

**PMA Module # M000023/PMA**

**Section 16 - Summary of Safety and Effectiveness**

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16.1 General Information

16.1.1 Device Generic Name: *In vitro* reagent system for a rapid assay for Endotoxin Activity in human whole blood samples.

16.1.2 Device Trade Name: Endotoxin Activity Assay

16.1.3 PMA Submitter's Name and Address: Sepsis, Inc.  
135-9 The West Mall  
Toronto, Ontario, M9C 1 C2, Canada

16.1.4 Manufacturing Site Name and Address: Spectral Diagnostics, Inc.  
135-9 The West Mall  
Toronto, Ontario, M9C 1C2, Canada

16.2 Indications for Use

The Endotoxin Activity Assay is a rapid *in vitro* diagnostic test that utilizes a specific **monoclonal** antibody to measure the endotoxin activity in EDTA whole blood specimens. When used in conjunction with microbial cultures and other relevant diagnostic tests (i.e. ultrasound, bronchoscopy or CAT scan) the test is indicated for use in ruling out the presence of Gram negative bacterial infections. The Endotoxin Activity Assay is intended for patients admitted to the ICU at risk of or suspected of having an infection.

16.3 Device Description

The Endotoxin Activity Assay is a rapid manual **chemiluminescent** immunodiagnostic test kit that contains labeled glass tubes containing specific reagents and a container of liquid substrate. A qualified technologist is required to add a measured volume from the patient's whole blood sample into certain tubes. Several pipetting and incubation steps are required for the sample/reagent reactions to occur. The production of light results from the reagent interactions with the patient's sample, and is proportional to the relative Concentration of endotoxin in the sample. The measurement of the light emitted requires the use of a luminometer.

16.3.1 Components

A single Endotoxin Activity Assay uses five 12x75mm borosilicate glass test tubes containing specific dry reagents and a supply of the liquid substrate.

These five tubes are divided into two sets.

**Set 1.** Two Blood Sample Preparatory Tubes:

- 1) Aliquot Tube: An empty 12 x 75 mm glass tube.
- 2) LPS Max Tube: This tube contains a single lyophilized bead of LPS (endotoxin) from *E.coli 055:B5* and inert buffers and sugars.

**set 2.** Three Signal Generating Assay Tubes:

- 3) Tube 1: Two lyophilized beads composed of inert buffers and sugars.
- 4) Tube 2: Two lyophilized beads of a specific murine IgM monoclonal antibody against Gram negative LPS and inert buffers and sugars.
- 5) Tube 3: Two lyophilized beads of a specific murine IgM monoclonal antibody against Gram negative LPS and inert buffers and sugars.

1 bottle of Liquid. Substrate

The liquid Luminol substrate (5-amino-2,3-dihydro-1,4-phthalazinedione, free acid) contains yeast zymosan A and heparin,

Negative and Positive Control assays are provided with each kit. The Negative Control assay contains five 12x75mm borosilicate glass tubes containing specific dry reagents. The Positive Control assay contains six 12x75mm borosilicate glass tubes containing specific dry reagents. The same liquid substrate from the assay reagents is used for the control assays. The control assays each contain one tube that is different than those contained in the patient assay.

Negative Control assay:

Negative Control Tube: This tube contains two lyophilized beads of a non-specific mouse monoclonal antibody and inert buffers and sugars. This tube is used in place of the signal generating Tube 2.

Positive Control assay:

Positive Control Tube: This tube contains a single lyophilized bead containing endotoxin from *E.coli 055:B5* and inert buffers and sugars. This tube is an additional sample preparatory tube.

### 16.3.2 Principles of the Test

The assay measures the endotoxin activity in whole blood by the priming of host neutrophil respiratory burst activity via complement opsonized LPS-IgM immune complexes. The luminol reaction in the presence of immune complexes emits light

energy. This light energy is measured and recorded by a luminometer. The Relative Light Units (RLU) measured by the instrument are converted by calculation into an Endotoxin Activity (EAA) value which are expressed as decimals from 0.00 – 1.00.

### 16.3.3 Performance of the Test - Patient Assay

#### 16.3.3.1 Sample Collection and Storage

The assay is performed on whole blood, which is collected aseptically, by venipuncture or by indwelling catheter into sterile EDTA anti-coagulated tubes. The tubes containing the whole blood can either be stored on ice for up to three (3) hours or at room temperature for one and one half (1.5) hours prior to the initiation of the test.

#### 16.3.3.2 Sample Preparation

If the patient blood sample is stored on ice prior to the assay, a 15-minute incubation of the Aliquot Tube is required.

- After mixing the patient blood sample by gentle inversion, pipette 1ml into the Aliquot Tube and place in a heating block set at 37°C for a 15-minute incubation.
- At the completion of the incubation, vortex the Aliquot Tube and pipette 0.5mL into the LPS MAX Tube.

If the patient blood sample is stored at room temperature, the 15-minute incubation step is omitted.

#### 16.3.3.3 Test Procedure

- Vortex both the LPS MAX Tube and the Aliquot Tube and place in a heating block set at 37°C for a ten-minute incubation.
- With five minutes remaining in the ten-minute incubation, insert Tubes 1, 2 and 3 into heating block, mix the Liquid Substrate bottle vigorously (minimum 15 seconds) and pipette 1ml of the solution into Tubes 1, 2 and 3.
- After the ten-minute incubation is complete, vortex then pipette 40 µl of blood from the Aliquot Tube into Tubes 1 and 2.
- Vortex then pipette 40 µl of blood from the LPS MAX Tube into Tube 3.

#### 16.3.3.4 Measurement

- Vortex all three signal generating assay tubes and place in the chemiluminometer carousel starting with Tube 1, followed by Tube 2, then Tube 3.

The assay type is chosen and a workload is created. A spreadsheet is generated upon initiation of the run and displays the Relative Light Units per second (RLU/sec) values over time for each tube. Accessory calculation software is provided to calculate the EAA values for the assay. The results can be printed on a data page.

#### 16.3.4 Performance of the Test - Quality Control Assays

Quality Control assay reagents are provided with each kit and are intended to be used according to each laboratory's standard practice for quality control testing. The Quality Control assays should be used in conjunction with the Patient assay to determine if the operator has performed the assay procedure correctly.

The Quality Control assays utilize the same blood sample collected and stored for use in the Patient assay. One blood **sample** is therefore shared between the Patient assay and the Quality Control assay.

#### 16.4 Contraindications, Warnings and Precautions

There are no known contraindications for the Endotoxin Activity Assay.

Warnings and Precautions for use of the Endotoxin Activity Assay are stated in the respective product labeling.

#### 16.5 Alternate Practices and Procedures

Alternate methods for ruling out Gram negative infections have been described in the literature including physiologic measures such as the absence of a fever or normal white cell counts and diagnostic procedures such as bacterial cultures, ultrasound and/or bronchoscopy.

#### 16.6 Marketing History

The Endotoxin Activity Assay has not been marketed in the United States or any other country.

#### 16.7 Potential Adverse Effects on Health

A false positive endotoxin level could result in the physician administering antibiotic therapy when an infection is not present. The unnecessary use of antibiotics could increase the risk of the antibiotic resistance. A false negative endotoxin level could result in the physician withholding appropriate antibiotic therapy when an infection is present.

Because the Endotoxin Activity Assay is intended to be used in conjunction with diagnostic cultures and other diagnostic procedures to rule out the presence of Gram negative infections the risk of an adverse effect on health is no greater than the use of the adjunct diagnostic method.

## 16.8 Summary of Studies

### 16.8.1 Summary of Non-Clinical Studies

The performance characteristics of the Endotoxin Activity Assay (EAA) were evaluated by Sepsis, Inc. Product testing, analytical studies and useful life determinations were performed to establish the non-clinical characteristics of the assay. Product testing included hazard analysis, reliability and software testing. Analytical studies were performed to determine and verify the precision, specificity, sensitivity, potential interfering substances, analytical cut-off value, linearity and cross reactivity characteristics of the assay. Useful life determinations have been initiated to establish the shelf life of the assay reagents and to study the stability of human whole blood samples.

