ASSAY MIGRATION STUDIES ON THE BECKMAN COULTER DXI 9000 ACCESS IMMUNOASSAY ANALYZER†

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BACKGROUND

The U.S. FDA's guidance for industry and staff titled "Assay Migration Studies for In Vitro Diagnostic Devices"[1] provides a least burdensome approach for the transfer of previously-approved assays from an existing to a new system. This approach enables use of rigorous analytical performance data in place of full clinical data to implement a cleared product on a new platform. The Beckman Coulter DxI 9000 Access Immunoassay Analyzer† includes numerous updates and new features designed to improve laboratory workflows and provide quality results to support patient management. Such elements include improved pipetting capabilities, updated process monitoring, increased throughput, reliability enhancements, and software features focused on the needs of the operator. The analyzer also utilizes a new alkaline phosphatase substrate reagent that provides reduced time-to-result for every test as well as other benefits including improved signal-to-noise and reduced sensitivity to endogenous alkaline phosphatase interference. The existing menu of Beckman Coulter Access reagents is being transferred to this system.

Purpose: Data herein summarize results from analytical studies described within the assay migration guidance and obtained during verification testing of assays for high sensitivity cardiac troponin I (hsTnI) and alpha-fetoprotein (AFP) on the DxI 9000 Access Immunoassay Analyzer. Analytical studies for quantitative assays were performed as directed by the assay migration guidance to compare performance of the Access hsTnI and Access AFP assays across the existing Access 2 and new DxI 9000 systems.

METHODS

Reproducibility

A multi-site study was performed to evaluate the reproducibility of the Access hsTnI and Access AFP assays on the DxI 9000 and Access 2 systems using a protocol based on CLSI EP05-A3[2].

Reproducibility was evaluated on three DxI 9000 & three Access 2 instruments across three external clinical laboratories. Both serum and lithium heparin plasma samples spanning the range of the assay were measured for hsTnI, while serum samples were tested for AFP. Samples were tested in replicates of three (3) per run with two (2) runs per day over five (5) days on each of the three (3) instruments across the three (3) testing sites. Reproducibility was evaluated using three (3) different reagent pack lots and one calibrator lot. QC were tested daily.

Comparison Study

A method comparison study was completed to compare the Access hsTnI assay on DxI 9000 to the Access hsTnI assay on the Access 2 Immunoassay Analyzer for both serum and plasma sample types. A method comparison study was completed to compare the Access AFP assay on DxI 9000 to the Access AFP assay on the Access 2 Immunoassay Analyzer for serum. Each study used a protocol based on CLSI EP09C-ED3[3] and the Assay Migration guidance[1]. Method comparison studies were performed on three DxI 9000 Access Immunoassay Analyzers and three Access 2 instruments at three external laboratories.

>240 serum samples and >180 lithium heparin plasma samples containing hsTnI concentrations spanning the analytical measuring range of the assay were tested. >200 serum samples containing AFP concentrations spanning the analytical measuring range of the assay were tested. All samples were tested in replicates of one on each of three (3) reagents lots across the three external sites. Each reagent lot was tested at different sites on separate DxI 9000/Access 2 pairs. QC were tested daily

The Assay Migration guidance document provides instructions to calculate an "allowable total difference" or ATD zone, for which approximately 95% of individual sample differences are expected to fall (see Figure 1).

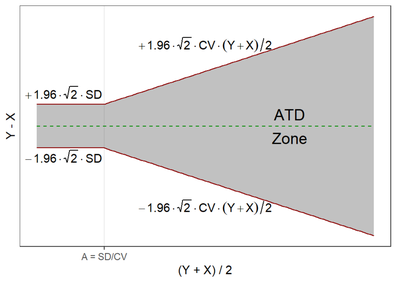


Figure 1 Allowable Total Difference (ATD) Zone Illustration – image adapted from Assay Migration guidance document[1].

Imprecision

Studies were performed to assess the imprecision of the Access hsTnI assay and the Access AFP assay on the DxI 9000 system using a protocol based on CLSI EP05-A3[2].

