Preliminary analytical performance data of a new D-dimer assay

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Introduction

D-dimer is a global indicator of coagulation activation and fibrinolysis and an indirect marker of thrombotic activity. The major diagnostic application of D-dimer testing lies in the exclusion of thromboembolic events, such as deep vein thrombosis (DVT) or pulmonary embolism (PE) and has been implemented into corresponding guidelines. The INNOVANCE D-Dimer 2.0 assay for the quantitative, non-standardized determination of D-dimer with liquid, ready to use reagents and controls was investigated for its key analytical performance characteristics on a variety of coagulation analyzers.

Methods

Analytical performance of the INNOVANCE D-Dimer 2.0 assay (Siemens Healthineers) was evaluated on the CN-3000/6000, CS-5100, CS-2500, and CA-660 systems.

Linearity (CLSI EP06-ED2)

- Measuring intervals: 0.19 to 7.5 mg/L FEU (extendable to 80 mg/L FEU by auto-redilution) for CN/CS systems; 0.27 to 7.5 mg/L FEU (extendable to 80 mg/L FEU) for the CA-660 system.
- Plasma samples were prepared with final concentrations across as well as below and above the measuring interval. Testing was performed with three reagent lots, two analyzers of each system, two runs/day, and four replicates/run.
- Comparison of deviations between measured and predicted values against predefined acceptance criteria to define the reportable linear range (Figure 1).

Detection Capability (CLSI EP17-A2)

- Five samples specific to the individual study, four replicates/sample, one run/day for three days, three reagent lots for a total of 180 measurements.
- Limit of Blank (LoB): Highest measurement result that is likely to be observed on a blank, analyte free sample with 95% probability (Table 1).
- Limit of Detection (LoD): Lowest concentration reliably detected for presence or absence of an analyte. LoD = lowest concentration detectable with 95% probability using a parametric analysis (classical approach), based on pooled SD across lots. The maximum lot-specific LoD was selected (Table 1).
- Limit of Quantitation (LoQ): Lowest concentration of analyte that can be quantitatively determined with samples at or below the intended lower measuring limit using total error (TE) calculations (TE = IBiasI + 2 SD). The lowest concentration meeting TE criteria defined the LoQ, with the highest lot-specific value reported (Table 1).

Precision (CLSI EP05-A3)

- Plasma pools (n=5) and INNOVANCE D-Dimer 2.0 Controls (n = 3) were tested with three reagent lots, ≥ 3 operators, 20 days, 2 runs/day, 2 replicates/run (n = 240 for each sample tested).
- To mimic routine, random access conditions, other samples were interspersed between precision samples.
- Precision estimates (Table 2) were generated using a three-factor nested analysis of variance (ANOVA).

Method Comparison (CLSI EP09c)

- Patient plasma samples (n ≥ 349) collected at clinical study sites in the United States of America (USA) and Germany representing the intended use population, including individuals with suspected or known DVT, PE, and DIC.
- Passing-Bablok regression and Bland-Altman plot for INNOVANCE D-Dimer 2.0 on CS-5100 versus CN-6000 are shown in Figure 2. Slopes, intercepts, and correlation coefficients for all comparisons are provided in Table 3.

Results

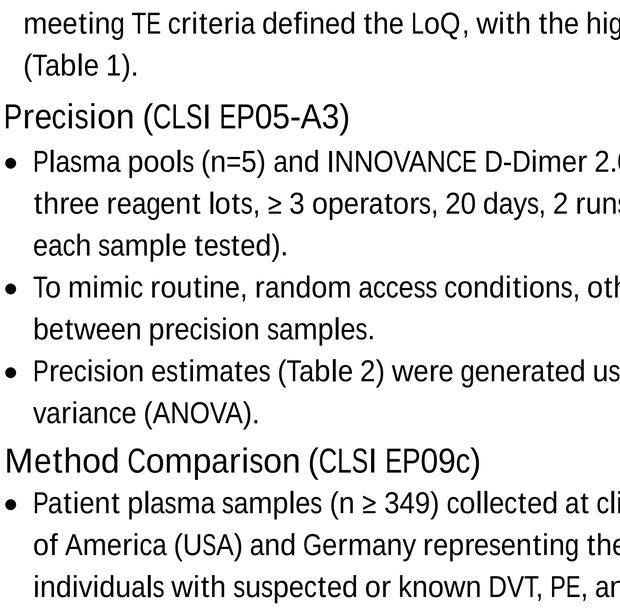

Figure 1. Linearity evaluation for the CN-6000 system. Regression equation: y = 0.98077x determined with weighted linear regression (weight = *R/SD2*; *R* = number of replicates).

For the CN/CS systems across three assay kit lots, the lower end of the evaluated linearity varied between 0.141 and 0.187 mg/L FEU (CA-660: 0.22 - 0.24 mg/L FEU) and the higher end between 81.329 and 87.608 (CA-660: 83.11 - 90.23 mg/L FEU).

Table 1. LoB, LoD, and LoQ results.

System	LoB (mg/L FEU)	LoD (mg/L FEU)	LoQ (mg/L FEU)	TE (mg/L FEU)
CN-6000	0.0217	0.0353	0.1270	0.03230
CN-3000	0.0210	0.0389	0.1342	0.04489
CS-5100	0.0277	0.0416	0.1345	0.04687
CS-2500	0.0200	0.0351	0.1240	0.04425
CA-660	0.090	0.120	0.166	0.0833

Detection capability studies support the lower limits of measuring intervals of 0.190 mg/L FEU for CN/CS systems and 0.27 mg/L FEU for the CA-660 System.