#### 16.8.1.1 Product Testing

##### 16.8.1.1.1 Hazard Analysis

Two risk analyses have been done during the development of this project. The first is a risk analysis of the EAA manufacturing processes. The second is a risk analysis for the test itself including the instrument and software. The company believes all plausible risks have been identified and appropriate controls have also been identified to mitigate these risks.

##### 16.8.1.1.2 Reliability

The data from reagent stability testing has verified that the product is reliable when stored and handled according to the recommended storage conditions. Final product labeling will indicate the appropriate storage conditions.

##### 16.8.1.1.3 Software Testing

The software validation protocol examined software performance from instrument startup to the generation of assay results and calculations. The validation protocol is divided into five sections encompassing the following areas:

- Section 1 – Startup and Shutdown
- Section 2 – Support Functions
- Section 3 – Assay and Measurement Setup
- Section 4 – Prepare Workload
- Section 5 – Reports and Assay Calculations

Each section is broken down into a series of testing steps. If a test step does not provide the expected results as defined, it is a failure. However, if the step is not the central functionality being tested and does not effect the safety and effectiveness of the device the step may be marked as failed and the test section marked as passed. All sections were tested and marked as passed.

## 16.8.1.2 Analytical Studies

### 16.8.1.2.1 Precision

The within-run, between-run and total precision of the EAA test was studied in a single multivariate experiment. Three independent operators using separate pipettes, heating blocks and chemiluminometers tested precision with each of three lots of reagents simultaneously. For each lot of reagents, a whole blood sample was tested both neat and spiked with 800 pg/ml of exogenous lipopolysaccharide (*E.coli 055:B5*). The use of neat and spiked blood samples permitted evaluation of the assay at the low (clinically negative) and high (clinically positive) EAA levels. Due to the dependence of the EAA on viable neutrophils and the consequent limited stability of blood samples, the potential for day to day variation within an individual blood donor, and the limited number of determinations allowed per run, the experimental designs used to assess precision were modifications of those suggested by the NCCLS in EPS-A. A summary of the precision results is presented in Table 16-1.

Between-lot variability was assessed by comparing three lots of reagents in a series of three experiments utilizing blood spiked with 1000 pg/ml of exogenous lipopolysaccharide (*E. coli 055:B5*).

Underlying instrument-to-instrument precision was assessed independently of the EAA. Stable luminescent standards were developed to mimic the luminescent activity typical of the EAA method and the precision of EAA values from analysis of these standards in five different instruments was determined.

#### 16.8.1.2.1.1 Within-Run Precision

Within-run precision was determined by analysis of the experimental results according to modifications of the NCCLS methods (EP5-A, Cl. 1.2). The results demonstrate that individual estimates of within-run precision for all three reagent lots tested exhibited CV% less than or equal to 15%. The EAA demonstrates acceptable within-run precision for a test of this type (Table 16-1).

#### 16.8.1.2.1.2 Total Precision

Total precision was similarly determined by analysis of the experimental results according to modifications of the NCCLS methods (EPS-A, C1.2). The total precision around the expected cut-off of 0.40 EAA in these studies incorporating three lots of reagents and three operators was less than 15% (Table 16-1) and is acceptable for a test of this type.

### 16.8.1.2.1.3 Between-Run Precision

Between-run precision was determined by analysis of the experimental results following modifications of the NCCLS methods (EP5-A, C2.3). Between-run precision was appreciable (16%) only at the lowest EAA levels tested (EAA = 0.11). At all other levels of EAA the between-run imprecision was small relative to the within-run precision, undetectable in four of five cases (Table 16-1). The EAA method demonstrates very little between-run variability and is acceptable for a test of this type.

**Table 16-1 Summary of Precision Studies**

E M Reagent Lot	Exogenous LPS Number (ng/ml)	N	M e a n EAA	Within-Run	Within-Run	Total	Total	Between-Run	Between-Run
				Std Dev	Precision (%CV)	Std Dev	Precision (%CV)	Std Dev	Precision (%CV)
1	0	24	0.11	0.015	14	0.023	22	0.017	16
1	800	24	0.52	0.036	7	0.034	6	0	0
2	0	24	0.20	0.030	15	0.029	14	0	0
2	800	24	0.50	0.059	12	0.064	13	0.031	6
3	0	24	0.30	0.043	15	0.042	14	0	0
3	800	24	0.59	0.050	8	0.046	8	0	0

### 16.8.1.2.1.4 Between-Lot Variability

In a series of three separate experiments, three different reagent lots were compared and EAA's measured using a whole blood sample spiked with 1000  $\mu\text{g}/\text{ml}$  of exogenous LPS. The data was evaluated using unpaired two-sided t-tests. Statistical analysis of the data indicated that the three lots generated EAA's that were equivalent. A summary of the data is presented in Table 16-2.

**Table 16-2 Summary of Between-Lot Variability**

	Experiment 1		Experiment 2		Experiment 3	
	Reagent Lot 1	Reagent Lot 2	Reagent Lot 1	Reagent Lot 3	Reagent Lot 2	Reagent Lot 3
	EAA's (N=7)	EAA's (N=7)	EAA's (N=7)	EAA's (N=7)	EAA's (N=7)	EAA's (N=7)
Mean EM	0.72	0.70	0.76	0.77	0.69	0.66
Std Dev	0.053	0.048	0.080	0.054	0.082	0.050
c v	7%	7%	11%	7%	12%	8%
	t calc	0.842	t calc	0.235	t calc	0.909
	T critical 95%	1.782	T critical 95%	1.782	T critical 95%	1.782
	interpret	not different	interpret	not different	interpret	not different
	P	0.416	P	0.818	P	0.381

### 16.8.1.2.1.5 Multi-Instrument Variability

In order to isolate and measure the variability of the EAA attributable to the LB 953 chemiluminometer, three sets of tritium standards were prepared. Each set is composed of three tubes containing tritiated glycerol at levels that would mimic the light emission from the three “signal generating” tubes of the EAA. The tubes were prepared to generate an EAA between 0.25-0.45 which approximates a sample near the cut-off range of the EAA. Five LB 953 chemiluminometers were then tested with these tritium standards under normal assay times and temperatures. The maximum amount of variability of the EAA attributable to instrument effects is in the range of 1.3% to 2.5% (Table 16-3) and is considered acceptable for an instrument of this type.

**Table 16-3 Tritium Standards - Multi-Instrument Variability**

Tritium Std	EAA's					SUM	Mean	Std Dev	CV
	Machine 1 (N=1)	Machine 2 (N=1)	Machine 3 (N=1)	Machine 4 (N=1)	Machine 5 (N=1)				
Set 1	0.34	0.33	0.34	0.34	0.34	1.7	0.34	0.004	1.3%
Set 2	0.34	0.34	0.34	0.33	0.34	1.7	0.34	0.004	1.3%
Set 3	0.34	0.32	0.33	0.33	0.34	1.7	0.33	0.008	2.5%

### 16.8.1.2.2 Analytical Specificity

Specificity studies were performed on purified lipoteichoic acid (LTA) from Gram positive bacteria, Gram positive cell wall extract and purified mannan from yeast.

#### 16.8.1.2.2.1 Gram Positive Bacteria Lipoteichoic Acid

LTA is a **highly conserved** bacterial cell wall component that has been implicated in inducing sepsis in patients infected with Gram positive bacteria. Purified LTA from two strains of Gram positive bacteria (*S. aureus* and *S. pyogenes*) were tested for reactivity in the EAA. LTA was diluted to a final working concentration of 2000 **pg/ml** in whole blood and compared to the same whole blood sample containing no exogenous LTA. Unpaired, two-sided t-tests were used to determine whether the two data sets were statistically different. Statistical analysis of the data indicates that addition of 2000 **pg/ml** of LTA from Gram positive bacteria did not increase EAA values compared to the control blood.

