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Comprehensive Overview of Preliminary Interference Data for a New D-dimer Assay

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Background

D-dimer is a soluble fibrin degradation product generated during the regulated breakdown of cross-linked fibrin by the fibrinolytic system and represents a key biomarker in hemostasis. Measurement of D-dimer is of major importance in clinical diagnostics, particularly for the assessment of thrombotic events, such as deep vein thrombosis (DVT) and pulmonary embolism (PE). The accuracy of D-Dimer assay results, especially at the cutoff level, is crucial for diagnosis. Specimen-specific inaccuracy due to the presence of interfering substances could be interpreted as a change in patient condition.[1,2] Consequently, understanding the interfering potential of endogenous and exogenous substances is of substantial importance.

The INNOVANCE D-Dimer 2.0 assay* (Siemens Healthineers) is an automated, quantitative latex-enhanced immunoassay employing liquid, ready to use reagents and controls. In this study, we investigated the impact of potentially interfering substances on its analytical performance.

Methods

INNOVANCE D-Dimer 2.0 assay interference was evaluated using the Automated Blood Coagulation Analyzer CS-5100 System (CS-5100) according to the CLSI EP07-ED3 guideline.[3] In addition, interference related to hemolysis, icterus, or lipemia (HIL), as well as other endogenous and exogenous interferents were evaluated using the Automated Blood Coagulation Analyzer CN-3000, CN-6000, CS-2500 and CA-660 Systems. The INNOVANCE D-Dimer 2.0 assay includes liquid and ready-to use reagents and controls (three levels at approx. 0.4, 0.8, 3.0 mg/L Fibrinogen Equivalent Unit [FEU]), as well as a lot-specific plasma-based calibrator to be used for the quantitative determination of D-dimer. The measuring interval for the CS-5100 System is 0.19 to 7.5 mg/L FEU and can be extended to 80 mg/L FEU by auto-redilution.

- Interference studies were conducted using four contrived plasma samples containing D-dimer spanning the 0.19–7.5 mg/L FEU measuring interval—one normal, one cut-off, and two pathological levels. For paired difference testing, each sample was used to prepare a 0% and a 100% interferent test pool. See Tables 1 and 2 for study exceptions.
- For the paired difference analyses, primary evaluation was per the CLSI EP07-ED3 guideline using paired differences of high and low pools (both absolute and relative values), including standard deviations and 90% 2-sided confidence intervals of mean differences (checked to fall within ±10% of the mean values obtained with the low pools).
- Additional testing using a 5- or 11-level dose response method per the CLSI guideline is noted in Tables 1 and 2.
- For dose response testing, differences to the regression intercept were plotted vs. interferent concentration results and the maximum allowable interferent concentration was defined as the intersection of the confidence limits with the allowable bias.
- The acceptance criterion for HIL interferents was an absolute difference of ± 0.070 mg/L FEU for the range of 0.270 to ≤ 0.600 mg/L FEU, or a relative difference of ±15 % within the D-dimer concentration range of >0.600 to ≤ 7.500 mg/L FEU. For other endogenous and exogenous interferents, the acceptance criterion was a relative difference of ± 10% for the range of 0.190 to ≤ 7.500 mg/L.

Table 1. Testing specifications for endogenous interferents.

	Interferent	Analysis	Samples (n) or levels	Replicates (n)	Reagent lots (n)	Analyzers (n)
HIL[a]	Hemoglobin, Conjugated bilirubin, Unconjugated bilirubin	Paired[b]	4	4	1	1
	Lipids (Intralipid[c])	Paired	4	4	1	1
		Dose response[d]	4 samples, 11-levels each[e]	5	1	1
Other	Creatinine, Cholesterol, Urea, Uric acid	Paired	4	4	1	1
	Albumin, Immunoglobulin G (IgG)	Dose response	4 samples, 5 levels each[f]	5	1	1

a. Hemolysis, icterus, and lipemia; b. Paired difference per CLSI EP07-ED3 guideline; c. Intralipid Fresenius Kabi, Toronto, CA; d. Dose response testing was done using a CS-5100 analyzer; e. The 11 interferent levels are 0%, 2%, 4%, 7%, 10%, 15%, 20%, 40%, 60%, 80%, and 100; f. The 5 interferent levels are 0%, 25%, 50%, 75%, and 100%.

Table 2. Testing specifications for exogenous interferents.

Interferent	Analysis	Samples (n) or levels	Replicates (n)	Reagent lots (n)	Analyzers (n)
40 OTC drugs[a,b]	Paired	4	5	1	1
Dextran 40, Valproic acid	Dose response	4 samples, 5 levels each[c]	5	1	1

a. Over-the-counter medications were investigated at concentrations exceeding the highest respective therapeutic range recommended for their specific intended-use population(s); b. The specific drugs are listed in Table 4; c. The 5 interferent levels are 0%, 25%, 50%, 75%, and 100%.

