510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K232095

B Applicant

Immunexpress, Inc

C Proprietary and Established Names

SeptiCyte RAPID

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

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for addition of EDTA venous whole blood as a sample type for the previously cleared SeptiCyte RAPID test (K203748).

B Measurand:

mRNA transcripts of host response genes PLA2G7 and PLAC8.

C Type of Test:

The SeptiCyte RAPID test is a quantitative reverse transcription polymerase chain reaction-based test (qRT-PCR).

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The SeptiCyte 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 Tubes, K2-EDTA blood tubes, or K3-EDTA blood tubes. The SeptiCyte 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 SeptiCyte 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. SeptiCyte RAPID is intended for in-vitro diagnostic use on the Biocartis Idylla System.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use on the Biocartis Idylla System.

IV Device/System Characteristics:

A Device Description:

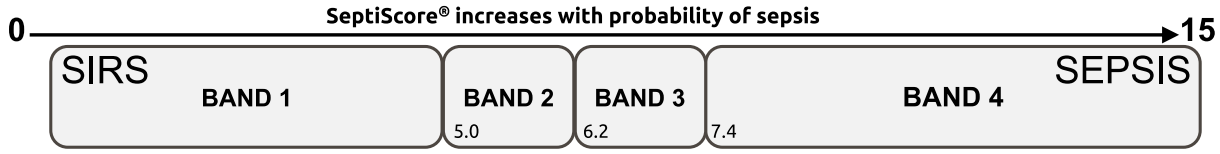
The SeptiCyte RAPID test was cleared previously for use with venous blood samples collected in PAXgene Blood RNA tubes (K203748) on the Biocartis Idylla System (K163628). The purpose of this submission is to validate use of venous whole blood samples collected in K2-EDTA and K3-EDTA tubes for use with the device.

The SeptiCyte RAPID test uses quantitative reverse transcription - polymerase chain reaction (qRT-PCR) to measure the expression levels of the host response genes PLA2G7 and PLAC8. Each SeptiCyte RAPID test kit includes six cartridges. Cartridges are individually packaged in sealed pouches. Each cartridge contains the necessary reagents to perform a single test.

B Principle of Operation:

Amplicons generated by qRT-PCR are detected and quantitated by fluorescence generated upon exonucleolytic release of dyes from oligonucleotide probes that specifically bind to the amplicons. The Idylla Cartridge contains all reagent components needed by the SeptiCyte RAPID test to generate patient results and automates all SeptiCyte RAPID test steps, including

sample extraction/purification and qRT-PCR for the detection and relative quantification of the two human mRNA targets PLAC8 and PLA2G7. These values are combined to produce a SeptiScore which is interpreted by means of four discrete bands which reflect a monotonically increasing likelihood of sepsis as shown in the following figure:



Sample Type

PAXgene blood sample: Whole blood (2.5 mL) is collected into a PAXgene Blood RNA tube (US FDA 510(k) number K042613) containing 6.9 mL stabilizing solution. A 0.9 mL volume of the resultant PAXgene-stabilized blood sample is then used as input for the SeptiCyte RAPID test.

EDTA blood sample: Whole blood is collected into either a K3-EDTA or K2-EDTA lavender top vacutainer, according to manufacturer's instructions. After mixing thoroughly by gentle inversion 10x, a volume of 240 µL EDTA blood sample is run fresh on the SeptiCyte RAPID test. Freeze/thawing of EDTA blood samples is not recommended and samples should be run fresh.

V Substantial Equivalence Information:

A Predicate Device Name(s):
SeptiCyte RAPID

B Predicate 510(k) Number(s):
K203748

C Comparison with Predicate(s):

Device & Predicate Device(s):	K232095	K203748
Device Trade Name	SeptiCyte RAPID	SeptiCyte RAPID
General Device Characteristic Similarities		
Intended Use/Indications For Use	The SeptiCyte 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 Tubes, K2-EDTA blood tubes, or K3-EDTA blood tubes. The SeptiCyte RAPID	The SeptiCyte 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 SeptiCyte RAPID test is used in conjunction with clinical assessments and

