



Immunoluminometric assay (ILMA) for the determination of PCT (procalcitonin) in human serum and plasma (Coated Tube System)

Article number: 354.1 (100 determinations)

Intended Use

The B·R·A·H·M·S PCT LIA is an immunoluminometric assay (ILMA) used to determine the concentration of PCT (procalcitonin) in human serum and plasma.

The B·R·A·H·M·S PCT LIA is intended for use in conjunction with other laboratory findings and clinical assessments to aid in the risk assessment of critically ill patients on their first day of ICU admission for progression to severe sepsis and septic shock.

Summary and Explanation

Sepsis is a daily challenge in intensive care units. Today various therapeutic strategies are known to improve survival in patients with sepsis. Early assessment is important for determination of the appropriate treatment.

PCT is the prohormone of the hormone calcitonin, but PCT and calcitonin are distinct proteins. Calcitonin is exclusively produced by C-cells of the thyroid gland in response to hormonal stimuli, whereas PCT can be produced by several cell types and many organs in response to pro-inflammatory stimuli, in particular by bacterial products.¹

In healthy people, plasma PCT concentrations are found to be below 0.3 ng/ml. PCT levels rise rapidly (within 6 – 12 hours) after a bacterial infectious insult with systemic consequences.² Early after multiple traumas, major surgery, severe burns, or in neonates, PCT levels can be elevated independently of an infectious process, but the return to baseline is usually rapid. Viral infections, bacterial colonization, localized infections, allergic disorders, autoimmune diseases, and transplant rejection do not usually induce a significant PCT response (values < 0.5 ng/ml). Therefore, by evaluating PCT concentrations, the physician may use the findings to aid in the risk assessment for progression to severe sepsis and septic shock.

The results of the B·R·A·H·M·S PCT LIA should be evaluated in context of all laboratory findings and the total clinical status of the patient. In cases where the laboratory results do not agree with the clinical picture or history, additional tests should be performed.

Principle

B·R·A·H·M·S PCT LIA is an immunoluminometric assay (ILMA) used to determine the concentration of Procalcitonin (PCT) in human serum and plasma. Two antigen-specific monoclonal antibodies that bind PCT (the antigen) at two different binding sites (the calcitonin and katacalcin segments) are added in excess. One of these antibodies is luminescence labeled (the tracer), and the other is fixed to the inner walls of the tube (coated tube system).

During the course of incubation, both antibodies react with PCT molecules in the sample to form "sandwich complexes". As result the luminescence labeled antibody is bound to the inner surface of the tube. Once the reaction is completed, the excess tracer is completely removed from the tube and discarded.

Then, the amount of residual tracer on the test tube wall is quantified by measuring the luminescence signal using a suitable luminometer and the B·R·A·H·M·S Basiskit LIA reagents. The intensity of the luminescence signal (RLU) is directly proportional to the PCT concentration in the sample. After a standard curve has been established using standards with known antigen concentrations (calibrated against recombinant intact human PCT), the unknown PCT concentrations in patient serum or plasma samples can then be quantitated by comparison of test values with the curve.

Reagents

The B·R·A·H·M·S PCT LIA kit contains sufficient reagents for 100 determinations. NOTE: B·R·A·H·M·S recommends running the calibrators, controls, and patient samples in duplicate.

Materials Provided:

Reagent	Quantity for 100 det.	Contents
A	1 vial lyophilized	Tracer, luminescence labeled (acridinium derivate) anti-PCT antibody (monoclonal, mouse), blue colored solution, 29 ml after reconstitution with buffer B.
B	1 x 29 ml vial	Buffer, for reconstituting tracer A, ready for use.
C	2 x 50 tubes	Coated tubes (test tubes), coated with anti-PCT antibody (monoclonal, mouse), ready for use.
G	1 x 4 ml vial	Zero serum (human serum), for reconstituting the standards resp. calibrators and controls, ready for use.
W	2 x 11 ml vials	B·R·A·H·M·S Washing solution universal, concentrate, 11ml.
S1, S2/C1, S3, S4/C2, S5, S6	6 vials lyophilized	PCT standards (recombinant), reconstitute each with 0.25 ml zero serum G before use. Concentration ranges: 0.08 (def.); 0.3 – 0.7; 1.5 – 2.5; 16 – 24; 160 – 240; 400 – 600 ng/ml. Precise concentrations see leaflet enclosed.
K1, K2	2 vials lyophilized	PCT controls 1 and 2, reconstitute each with 0.25 ml zero serum G before use. Concentrations see leaflet enclosed.