#### 16.8.1.2.2.2 Gram Positive Bacteria Cell Wall Extract

To ensure that the EAA does not react with other cell wall antigens from Gram positive bacteria, a pooled cell wall extract purified from *S. aureus*, *S. faecalis*, *S. pyogenes*, *L. monocytogenes*, *S. sanguis*, *P. strep*, *S. pneumoniae*, *S. epidermidis*, and *B. subtilis* was tested for cross reactivity in the EAA. The cell wall extract was diluted to a final

concentration of 2000 pg/ml in whole blood and tested in the EAA. Results were compared to the same blood sample containing no exogenous cell wall extract. Unpaired, two-sided t-tests were used to determine whether the two data sets were statistically different. The addition of 2000 pg/ml of Gram positive cell wall extract did not increase the EAA values compared to the control blood.

#### 16.8.1.2.2.3 Yeast Mannan

Many fungi, especially the yeasts, have soluble peptido-mannans as a component of their outer cell wall in a matrix of  $\alpha$ - and  $\beta$ -glucans. The EAA was tested for reactivity to purified yeast mannan. Purified mannan (from *S. cerevisiae*) was diluted to a final concentration of 2000 pg/ml in whole blood and tested for reactivity in the EAA. The results were compared to the same whole blood sample containing no exogenous mannan. Unpaired, two-sided t-tests were used to determine whether the two data sets were statistically different. There was no statistical difference between the two data sets indicating mannan spiked at 2000 pg/ml in a whole blood sample did not increase EAA values compared to the control blood.

#### 16.8.1.2.3 Analytical Accuracy

The EAA measures the degree of stimulation of a patient's neutrophils under certain controlled conditions designed to indicate the endotoxin activity in whole blood. Presently there is no known test against which this test can be compared. Further, the endotoxin activity in any given individual's blood is a result of not only the concentration of endotoxin but the individual's cellular and humoral responsiveness to endotoxin-antibody immune complexes. There is therefore no known test of analytical accuracy,

#### 16.8.1.2.4 Analytical Sensitivity

Sensitivity for an assay with a quantitative readout may be established by analyzing repeat determinations of the zero calibrator, calculating the mean and adding two standard deviations. Alternatively the analytical sensitivity can be estimated by serially diluting a sample with a known amount of analyte until the sample no longer renders a positive result. The lowest concentration, which is statistically different from "zero", is reported as the sensitivity. These scenarios cannot be achieved with the EAA, therefore the analytical sensitivity for the EAA was estimated from the precision observed on a sample with a low EAA level. The sensitivity was calculated based on extrapolation of the observed standard deviation in a low level sample to a "zero" EAA level. Based on the calculation presented for the total precision of the EAA the overall EAA mean was 0.11 with a standard deviation of 0.023 EAA units. The analytical sensitivity can be estimated by applying the standard deviation to a hypothetical measurement of "zero" EAA where the analytical sensitivity is equal to 2 times the SD or 0.046 EAA units.

#### 16.8.1.2.5 Interfering Substances

Interference testing was performed to evaluate the effects of triglycerides, hemoglobin, bilirubin, human serum albumin, steroids and immunosuppressants, and blood components on the Endotoxin Activity Assay. Where appropriate, interference studies were performed following NCCLS protocols described in EP7-P.

##### 16.8.1.2.5.1 Triglycerides

For studies on the effects of lipemia and turbidity, whole blood samples were analyzed neat and with a parallel spike of the sample with 5% and 10% v/v Intralipid at triglyceride levels of 750 and 1500 mg/dL, respectively (8.5 and 17 mM triglyceride in SI units). Samples were assayed without exogenous LPS and with LPS spiked from 400 pg/ml to 1000 pg/ml. Unpaired, two-sided t-tests were used to determine whether the addition of triglycerides to a blood sample generated EAA's statistically different from the control blood. Overall no inhibition of baseline signal was detected up to triglyceride levels of 1500 mg/dL, which is approximately five times the upper limit of the normal range. Intralipid at a final concentration of 10% showed elevated EAA values above the control when blood was supplemented with 1000 pg/ml of LPS. This enhancement was not evident in a second experiment where blood was supplemented with LPS.

Overall, triglycerides at a final concentration of 750 mg/dL (5% Intralipid) did not interfere with baseline blood samples containing no exogenous LPS or with blood samples containing exogenous LPS. Additional experiments are being performed to determine if triglycerides at higher concentrations affect the EAA.

##### 16.8.1.2.5.2 Hemoglobin

For studies on the effects of hemoglobin, the donor's own erythrocytes were lysed to produce a source of hemoglobin. The donor's blood was then supplemented with two doses of hemoglobin at either 1.0 g/L or 5.0 g/L. The samples were then analyzed with no exogenous LPS and a 400 pg/ml LPS spike. Unpaired, two-sided t-tests were used to determine whether the addition of high levels of hemoglobin to a blood sample generated EAA's statistically different from the control. Hemoglobin at levels of 1.0 g/L did not interfere with the EAA test. Hemoglobin at a final concentration of 5.0 g/L interfered with assay performance by lowering EAA values. Hemoglobin levels between 1.0 g/L and 5.0 g/L are being investigated to further define the concentration at which interference occurs.

##### 16.8.1.2.5.3 Bilirubin

Bilirubin (unconjugated) was dissolved at concentrations of 10 and 20 mg/dL of blood. In order to accomplish this the bilirubin was initially dissolved in endotoxin free DMSO at a concentration of 30 mg/ml and then added to blood to yield the requisite final bilirubin concentrations. The control consisted of an equivalent volume of DMSO added to blood alone. Blood samples were analyzed with no exogenous LPS and a 400 – 500

pg/ml spike. Unpaired, two-sided t-tests were used to determine whether the addition of bilirubin interfered with the EAA. Bilirubin concentrations of 10 and 20 mg/dL did not interfere with the measurement of EAA's in neat whole blood samples or in samples containing exogenous doses of LPS (400-500 pg/ml).

#### 16.8.1.2.5.4 Human Serum Albumin

For these studies it was imperative to find a source of albumin that was endotoxin free. Medical grade albumin for human parenteral use was the only commercial grade albumin, which was found to be essentially endotoxin free. All other commercial sources of albumin, which when tested, were found to be contaminated with endotoxin and gave large increases in endotoxin activity when added to whole blood.

The effects of albumin supplementation to whole blood were conducted at additions of 10, 20 and 30 mg/ml of albumin to whole blood above the ambient albumin concentration (generally 4-5 mg/ml). Blood samples were analyzed with no exogenous LPS and a 500 pg/ml spike. Unpaired, two-sided t-tests were used to determine whether the addition of high levels of albumin interfered with the EAA. Albumin is known to bind endotoxin at its fatty acid binding site via lipid A and acts as a low affinity compartment for some of the endotoxin in whole blood. As tested, human serum albumin at concentrations from 10-30 mg/ml influenced the measurement of LPS in a dose dependent manner. However, albumin interaction with endotoxin reduces its toxicity in vivo and therefore lower EAA levels are appropriate.

#### 16.8.1.2.5.5 Steroids and Immunosuppressants

The EAA, being dependent on a patient's endogenous neutrophil activity, will potentially be affected by drugs that are designed to suppress immune activity. Drugs that suppress neutrophil activity such as corticosteroids will render these patients as non-responders. Patients receiving high doses of the steroid Solu-cortef (50-100 mg) presenting to the ICU failed to respond to LPS-antibody complexes. In this situation the signal generated in Tube 1 and the signal generated in Tube 3 overlap. *In vitro* studies using hydrocortisone added to whole blood samples at a final concentration of 20 µg/ml confirmed the suppressive effect of signal generation. These patients are considered non-responders and the EAA records this as a non-test. Although safety and effectiveness is not compromised as no test result is generated, labeling will indicate that immunosuppressive drugs may result in a non-test.