Table 2. INNOVANCE D-Dimer repeatability and within-device precision.

System	Sample	Mean (mg/L FEU)	Repeat-ability CV (%)	Within-Device CV (%)
CN-6000	P1	0.237	3.1	3.9
	P2	0.555	1.3	1.6
	P3	6.199	1.4	1.8
	P4	25.455	0.6	1.2
	P5	62.254	0.9	1.4
	C1	0.350	2.7	3.0
	C2	0.760	0.9	1.4
	C3	3.116	0.9	1.5
CN-3000	P1	0.247	2.7	2.9
	P2	0.561	1.2	1.7
	P3	6.255	1.8	2.2
	P4	25.411	0.9	1.2
	P5	61.926	1.0	1.4
	C1	0.358	1.6	2.3
	C2	0.774	0.8	1.5
	C3	3.210	1.1	1.5
CS-5100	P1	0.244	2.9	3.0
	P2	0.551	1.0	1.3
	P3	6.239	1.7	2.0
	P4	25.827	1.0	1.3
	P5	64.076	1.5	1.9
	C1	0.352	1.7	1.9
	C2	0.764	0.7	1.2
	C3	3.159	1.1	1.4
CS-2500	P1	0.254	1.9	2.2
	P2	0.538	1.0	1.3
	P3	5.887	1.3	1.9
	P4	27.849	1.2	1.6
	P5	62.926	0.9	1.2
	C1	0.353	1.4	1.8
	C2	0.755	0.7	1.3
	C3	3.157	1.0	1.2
CA-660	P1	0.30	4.9	5.0
	P2	0.56	2.7	2.9
	P3	6.62	3.0	3.3
	P4	23.05	2.9	3.3
	P5	63.99	5.0	5.4
	C1	0.41	2.9	3.2
	C2	0.81	1.3	1.8
	C3	3.09	2.2	2.8

P, Plasma sample; C, INNOVANCE D-Dimer 2.0 Control.

For the CN/CS systems, repeatability was ≤3.1% CV and within-device/lab was ≤3.9% CV. For the CA-660 system, repeatability was ≤5.0% CV and within-device/lab CV was ≤5.4%.

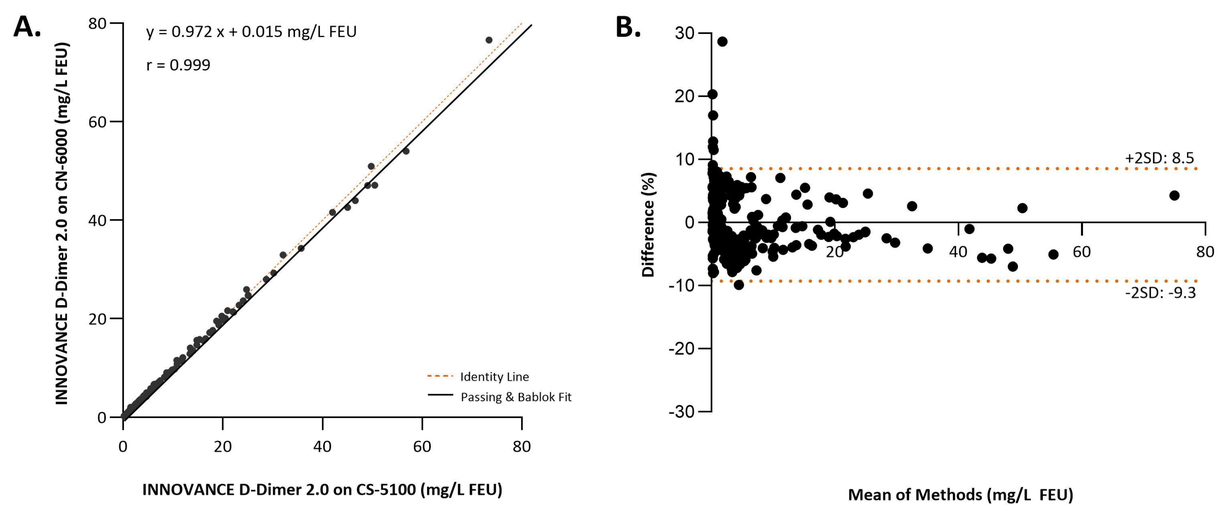

Figure 2. Method comparison (A) and Bland-Altman plot (B) for INNOVANCE D-Dimer 2.0 assay on CN-6000 System versus INNOVANCE D-Dimer 2.0 assay on CS-5100 System.

Table 3. Method comparison results.

Method 2 (y-axis)		Method 1 (x-axis)	n	Slope	Intercept (mg/L FEU)	Correlation Coefficient (r)
CN-6000	vs.	CS-5100	349	0.972	0.015	0.999
CN-3000	vs.	CS-5100	349	1.003	0.013	0.999
CS-2500	vs.	CS-5100	350	0.989	0.000	0.999
CA-660	vs.	CS-5100	336	0.977	0.025	0.995

Conclusions

The INNOVANCE D-Dimer 2.0 assay applications demonstrate strong analytical performance and high comparability across all evaluated analyzers. These results confirm the assay's suitability for the quantitative measurement of D-dimer concentrations in daily routine.

The INNOVANCE D-Dimer 2.0 assay mentioned herein is not commercially available in all countries. Currently it is under FDA's 510(k) review. Its future availability cannot be guaranteed.

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