Additional Materials Required but not provided:

- Luminometer with two injectors
- B·R·A·H·M·S Basiskit LIA Reagents for the generation of the luminescence signal in B·R·A·H·M·S LIA immunoassays
Article number: 371.0
(1 000 chemiluminescence measurements)
- Dispenser (5 ml) for B·R·A·H·M·S washing solution universal
- Micropipettes (20 µl, 250 µl) with disposable plastic tip
- Sample mixer
- Horizontal rotator
- Distilled water

Warnings and Precautions:

For *in vitro* diagnostic use only.

This kit contains materials of human origin (e.g. human serum). These materials have been screened for HBsAg, HIV I/II antibodies, and HCV antibodies; all tests were negative. However, the reagents and patient samples should be handled with care, as all materials of human origin are potentially hazardous.

The following kit reagents contain the preservative sodium azide at concentrations of < 0.1 percent by weight: buffer C and zero serum. These reagents should not be swallowed or allowed to come in contact with the skin or mucous membranes.

B·R·A·H·M·S Customer Service will gladly send the reagent-specific Safety Data Sheets upon request.

Tel.: (1) 770 449 7738

Fax: (1) 770 449 7739

E-Mail: service@brahms-usa.com

In large test series, reagents of the same batch should be pooled.

Because glass vials are included in the reagent kit, we explicitly point out that there will be a breakage hazard, and consequently a risk of injury.

The reagents as well as waste originated by the test must be disposed of in accordance with the specifications of local authorities.

Stability and Storage Conditions

Store all reagents at 2 to 8 °C in their original shipping containers until directly prior to use. Observe the expiry dates specified on the main container and the vial labels. Do not use any reagents that have exceeded the expiration date printed on the label.

Diluted washing solution may be used for up to 4 weeks if stored at 2 – 8 °C. Contaminated washing solution must not be used. This is the case either if the liquid is clouded or the pH value is < 6.

If less than 100 determinations are to be carried out per assay, for reconstituted reagents the following storage conditions are valid: Store **reconstituted standards and controls** at – 20 °C (thawing is possible up to ten times); the reconstituted tracer keeps well for three days at 2 – 8 °C, otherwise, store the **reconstituted tracer** at – 20 °C (may be frozen and thawed four times).

Components from different batches should not be exchanged or mixed.

Specimen Collection and Preparation

Specimens Recommended: Serum or plasma may be used. B·R·A·H·M·S recommends the use of only one matrix, i.e., use the same material (either serum or plasma) throughout the patient's clinical course.

Specimen Collection: NCCLS guidelines should be followed for collecting, transporting, and processing patient samples. A slight difference in results was noted between the use of **glass and plastic collecting tubes**. For plastic tubes, a slight increase is noted if the sample remains in the collecting tube for more than 24 hours, if the filling volume is higher, or if plasma is used. B·R·A·H·M·S recommends the use of one type of collecting tube, i.e., either glass or plastic, throughout the patient's clinical course.

Even though a slight difference in results was noted between plasma/serum and glass/plastic collecting tubes, the results are interpreted in the same manner. The results still fall within the ranges provided in the **Expected Results and Interpretation of Results** section. In addition, the results of the B·R·A·H·M·S PCT LIA should be evaluated in context of all laboratory findings and the total clinical status of the patient. In cases where the laboratory results do not agree with the clinical picture or history, additional tests should be performed.

WARNING: Patient samples should be handled with care, as all materials of human origin are potentially hazardous.

Specimen Handling and Storage: Samples that are not used in an assay within 24 hours following the blood draw must be frozen and stored at –20 °C. Samples may be frozen and thawed three times.

Procedure

Test Procedure Overview:

1. Number	test tubes (a, b)		S1 – S6	K1, K2	P1 etc.
2. Pipette	standards	µl	20	–	–
	controls	µl	–	20	–
	patient samples	µl	–	–	20
3. Pipette	tracer	µl	250	250	250
4. Incubate	1 h – 1 h 15 min at RT (18 – 25 °C) on orbital shaker (170 – 300 rpm).				
5. Decant	Add 1 ml B·R·A·H·M·S Washing solution universal to each coated tube prior to decanting off the liquid.				
6. Wash	Add 1 ml B·R·A·H·M·S Washing solution universal to each coated tube four times and decant off the liquid. Turn the tubes upside down and allow them to drain on blotting paper for 5 – 10 min.				
7. Transfer	Place all coated tubes in luminometer.				
8. Measurement	Measure in luminometer with automatic injection of B·R·A·H·M·S Basiskit LIA reagents 1 and 2.				