Similarly drugs used to suppress the immune system in order to prevent host transplant rejection are additional potential interferents. Cyclosporin at clinical doses achieved in liver and kidney transplant patients had no effect on assay responsiveness and patients were able to generate signal in response to the presence of LPS-Antibody complexes. This is consistent with the cell type targeted by cyclosporin, which are T-cells of the adaptive immune system rather than neutrophils of the innate immune response.

#### 16.8.1.2.5.6 Blood Components

The EAA first published in 1998 describes the dependence of the EAA on neutrophil and erythrocyte concentration. Neutrophil counts varying from 0.5 to 20.0 x 10<sup>9</sup> cells/L had no effect on patient response values across a range of LPS concentrations (0 to 800 pg/ml). Similarly varying erythrocyte concentrations as measured by final hemoglobin concentrations of 0, 70 and 140 g/L had no effect on patient response values across a range of LPS concentrations (0 to 800 pg/ml).

Endotoxin levels have been successfully measured in patients receiving standard chemotherapeutic regimens (cyclophosphamide/methotrexate/5-fluoro-uracil, cyclophosphamide/epirubicin/5-fluoro-uracil). These agents typically induce neutropenia however they do not reduce neutrophil responsiveness.

#### 16.8.1.2.6 ROC Analysis and Linearity

##### 16.8.1.2.6.1 ROC Analysis

In order to affirm an appropriate analytical cut-off for the EAA to allow rule-out of Gram negative infection in patients suspected of infection, a pilot study was conducted. For the ROC analysis, patients with suspicion of infection had blood cultures drawn within eight hours of the first endotoxin assay and all culture results drawn from any body site on the same calendar day were recorded. Infection status on the first day of the study was compared to endotoxin activity. A total of 64 patients were evaluated, 11 of whom had Gram negative infections as defined in the clinical study protocol. All EAA's were performed in duplicate and the diagnostic parameters for a single, duplicate and average EAA value were tabulated. Based on the results obtained with an average EAA determination, a cut-off value of 0.40 was proposed.

##### 16.8.1.2.6.2 Linearity

Because of the nature of the EAA, neither the response of the test to varying amounts of blood or to increments of exogenous endotoxin are expected to be linear. Therefore the typical linearity studies, which offer support for the analytical accuracy of more traditional methods, are not applicable in evaluating the Endotoxin Activity Assay.

##### 16.8.1.2.7 Cross Reactivity

Purified LPS from eight different strains of Gram negative bacteria were tested in the EAA. LPS was diluted to a final working concentration of 100 pg/ml in whole blood and compared to the same blood sample without LPS. Unpaired, two-sided t-tests were used to determine whether the spiked blood samples generated elevated EAA's that were statistically different than basal EAA levels. Seven of the eight strains (*E. coli* 055, *P. aeruginosa*, *K. pneumonia*, *S. enteridis*, *E. coli* 0127:B8, *S. marcescans* and *S. flexneri*) tested generated EAA's that under statistical analysis were significantly higher than the control blood. This indicates positive recognition of each strain of LPS. *V. cholerae*

generated EAA values that were statistically lower than the control blood. The inhibitory effect of *V. cholerae* LPS on endotoxin activity is likely due to contaminating additives in the commercial LPS preparation which inhibit neutrophil oxidative burst activity. Further investigations are underway to discover the source of the inhibition.

### 16.8.1.3 Useful Life

#### 16.8.1.3.1 Reagent Kit Shelf Life

After 3 months of studies of the freeze dried kit reagents stored at 4°C, 20-25°C (RT), and 37°C, no significant drop in activity has been observed. Accelerated stability studies suggest that longer kit stability may be achieved. Therefore ongoing stability studies will be conducted and reviewed and stability claims will be extended if the real-time study data supports such claims.

The performance of frozen (-20°C) and refrigerated (4°C) liquid luminol substrate has shown no significant drop in activity after 3 months. Substrate stored at room temperature has shown a drop in activity (15-20%) after 3 months. Substrate stored at 37°C showed a drop in activity of 50% after 4 weeks. The stability studies at -20°C and 4°C are being continued to determine the real-time shelf life at these conditions.

#### 16X1.3.2 Blood Sample Stability

Endotoxin Activity Assay whole blood clinical specimen stability was evaluated at two storage conditions. It has been established in early studies that whole blood samples, when stored at room temperature (25°C), generated consistent EAA values across a 90 minute time period. In order to increase the time in which blood samples can be evaluated for EAA's, a study was initiated investigating the effect of blood samples stored on ice (2-8°C). Using a modified incubation protocol where each blood sample was pre-warmed for a period of 15 minutes after storage on ice, it was found that consistent EAA's could be generated for both normal and spiked blood samples (100 – 400 pg/ml LPS) for up to three hours. Therefore product labeling will indicate two possible storage conditions and protocols for each condition.

### 16.8.2 Summary of Clinical Studies

#### 16.8.2.1 Pilot Study

A pilot study for the pivotal trial was performed at three investigative sites over a 6-week period, from November 1, 1999 to December 16, 1999. The purpose of the pilot was three-fold. The first goal was to select an appropriate cut-off for the EAA which effectively ruled out Gram negative infection in patients with suspicion of infection. The second aim was to affirm the established sample size calculations outlined in the clinical protocol. Lastly, the pilot study would provide an opportunity to functionally test the adapted CDC infection outcome definitions as stated in the clinical protocol.

Several conclusions were drawn from the pilot study. First, a cut-off value of 0.40 for the EAA would be used in the pivotal trial. Second, the sample size, based on the proportion of true negative results achieved in the pilot study, substantiated the original estimates outlined in the clinical protocol. Lastly, the culture reports from evaluated patients required clinical interpretation. In order to maximize infection outcome accuracy, a Clinical Evaluation Committee would be established to independently review and evaluate culture reports and determine the patient's infection status.

#### 16.8.2.2 Pivotal Study

The MEDIC (Multi-Centre Endotoxin **D**etection **I**n Critical Illness) trial was conducted to evaluate the Endotoxin Activity Assay (EAA) in conjunction with cultures and other relevant diagnostic and clinical information for assessing Gram negative infection in critically ill patients.

##### 16.8.2.2.1 Study Objective

The primary objective was to determine whether the use of a rapid assay for endotoxin can reliably exclude the diagnosis of Gram negative infection in critically ill patients with suspected infection admitted to the Intensive Care Unit (ICU).

Referred to as the Longitudinal phase of the study in the clinical protocol, this group comprises the target population of the clinical study.