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Intended Use Population	Same	Patients suspected of sepsis on their first day of Intensive Care Unit admission
Assay Principle	Same	qRT-PCR amplification and quantification of host mRNA transcripts
Analytes	Same	Two mRNA transcript immune biomarkers: PLA2G7, PLAC8
Result Output	Same	SeptiScore, calculated from the expression levels of the two mRNA analytes PLA2G7, PLAC8. The SeptiScore is placed into four discrete bands that describe a monotonically increasing likelihood of sepsis vs. Systemic Inflammatory Response Syndrome (SIRS).
Controls	Same	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 qRT-PCR step. External controls not provided with the assay but are described in labeling with protocols available from sponsor.
Specimen Processing	Same	Automated extraction of material using the Idylla System
Instrument Platform	Same	Biocartis Idylla System
General Device Characteristic Differences		
Specimen Type	Whole blood collected in a PAXgene Blood RNA Tube, K2-EDTA, or a K3-EDTA tube	Whole blood collected in a PAXgene Blood RNA Tube
Sample Volume	EDTA blood (240µL/cartridge) and PAXgene blood RNA Samples (900µL/cartridge)	PAXgene blood RNA Samples (900µL/cartridge)

VI Standards/Guidance Documents Referenced:

- CLSI. Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures, 1st ed. CLSI guidelines EP35. Wayne, PA: Clinical and Laboratory Standards Institute; 2019.

- CLSI. Interference Testing in Clinical Chemistry, 3st ed. CLSI guidelines EP07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Lot-to-Lot Reproducibility

Lot-to-lot reproducibility of the SeptiCyte RAPID was assessed at a single site with three test lots using replicate EDTA whole blood samples from two healthy donors (**Table 1**). Replicate samples of EDTA whole blood from each donor were prepared. Control samples were prepared by mixing EDTA whole blood with PAXgene stabilizing solution to replicate PAXgene Blood RNA collection. Replicate samples of EDTA whole blood and PAXgene-stabilized blood were tested across three lots of the SeptiCyte RAPID. Acceptance criteria for this study included:

1. <1.0-unit difference in mean SeptiScore between the control PAXgene-stabilized samples and the corresponding EDTA blood samples from the same donor for each of the tested cartridge lots.
2. <1.0-unit difference in mean SeptiScore between the PAXgene-stabilized controls from the same donor tested with the three cartridge lots.
3. <1.0-unit difference in mean SeptiScore between the EDTA blood samples from the same donor tested with the three cartridge lots.

Table 1. Summary of Lot-to-Lot Reproducibility Data

		Subject 1			Subject 2		
		Lot Number			Lot Number		
		1	2	3	1	2	3
EDTA blood	Mean	4.60	4.88	4.83	5.13	5.43	5.30
	SD	0.22	0.21	0.15	0.17	0.17	0.24
	%CV	4.70%	4.23%	3.11%	3.33%	3.15%	4.62%
PAXgene blood	Mean	4.55	4.90	4.65	5.40	5.50	5.40
	SD	0.07	0.28	0.21	0.14	0.00	0.42
	%CV	1.55%	5.77%	4.56%	2.62%	0.00%	7.86%
	Delta Mean	0.05	0.02	0.18	0.27	0.07	0.10

For each subject, the change in SeptiScore among the sample matrices and test lots was less than 1.0 and met the study acceptance criteria. These data are acceptable.

Additional estimates of assay precision were determined by analyzing data generated during specimen stability testing of EDTA whole blood samples from healthy donors (**Table 2**) and are acceptable.

Table 2. Summary of Intermediate Precision Estimates with EDTA Blood Samples

Donor	N	Avg	Repeatability		Between Instrument	
		SeptiScore	SD	CV (%)	SD	CV (%)
1	14	6.24	0.18	2.8%	0.18	2.8%
2	13	5.36	0.34	6.2%	0.07	1.3%

2. Linearity:

The SeptiCyte RAPID test was cleared previously under K203748. Please refer to the published decision summary for additional information. Equivalent performance in whole blood specimens collected in EDTA and PAXgene RNA tubes was evaluated in a method comparison study described below.