Step by Step Procedure:

Notes on B·R·A·H·M·S PCT LIA Execution:

- A double assessment, i.e., a **duplicate** run, of calibrators, controls, and patient samples is recommended.
- The indicated sequence of steps must be followed.
- **Carefully follow the manufacturer's instructions.** Improper handling of the reagents may falsify the test results. B·R·A·H·M·S Aktiengesellschaft is not liable for faulty test results arising from improper storage, use, or handling.

Sample Dilution: Patient samples with a concentration above the measuring range are to be rated as “> highest standard”. The result must not be extrapolated. The patient sample in question should be diluted and retested. Further information can be obtained from B·R·A·H·M·S Aktiengesellschaft customer services.

1. Preparations

- Allow all kit components and patient samples to warm up to controlled room temperature or up to 25 °C.
- Reconstitute Tracer (A) with Buffer (B). Reconstitute standards and controls with 0.25 ml Zero serum (G).
- Agitate all liquid reagents – including patient sera – gently before use (avoid foam formation).
- Number the coated tubes (preferentially using a, b for duplicates).
- Prepare B·R·A·H·M·S Washing solution universal: dilute 11ml concentrate with distilled water to yield 550 ml. We strongly recommend contacting the manufacturer or distributor before using other washing solutions.
- Prepare the luminometer for use.

2. Pipette **20 µl PCT standards** of increasing concentrations into the test tube bottoms S1 a, b ... S6 a, b. Pipette **20 µl of each control** into the test tubes K1 a, b, K2 a, b, and **20 µl of each patient serum sample or plasma** into the tubes P1 a, b, etc. To avoid any carryover into subsequent samples, a new plastic micropipette tip should be used for each sample.

3. Pipette **250 µl tracer** into all test tubes.

Note: In order to minimize possible shift effects, the recommended total working time for steps 2 and 3 should be 15 minutes.

4. Mix the tubes for a short period of time on a sample mixer to ensure homogeneity of the liquid. Cover the test tubes with adhesive foil **and incubate them on a horizontal rotator (170 – 300 rpm) for 1 hour to 1 hour 15 min at room temperature (18 – 25 °C).**

Caution! **The test tubes should be protected from light during incubation.**

5. When the incubation is finished add **1 ml of the B-R-A-H-M-S Washing solution universal** to each tube prior to decanting the liquid off completely.

Ensure that the upper section of the test tube wall is completely wet with washing solution. This ensures that any residual tracer which may be bound to the upper tube walls will also be removed.

6. Subsequently, add **1 ml of the B-R-A-H-M-S Washing solution universal four more times** to all test tubes (as described in 5) and decant off the liquid completely after each washing step. A total of 5 washing steps will be performed.

After the **last rinsing step**, turn the tubes upside down and allow tubes to drain for 5 – 10 minutes on clean blotting paper. Then tap the tubes gently on the blotting paper to remove any remaining liquid.

7. Place all tubes in the luminometer in the order defined by the numbered sequence.
8. Start luminescence measurement with automatic injection of 300 µl B-R-A-H-M-S Basiskit LIA reagents 1 and 2. **Recommended measuring time is 1 second per tube.**

Calibration

For each test series a new standard curve needs to be performed. The standards are provided in defined PCT concentrations, the range is between 0.08 - 500ng/ml. The concentrations for calibrators were validated by Mass analysis. The material used is recombinant human PCT. The calibration is performed in conjunction with the test procedure for each run. Using the standard curve, the measured luminescence signal values can then be used to directly determine the PCT concentration of the unknown samples in ng PCT/ml.

The range where PCT concentrations can be measured is 0.3ng/ml – 500ng/ml.

Quality Control

The control material contained in the kit (K1, K2) is recombinant human PCT. The controls should be applied to every batch and results should lie within the acceptable ranges listed in the package insert. If unacceptable control values are obtained, proceed as outlined in standard laboratory diagnostic procedures to determine the cause and implement corrective measures. The 2 level positive controls are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut off. Test accuracy and precision must be monitored by means of laboratory in-house and/or commercially available control materials.