Human anti-mouse antibodies (HAMAs) and Rheumatoid Factors (RF)

- **HAMA samples:** 20 negative control specimens (0 ng/mL) and 20 positive test specimens (100.1–901.2 ng/mL HAMA) containing D-dimer spanning the 0.19–7.5 mg/L FEU measuring interval.
- **RF samples:** 10 negative control specimens (0 IU/mL) and 10 positive test specimens (554–2141 IU/mL RF) spanning the 0.19–7.5 mg/L FEU measuring interval.
- For both HAMA and RF testing, additional treatment of a separate aliquot of each sample with a heterophilic blocking tube as a reference method.
- **Testing method:** patient calculations per the CLSI guideline.
- **HAMA testing scheme:** two reagent lots on two analyzers, four replicates per each test and reference method sample.
- **RF testing scheme:** one reagent lot on one analyzer, four replicates per each test- and reference method sample.
- For both HAMA and RF, the bias between the interferent-positive sample (test and the interferent-positive sample treated with HBT [reference method]) determined the level of HAMA and RF interference.

Results

For paired difference analyses, interference was determined as the difference between high (100% interferent) and low (0% interferent) pools. The maximum deviation and respective acceptance criteria are reported as absolute (mg/L FEU) or relative (%) values depending on the level of analyte investigated.

Table 3. For HIL and other endogenous interferents, the lowest allowable result at which no interference of the INNOVANCE D-Dimer 2.0 assay was observed is listed along with the highest concentration tested for each interferent.

Endogenous interfering substance	Unit	Highest concentration tested	Lowest allowable result
Hemoglobin	mg/dL	1000	1000
Bilirubin, unconjugated	mg/dL	60	60
Bilirubin, conjugated	mg/dL	40	40
Lipids (Intralipid)	mg/dL	300	300
Lipids (Intralipid) on CS-5100	mg/dL	2000	400
Lipids (Intralipid) on CA-660	mg/dL	400	400
Creatinine	mg/dL	75	75
Cholesterol	mg/dL	379	379
Urea	mg/dL	500	500
Uric acid	mg/dL	31	31
Albumin	g/dL	6.0	5.8
Immunglobulin G (IgG)	g/dL	5.0	3.3

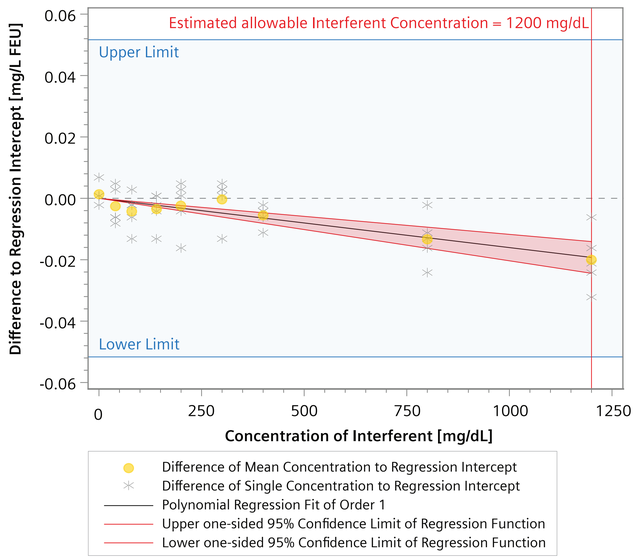

Figure 1. Example of a scatter plot used to interpret dose response analyses. For the Intralipid 11-level dose response analysis, the maximum allowable interferent concentration (2000 mg/dL) for the cutoff pool measured on the CS-5100 System was determined by the intersection of the confidence limits with the allowable bias (red bar). Note: only nine levels shown; the two highest levels reported no results due to excessive turbidity.

Exogenous Interference

Table 4. For over-the-counter-drugs and prescription drugs, the lowest allowable result at which no interference of the INNOVANCE D-Dimer 2.0 assay was observed is listed along with the highest concentration tested for each interferent.

