Determination of Analytical Performance Characteristics of RAMP® Procalcitonin

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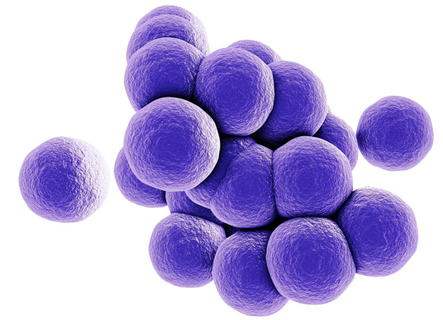
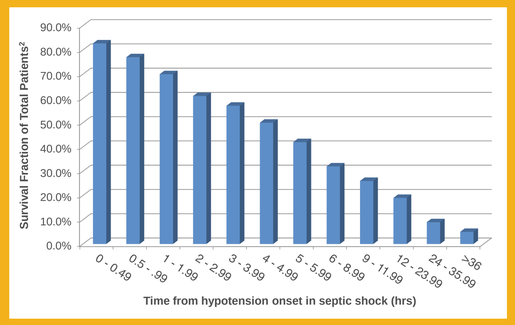


SEPSIS FACTS

GLOBAL: More than **30 million cases** and **6 million deaths** estimated worldwide each year.[1]

URGENT: Early detection and treatment of sepsis has been proven to improve patient outcomes and reduce healthcare costs.[2,3]

DEADLY: **One-third** of ICU patients with sepsis die before leaving the hospital.[4]



COSTLY: Sepsis is one of the most expensive conditions to treat, with costs exceeding **$20 billion USD** annually in the US alone.[5]

PROCALCITONIN (PCT): is a biomarker elevated in the blood of patients suffering from sepsis, the result of the body's excessive inflammatory response to an infection. Physicians use PCT to distinguish bacterial sepsis from other causes of similar symptoms.[6]

Whereas the traditional method of sepsis diagnosis relies on bacterial culture, which can take upwards of 24 hours and lacks sensitivity and specificity, PCT is highly specific for bacterial infections, and correlates strongly with their extent and severity.[6,9-13]

RAMP® PROCALCITONIN

The RAMP® System is a lateral flow immunoassay platform that provides accurate and precise diagnostic information in ~**15 minutes**. RAMP Procalcitonin is a quantitative *in vitro* diagnostic test used with the RAMP System to measure levels of the prohormone procalcitonin (PCT) in human EDTA anticoagulated whole blood.

The objective of these studies was to determine the analytical performance characteristics of the RAMP Procalcitonin test.

Detection Limits

Detection Limits of the RAMP Procalcitonin test were determined based on methods outlined in CLSI document *EP17-A* (2004). LoB was determined in K_2-EDTA pooled plasma; LoD and LoQ were determined in EDTA whole blood. The determined values are presented below:

Limit of Blank (LoB) = 0.18 ng/mL

Limit of Detection (LoD) = 0.36 ng/mL

Limit of Quantitation (LoQ) 20% = 0.64 ng/mL
10% = 1.28 ng/mL

Linearity

The RAMP Procalcitonin test was evaluated for linearity based on methods described in CLSI document *EP6-A* (2003) using EDTA whole blood samples containing commercially available PCT antigen at concentrations up to 250 ng/mL. 10 dilutions of a high concentration sample and a blank were prepared and tested at 3 replicates. A linearity plot of analytical result (y) versus sample concentration (x) is presented in Figure 1. Only samples below the upper limit of the analytical measurement range (≤ 200 ng/mL) were included in the analysis, giving a linear regression slope (95%CI) of 0.97 (0.93 to 1.02) and an R-value (95%CI) = 1.00 (0.99 to 1.00).

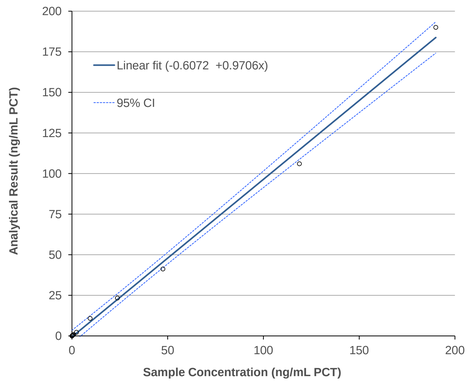


Figure 1. Linearity Plot for the RAMP Procalcitonin test

Hook Effect

The RAMP Procalcitonin test was evaluated for hook effect using whole blood samples containing commercially available PCT antigen at concentrations up to 2000 ng/mL. No evidence of high dose hook effect was observed in the raw instrument signals, with samples having estimated concentrations above the upper limit of the test reporting >200 ng/mL on the RAMP instrument.

Repeatability and Total Precision

Repeatability (within-run) and total precision of the RAMP Procalcitonin test were determined based on methods outlined in CLSI document *EP5-A3* (2014) by testing three levels of frozen plasma control materials in duplicate, twice per day for 20 days on three lots of test materials. Within-run precision was also determined for whole blood samples by testing three levels at 10 replicates in a single run on a single lot of test materials.