B-R-A-H-M-S PCT LIA should be performed in accordance with local, state and/or federal and accreditation requirements.

Calculation of Results

Select a spline/unsmoothed evaluation program. Using the standard curve, the measured luminescence signal values can then be used to directly determine the PCT concentration of the unknown samples in ng PCT/ml.

B·R·A·H·M·S recommends running the standards, controls, and patient samples in duplicate. Report the mean value of the duplicate PCT concentration as calculated by the luminometer as the final result. If the CV of the duplicate results exceeds 10%, the run is invalid and needs to be repeated. For technical support, please contact B·R·A·H·M·S Customer Service.

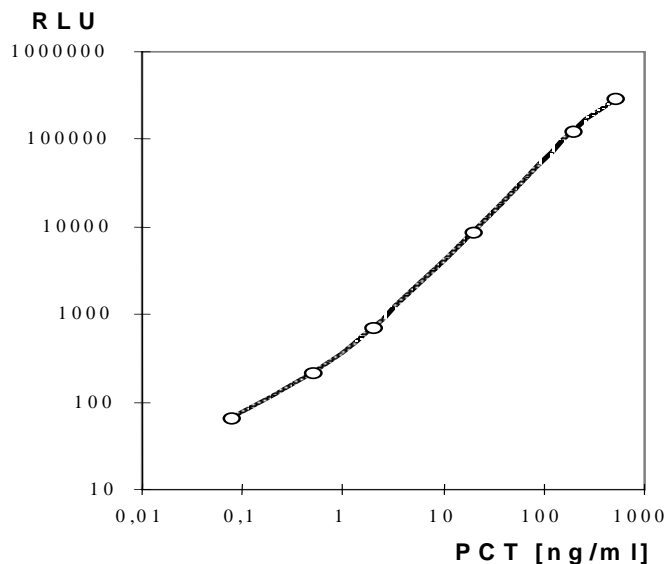
Example:

Signal values (RLU = relative light units) obtained with an AutoCliniLumat LB 952 (Laboratory Prof. Berthold, Germany).

Test tubes	RLU (1 st result)	RLU (2 nd result)	RLU (mean)	ng/ml (mean)
Standard S1	75	56	66	(def.) 0.08
Standard S2	212	207	210	0.5
Standard S3	696	693	694	2.0
Standard S4	9 022	8 644	8 833	20
Standard S5	123 430	127 747	125 588	200
Standard S6	295 145	288 988	292 066	500
Patient sample P1	3 873	3 979	3 926	10.6

The measured signal values vary from one luminometer to another. Therefore, these values are for orientation purposes only.

Standard Curve:



Interfering Substances:

Based on NCCLS testing, the following substances were evaluated in the B·R·A·H·M·S PCT LIA at the concentrations listed and were found not to affect test performance.

Interfering Substance	Non-Interfering Concentration
Bilirubin (conjugated)	40 mg/dl
Triglyceride	634 mg/dl
Hemoglobin	500 mg/dl
Protein (Albumin)	1 g/dl
Imipenem	1.18 mg/ml
Cefotaxim	90 mg/dl
Vancomycin	3.5 mg/ml
Dopamine	13 mg/dl
Noradrenaline	2 µg/ml
Dobutamine	11.2 µg/ml
Heparin	8000 U/l
Furosemide	2 mg/dl
Calcitonin	8 ng/ml
Katacalcin	30 ng/ml
α-CGRP*	30 ng/ml
β-CGRP*	30 ng/ml
Calcitonin Salmon	30 µg/ml
Calcitonin Eel	30 µg/ml

*Calcitonin Gene Related Peptide

Linearity / High Dose Hook Effect:

A High Dose Hook Effect occurs in immunometrical assay systems and yields erroneously low PCT results in cases of very high PCT concentrations (beyond 900 ng/ml after calibration).

Therefore, if a PCT result above the highest standard is obtained, the samples should be diluted with the dilution serum (Zero serum) contained in the assay kit and the test should be re-run in order to obtain the correct PCT concentration. PCT concentrations up to 4000 ng/ml do not have an effect on the assignment of the patient to the reference ranges described above.