Each study was run on three DxI 9000 Immunoassay systems, three reagent lots and three calibrator lots. Both serum and lithium heparin plasma samples spanning the range of the assay were measured for hsTnI, while serum samples were tested for AFP. Each sample was tested in replicates of two per run for hsTnI and three per run for AFP. Two runs per day were completed over 20 days on each instrument and reagent lot combination. Three commercial quality controls were run in duplicate for each assay on each day to verify the system was in control.

Detection Capability

Studies were performed to determine the Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) for the Access hsTnI assay and Access AFP assay using a protocol based on CLSI EP17-A2[4].

For the estimation of LoB, three DxI 9000 Immunoassay Systems were used in the study design with three reagent lots and one calibrator lot. Four S0 calibrator preparations for each respective assay were used for the LoB determination. Blank samples were tested over three days one run per day, five replicates per run, for each pack lot.

For estimation of LoD and LoQ, three DxI 9000 Immunoassay Systems were used in the study design with three reagent lots and one calibrator lot. Both serum and lithium heparin plasma samples containing low levels of analyte were measured for hsTnI, while serum was tested for AFP. Samples were tested in replicates of nine per run with one run per day and five total days on each pack lot and instrument. This resulted in a minimum of 40 replicates for each sample on each pack lot tested.

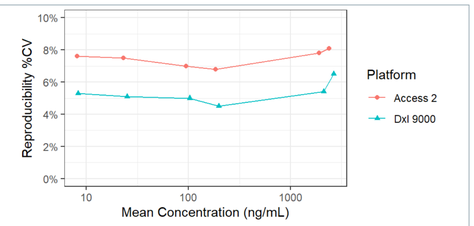
Three quality controls were run in replicates of two for each assay on each day to verify the systems were in control.

Linearity

Studies were performed to assess the linearity of the Access hsTnI assay and the Access AFP assay on the DxI 9000 Access Immunoassay Analyzer based on CLSI EP06-Ed2[5].

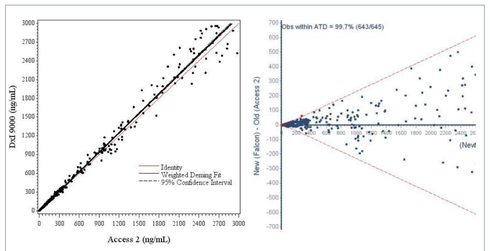
Samples covering the full analytical measuring range of each assay were used for the linearity determination. Both serum and lithium heparin plasma sample types were evaluated independently for hsTnI, while serum was tested for AFP. A native sample containing a concentration at the low end of the measuring interval was obtained. A high sample was prepared by spiking antigen into a low sample until a concentration above the highest commercial calibrator was achieved. In addition to the high and low concentration samples, seven mixtures were tested in this study. These samples were prepared independently by using incrementally larger proportions of the high sample diluted with the low sample, in order to achieve concentrations that covered the range of the assay. The low sample was run in replicates of eight, and all other samples were run in replicates of four. This study was run on one DxI 9000 Immunoassay System, using three reagent lots and one calibrator lot. Three quality controls were run in replicates of two for each assay on each day to verify the system was in control.

RESULTS – Access AFP



Sample	Access 2 N	Access 2 Mean (ng/mL)	Access 2 Reproducibility SD	Access 2 Reproducibility %CV	DxI 9000 N	DxI 9000 Mean (ng/mL)	DxI 9000 Reproducibility SD	DxI 9000 Reproducibility %CV
1	270	8.1	0.62	7.6%	269	8.3	0.43	5.3%
2	270	23	1.8	7.5%	268	25	1.3	5.1%
3	270	95	6.7	7.0%	268	103	5.1	5.0%
4	270	184	12.6	6.8%	269	199	9.1	4.5%
5	270	1,908	148.2	7.8%	268	2,117	114.4	5.4%
6	270	2,396	193.7	8.1%	266	2,662	172.1	6.5%

Figure 2 Reproducibility of the Access AFP assay was evaluated following EP05-A3 on both DxI 9000 and Access 2 including between-site, between-lot, between-day, between-run, and within-run variance components. DxI 9000 reproducibility was markedly improved compared to Access 2.



