



DEVELOPMENT OF AN ASSAY FOR MEASUREMENT OF TOTAL IMMUNOGLOBULIN E (IGE) ON BECKMAN COULTER AU CHEMISTRY ANALYZERS

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BACKGROUND

IgE is a member of the immunoglobulin family of proteins that are produced by plasma cells in response to antigenic stimuli. IgE differs from the other immunoglobulins in certain structural aspects, its role in allergic diseases, and the extremely low normal levels of circulating IgE in comparison to other immunoglobulins. Elevated levels are commonly seen in cases of allergic diseases and other conditions. Measurement of serum total IgE aids in the diagnosis of IgE-mediated allergic disorders in conjunction with other clinical findings.

INTRODUCTION

An assay to measure total IgE in human serum and plasma was developed for use on AU chemistry analyzers*. Our development studies aimed to evaluate the performance of the Total IgE assay on AU chemistry analyzers.

*The assay is 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.

METHODS

Assay Methodology

Anti-IgE antibody-coated particles bind to IgE in the serum/plasma patient sample resulting in the formation of insoluble aggregates, causing turbidity. The amount of particle aggregation is directly proportional to the concentration of IgE in the sample. The assay was standardized to the 3rd WHO International Standard for human serum IgE.

Precision

Within-run and total imprecision were evaluated with human samples and IgE controls. An abbreviated CLSI EP05-A3 study determined within-run and total imprecision from recoveries of 40 replicates obtained in 20 runs over 10 days.

Linearity

High and low IgE human samples were inter-diluted to span the analytical range. Data was analyzed by linear regression per CLSI EP06-A.

Sensitivity

Four Limit of Blank, four Limit of Detection and four Limit of Quantitation human samples were evaluated in three runs over three test days. Data were analyzed per CLSI EP17-A2.

Methods Comparison

Sixty-eight (68) human samples were compared against a competitive method. Results were analyzed by weighted Deming regression analysis (CLSI EP09-A3).

On-board / Calibration Stability

On-board stability and calibration frequency were assessed by monitoring IgE recoveries over 29 days with calibration at days 0 and 15. On-board stability was analyzed by recovery difference or % difference against day 0 per CLSI EP25-A.

Interferences

High, mid and low human IgE samples were spiked with bilirubin, Intralipid and hemoglobin. Results were compared per CLSI EP07-A2 against IgE samples containing no interferent.

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RESULTS

Precision

AU480	Human Sample 1	Human Sample 2	Human Sample 3	Control L1	Control L2
IgE Mean (IU/mL)	57.0	170.2	482.2	110.0	289.4
Within-run SD (IU/mL) or CV (%)	1.5 IU/mL	0.9%	0.7%	1.0%	0.7%
Total SD (IU/mL) or CV (%)	2.1 IU/mL	1.2%	0.9%	1.4%	0.9%

AU680	Human Sample 1	Human Sample 2	Human Sample 3	Control L1	Control L2
IgE Mean (IU/mL)	51.5	172.2	479.7	110.8	287.3
Within-run SD (IU/mL) or CV (%)	3.9 IU/mL	1.1%	0.8%	2.1%	0.7%
Total SD (IU/mL) or CV (%)	3.6 IU/mL	1.3%	0.8%	2.4%	1.0%

AU5800	Human Sample 1	Human Sample 2	Human Sample 3	Control L1	Control L2
IgE Mean (IU/mL)	52.8	169.4	461.2	110.4	274.3
Within-run SD (IU/mL) or CV (%)	1.4 IU/mL	2.8%	2.0%	1.6%	1.1%
Total SD (IU/mL) or CV (%)	1.8 IU/mL	3.2%	3.3%	2.0%	1.1%

DxC 700 AU	Human Sample 1	Human Sample 2	Human Sample 3	Control L1	Control L2
IgE Mean (IU/mL)	54.5	166.4	467.4	105.6	276.6
Within-run SD (IU/mL) or CV (%)	2.8 IU/mL	1.0%	0.7%	1.3%	0.5%
Total SD (IU/mL) or CV (%)	2.8 IU/mL	1.2%	0.9%	1.8%	0.8%