Limitations

- Precise technique is required. Carefully follow the manufacturer's instructions. Improper handling of the reagents may falsify the test results.
- The B·R·A·H·M·S PCT LIA should be used for ICU patients on their first day of ICU admission.
- Increased PCT levels may not always be related to systemic infection. These conditions include, but are not limited to
 - the first days after a major trauma, major surgical intervention, burns, treatment with OKT3 antibodies and other drugs stimulating the release of pro-inflammatory cytokines, neonates (first 2 days of life).^{3, 4, 5}
 - patients with prolonged or severe cardiogenic shock, prolonged severe organ perfusion anomalies.
- The results of the B·R·A·H·M·S PCT LIA should be evaluated in context of all laboratory findings and the total clinical status of the patient. In cases where the laboratory results do not agree with the clinical picture or history, additional tests should be performed.

Interpretation of Results

The B·R·A·H·M·S PCT LIA is intended to aid in the risk assessment of critically ill patients on their first day of ICU admission for progression to severe sepsis and septic shock. The likelihood of progressing to severe sepsis and septic shock was investigated in two independent, controlled prospective studies^{6, 7} (see **Clinical Performance** section below). SIRS, Sepsis, Severe Sepsis, and Septic Shock were categorized according to the criteria of the consensus conference of the American College of Chest Physicians/Society of Critical Care Medicine.⁸

PCT should always be interpreted in the clinical context of the patient. Therefore, clinicians should use the PCT results in conjunction with other laboratory findings and clinical signs of the patient.

The test should be run in duplicate. If the duplicate results are above 20% CV, the sample should be re-run. When a control is out of range, the run is invalid and should be re-run.

The data from the two studies supported the following interpretative risk assessment criteria:

PCT > 2 ng/ml

PCT levels above 2.0 ng/ml on the first day of ICU admission represent a high risk for progression to severe sepsis and/or septic shock.

PCT < 0.5 ng/ml

PCT levels below 0.5 ng/ml on the first day of ICU admission represent a low risk for progression to severe sepsis and/or septic shock.

Note: PCT levels below 0.5 ng/ml do not exclude an infection, because localized infections (without systemic signs) may also be associated with such low levels. If the PCT measurement is done very early after the systemic infection process has started (usually < 6 hours), these values may still be low.

As various non-infectious conditions are known to induce PCT as well, PCT levels between 0.5 ng/ml and 2.0 ng/ml should be reviewed carefully to take into account the specific clinical background and condition(s) of the individual patient.

Expected Results:

In normal subjects, PCT concentrations are < 0.3 ng/ml, thus below the detection limit of the assay. In a population of 144 healthy subjects, 143 had a PCT value < 0.3 ng/ml.

Performance Characteristics:

The clinical data for the B·R·A·H·M·S PCT LIA were obtained from a total of 179 patients in two independent, controlled prospective studies performed in ICUs of academic hospital settings (see below). The data from the two studies have been pooled and re-evaluated using the cut-offs recommended by B·R·A·H·M·S. In 44 patients with a PCT level < 0.5 ng/ml, no patient had severe sepsis or septic shock. In 77 patients with severe sepsis or septic shock, only one (1) had a PCT level \leq 2.0 ng/ml.

The data from the two studies is summarized in the following graph and tables. The 2x2 tables below show the PCT results for SIRS and Sepsis compared to Severe Sepsis and Septic Shock on the first day of ICU admission.

Study 1: Calcitonin precursors are reliable markers of sepsis in a medical intensive care unit⁶

Patients: 101 consecutive critically ill patients of representative population of unselected, well-defined patients in a medical ICU in the Switzerland. The median age of the study population was 59 years (age range, 23-86 years). There were 55 men and 46 women in this study.

Study 2: Diagnostic Value of Procalcitonin, Interleukin-6 and Interleukin-8 in Critically Ill Patients admitted with suspected Sepsis⁷

Patients: 78 consecutive critically ill patients newly admitted to a medical and surgical ICU in the Switzerland, including also neutropenic and immunosuppressed patients, with suspected diagnosis of infection. Patients had to fulfill at least 2 criteria of SIRS. Source of infection was the respiratory tract, intra-abdominal space and bloodstream infection. The mean ages were as follows: SIRS, 51 ± 18 years; sepsis, 51 ± 21 years; severe sepsis, 59 ± 18 years; and septic shock, 54 ± 15 years. There were 57 men and 21 women in this study.

