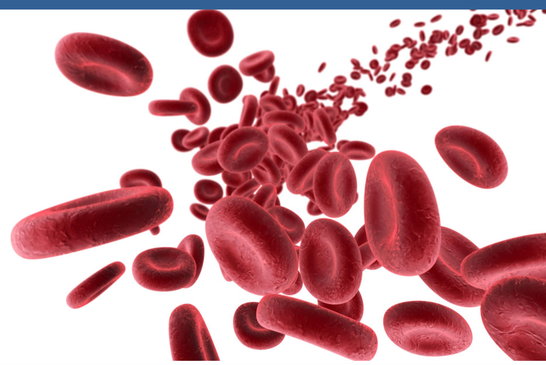
Determination of Analytical Performance Characteristics of the RAMP® D-dimer Test

J.F. Wilson, S. Bie, P. Tan, V. Ng, E. Siu, M.L. Zaharik

Response Biomedical Corp. Vancouver, Canada



Introduction

The RAMP® D-dimer test is a quantitative immunochromatographic test for the determination of the fibrinogen degradation product (FDP) D-dimer in human EDTA anti-coagulated whole blood. D-dimer is considered to be a marker of coagulation activation and is present in the circulation as part of the normal wound healing process. It is also valuable as a diagnostic marker for Disseminated Intravascular Coagulation (DIC) and as an aid to the rule-out of Venous Thromboembolism (VTE), a spectrum of diseases that include Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE). The objective of these studies was to determine the analytical performance characteristics of the RAMP D-dimer test.

Detection Limits

Detection Limits of the RAMP D-dimer test were determined based on methods outlined in EP17-A Volume 24, Number 34 *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline – First Edition* (ISBN1-56238-551-8). The determined values as well as a diagram of the results distribution for 60 replicates of each the LoB and LoD samples is presented below in Figure 1.

Limit of Blank (LoB) = 51 ng/mL FEU

Limit of Detection (LoD) = 89 ng/mL FEU

Limit of Quantitation (LoQ) 20% = 419 ng/mL FEU
10% = 839 ng/mL FEU

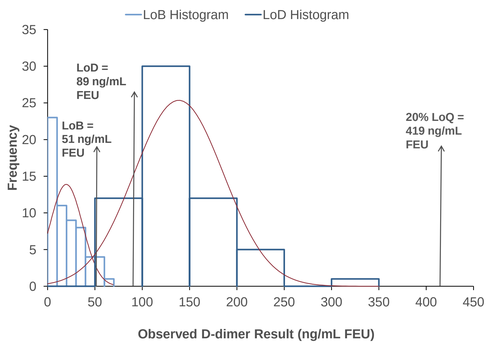


Figure 1. Distribution of Blank and Low Positive Sample Replicates

Linearity

The RAMP D-dimer Test was evaluated for linearity based on methods described in *EP6-A Volume 23, Number 16 Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline – (ISBN1-56238-498-8)* on three lots of test materials using whole blood samples containing commercially available D-dimer antigen at concentrations up to 6500 ng/mL FEU. 11 dilutions of a high concentration sample were prepared and tested at a minimum of 4 replicates. A linearity plot of analytical result (y) versus sample concentration (x) is presented in Figure 2. Linearity of the RAMP D-dimer test was demonstrated between 100 and 5000 ng/mL FEU, thus defining the analytical measurement range.

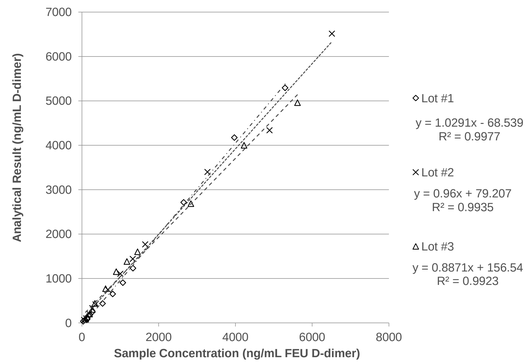


Figure 2. Linearity Plot for Three Lots of RAMP D-dimer Tests

Hook Effect

The RAMP D-dimer Test was evaluated for hook effect on three lots of test materials using whole blood samples containing commercially available D-dimer antigen at concentrations up to 250,000 ng/mL FEU. No evidence of high dose hook effect was observed; samples with estimated concentrations above the upper limit of the test reported >5000 ng/mL FEU on the RAMP instrument, as shown in Figure 3 (values >5000 ng/mL FEU are represented as 5000 ng/mL).