Figure 1. Within-run and total imprecision were evaluated on AU480, AU680, AU5800 and DxC 700 AU systems. Samples were assayed in 2 sample cups with a single replicate for each cup per run and two runs per day for 10 days. Within-run imprecision was ≤ 3.9 IU/mL SD or $\leq 2.8\%$ CV (COV = 71.4 IU/mL) and total imprecision was ≤ 3.6 IU/mL SD or $\leq 3.3\%$ CV (COV = 93.3 IU/mL) in the sample concentration range 51.5 – 482.2 IU/mL.

Linearity

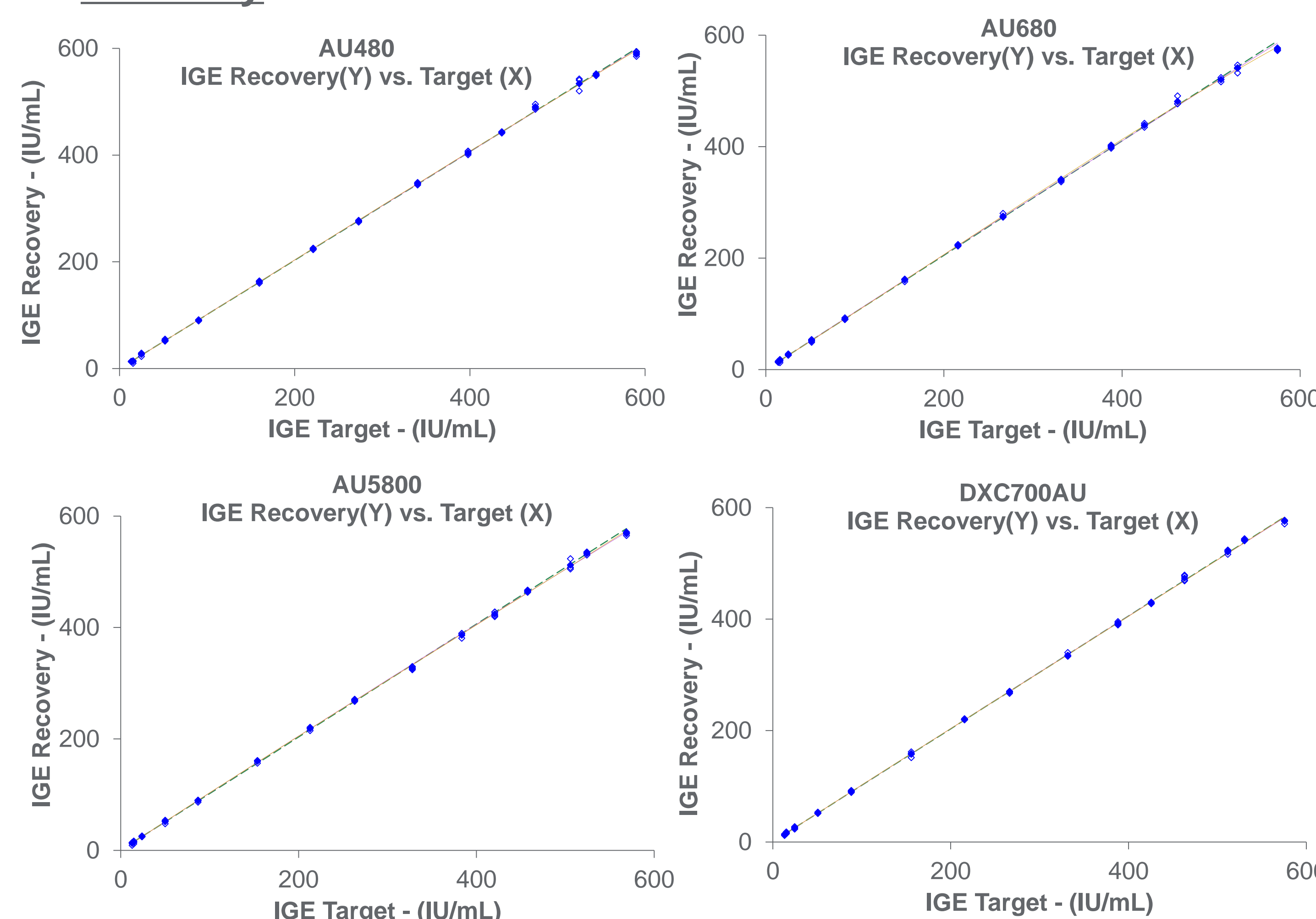


Figure 2. Linearity was evaluated on AU480, AU680, AU5800 and DxC 700 AU systems with a set of 13 dilutions with IgE concentrations between 12.8 and 590.2 IU/mL. IgE recovery was analyzed per CLSI EP06-A.

Sensitivity

Parameter	Result			
	AU480	AU680	AU5800	DxC 700 AU
Limit of Blank (LoB)	2.2 IU/mL	5.2 IU/mL	4.5 IU/mL	5.9 IU/mL
Limit of Detection (LoD)	4.4 IU/mL	9.4 IU/mL	6.7 IU/mL	9.1 IU/mL
Limit of Quantitation (LoQ)	7.9% CV at 18.6 IU/mL	12.2% CV at 18.7 IU/mL	9.9% CV at 18.8 IU/mL	11.9% CV at 19.6 IU/mL

Figure 3. Sensitivity was evaluated on AU480, AU680, AU5800 and DxC 700 AU systems using IgE-depleted serum samples for LoB and patient samples for LoD and LoQ.

RESULTS (continued)