N	Range (ng/mL)	Slope [95% CI]	Intercept [95% CI]	R
645	1.2 - 2,976	1.04 [1.03, 1.05]	-0.12 [-0.16, -0.09]	0.994

Data Range	Obs Within ATD Zone [90% Bootstrap CI]
Full	99.7% [99.2% - 100%]
Low	100% [100% - 100%]
Medium	99.6% [98.9% - 100%]
High	99.5% [98.5% - 100%]

Figure 3 A quantitative comparison study was completed to compare the Access AFP assay on DxI 9000 to the Access AFP assay on Access 2. Regression analysis following EP09C-ED3 was completed in addition to comparison to Allowable Total Difference (ATD) zones prescribed within the Assay Migration guidance.

Concentration (ng/mL)			Repeatability (Within-run)		Between-run		Between-day		Within-Laboratory (Total)	
Sample	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	120	8.1	0.15	1.8	0.09	1.1	0.10	1.2	0.20	2.4
Sample 2	120	24	0.4	1.7	0.3	1.2	0.5	2.2	0.7	3.0
Sample 3	120	99	2.0	2.0	1.0	1.1	2.5	2.6	3.4	3.4
Sample 4	120	192	4.0	2.1	3.3	1.7	5.6	2.9	7.7	4.0
Sample 5	120	2006	55.5	2.8	29.3	1.5	36.3	1.8	72.5	3.6
Sample 6	120	2561	58.8	2.3	85.9	3.4	24.3	0.9	106.9	4.2

Figure 4 Access AFP assay imprecision was evaluated following EP05-A3 on DxI 9000 on each of three reagent lots. A representative reagent lot is shown for illustration; all lots yielded acceptable performance.

Study	ng/mL LoB	LoD	LoQ
1	0.08	0.22	0.13
2	0.09	0.24	0.19
3	0.16	0.23	0.20

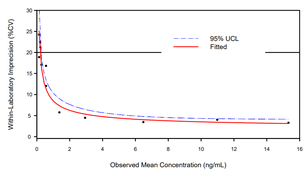
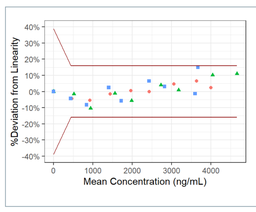


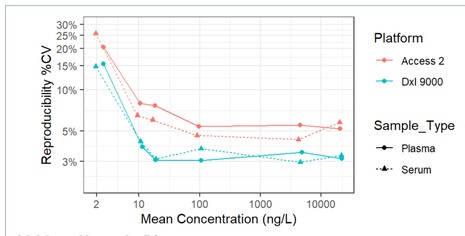
Figure 5 Access AFP assay LoB, LoD, and LoQ were evaluated following EP17-A2 on DxI 9000 on each of three reagent lots. A representative precision profile at low concentrations is shown. Assay detection capability was shown to be acceptable on DxI 9000.



% of High Sample	Approximate Mean Conc (ng/mL)	Maximum Nonlinearity
0.0	0.37	0.00 ng/mL
12.5	473	-4%
25.0	900	-10%
37.5	1,466	3%
50.0	1,887	-6%
62.5	2,530	7%
75.0	3,019	5%
87.5	3,782	15%
100.0	4,085	11%

Figure 6 Access AFP assay linearity was evaluated following EP06-Ed2 on DxI 9000 across 3 reagent lots. Acceptable nonlinearity was observed for each individual assessment.

RESULTS – Access hsTnI



Lithium Heparin Plasma

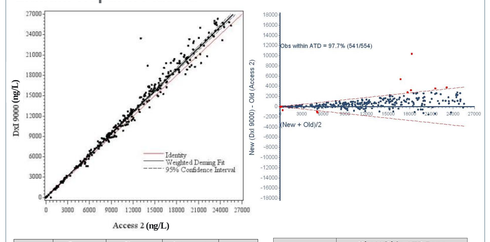
Sample	Access 2 N	Access 2 Mean (ng/L)	Access 2 Reproducibility SD	Access 2 Reproducibility %CV	DxI 9000 N	DxI 9000 Mean (ng/L)	DxI 9000 Reproducibility SD	DxI 9000 Reproducibility %CV
1	269	2.6	0.54	20.5%	270	2.6	0.40	15.5%
2	270	10.5	0.83	8.0%	270	11.3	0.43	3.8%
3	270	18.5	1.41	7.6%	270	18.9	0.58	3.1%
4	270	98.0	5.26	5.4%	270	105	3.2	3.0%
5	270	4,559	251.7	5.5%	270	4,846	169.5	3.5%
6	270	20,335	1,055.4	5.2%	270	22,045	691.5	3.1%