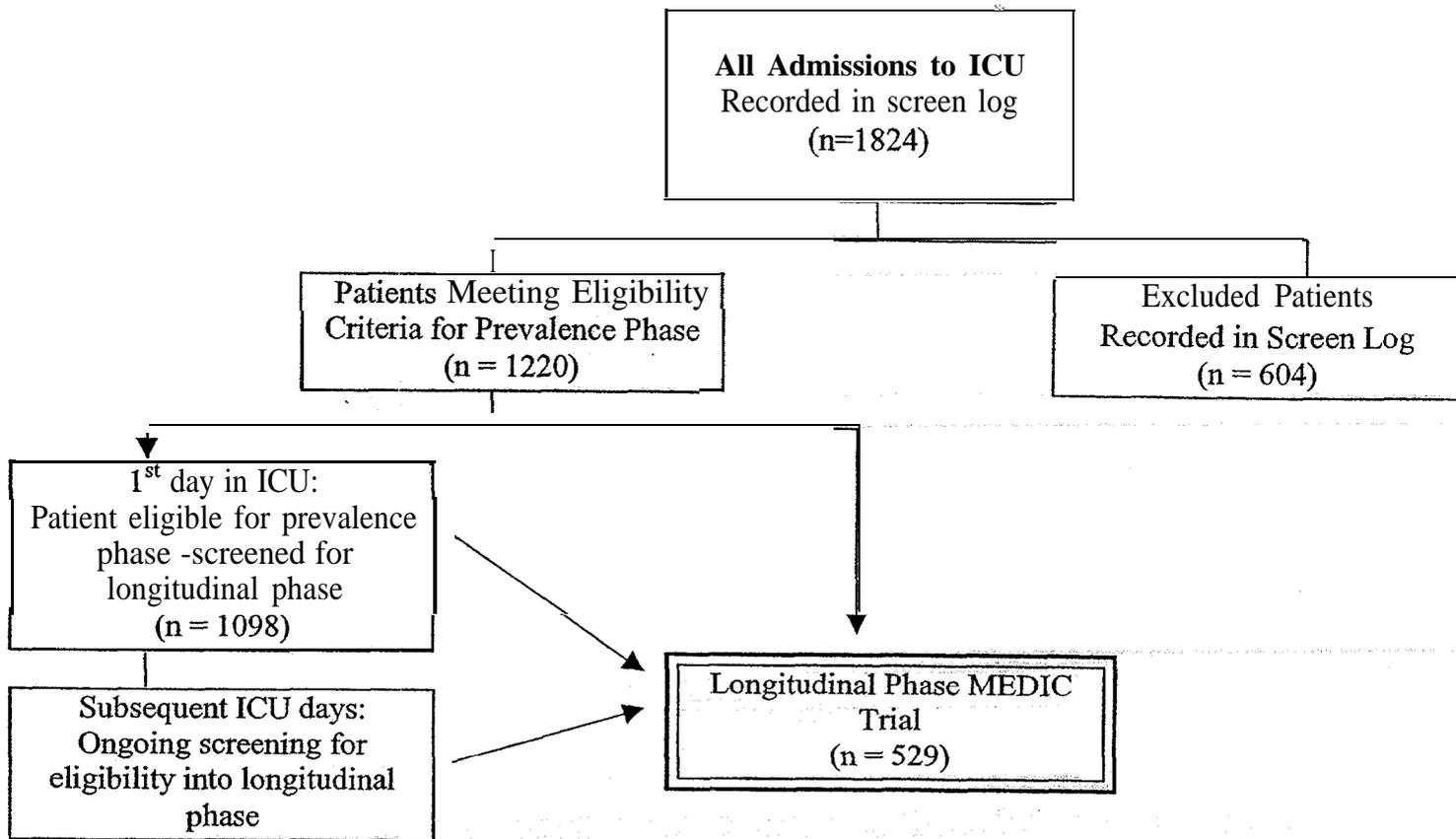
##### 16.8.2.2.2 Study Design

The MEDIC trial was a blinded, multi-centre, prospective observational study, performed in 10 intensive care units (ICU's) of academic hospital settings in North America and Europe. Participating hospitals included two centers located in Europe; four centers located in the U.S. and four centers located in Canada. The study ran from January 5, 2000 to September 25, 2000.

##### 16.8.2.2.3 Patient Selection

All admissions to the Intensive Care Unit of participating study sites were screened for entry into the study (Figure 16-1). A detailed electronic screen log to record eligible and excluded patients was collected. Daily screening for study eligibility was performed for patients not meeting study entry criteria on the day of ICU admission. When a written order for cultures appeared on the patient's medical record, the patient became eligible for the study. If no exclusion criteria were met, and consent (if necessary) was obtained, those patients were followed daily for up to 7 consecutive days. Endotoxin levels, a subset of routine ICU bloodwork, vital signs, daily organ dysfunction scores, and culture results were collected.

**Figure 16-1 Flow Diagram of Patient Screening And Allocation For MEDIC! Trial**



Patients were eligible for the target longitudinal group at any time during their ICU stay. If entry criteria were met on the day of ICU admission, patients could be enrolled in both the prevalence and longitudinal phase. If entry criteria were met after the first day, patients were **enrolled in** the longitudinal phase irrespective of previous prevalence phase eligibility.

#### 16.8.2.2.4 Ethics

All clinical investigative sites had ethical approval for the MEDIC protocol, from either the Institutional Review Board (IRB), for U.S. sites, the Research Ethics Board (REB) for Canadian sites, or the Ethics Committee (EC) for European sites. As required by FDA regulation 21 CFR 8 12.11'0 (a), approval was received prior to their participation in the study. The study was conducted in accordance with the International Code of Harmonization (ICH) guidelines and used principles of Good Clinical Practice (GCP). The MEDIC study was conducted in accordance with the ethical principles contained in the 1989 version of Declaration of Helsinki as set out in 21 CFR 3 12.120 (c)(4). The protocol and informed consent documents (where necessary) were approved by the IRB/REB/EC prior to study initiation at each site. Informed consent was obtained from each study participant, where necessary, prior to entry into the study.

#### 16.8.2.2.5 Inclusion Criteria

ICU patients in whom there was a clinical suspicion of infection were screened for entry into the target population (longitudinal phase) of the clinical study. Clinical suspicion of infection was defined by the existence of an order for, or the performance of a diagnostic test for infection (bacterial cultures or other diagnostic tests such as bronchoscopy, CT scan or surgery) in the patient's medical record.

#### 16.8.2.2.6 Exclusion Criteria

Patients were not eligible for **enrolment** into the target population (longitudinal phase) if any one of the following were present during the time of screening: von Willebrand's disease; concomitant **plasmapheresis**; massive blood transfusion defined as  $> 30\text{g/L}$  decrease in hemoglobin measurement and/or requiring 3 or more units of PRBC's (packed red blood cells) within 6 hours preceding study entry; or receipt of investigational **anti-endotoxin** therapy within 72 hours of MEDIC study entry.

#### 16.8.2.2.7 Study Population

The study population of the target longitudinal group consists of all patients meeting the study eligibility criteria at any time during the ICU stay who have signed informed consent documents (may be waived depending on site IRB/REB/EC). Enrolled patients must have at least one set of blood cultures plus any of other diagnostic cultures or a diagnostic test or procedure to detect an infectious process.

#### 16.8.2.2.8 Blinding of Patient EAA Results

Assay results were collected and sent weekly by electronic data transfer to the data management center (Toronto, Canada) by the laboratory technicians at each of the participating sites. Results of the Endotoxin Activity Assay were blinded to the investigators, study coordinators and ICU staff involved in the care of the patients,

#### 16.8.2.2.9 Sampling Procedures

##### 16.8.2.2.9.1 Collection of Blood Sample for EAA

Blood was drawn for **EAA** analysis within 8 hours of the initial culture set. In addition, daily blood samples for EAA analysis were drawn for 7 consecutive days or until ICU discharge, whichever occurred first. There were no other interventions required by the MEDIC protocol.

#### 16.8.2.2.9.2 Collection of Blood Sample for Day 1 Blood Culture

For all patients with suspicion of infection, regardless of the anatomic site of suspicion, one set of blood cultures was obtained in addition to cultures that may have been taken at other anatomic sites.

#### 16.8.2.2.10 Primary Outcome Measure

The primary outcome of the MEDIC study was the documentation of Gram negative infection. In order to demonstrate the EAA's ability to rule out Gram negative infection, it was first necessary to apply appropriate criteria for identifying Gram negative infection when it occurred. Infections were classified according to 'definitions in the MEDIC clinical protocol which are adapted from the CDC definitions for nosocomial infections. The authors stipulate that information used to determine the presence and classification of infection involves combinations of clinical findings, and results of laboratory and other diagnostic tests. Clinical evidence came from direct observation of the patient, laboratory evidence consisting of cultures and supportive data coming from other diagnostic tests such as x-rays, CT s&n or biopsies.

