	1	0.5
	Moderate	14	12	2	0.2
	Low	9	9	0	n/a
	Very Low	8	7	1	0.2

n/a; not applicable

Numbers for influenza A and B infections (N = 27) were too low to present as a separate analysis (reflecting the epidemiology of viral diseases in the US during the study phases). A limitation statement has been included in the labeling that this test is not meant to diagnose influenza.

D Clinical Cut-Off:

Following training of the TriVerity classifier, thresholds between the interpretation bands for each result were set based on data collected on the cartridge. Cut-off values for the TriVerity test were established prior to the clinical trial. Samples used for setting the thresholds (n = 399) were collected from single site and multicenter Emergency Departments and ICUs that were independent from the pivotal clinical study. Thresholds were optimized using the procedure outlined below:

- Set initial threshold targeting 95% Positive Percent Agreement (PPA) in band 1 (very low likelihood), 95% Negative Percent Agreement (NPA) in band 5 (very high likelihood), and 10% moderate band coverage on each axis (bacterial, viral, illness severity)
- For each score, examine band 1/5 fraction (i.e., percentage of results in bands 1 and 5 combined) and consider relaxing initial thresholds between bands 1 and 2 and between bands 4 and 5 to ensure maximal outer coverage. This was noted as minor reductions in PPA or NPA below 95% may give significant increases in outer band coverage, which in turn may provide greater benefit to more patients.
- Examine and adjust for monotonicity, aiming for roughly likelihood ratios 0.3-0.5 in band 2, roughly 0.9-1.2 in band 3, and 1.8-2.5 in band 4, to ensure clear gradations of separability.
- Examine standard deviation per band, ensuring none is substantially greater than 4.5 and adjust thresholds if necessary.
- Globally re-examine all metrics (PPA/NPA in outer bands; band fraction; moderate coverage; LR monotonicity; and standard deviation) and perform one final refinement to balance competing metrics if needed. Where band fraction is the percentage of patients in a one or more given bands, moderate coverage is the percentage of patients in the middle band, and LR monotonicity is likelihood monotonicity.

E Expected Values/Reference Range:

A reference range study was conducted to establish the performance of the TriVerity Acute Infection and Sepsis Test with a healthy population. Samples were collected from diverse geographical locations in the U.S. and India from individuals who self-reported their healthy status. A total of 120 remnant samples were obtained from previous clinical studies and commercial bio-banked samples. The presumed healthy population included male and female patients 18 – 71 years old with a mean age of 41 years. Results from the study are included in Tables 43 – 46 and stratified by demographic group.

Table 43. Reference Range Healthy Population Demographics

Race	Asian	27 (22.5%)
	Black or African American	27 (22.5%)
	White	54 (45.0%)
	N/A	12 (10.0%)
Ethnicity	Hispanic	12 (10.0%)
	Not Hispanic or Latino	108 (90.0%)
Gender	Female	58 (48.3%)
	Male	62 (51.7%)

Table 44. Reference Range Results by Demographics, Bacterial Score

Demographics		TriVerity Bacterial Score Band				
		Very Low (0-10)	Low (11-20)	Moderate (21-30)	High (31-40)	Very High (41-50)
Gender						
Female	N	12	37	7	2	0
	%	20.7%	63.8%	12.1%	3.4%	0.0%

Male	N	15	45	2	0	0
	%	24.2%	72.6%	3.2%	0.0%	0.0%
Race						
Asian	N	3	20	4	0	0
	%	11.1%	74.1%	14.8%	0.0%	0.0%
Black	N	14	12	1	0	0
	%	51.9%	44.4%	3.7%	0.0%	0.0%
White	N	6	44	3	1	0
	%	11.1%	81.5%	5.6%	1.9%	0.0%
Ethnicity						
Hispanic	N	4	6	1	1	0
	%	33.3%	50.0%	8.3%	8.3%	0.0%
Not Hispanic or Latino	N	23	76	8	1	0
	%	21.3%	70.4%	7.4%	0.9%	0.0%
Total	N	27	82	9	2	0
	%	22.5%	68.3%	7.5%	1.7%	0.0%

Table 45. Reference Range Results by Demographics, Viral Score

Demographics		TriVerity Viral Score Band				
		Very Low (0-10)	Low (11-20)	Moderate (21-30)	High (31-40)	Very High (41-50)
Gender						
Female	N	11	23	13	8	3
	%	19.0%	39.7%	22.4%	13.8%	5.2%
Male	N	17	26	16	1	2
	%	27.4%	41.9%	25.8%	1.6%	3.2%
Race						
Asian	N	6	11	8	2	0
	%	22.2%	40.7%	29.6%	7.4%	0.0%
Black	N	3	11	6	3	4
	%	11.1%	40.7%	22.2%	11.1%	14.8%
White	N	17	22	11	3	1
	%	31.5%	40.7%	20.4%	5.6%	1.9%
Ethnicity						
Hispanic	N	2	5	4	1	0
	%	16.7%	41.7%	33.3%	8.3%	0.0%
Not Hispanic or Latino	N	26	44	25	8	5
	%	24.1%	40.7%	23.1%	7.4%	4.6%
Total	N	28	49	29	9	5
	%	23.3%	40.8%	24.2%	7.5%	4.2%

Table 46. Reference Range Results by Demographics, Illness Severity Score

Demographics	TriVerity Severity Score Band				
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		Very Low (0-10)	Low (11-20)	Moderate (21-30)	High (31-40)	Very High (41-50)
Gender						
Female	N	47	10	0	1	0
	%	81.0%	17.2%	0.0%	1.7%	0.0%
Male	N	57	5	0	0	0
	%	91.9%	8.1%	0.0%	0.0%	0.0%
Race						
Asian	N	22	4	0	1	0
	%	81.5%	14.8%	0.0%	3.7%	0.0%
Black	N	25	2	0	0	0
	%	92.6%	7.4%	0.0%	0.0%	0.0%
White	N	45	9	0	0	0
	%	83.3%	16.7%	0.0%	0.0%	0.0%
Ethnicity						
Hispanic	N	12	0	0	0	0
	%	100.0%	0.0%	0.0%	0.0%	0.0%
Not Hispanic or Latino	N	92	15	0	1	0
	%	85.2%	13.9%	0.0%	0.9%	0.0%
Total	N	104	15	0	1	0
	%	86.7%	12.5%	0.0%	0.8%	0.0%

The reference interval was determined to be 7.0 – 27.1 for the Bacterial score, 7.0 – 41.0 for the Viral score, and 3.0 – 19.0 for the Severity score. Viral scores appear to be elevated in the presumed healthy population, with a high proportion of African Americans falling into the High and Very High interpretation bands. A limitation statement has been included in the labeling that this test is not indicated for patients who are presumed to be in good health.

F Other Supportive Instrument Performance Characteristics Data:

Not applicable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.