Serum

Sample	Access 2 N	Access 2 Mean (ng/L)	Access 2 Reproducibility SD	Access 2 Reproducibility %CV	DxI 9000 N	DxI 9000 Mean (ng/L)	DxI 9000 Reproducibility SD	DxI 9000 Reproducibility %CV
1	270	2.0	0.51	25.8%	270	1.9	0.29	14.8%
2	270	9.4	0.61	6.5%	270	10.6	0.44	4.2%
3	270	16.9	1.02	6.0%	270	19.2	0.59	3.1%
4	270	91.00	4.22	4.6%	270	105	3.9	3.7%
5	270	4,219	182.5	4.3%	270	4,548	134.4	3.0%
6	270	20,114	1,163.2	5.8%	270	21,567	712.0	3.3%

Figure 7 Reproducibility of the Access hsTnI assay was evaluated following EP05-A3 on both DxI 9000 and Access 2 including between-site, between-lot, between-day, between-run, and within-run variance components. Independent studies were completed for both serum and lithium heparin plasma. DxI 9000 reproducibility was markedly improved compared to Access 2.

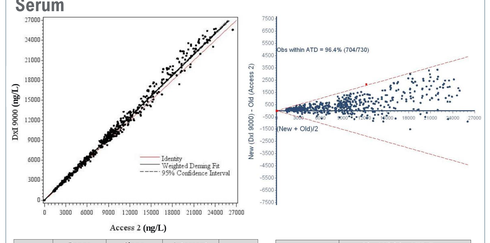
Lithium Heparin Plasma



N	Range (ng/L)	Slope [95% CI]	Intercept [95% CI]	R
566	2.0 - 25,496	1.05 [1.03, 1.06]	-0.48 [-0.66, -0.31]	0.996

Data Range	Obs Within ATD Zone [90% Bootstrap CI]
Full	97.7% [96.6% - 98.7%]
Low	99.2% [97.6% - 100%]
Medium	98.4% [96.9% - 100%]
High	96.7% [95.0% - 98.3%]

Serum



N	Range (ng/L)	Slope [95% CI]	Intercept [95% CI]	R
730	2.1 - 26,490	1.04 [1.04, 1.05]	-0.49 [-0.66, -0.32]	0.997

Data Range	Obs Within ATD Zone [90% Bootstrap CI]
Full	96.4% [95.2% - 97.5%]
Low	93.5% [89.8% - 97.2%]
Medium	85.4% [79.7% - 90.2%]
High	99.8% [99.4% - 100%]

Figure 8 A quantitative comparison study was completed to compare the Access hsTnI assay on DxI 9000 to the Access hsTnI assay on Access 2. Regression analysis following EP09C-ED3 was completed in addition to comparison to Allowable Total Difference (ATD) zones prescribed within the Assay Migration guidance. Study design criteria were met.

Lithium Heparin Plasma

Concentration (ng/L)			Repeatability (Within-run)		Between-run		Between-day		Within-Laboratory (Total)	
Sample	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	80	2.4	0.15	6.3	0.08	3.2	0.05	2.0	0.18	7.3
Sample 7	80	7.6	0.16	2.1	0.09	1.1	0.09	1.2	0.20	2.6
Sample 8	80	9.4	0.22	2.3	0.00	0.0	0.11	1.2	0.25	2.6
Sample 9	80	13	0.3	2.3	0.2	1.2	0.1	1.2	0.4	2.9
Sample 3	80	19	0.4	2.0	0.3	1.7	0.7	3.6	0.9	4.4
Sample 4	80	96	1.4	1.5	0.4	0.5	0.8	0.8	1.7	1.8
Sample 5	80	4,557	76.0	1.7	21.2	0.5	42.1	0.9	89.4	2.0
Sample 6	80	23,533	368.0	1.6	143.4	0.6	203.8	0.9	444.4	1.9

