



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

I Background Information:

A 510(k) Number

K250912

B Applicant

Cytovale, Inc.

C Proprietary and Established Names

IntelliSep test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QUT	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 modifications to the Cytovale IntelliSep test exterior cartridge housing, inlet filter assembly, and cartridge primary packaging.

B Measurand:

White blood cells in K2 EDTA venous whole blood

C Type of Test:

Semi-quantitative *in vitro* assay that measures deformability cytometry of leukocytes

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Cytovale IntelliSep test is a semi-quantitative test that assesses cellular host response via deformability cytometry of leukocyte biophysical properties and is intended for use in conjunction with clinical assessments and laboratory findings to aid in the early detection of sepsis with organ dysfunction manifesting within the first 3 days after testing. It is indicated for use in adult patients with signs and symptoms of infection who present to the Emergency Department. The test is performed on an EDTA anticoagulated whole blood sample.

The IntelliSep test generates an IntelliSep Index value that falls within one of three discrete interpretation bands based on the probability of sepsis with organ dysfunction manifesting within the first three days after testing. The IntelliSep test represents the probability of the clinical syndrome of sepsis and is intended to be used alongside other clinical information and clinical judgement. It does not identify the causative agent of infection and should not be used as the sole basis to determine the presence of sepsis. The IntelliSep test is intended for *in vitro* diagnostic use.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

The IntelliSep test is intended for use on the Cytovale System instrument.

IV Device/System Characteristics:

A Device Description:

The Cytovale IntelliSep test is an *in vitro* clinical test to aid in the early detection of sepsis with organ dysfunction manifesting within the first three days after testing. It assesses the state of immune activation in patients with clinical suspicion of infection who present in the Emergency Department (ED). The IntelliSep test and Cytovale System instrument were previously cleared in K250513. Refer to the published decision summary for additional information. The purpose of these 510(k) submissions was to validate modifications to the Cytovale IntelliSep test exterior cartridge housing, inlet filter assembly, and cartridge primary packaging.

B Principle of Operation:

Laboratory operators transfer 100 µL of whole blood into a sample preparation tube. The operator inserts the sample preparation tube into the Cytovale System instrument, which automatically lyses erythrocytes and washes purified leukocytes in a diluent producing a total volume of approximately 1mL of prepared sample. The operator transfers the prepared sample to the IntelliSep test cartridge for imaging and analysis on the Cytovale System.

The biophysical properties of individual leukocytes are measured using a microfluidic deformability cytometry technique. Based on these measurements, the test assigns a numerical value, the IntelliSep Index, ranging from 0.1-10.0. Results are stratified into three discrete interpretation bands (Band 1, Band 2, Band 3) of increasing sepsis likelihood.

C Instrument Description Information:

1. Instrument Name:

Cytovale System

2. Specimen Identification:

The IntelliSep test on the Cytovale System is validated for use only with K2 EDTA anticoagulated whole blood.

3. Specimen Sampling and Handling:

The Sample Processing Module of the Cytovale System receives 100 µL of K2 EDTA anticoagulated whole blood in the Sample Preparation Tube and processes the specimen into a white blood cell sample suspended in Cytovale Diluent. The operator injects 1.0 mL of the prepared sample into the IntelliSep test cartridge inlet well and inserts the cartridge into the Cell Imaging Module for analysis. Once the analysis is complete, the Cartridge is disposed of according to institutional guidelines on biohazardous waste.

4. Calibration:

All necessary configuration, qualification, and calibration (where applicable) is performed by a Cytovale technician during installation. Further configuration and calibration procedures are not required by the operator.

Cytovale recommends that the System be serviced every three months of use, based on the initial installation date (or based on the previous service date), or every 3500 tests, whichever comes first.

5. Quality Control:

The IntelliSep test on the Cytovale System has a two-level quality control (i.e., L1 and L2) set called the IntelliSep Quality Control Kit, derived from stabilized whole blood. The kit contains assayed controls with values within the established measuring range. This test-specific Quality Control Kit is tested the same as patient specimens during processing. The mean and range of assay values for a particular lot of the IntelliSep Quality Control Kit (L1 and L2) are reported on the IntelliSep Quality Control Assay Sheet. An Assay Sheet is

provided with each lot of materials. They are lot specific and are derived from replicate analyses on Cytovale Systems.