#### 16.8.2.2.11 Distribution of All Enrolled Patients

In the longitudinal phase, there were 529 patients who constituted the target population for the MEDIC trial. The following table provides a breakdown of recruitment by investigative site.

**Table 16-4. Patients Enrolled In The MEDIC Trial By Investigative Site And Number Of Months Of Participation**

Site Code	Principal Investigator	Location	Duration of Enrollment (months)	# of Patients Enrolled (% of Total Population)
Site 1	Dr. John Marshall	Toronto General Hospital University Health Network 9EN 234 – 200 Elizabeth St Toronto, Ontario, Canada,	9	117 (22.1%)
Site 2	Dr. Deborah Cook	St. Joseph's Hospital McMaster University – Dept of Medicine 50 Charlton Ave E Hamilton, Ontario, Canada	4	68 (12.9%)
Site 3	Dr. Steven Opal	Memorial Hospital of Rhode Island Infectious Diseases Division 111 Brewster St. Pawtucket, Rhode Island, U.S.A.	2	2 (0.4 %)
Site 4	Dr. Edward Abraham	University of Colorado Health Sciences Center 4200 East Ninth Ave. Box C-272 Denver, Colorado, U.S.A.	8	42 (7.9 %)
Site 5	Prof. Jean-Louis Vincent	Erasmé Hospital Université Libre de Bruxelles 808 Route de Lennik Brussels, Belgium	6	113 (21.4%)
Site 6	Prof. Jonathan Cohen (Co-Inv: Dr. Stephen Brett)	The Hammersmith Hospital Imperial College of Science Technology and Medicine London, United Kingdom	4	29 (5.5%)
Site 7*	Dr. John Marshall	Toronto Western Hospital University Health Network Toronto, Ontario, Canada	n/a	n/a
Site 8	Dr. Sangeeta Mehta	Mount Sinai Hospital 600 University Ave # 18 18 Toronto, Ontario, Canada	4	10 (1.9%)
Site 9a	Dr. R.P. Dellinger	Rush Presbyterian, St. Luke's Medical Center Rush University . 1653 West Congress Pkwy. Chicago, Illinois, U.S.A.	3.5	30 (5.7%)
Site 9b	Dr. Kimberly Nagy	Cook County Hospital 1835 W. Harrison St. Chicago, Illinois, U.S.A.	3.5	1 (0.2%)
Site 10	Dr. Terry Smith	Sunnybrook and Women's College Health Sciences Centre 2075 Bawiew Ave. Toronto, Ontario, Canada	3.5	117 (22.1%)

\* Site 7 participated only in the pilot study.

Participating study sites began study enrollment at varying times Sites 1, 2, 3, 4 & 6 began in January 2000 followed by sites 5 & 8 in February 2000. Each participating site agreed to a minimum of 4 months of recruitment. However, Site 3 was dropped when enrollment did not meet minimum expectations. Sites 9a, 9b and 10 were added to boost recruitment within the timeline constraints for the end of the study.

### 16.8.2.2.12 Demographics of Enrolled Patients

Demographic summaries for the enrolled group of patients (n=529) in the MEDIC trial are included in Tables 16-5, 16-6, 16-7 and 16-8.

Sepsis populations are characteristically defined in terms of parameters such as: age, race, mortality and severity of illness indicators. The Acute Physiologic, Age and Chronic Health Evaluation II (APACHE II) score has been validated as a severity of illness measure and predictor of mortality in ICU patients. Additional measures of morbidity are: the Multiple Organ Dysfunction (MOD) score, the Sequential Organ Failure Assessment (SOFA) score and the patients' lengths of hospital and ICU stay,

The development of the APACHE score, as discussed by Knaus and colleagues, was based on the hypothesis that the severity of acute disease can be measured by quantifying the degree of abnormality of a number of physiologic variables. APACHE II classification of patients, performed at the time of admission to the intensive care unit provides a clinically validated severity classification independent of therapy.

The MOD score describes clinical outcome using the constructs of organ dysfunction. The MOD score is calculated based on physiological measures of dysfunction in multiple organ systems, and correlates with risk of ICU mortality and hospital mortality.

The SOFA score is another measure used to describe organ dysfunction or failure. The SOFA score was designed to complement existing organ failure scores. Where traditional severity of illness scores assess the mortality risk, the SOFA score was designed to describe a sequence of complications in critically ill patients following ICU entry.

**Table 16-5. Severity of Illness Scores and Length of Stays for Enrolled Patients (n=529)**

	Mean	Median	Std Dev	Range
Age (y)	58	60	18.2	12-96
APACHE II	21	20	9.8	0-51
MOD score	5.2	5.0	3.2	0-18
SOFA score	6.9	6.0	3.9	0-18
ICU length of stay (d)	14	7	22.7	0-228
Hospital length of stay (d)	39	25	41.7	0-293

Y, year; d, days; APACHE II, acute physiologic, age and chronic health evaluation; MOD, multiple organ dysfunction; SOFA, sequential organ failure assessment.

Table 16-6. Race of Enrolled Patients (n=529)

	Caucasian	Asian	Black	Other	Unknown
Race	409	17	19	26	58

Table 16-7. Gender of Enrolled Patients (n=529)

	Male	Female
Total	322	207

Mortality rates for sepsis patients, as reported in the literature, range from 20% to 50%. Mortality rates in the MEDIC trial are listed in Table 16-8. Significant mortality from sepsis is indisputable. From 1979-1987 CDC MMWR reported substantial increases in the incidence of sepsis in the United States. In 1987, sepsis became the 13th leading cause of death in the general population. In addition, sepsis was reported as the most common cause of death in non-cardiac intensive care units (ICUs) in 1990.

Table 16-8. Mortality Rates of Enrolled Patients (n=529)

	Mortality Rate	Alive	Dead	Unknown *	Missing **
ICU mortality	22.5 %	410	119	0	0
28 day mortality	27.8 %	361	147	19	2
Hospital mortality	32.3 %	357	171	0	1

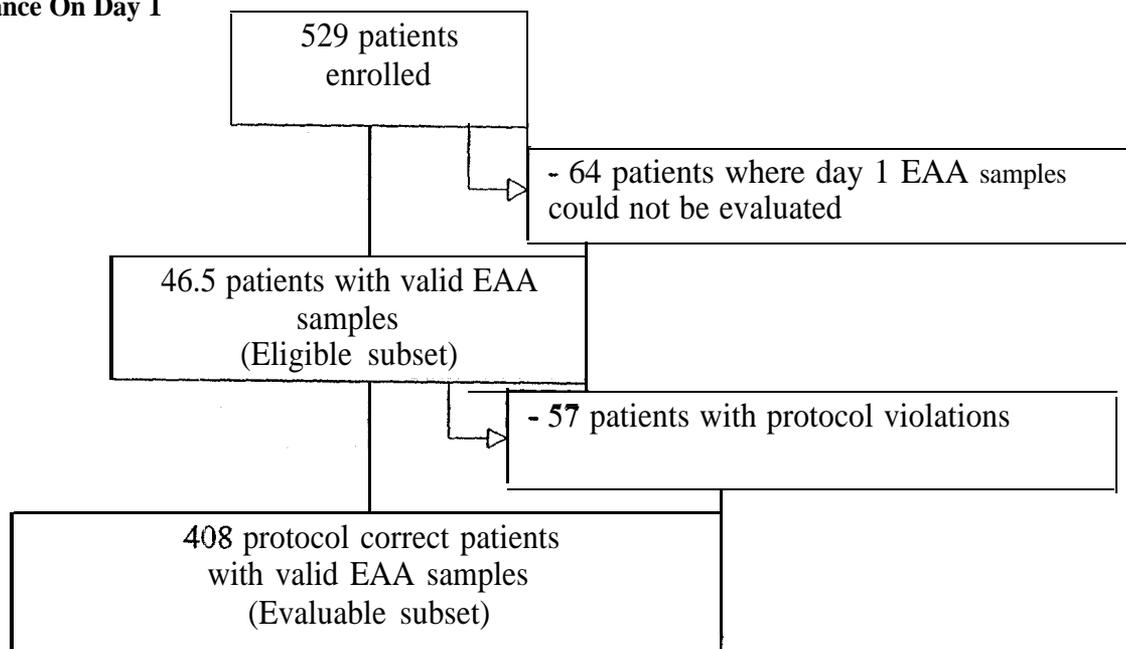
\* Unknown: Patient Discharged Prior to 28 Days