Serum

Concentration (ng/L)			Repeatability (Within-run)		Between-run		Between-day		Within-Laboratory (Total)	
Sample	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	80	1.0	0.15	14.8	0.09	8.5	0.00	0.0	0.17	17.1
Sample 2	80	9.6	0.26	2.7	0.11	1.1	0.12	1.2	0.31	3.2
Sample 3	80	24	0.6	2.3	0.2	0.7	0.2	1.0	0.6	2.6
Sample 4	80	88	1.3	1.4	0.8	0.9	1.6	1.8	2.2	2.5
Sample 5	80	4,889	62.4	1.3	75.9	1.6	92.6	1.9	135.0	2.8
Sample 6	80	22,963	404.1	1.8	556.7	2.4	1,211.4	5.3	1,393.2	6.1

Figure 9 Access hsTnI assay imprecision was evaluated following EP05-A3 on DxI 9000 on each of three reagent lots. Serum and lithium heparin plasma were evaluated individually. A representative reagent lot is shown for illustration; all lots yielded acceptable performance.

Study	ng/L LoB	LoD Plasma	LoD Serum	20% CV LoQ Plasma	20% CV LoQ Serum	10% CV LoQ Plasma	10% CV LoQ Serum
1	0.2	0.7	0.6	0.3	0.3	0.7	0.7
2	0.4	0.6	0.6	0.4	0.3	0.7	0.7
3	0.5	0.8	0.9	0.8	1.0	2.4	2

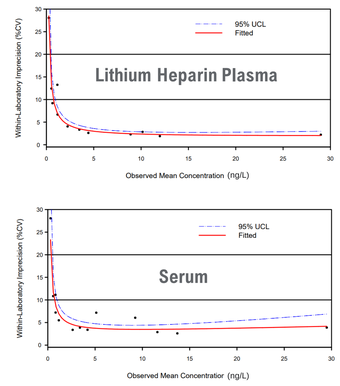
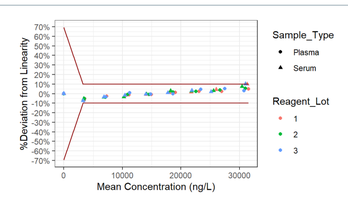


Figure 10 Access hsTnI assay LoB, LoD, and LoQ were evaluated following EP17-A2 on DxI 9000 on each of three reagent lots. Serum and lithium heparin plasma were evaluated individually. A representative precision profile at low concentrations is shown. Assay detection capability was shown to be acceptable on DxI 9000.



% of High Sample	Plasma Approximate Mean Conc (ng/L)	Plasma Maximum Nonlinearity	Serum Approximate Mean Conc (ng/L)	Serum Maximum Nonlinearity
0.0	1.46	0.00 pg/mL	1.03	0.00 pg/mL
12.5	3,501	-7%	3,312	-7%
25.0	7,183	-4%	6,831	-4%
37.5	11,106	-1%	10,374	-3%
50.0	14,752	-1%	14,078	-1%
62.5	18,772	1%	17,992	3%
75.0	23,018	5%	21,818	4%
87.5	27,046	5%	25,572	4%
100.0	31,100	5%	30,922	10%

Figure 11 Access hsTnI assay linearity was evaluated following EP06-Ed2 on DxI 9000 across 3 reagent lots. Independent studies were completed for serum and lithium heparin plasma. Acceptable nonlinearity was observed for each individual assessment.

CONCLUSION

Individual assay data generated for the Access hsTnI and Access AFP assays on the DxI 9000 Access Immunoassay Analyzer† including accuracy, imprecision, detection capability, and linearity met US FDA assay migration guidance study design criteria and demonstrate acceptable assay performance.

† Pending submission and clearance by the United States Food and Drug Administration; not yet available for in vitro diagnostic use in the US. For Investigational Use Only. The performance characteristics of this product have not been established.

In development, pending achievement of CE compliance; not available for in vitro diagnostic use.

Not available in all countries.

References

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