On-board Stability

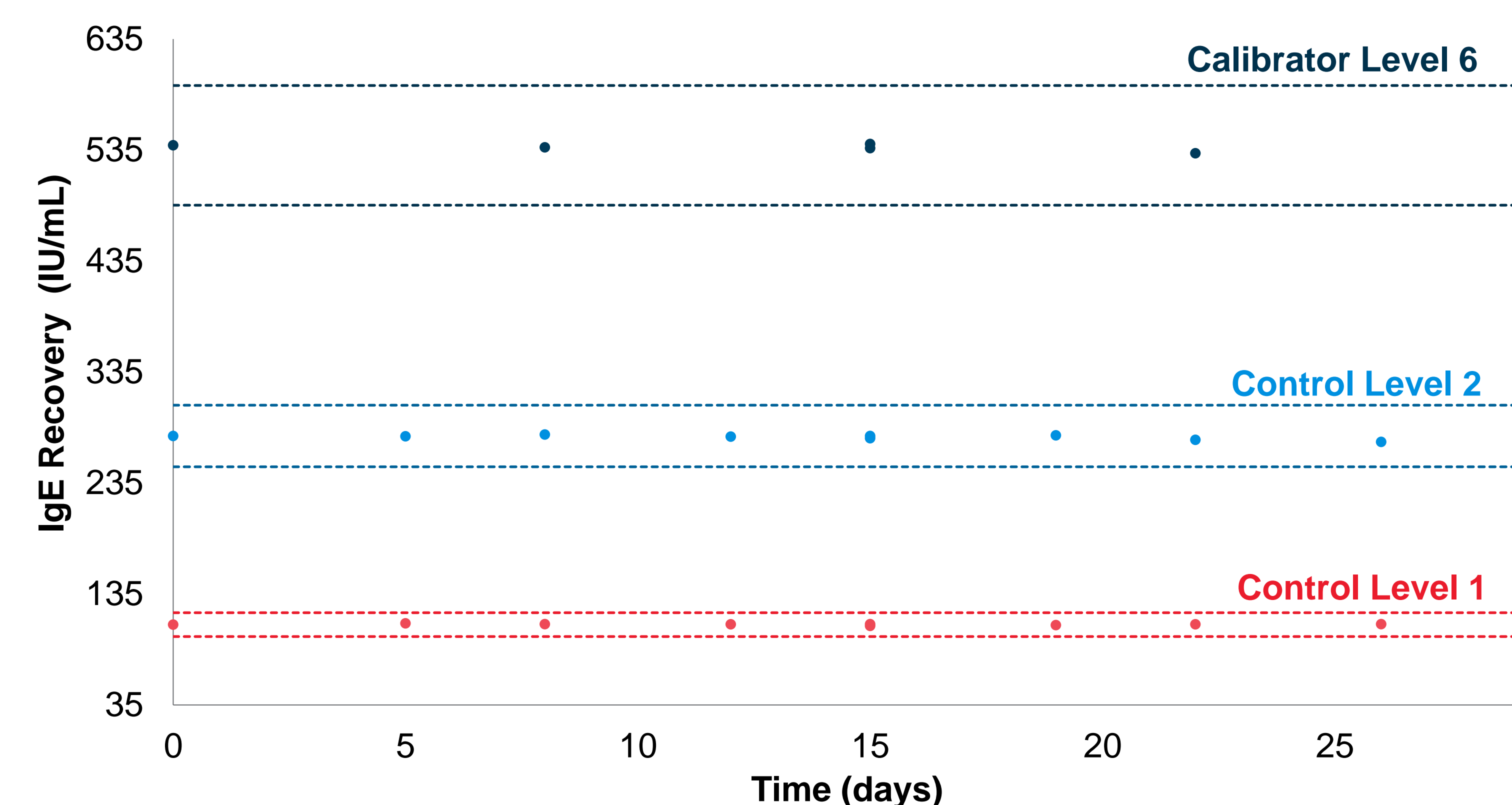


Figure 4. On-board stability and calibration frequency were assessed on a DxC 700 AU system over a 29-day period. The IgE assay was calibrated on days 0 and 15. IgE recoveries were assayed at each time point (n = 6 on day 0, then n = 3 on following days). All IgE recoveries were within ± 10 IU/mL or $\pm 10\%$ of day 0. Calibration stability at day 15 was within ± 10 IU/mL or $\pm 10\%$ of day 0.