\*\* Missing: Data not provided

#### 16.8.2.2.13 Determination of Evaluable Patients

The MEDIC trial enrolled a total of 529 patients in the longitudinal phase throughout the course of the study. Of the 529 patients enrolled in the trial, there were 64 cases where the first EAA sample could not be evaluated. The remaining pool of 465 patients are the eligible patients. Figure 16-2 contains a summary of subject accountability for the MEDIC trial. Of these remaining 465 patients, there were 57 cases of non-compliance with the clinical protocol. The patient dataset analyzed for the primary study endpoint consists of 408 patients.

**Figure 16-2. Sub-Sets Of Enrolled Patients Based On EM Sample And Clinical Protocol Compliance On Day 1**



16X2.2.14 Demographics of Evaluable Patients

Demographic summaries for the evaluable group of patients (n=408) in the MEDIC trial are included in Tables 16-9, 16- 10, 16-I 1 and 16- 12.

**Table 16-9. Severity of Illness Scores and Length of Stays for Evaluable Patients (n=408)**

	Mean	Median	Std. Dev	Range
Age (y)	57	60	18.4	14-96
APACHE II	21	21	9.9	0-51
MOD score	5.3	5.0	3.3	0-18
SOFA score	7.0	6.0	3.9	0-18
ICU length of stay (d)	15	7	24.7	0-228
Hospital length of stay (d)	38	24	41.4	0-293

Y, year; d, days; APACHE II, acute physiological, age and chronic health evaluation; MOD, multiple organ dysfunction; SOFA, sequential organ failure assessment.

**Table 16-10. Race of Evaluable Patients (n=408)**

	Caucasian	Asian	Black	Other	Unknown
Race	302	17	12	21	56

**Table 16-11. Gender of Evaluable Patients (n=408)**

	Male	Female
Total	241	167

**Table 16-12 Mortality Rates of Evaluable Patients (n=408)**

	Mortality Rate	Alive	Dead	Unknown *	Missing **
ICU mortality	22.3 %	317	91	n/a	0
28 day mortality	27.0 %	285	110	12	1
Hospital mortality	31.4 %	279	128	n/a	1

\* Unknown: Patient Discharged Prior to 28 Days

\*\* Missing: Data not provided

There are no significant differences in the characteristics of all patients enrolled (n=529) in the longitudinal phase of the MEDIC trial compared to those in the evaluable subset (n=408). Both the enrolled population and the evaluable subset are representative of a typical ICU population with suspicion of infection.

#### 16.8.2.2.15 MEDIC Primary Outcome Results

A high rate of suspicion of infection in ICU patients was confirmed in the MEDIC trial as shown in Table 16-13.

**Table 16-13. Flow of Patients in the MEDIC Trial**

	Number of Patients	Percent of Screened Patients
Patients Admitted to ICU during Study Period	1824*	n/a
Screened ICU Patients	1220	100%
Patients with Presumption of ICU Infection (Enrolled Patients for Longitudinal Phase)	529	43.4%

\* 604 patients were admitted to the ICU during times when study coordinators were not available to screen patients for the MEDIC trial.

On study day 1, 73 patients had Gram negative bacterial growth on any culture from any anatomic site (Table 16-14). Only 54 patients with Gram negative bacterial growth met CDC criteria for infection which confirms that the actual incidence of Gram negative infection is low.

**Table 16-14. Patients With Gram Negative (GN) Bacterial Growth on Cultures From Study Day 1**

	Number of Patients	Percent of Evaluable Patients
AH Enrolled Study Patients	<b>529</b>	n/a
All Evaluable Study Patients	<b>408</b>	100%
Evaluable Patients with Gram negative bacterial growth	<b>73</b>	<b>17.9%</b>
Evaluable Patients with Gram negative growth that meets CDC criteria for infection	<b>54</b>	<b>13.2%</b>

The presence of Gram negative bacterial growth on a culture is not synonymous with the presence of infection. Information used to determine the presence of infection involves a combination of clinical findings and results of other laboratory and diagnostic tests. The same logic can be applied to the finding of no growth on a culture. It must be taken into context with clinical findings from the patient, yet at this time there are no supporting/ contributing laboratory tests to refine the clinician's certainty of the absence of infection.

Table 16-15 is a presentation of MEDIC trial primary outcome results and Endotoxin Activity Assay test characteristics. An EAA level of less than 0.40 is considered to be negative for endotoxin activity and an EAA level of greater than or equal to 0.40 is positive for endotoxin activity. EAA results are based on an average of duplicate samples.

**Table 16-15. Frequency of Cases With and Without CDC Evaluated Gram Negative: Infection Versus EM Level at the 0.40 Cut-Off and EAA Test Characteristics in the Evaluable Subset With 95% Confidence Interval. Using Average of Duplicate Samples (N= 408)**

	No Gram Negative Infection	Gram Negative Infection Meeting CDC Criteria	Total
< 0.40 EAA	117	11	128
≥ 0.40 EAA	237	43	280
<b>Total</b>	354	54	408

NPV (95% CI)	91.4 (84.2 - 96.1)
Sensitivity (95% CI)	79.6 (62.8 - 91.3)
Specificity (95% CI)	33.1 (26.4 - 40.3)
PPV (95% CI)	15.4 (11.3 - 20.2)

## 16.9 Conclusion Drawn from Studies

Non clinical study results support that acceptable performance is obtained with the Endotoxin Activity Assay (EAA).

The EAA demonstrated a total precision of <15% at the expected cutoff value of 0.40 EAA units. The analytical sensitivity of the EAA, determined utilizing a normal sample with a low EAA value was estimated to be 0.046 EAA units.

Cross reactivity studies were performed on LPS isolated from eight different strains of Gram negative bacteria. At LPS levels of 100 pg/ml, seven of the eight strains generated statistically higher EAA values compared to a control, indicating positive recognition of each of these strains. One strain of LPS (from *V.cholera*) generated significantly lower EAA values. The lower EAA values may be due to contaminating additives in the *V. cholera* preparation.

The analytical specificity of the EAA was measured by evaluating cell wall components from Gram positive bacteria and from fungi. There was no reactivity to either purified lipoteichoic acid or from cell wall extracts at levels as high as 2000 pg/ml. No reactivity to purified mannan from yeast was observed at levels as high as 2000 pg/ml.

Interference studies were performed with triglycerides, hemoglobin, bilirubin, and human serum albumin at levels recommended by NCCLS guidelines. Triglycerides up to levels

of 750 mg/dL did not interfere with assay performance. Similarly hemoglobin at 1.0 g/L, and bilirubin at 20 mg/dL, did not interfere with EAA values. At levels of 10-30 mg/ml, human serum albumin, a protein known to bind endotoxin, attenuated EAA values in a dose dependent manner.

Patients receiving high doses (50-100 mg) of the steroid Solucortef generated non-test results in the EAA. This is consistent with the known suppressive effect that steroids have on neutrophil activity. Immunosuppressive drugs such as cyclosporin, did not interfere with EAA performance. Neutrophil levels from  $0.5-20.0 \times 10^9$  cells/L had no effect on patient EAA values and erythrocyte levels as measured by final hemoglobin concentrations (0, 70 and 140 g/L) also did not affect EAA values.