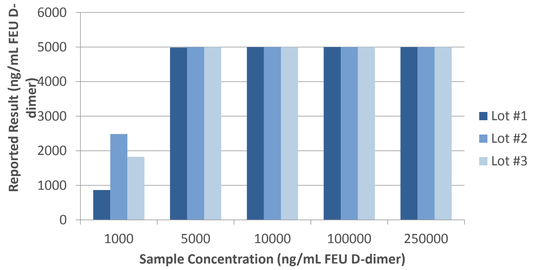


Figure 3. Hook Effect Results

Repeatability and Total Precision

Repeatability and total precision were determined based on methods outlined in *EP5-A2–Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline–Second Edition (ISBN 1-56238-542-9)* by testing three levels of frozen plasma control materials in duplicate, twice per day for 12 days on three lots of RAMP D-dimer tests. Repeatability and total precision were also determined for whole blood samples by testing three levels in triplicate, in five runs over three days on three lots of RAMP D-dimer test.

Sample Type	[D-dimer] ng/mL FEU	Repeatability	Total Precision
Plasma Controls	363	6.6%	8.5%
	656	5.4%	6.3%
	4044	6.5%	6.9%
Whole Blood	174	19.7%	22.6%
	465	10.3%	12.3%
	2753	7.8%	10.0%

Interference

Potentially interfering substances were evaluated in the D-dimer test based on methods outlined in *EP7-A2 - Interference Testing in Clinical Chemistry; Approved Guideline–Second Edition (ISBN 1-56238-584-4)*. Interferents were added to EDTA whole blood samples containing commercially available D-dimer antigen at two concentrations. No interference was observed as the result of 6 common endogenous interferents (hemoglobin, bilirubin (conjugated), bilirubin (unconjugated), cholesterol, triglycerides, or gamma (γ)-globulins) or 6 common anticoagulants (acetylsalicylic acid (ASA), clopidogrel, heparin, ibuprofen, plasminogen (human) or warfarin), as well as 45 common pharmaceutical compounds (tested at 3 x MRTD, data not shown).

Interferent	Interferent Concentration	D-dimer Result (ng/mL FEU)		Interference (%)	D-dimer Result (ng/mL FEU)		Interference (%)
		Control	Interferent		Control	Interferent	
Endogenous Interferents							
Hemoglobin	200 mg/dL	383	357	-6.7	2762	2696	-2.4
Bilirubin (conjugated)	5 mg/dL	383	384	0.4	2762	2873	4.0
Bilirubin (unconjugated)	15 mg/dL	367	362	-1.5	2924	2914	-0.3
Cholesterol	500 mg/dL	438	388	-11.5	3312	3265	-1.4
Triglycerides	500 mg/dL	438	400	-8.7	3312	3246	-2.0
Gamma (γ)-globulins	60 mg/dL	383	352	-7.9	2762	2606	-5.6
Anticoagulants							
ASA	4.00 mg/mL	543	544	0.2	3099	3076	-0.7
Clopidogrel hydrogen sulfate	0.25 mg/mL	270	250	-7.4	1980	1926	-2.7
Heparin Sodium Salt	0.03 mg/mL	349	344	-1.5	2943	2823	-4.1
Ibuprofen	0.35 mg/mL	543	568	4.7	3099	3419	10.3
Plasminogen	1.00 mg/mL	432	405	-6.2	3118	3496	12.1
Warfarin	0.015 mg/mL	396	418	5.5	3195	3132	-2.0

Cross-Reactivity

Potentially cross-reacting substances Fibrinogen, Fragment D and Fragment E were evaluated in the D-dimer test with reference to methods outlined in *EP7-A2.* Cross-reacting substances were added to EDTA whole blood samples containing commercially available D-dimer antigen at two concentrations. No cross reactivity was observed as the result of Fibrinogen (1 mg/mL), but there was evidence of cross-reactivity for both Fragment D and Fragment E at 20 µg/mL.

Dose-response evaluation of Fragment D and Fragment E was performed in order to characterize the observed cross-reactivity. Fragment D showed a bias of 0.009 ng/mL per unit at ~500 ng/mL FEU D-dimer and a bias of 0.030 ng/mL per unit at ~3000 ng/mL FEU D-dimer as shown in Figure 4 below. Cross-reactivity bias observed with Fragment E was not statistically significant; however, elevated levels of either Fragment D and Fragment E, as may be present during thrombolytic therapy, may lead to elevated measurement values.

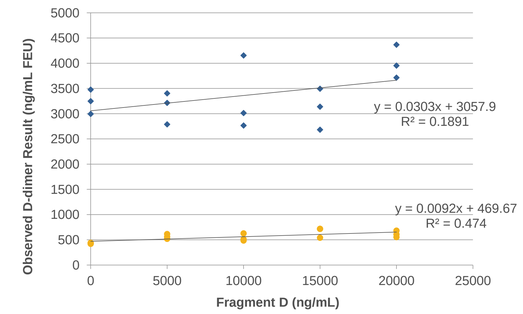


Figure 4. Cross-Reactivity Bias Determination of Fragment D

Conclusion

The RAMP D-dimer test demonstrated acceptable analytical performance for the quantification of D-dimer, based on methods outlined in applicable CLSI guidelines.