Drug class	Exogenous interfering substance	Unit	Highest concentration tested	Lowest allowable result
Analgesics / anti-inflammatory / common OTC agents	Acetaminophen	mg/dL	20	20
	Acetylsalicylic acid	mg/dL	60	60
	Ibuprofen	mg/dL	50	50
	Ascorbic acid	mg/dL	5.3	5.3
	Caffeine	mg/dL	11	11
	Ethanol	mg/dL	600	600
Antibiotics / anti-infectives	Amikacin	mg/dL	15	15
	Ampicillin	mg/dL	7.5	7.5
	Gentamycin	mg/dL	12	12
	Penicillin G	U/mL	25	25
	Chloramphenicol	mg/dL	7.8	7.8
	Erythromycin	mg/dL	13.8	14
Anticoagulants / antithrombotics	Enoxaparin	IU/mL	15	15
	Heparin, sodium	IU / mL	3.3	3.3
	Warfarin	mg/dL	11	11
	Apixaban	ng/mL	990	990
	Rivaroxaban	ng/mL	1250	1250
	Edoxaban	ng/mL	1560	1560
	Dabigatran	ng/mL	900	900
Drug class	Exogenous interfering substance	Unit	Highest concentration tested	Lowest allowable result
Antiseizure medications / CNS drugs / sedatives	Carbamazepine	mg/dL	4.5	4.5
	Phenobarbital	mg/dL	69	69
	Phenytoin	mg/dL	6.0	6.0
	Primidone	mg/dL	5.7	5.7
	Diazepam	mg/dL	3.0	3.0
	Valproic acid	mg/dL	50	19
	Ethosuximide	mg/dL	30	30
	Lidocaine	mg/dL	1.5	1.5
	Theophylline	mg/dL	6.0	6.0
	Digoxin	ng/mL	39	39
Cardiovascular drugs (non-anticoagulant)	Atorvastatin	mg/L	0.76	0.76
	Furosemide	mg/dL	6.0	6.0
	Captopril	mg/dL	20	20
	Eplerenone	mg/dL	0.03	0.03
	Losartan	mg/mL	0.09	0.09
	Propranolol	mg/dL	0.50	0.50
	Verapamil	mg/mL	0.288	0.29
Metabolic / endocrine / lifestyle compounds / Immuno-suppressant / Plasma expanders	Cyclosporin A	mg/dL	35	35
	Metformin	mg/dL	1.2	1.2
	Nicotine	mg/dL	0.10	0.10
	Lithium chloride	mg/dL	2.3	2.3
	Dextran 40	mg/dL	2400	1200
	Cimetidine	mg/dL	3.0	3.0

Table 5. The highest concentration tested for HAMA and RF and the lowest allowable result at which no interference of the INNOVANCE D-Dimer 2.0 assay was observed.

Interfering substance	Unit	Highest concentration tested	D-dimer concentrations in sample	Lowest allowable result
HAMA	ng/mL	901.19	up to 0.7353 mg/L FEU	896.44
			up to 6.8575 mg/L FEU	901.19
RF	IU/mL	2141	up to 0.7048 mg/L FEU	1246.93
			up to 6.9233 mg/L FEU	2141

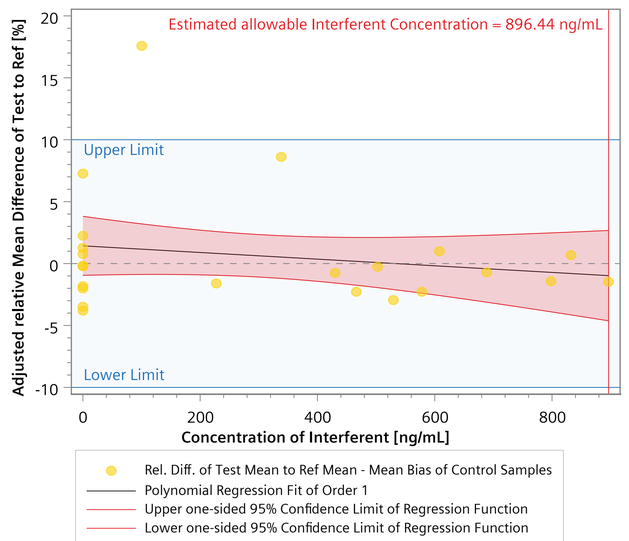

Figure 2. Example of a scatter plot using patient calculations: Analysis of HAMA positive plasma samples containing D-dimer up to 0.7353 mg/L FEU with the INNOVANCE D-Dimer 2.0 assay on CS-5100 System. The maximum allowable interferent concentration (896.44 ng/mL) is defined as the intersection of the confidence limits with the allowable bias (red bar).

Conclusion

Overall, the INNOVANCE D-Dimer 2.0 assay demonstrated robust performance across a wide range of potentially interfering substances. The assay provides reliable results for the quantitative determination of D-dimer.

References

1. *Weitz JI, et al. A Test in Context: D-Dimer. J Am Coll Cardiol.2017;70(19):2411-20. DOI 10.1016/j.jacc.2017.09.024*
2. *Tripodi A. D-dimer testing in laboratory practice. Clin Chem. 2011;57(9):1256-62. DOI 10.1373/clinchem.2011.166249*
3. *The CLSI EP07-ED3 guideline can be found on the CLSI website: https://clsi.org*

** INNOVANCE D-Dimer 2.0 assay is pending 510(k) clearance and not yet commercially available in the United States or other countries. Its future availability cannot be guaranteed.*

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