The results of the MEDIC clinical study clearly support the role of the Endotoxin Activity Assay (EAA) in addressing an important unmet medical need related to diagnosing patients in the ICU with the suspicion of infection. The current practice limitations resulting from the demonstration of an infecting microorganism by means of diagnostic culture creates difficulties due to several factors inherent in the process.

#### **EAA test characteristics support presumptive rule out of Gram negative infection.**

Sepsis, Inc. believes that the Negative Predictive Value (NPV) (Table 16-15) is the key performance parameter that best supports the intended use of the EAA test. The negative predictive value represents the ability to predict a negative disease state from a negative test result. With a 91% NPV (117/128), it is valuable as a presumptive indicator to rule out the presence of Gram negative infection in ICU patients when the assay is used in conjunction with culture and other diagnostic procedures. 128 out of 408 subjects (31.4%) had negative EAA values (less than 0.40 EAA units). Of those 128 subjects, the absence of Gram negative infection was confirmed in 117 subjects. The high predictive value of a negative EAA result demonstrates the ability to presume a negative EAA value correlates with the absence of Gram negative infection. Because there is a high presumption of infection in ICU patients, compared to true rates of infection, as shown in Table 16-14, the ability to identify patients that do not have Gram negative infections becomes important.

Specificity is defined as the probability that a patient that does not have the disease or condition of interest given the test is negative. The specificity of the EAA is 33% ( $117/[237+117]$ ), (Table 16-15). This is interpreted as the test's ability to accurately identify one third of all patients presumed to have Gram negative infection, who in fact, do not have Gram negative infection. When specificity is considered in the context of other tests commonly used in the clinical laboratory, an EAA specificity of 33% might be judged as very modest. However, most other laboratory tests are developed and used with the intention of ruling in a disease or condition of interest where minimizing false positive tests is desirable. The premise of the EAA, unlike most other tests, is primarily intended to rule out rather than rule in a clinical condition of interest. In the MEDIC trial, there is a low true incidence of Gram negative infection compared to what is initially presumed (Tables 16-13 and 16-14), so that the ability to identify any

patients that do not have Gram negative infection becomes crucial. Specificity becomes a key EAA performance parameter in the context of ruling out infections, since it enables clinicians to identify 33% of patients with an initial presumption of infection, who actually did not have Gram negative infections.

In the clinical situation where the presumption of disease is high, where the disease is important and should be treated, yet confirmatory diagnostic methods and test results are delayed and of uncertain significance, the EAA test can play an important role by aiding clinician's ability to rule out about 1/3 of all patients who do not have the disease.

EAA sensitivity was observed to be 79.6% which indicates that 79.6% of subjects with CDC criteria based Gram negative infections also had EAA values  $>0.40$  units. However, as mentioned previously, the presence of endotoxin in the circulation can occur in circumstances other than Gram negative infection. While endotoxin may be a sensitive marker for the detection ("rule-in") of Gram negative infection, it is not likely to be specific (*vis-à-vis* EAA specificity of 33%). The study was not intended to evaluate the role of endotoxin and endotoxemia from sources other than acute Gram negative infection, therefore, no conclusions should be drawn from EAA values greater than or equal to the established cut-off (EAA  $\geq 0.40$ , positive EAA values) relative to detecting the presence of Gram negative infection.

#### **The EAA provides presumptive results in a rapid timeframe.**

The diagnostic culture process by design requires a significant lapse of time between the physician's presumption of infection from the clinical findings of acute inflammatory response until the results become available demonstrating microbiological growth demonstration in a laboratory setting. This delay can range upwards of 7 days in the case of no confirmed growth, in order to rule-out the presence of slow-growing organisms. A sample of the MEDIC clinical trial data shows that negative culture results are not available for an average of 4.2 days (median 3 days) from the time a culture is ordered with a range of 0 days to 48 days. This delay requires that the clinician make judgements regarding the patient's status of infection without the benefits of supporting diagnostic information. EAA values are available less than 4 hours from the suspicion of infection.

#### **The EM adds confidence to diagnostic uncertainty.**

In a significant number of critically ill patients, the presumption of infection is high. The current course of action is to presume infection is present and provide empiric antibiotics while awaiting confirmatory diagnostic information. The morbidity and mortality associated with infection in ICU patients drives this practice despite potential consequences associated with the unnecessary use of antibiotic therapy.

1. There is a high prevalence of presumption of infection in ICU patients (43.4%) (Table 16-13).
2. There is a low incidence of Gram negative infection (13.2%) (Table 16-14).
3. In conjunction with culture, negative EAA values can reliably exclude the presence of Gram negative infection (91%).

In reality, the true incidence of infection in the total ICU population, as well as the incidence in patients with the presumption of infection, are significantly lower than expected. When culture results are finally reported as negative, doubt is often created in the validity of the culture outcome. This uncertainty is due in part to the delay between presumption of infection and confirmation by negative culture results. The potential impact of a delay in treatment from an incorrect negative culture result drives the initial suspicion to presumption and institution of empiric therapy. Therefore, the improved ability to identify the absence of infection with a high degree of confidence in a timely manner is important.

## Conclusion

**WHEN USED IN CONJUNCTION WITH CULTURES AND OTHER RELEVANT DIAGNOSTIC TESTS, THE ENDOTOXIN ACTIVITY ASSAY CAN RELIABLY RULE-OUT THE PRESENCE OF GRAM NEGATIVE INFECTION IN ICU PATIENTS SUSPECTED OF INFECTION.**

### 16.9.1 Safety

As a rule-out *in vitro* diagnostic assay, the EAA involves the removal of blood for testing purposes. The test, therefore, presents no more safety hazard than other tests where blood is removed from subjects including diagnostic blood culture.

In the study population with suspected infection (408 patients), there were 11 episodes (2.7% of the entire study population) that were considered to represent false negatives, because CDC criteria for Gram negative infection were met, but the EAA level was less than 0.40 at study baseline.

The false negatives for the EAA can be attributed to the following:

- Inaccuracy of infection diagnosis as determined by the CDC criteria. The criteria for systemic infection were not really met in a small group of patients as determined by CEC criteria.
- Gram negative bacteria from a non-hematogenous site of infection that has not attained a systemic state.

The false positive rate of the EAA test was not reviewed as the study was not intended to evaluate the role of endotoxin and endotoxemia from sources other than Gram negative infection.

### 16.9.2 Effectiveness

The target population for diagnostic culture in the TCU is those patients suspected of infection. The same target population was selected for this device to demonstrate the relationship between negative EAA results and the absence of culture supported Gram negative infection. In the sampled population the NPV of the EAA when compared to the absence of culture supported Gram negative infection was 91.4% and was available a

median of 3 days earlier than negative culture results. Therefore it can be concluded that a negative EAA result (<0.40) is presumptive of the absence of Gram negative infection until confirmed when culture results become available.

### 16.9.3 Risk Benefit Analysis

An elevated EAA result has not been correlated to the presence of Gram negative infection. Interpretation of infection status should not be made for EAA results greater than or equal to 0.40. False negative results (2.7% in the current study) may result in the presumption of the absence of Gram negative infection prior to the receipt of culture results. The physician should utilize the EAA test result in conjunction with culture, other diagnostic procedures and the overall clinical assessment. Therefore, assessment of patient status should not be based exclusively on an EAA result.

It is reasonable to conclude that the benefits of use of the device for the target population outweigh the risk of illness or injury when used in accordance with the directions for use.

### 16.10 Panel Recommendations

[TO BE COMPLETED]

### 16.11 CDRH Actions on the Application

[TO BE COMPLETED]

### 16.12 Approval Specifications

[TO BE COMPLETED]

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