3. Analytical Specificity/Interference:

Potentially interfering substances in venous blood samples were evaluated for potential impact on SeptiCyte RAPID test results. Potential interferents were added to pooled EDTA

whole blood from healthy donors at concentrations higher than the maxima of their normal or expected reference ranges. Three replicates of EDTA blood were tested at each concentration of potential interferent and compared to matched EDTA blood controls (containing the appropriate solvent but no interferent). A change in the mean SeptiScore values between EDTA blood samples containing the potential interferent and matched EDTA blood controls (containing the appropriate solvent but no interferent) was evaluated. The acceptance criteria required any difference in mean SeptiScore be less than 1.5 score units when calculated between replicates of each potential interferent, and its respective solvent control replicates. None of the potential interferents evaluated affected the SeptiScore by more than 0.65 score units satisfying the study acceptance criteria (**Table 3**). The results of the interference study are acceptable.

Table 3. Effect of Potential Interferants on SeptiScore Results

Potential Interferent	Mean	SD	N	Delta SeptiScore
Rheumatoid Factor	5.70	0.17	3	0.23
Heparin	5.37	0.12	3	0.10
Imipenem	5.53	0.12	3	0.06
Bilirubin	5.72	0.47	3	0.65
Triglycerides	5.87	0.25	3	0.40
Vancomycin	5.90	0.10	3	0.43
Cefotaxime	5.00	0.20	3	0.47
Dopamine	5.27	0.32	3	0.20
CRP	5.30	0.17	3	0.17
Noradrenaline	5.30	0.10	3	0.17
Dobutamine	5.53	0.25	3	0.06
Hemoglobin	5.63	0.06	3	0.16
Albumin	5.67	0.15	3	0.20
Furosemide	5.33	0.23	3	0.00
sCD14	5.80	0.22	3	0.33
IL-6	5.93	0.15	3	0.46
LBP	5.37	0.15	3	0.14
Solvent Control - Methanol/Blood	5.33	0.12	3	N/A
Solvent Control - Water/Blood	5.53	0.13	3	N/A
Solvent Control - PBS/Blood	5.23	0.12	3	N/A

4. Assay Reportable Range:

The assay reportable range has not been altered from the original clearance. Please refer to the published decision summary for K203748 for additional information.

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

Sample Stability

Sample stability was evaluated using EDTA whole blood collected from two healthy donors. Blood from each donor was collected directly in both a K2-EDTA tube and a PAXgene blood RNA collection tube and transported to an external laboratory for testing. K2-EDTA samples were transported either on ice (0-4°C) or at ambient room temperature. Samples were held on ice or at room temperature and tested at timepoints ranging from 0 hours to 24 hours. PAXgene whole blood samples were tested at 1.2h and 24.7h post-phlebotomy as a control. Acceptance criteria for this study required SeptiScore values for each healthy donor to not differ by more than 1.0 score units among incubation time points.

Results from stability testing of EDTA whole blood samples met the study acceptance criteria since SeptiScore values did not differ by more than 1.0 score units among time points. No appreciable difference in SeptiScore values was observed between samples collected in EDTA whole blood tubes and PAXgene Blood RNA tubes over the evaluated time points. These data support SeptiScore testing within 6 hours after collection as recommended in the instructions for use.

Sample Processing Control

A sample processing control (SPC) is present in each cartridge. The SPC consists of a predefined quantity of inactivated bacteriophage MS2 particles, which are mixed with the sample after injection prior to further processing. The SPC is taken through the entire sample processing path, ultimately generating a PCR curve and Cq value, thereby serving as a positive control for both the extraction process and for the proper general functioning of the RT-qPCR enzymology.

6. Detection Limit:

The limit of detection in EDTA whole blood was determined using serial dilutions of white blood cells (WBC) in platelet-depleted plasma. Twenty sample replicates of WBC at each concentration were tested with SeptiCyte RAPID and the lowest concentration at which 19/20 replicates generated a SeptiScore was reported as the limit of detection. The limit of detection in EDTA whole blood samples using the SeptiCyte RAPID was determined to be 400 cells/µL (**Table 4**). The limit of quantitation, the lowest WBC concentration for which 19/20 replicates generate a SeptiScore with a standard deviation of <1.0 Score units, was also determined to be 400 cells/µL. The results from the limit of detection study are acceptable.