External controls should be tested in accordance with laboratory practices and accreditation requirements per 42 CFR 493.1256.

V Substantial Equivalence Information:

A Predicate Device Name(s):

IntelliSep test

B Predicate 510(k) Number(s):

K220991

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K250912</u>	<u>K220991</u>
Device Trade Name	IntelliSep test	IntelliSep test
General Device Characteristic Similarities		
Intended Use/Indications For Use	<p>The Cytovale IntelliSep test is a semi-quantitative test that assesses cellular host response via deformability cytometry of leukocyte biophysical properties and is intended for use in conjunction with clinical assessments and laboratory findings to aid in the early detection of sepsis with organ dysfunction manifesting within the first 3 days after testing. It is indicated for use in adult patients with signs and symptoms of infection who present to the Emergency Department. The test is performed on an EDTA anticoagulated whole blood sample.</p> <p>The IntelliSep test generates an IntelliSep Index value that falls within one of three discrete interpretation bands based on the probability of sepsis with organ dysfunction manifesting within the first three days after testing. The IntelliSep test represents the probability of the clinical syndrome of sepsis and is intended to be used alongside other clinical information and clinical judgement. It does not identify the causative agent of</p>	<p>The Cytovale IntelliSep test is a semi-quantitative test that assesses cellular host response via deformability cytometry of leukocyte biophysical properties and is intended for use in conjunction with clinical assessments and laboratory findings to aid in the early detection of sepsis with organ dysfunction manifesting within the first 3 days after testing. It is indicated for use in adult patients with signs and symptoms of infection who present to the Emergency Department. The test is performed on an EDTA anticoagulated whole blood sample.</p> <p>The IntelliSep test generates an IntelliSep Index value that falls within one of three discrete interpretation bands based on the probability of sepsis with organ dysfunction manifesting within the first three days after testing. The IntelliSep test represents the probability of the clinical syndrome of sepsis and is intended to be used alongside other clinical information and clinical judgement. It does not identify the causative</p>

	infection and should not be used as the sole basis to determine the presence of sepsis. The IntelliSep test is intended for in vitro diagnostic use.	agent of infection and should not be used as the sole basis to determine the presence of sepsis. The IntelliSep test is intended for in vitro diagnostic use.
Assay Method	Microfluidic deformability cytometry	same
Analyte	Leukocyte biophysical properties	same
Specimen Type	Human venous whole blood (K2 EDTA)	same
Instrument Platform	Cytovale System	same
Result Output	IntelliSep Index	same
General Device Characteristic Differences		
Filter Material	proprietary synthetic polyamide	nylon
Filter Size	10 µm	25 µm
Cartridge Material	acrylic	cyclo olefin polymer
Cartridge Cover Material	cyclo olefin polymer	acrylic
Packaging	thermoformed sealed pouch	thermoformed clamshell

VI Standards/Guidance Documents Referenced:

- ISO 14971:2019 Medical devices - Application of risk management to medical devices
- ISO TR24971:2013 Medical devices – Guidance on the application of 14971
- ISO 1348:2016 Medical devices – Quality Management Systems – Requirements for regulatory purposes

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Within-Laboratory Precision with Donor Samples

A study was conducted to evaluate the within-laboratory precision for the modified assay cartridges. Specifically, five donor samples were tested in triplicate by two operators on two instruments for two separate runs spaced at least one hour apart for a total of twenty-four tests per donor. For each sample, one lot of IntelliSep test cartridges, one lot of Cytovale Reagent Kit, one lot of Cytovale Diluent, and one lot of Cytovale Cleanse was used.

A total of 123 assays were performed for the study with four run failures encountered (3.3%). Three run failures were clog errors on the same instrument and Band 3 sample combination. One error was caused by a facility power outage. Due to the power outage, no ISI was reported for this assay. The assay was successfully rerun within the study design and time parameters. Table 1 below, summarizes the within-laboratory precision study results.