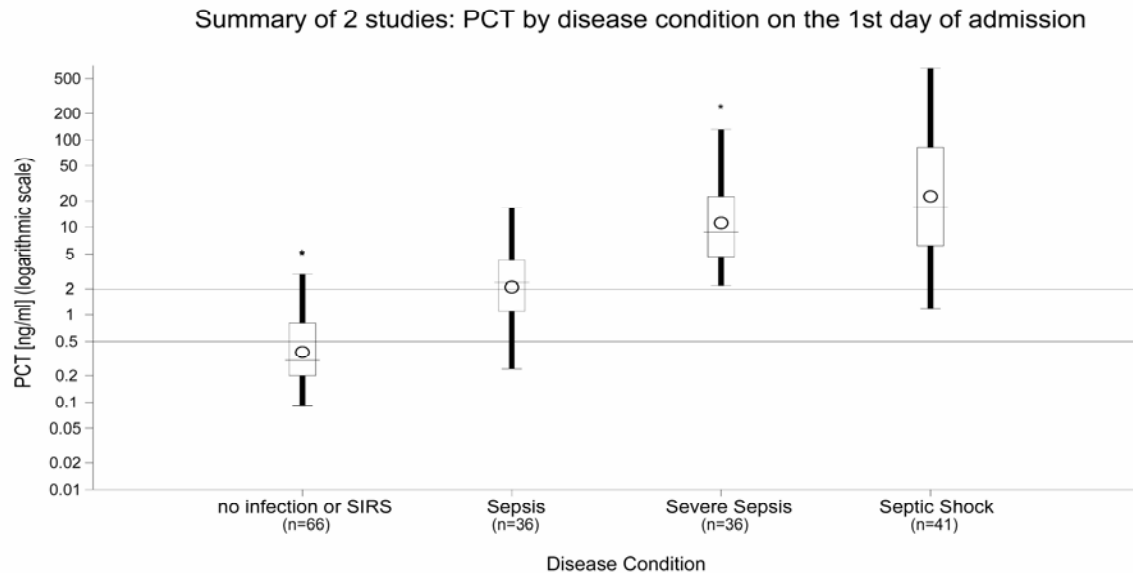
PCT by no infection or SIRS, Sepsis versus Severe Sepsis or Septic Shock Cut Off 0.5 ng/ml

PCT Result Study 1	No infection or SIRS/Sepsis	Severe Shock/ Septic Shock	Totals
PCT < 0.5	36	0	36
PCT > 0.5	34	31	65
Totals	70	31	101
PCT Result Study 2	SIRS/Sepsis	Severe Shock/ Septic Shock	Totals
PCT < 0.5	8	0	8
PCT > 0.5	24	46	70
Totals	32	46	78

PCT by no infection or SIRS, Sepsis versus Severe Sepsis or Septic Shock Cut Off 2.0 ng/ml

PCT Result Study 1	No infection or SIRS/Sepsis	Severe Shock/ Septic Shock	Totals
PCT < 2.0	60	0	60
PCT > 2.0	10	31	41
Totals	70	31	101
PCT Result Study 2	SIRS/Sepsis	Severe Shock/ Septic Shock	Totals
PCT < 2.0	19	1	20
PCT > 2.0	13	45	58
Totals	32	46	78

The 4 box and whisker diagrams below summarize the individual PCT results of the 4 subgroups of patients on the first day of ICU admission.



Precision:

The **analytical assay sensitivity** is **approximately 0.1 ng/ml**. The **functional assay sensitivity** (20 % inter-assay variation coefficient) is **approximately 0.3 ng/ml**.

Aliquots of 14 samples distributed over the measuring range were assayed in duplicates on 20 days by 4 different operators using various reagent lots. The profiles of **total and within run** precision (mean of PCT concentration vs. CV %) are as follows:

	Mean (ng/ml)	Total Precision CV %	Within run Precision CV %
00/20	0.54	13.4	4.0
00/21	2.61	8.7	3.1
Sample 1	0.14	16.6	10.0
Sample 2	0.43	13.9	6.3
Sample 3	1.00	9.6	4.0
Sample 4	2.46	7.9	3.4
Sample 5	2.44	9.6	4.3
Sample 6	2.17	9.5	3.3
Sample 7	4.92	8.9	2.5
Sample 8	14.1	7.1	2.4
Sample 9	43.2	5.3	2.7
Sample 10	34.2	7.1	2.5
Sample 11	66.6	5.7	2.8
Sample 12	184	6.7	3.2

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Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis

Revision History

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(This version supersedes all earlier instruction manuals.)

Date of Revision	Version	Description of Changes
[insert date]	Version 1.0	First release of document



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