Sample Type	[PCT] ng/mL	Repeatability (Within-run Precision)	Total Precision
Plasma Controls	0.58	16.0 – 18.7%	15.8 – 19.3%
	2.72	8.0 – 9.0%	7.7 – 9.3%
	92.7	6.6 – 8.5%	7.2 – 9.4%
Whole Blood	0.75	14.4%	-
	3.00	6.8%	-
	103.2	8.2%	-

Interference

The RAMP Procalcitonin test was evaluated for potential interference with 5 endogenous substances and 11 common pharmaceutical compounds based on methods outlined in CLSI document *EP7-A2* (2005). Interfering substances were added to EDTA whole blood samples containing commercially available PCT antigen at two concentrations; data for the highest concentration of interfering substance tested for which no interference was observed (i.e. interference not exceeding ±20%) are presented in the table below.

Interfering Substance	Substance Conc.	PCT Result (ng/mL)		Interference (%)	PCT Result (ng/mL)		Interference (%)
		Control	+ Sub		Control	+ Sub	
Endogenous Interferents							
Bilirubin conj.	40 mg/dL	0.57	0.51	-10.5%	12.40	12.45	0.4%
Bilirubin unc.	40 mg/dL	0.60	0.58	-3.3%	11.30	10.80	-4.4%
Triglycerides	3260 mg/dL	0.36	0.40	11.1%	13.37	12.83	-4.0%
Hemoglobin	500 mg/dL	0.57	0.48	-15.8%	12.40	12.65	2.0%
HSA	6.0 g/dL	-	-	-	13.37	14.21	6.3%
HSA	2.44 g/dL	0.46	-	20.0%	-	-	-
Pharmaceutical Compounds							
Acetaminophen	180 mg/dL	0.61	0.51	-16.4%	10.73	10.53	-1.9%
ASA	240 mg/dL	0.68	0.57	-16.2%	12.01	9.73	-19.0%
Cefotaxime	200 mg/dL	0.62	0.68	9.7%	7.77	7.65	-1.5%
Dobutamine	25 µg/mL	0.62	0.63	1.6%	7.77	7.64	-1.7%
Dopamine	30 mg/dL	0.62	0.58	-6.5%	11.10	11.60	4.5%
Furosemide	40 mg/dL	0.52	0.58	11.5%	16.68	18.41	10.4%
Heparin	28.8 U/mL	0.57	0.51	-10.5%	19.30	20.25	4.9%
Ibuprofen	144 mg/dL	0.61	0.53	-13.1%	10.73	9.70	-9.6%
Imipenem	100 mg/dL	0.76	0.88	15.8%	7.96	7.71	-3.1%
Noradrenalin	5 µg/mL	0.62	0.64	3.2%	7.77	7.91	1.8%
Vancomycin	5.5 mg/mL	0.50	-	20.0%	-	-	-
Vancomycin	1.8 mg/mL	-	-	-	8.56		20.0%

Cross-Reactivity

The RAMP Procalcitonin test was evaluated for cross-reactivity with calcitonin, katacalcin, α-CGRP and β-CGRP. These substances were added to EDTA whole blood samples containing commercially available Procalcitonin antigen at two concentrations. Percent cross-reactivity was calculated as: *[(test result – control result)/(cross-reacting substance concentration)] x 100%,* with an acceptance criteria of ±20%.

Cross-Reacting Substance	Substance Concentration	PCT Result (ng/mL)		Cross-reactivity	PCT Result (ng/mL)		Cross-reactivity
		Control	+ Sub		Control	+ Sub	
Catacalcin	5 ng/mL	0.62	0.72	1.83%	9.63	9.03	-12.00%
Katacalcin	10 ng/mL	0.62	0.81	1.90%	10.7	9.76	-9.15%
α-CGRP	30 ng/mL	0.62	0.64	0.05%	10.7	9.59	-3.61%
β-CGRP	30 ng/mL	0.62	0.62	-0.03%	10.7	9.79	-2.95%

Expected Values

The upper reference limit (URL) of the RAMP Procalcitonin test was determined in EDTA anti-coagulated whole blood as the 95th percentile of the normal range from a healthy reference population.

A total of 125 healthy individuals (66 males, 59 females), 18 years of age and older, were enrolled in the study. An EDTA whole blood sample was collected from each subject and tested within 2 hours of collection. Nonparametric analytical methods were used for data analysis, as outlined in CLSI document *C28-A3c* (2010).

The 95th percentile was found to be 0.36 ng/mL (95% CI: 0.25 to 0.98 ng/mL), with no significant differences between gender, age or race.

Conclusion

RAMP Procalcitonin demonstrated robust analytical performance for the quantification of procalcitonin. Detection limits, linearity, hook effect, repeatability and total precision, interference and cross-reactivity, and expected values (reference range; 95th percentile) were determined for the RAMP Procalcitonin test based on methods outlined in applicable CLSI guidelines.

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