Table 1: Within-Laboratory Precision Results

Sample	N	Mean	Between-operator (SD)	Between-instrument (SD)	Between-run (SD)	Repeatability (SD)	Within-lab precision (SD)
1	24	6.37	0.00	0.00	0.03	0.22	0.22
2	24	3.84	0.00	0.13	0.10	0.16	0.23
3	24	7.29	0.06	0.18	0.30	0.44	0.56
4	23	7.37	0.00	0.42	0.00	0.49	0.65
5	24	5.53	0.00	0.21	0.00	0.22	0.30
Total	119	-	0.00	0.04	0.09	0.38	0.39

The acceptance criterion for within-laboratory precision is standard deviation ≤ 1.0 units of IntelliSep Index. The study design and results from within-laboratory precision testing are acceptable.

Reproducibility with Quality Control Materials

Since samples must be tested immediately after collection on the IntelliSep test, a site-to-site reproducibility study was conducted with IntelliSep Quality Control materials on the Cytovale System. In summary, five replicates of two Quality Control level materials (Level 1 and 2) were tested over five days at two independent sites for a total of 25 valid test results per control at each site. A single lot of modified IntelliSep test cartridges was used across all test sites and replicates. Table 2 summarizes the reproducibility study results at each site and both sites combined.

Table 2: Reproducibility Results

Sample	Site	Mean ISI	N	SD
QC Level 1	1	3.63	25	0.39
QC Level 2		7.87	25	0.37
QC Level 1	2	3.16	25	0.43
QC Level 2		7.27	25	0.56
QC Level 1	Both Sites	3.4	50	0.47
QC Level 2		7.57	50	0.56

The study acceptance criteria was a reproducibility standard deviation of 1.5 ISI values. The estimated site-to-site reproducibility for the modified cartridges is acceptable.

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

Not applicable.

4. Assay Reportable Range:

The measuring range of the IntelliSep test is between 0.1 and 10.0 units, divided into three interpretation bands: Band 1 (ISI = 0.1-4.9), Band 2 (ISI = 5.0-6.2), and Band 3 (6.3-10.0).

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

There were no changes that affect stability claims or calibration of the device. For additional information, refer to the published decision summary for K220991.

6. Detection Limit:

Not applicable.

7. Assay Cut-Off:

There were no changes to the assay cutoff. Refer to the published decision summary for K220991 for additional information.

8. Accuracy (Instrument):

Not applicable.

9. Carry-Over:

A carry-over study was conducted to support the original clearance of the device. Refer to the published decision summary for K220991 for additional information.

B Comparison Studies:

Clinical validation of the Cytovale IntelliSep test in the intended use population was previously established in K220991. Refer to the published decision summary for additional information. The purpose of these 510(k) submissions is to establish equivalence between the modified Cytovale IntelliSep test cartridge and the originally cleared predicate device.

1. Method Comparison with Predicate Device:

A method comparison study was performed with 109 donor samples, of which 51 were adjudicated septic and 57 were adjudicated as not septic. For each donor sample, two replicates were performed on one cleared predicate cartridge lot and one modified cartridge lot for a total of four tests per donor and 432 total valid tests.

Weighted Deming regression analysis was performed on the dataset to assess the equivalence between the two cartridges. Regression analysis was used to calculate systematic bias at the two medical decision boundaries, corresponding to $X=5.0$ ISI and $X=6.2$ ISI. Equivalence was assessed at each boundary using two one-sided tests conducted at $\alpha = 0.05$. Results from this study are summarized in Table 3 and Table 4 below.

The acceptance criterion for this study was that equivalence tests pass at the 95% confidence level for each medical decision point. Table 3 below summarizes the Deming regression results.

Table 3: Medical decision boundary assessment with 95% Confidence Interval for weighted Deming regression using the entire data set.