Table 4. Results for Limit of Detection

WBC Concentration (cells/µl)	Invalid Tests (N)	Total Tests (N)	Failure Rate	Result
6000	0	2	0%	Above LoD
4000	0	2	0%	Above LoD
2100	0	2	0%	Above LoD

WBC Concentration (cells/µl)	Invalid Tests (N)	Total Tests (N)	Failure Rate	Result
690	0	2	0%	Above LoD
400	0	22	0%	LoD, LoQ
220	2	2	100%	Below LoD
130	2	2	100%	Below LoD
74	2	2	100%	Below LoD
45	2	2	100%	Below LoD
25	2	2	100%	Below LoD

7. Assay Cut-Off:

   The assay cut-off has not changed from the original clearance. Please refer to the published decision summary for K203748 for additional information.

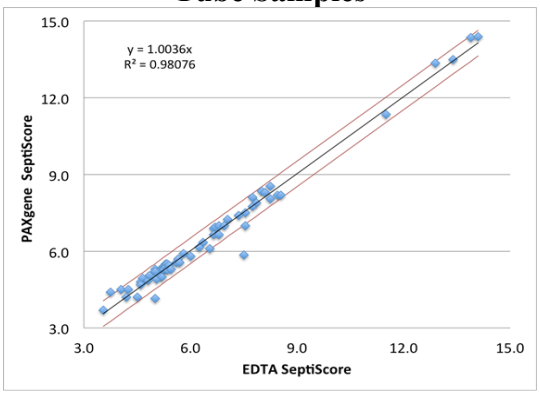
B Comparison Studies:

1. Method Comparison with Predicate Device:
   N/A

2. Matrix Comparison:

   The matrix comparison study was conducted at two US sites and evaluated whole blood collected from a total of 60 patients in matched EDTA or PAXgene blood RNA tubes (1 EDTA blood tube and 1 PAXgene blood RNA tube). Study participants were adults suspected of non-infectious systemic inflammation (i.e., systemic inflammatory response syndrome (SIRS)) or infection-positive systemic inflammation (i.e., sepsis) by the treating physician. Samples were collected at the time of enrollment and processed immediately in duplicate on the SeptiCyte RAPID. SeptiScores of matched samples of blood collected in PAXgene blood RNA tubes and EDTA blood tubes from the same subjects were compared using linear regression analysis. For matched EDTA and PAXgene blood samples from individual patients SeptiScores were within 1.0 score units among replicates resulting in interpretation bands that were either identical or adjacent for both matrices. The method comparison study results support agreement between SeptiScores from matched patient blood samples collected in EDTA and PAXgene tubes (**Table 5**) and are acceptable.

Table 5. Linear Regression Analysis SeptiScore from Matched EDTA and PAXgene Tube Samples



3. K2-EDTA/K3-EDTA Comparison:

Blood samples from 7 donors were drawn into both K2-EDTA and K3-EDTA blood collection tubes. The two EDTA sample types were directly compared by testing 8 K2-EDTA replicates and 7 K3-EDTA replicates from each donor on 15 different Idylla instruments using the EDTA whole blood protocol (**Table 6**). The mean SeptiScore for samples collected in K2-EDTA compared to samples collected in K3-EDTA for each patient did not differ by more than 0.2 score units and support equivalent performance of both sample types on the SeptiCyte RAPID test. The data from this study are acceptable.

Table 6. Results from K2-EDTA and K3-EDTA Comparison Study

Donor	Sample Type	Mean	SD	% CV	Delta Mean
1	K2	5.1	0.20	3.85%	0.2
	K3	4.9	0.24	4.78%	
2	K2	3.7	0.25	6.70%	0.1
	K3	3.6	0.22	6.16%	
3	K2	3.7	0.12	3.18%	0.0
	K3	3.7	0.13	3.54%	
4	K2	3.8	0.22	5.84%	0.1
	K3	3.7	0.21	5.68%	
5	K2	3.2	0.19	5.87%	0.2
	K3	3.3	0.18	5.37%	
6	K2	4.5	0.21	4.67%	0.2
	K3	4.3	0.14	3.23%	
7	K2	5.5	0.16	2.86%	0.1
	K3	5.7	0.14	2.42%	

C Clinical Studies:

1. Clinical Sensitivity:

N/A

2. Clinical Specificity:

N/A

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

N/A

D Clinical Cut-Off:

Clinical cut-offs remain unchanged from the previous clearance. Please refer to the published decision summary for K203748 for additional details.

E Expected Values/Reference Range:

The reference range for the SeptiCyte RAPID test were established in presumably healthy individuals in the original clearance. Please refer to the published decision summary for K203748 for additional details.

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.