Medical Decision Boundary	Regression Value [95% CI] (test cartridge ISI)	Acceptance Bounds
5.0	5.3 [5.2, 5.5]	[4.5, 5.5]
6.2	6.5 [6.4, 6.6]	[5.7, 6.7]

Table 4 summarizes the comparison between the cleared predicate device results and the modified IntelliSep test results by bin including overall agreement.

Table 4: Modified IntelliSep test Bin Results – Comparison to the Predicate Device

Modified IntelliSep test	Cleared Predicate Device		
	Bin 1	Bin 2	Bin 3
Bin 1	20	0	0
Bin 2	5	26	0
Bin 3	0	8	49
Agreement	80.00%	76.47%	100.00%
	(20/25)	(26/34)	(49/49)

No clinical samples evaluated shifted to a non-adjacent reporting bin when measured by the modified IntelliSep test. Cumulatively, the data summarized establishes equivalent performance between the modified IntelliSep test and the cleared predicate device.

Clinical data and adjudication results were collected from the patient Electronic Health Record (EHR). A structured adjudication process was used to classify the presence or absence of sepsis by Sepsis-3 criteria: To be considered positive for Sepsis-3, patients must have each of three components: (1) infection (present on presentation to the ED), (2) organ dysfunction (manifesting within 3 days of the ED visit), and (3) organ dysfunction caused by a dysregulated host response to the infection.

The Site Principal Investigator, with access to the medical records but blinded to study ISI values, performed a retrospective clinical adjudication by completing a structured adjudication process in the REDCap system, recording pertinent clinical information about the determination. Table 5 summarizes the method comparison study results when comparing IntelliSep test modified cartridge results to a physician adjudication of sepsis.

Table 5: Modified IntelliSep test – Comparison to Sepsis Adjudication

IntelliSep Result (Modified Cartridge)	Adjudicated Septic (+)	Adjudicated Not-Septic (-)	Sepsis Predictive Value (80% CI)	Sepsis Likelihood Ratio
Band 1	1	19	5.0% (0.5%, 18.1%)	0.06
Band 2	13	18	41.9% (29.7%, 55.0%)	0.81
Band 3	37	20	64.9% (55.6%, 73.4%)	2.07
			Sepsis Prevalence	
Total	51	57	47.20%	N/A

Table 6 summarizes the method comparison study results when comparing predicate device results to a physician adjudication of sepsis.

Table 6: IntelliSep Tests Original and Modified Cartridge - Sepsis Predictive Values and Sepsis Likelihood Ratios

	Modified Cartridge		Original Cartridge	
IntelliSep Result	Sepsis Predictive Value (80% CI)	Sepsis Likelihood Ratio	Sepsis Predictive Value (80% CI)	Sepsis Likelihood Ratio
Band 1	5.0% (0.5%, 18.1%)	0.06	8.0% (2.1%, 19.9%)	0.10
Band 2	41.9% (29.7%, 55.0%)	0.81	47.1% (35.0%, 59.4%)	0.99
Band 3	64.9% (55.6%, 73.4%)	2.07	67.3% (57.3%, 76.3%)	2.07

The predictive value of the ISI band results for identifying patients with sepsis in the modified assay cartridge increased with increasing score value with non-overlapping confidence intervals for non-adjacent bands. The results for the modified assay cartridge were also similar to results obtained from the original unmodified version. These data support equivalent performance of the modified IntelliSep test cartridge.

2. Matrix Comparison:

Not applicable.

C Clinical Studies:

1. Clinical Sensitivity:

The clinical performance in the intended use population was established in the original 510(k) for the device. Refer to the published decision summary for K220991 for additional information.

2. Clinical Specificity:

The clinical performance in the intended use population was established in the original 510(k) for the device. Refer to the published decision summary for K220991 for additional information.

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

Not applicable.

D Clinical Cut-Off:

There were no changes to the assay cut-offs or bin interpretations. Refer to the published decision summary for K220991 for additional information.

E Expected Values/Reference Range:

The expected values and reference ranges for the assay were established in the original 510(k) submission for the device. Refer to the published decision summary for K220991 for additional information.

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.