Methods Comparison

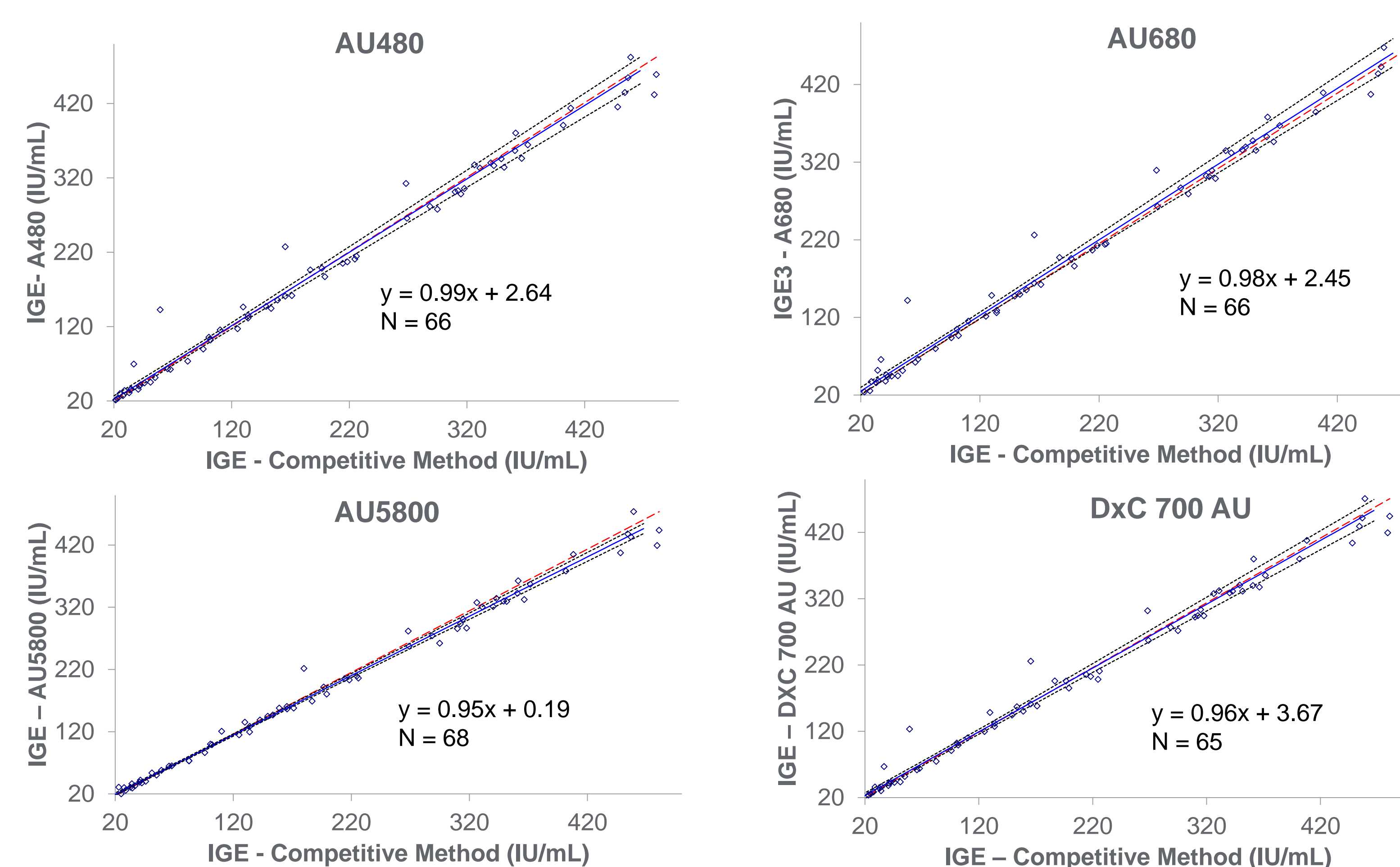


Figure 5. AU480, AU680, AU5800 and DxC 700 AU systems were evaluated against a competitive assay. Weighted Deming regression yielded slopes ranging from 0.95 to 0.99 and intercepts ranging from 0.19 to 5.59.

Interferences

Sample	Human Sample 1	Human Sample 2	Human Sample 3	
Bilirubin (mg/dL)	0	60	0	60
Average IgE Recovery (IU/mL)	47.6	46.9	156.1	154.6
%Bias	-	-1.5	-	-1.0

Sample	Human Sample 1	Human Sample 2	Human Sample 3	
Hemoglobin (mg/dL)	0	1000	0	1000
Average IgE Recovery (IU/mL)	43.1	44.2	145.7	145.3
%Bias	-	2.4	-	-0.3

Sample	Human Sample 1	Human Sample 2	Human Sample 3	
Intralipid (mg/dL)	0	1000	0	1000
Average IgE Recovery (IU/mL)	39.9	42.8	149.3	155.6
%Bias	-	6.0	-	4.2

Figure 6. High, mid and low human IgE samples were spiked with bilirubin (up to 60 mg/dL), Intralipid (up to 1000 mg/dL) and hemoglobin (up to 1000 mg/dL) and tested on a DxC 700 AU. Results were compared against IgE samples containing no interferent. Biases of $\leq 6\%$ were observed up to the indicated interferent levels.

CONCLUSIONS

- Within-run imprecision was ≤ 3.9 IU/mL SD / $\leq 2.8\%$ CV (COV = 71.4 IU/mL) and total imprecision was ≤ 3.6 IU/mL SD / $\leq 3.3\%$ CV (COV = 93.3 IU/mL).
- Linearity was demonstrated between 20 – 500 (Cal6) IU/mL.
- Sensitivity studies demonstrated LoB ≤ 5.9 IU/mL, LoD ≤ 9.4 IU/mL and LoQ $\leq 12.2\%$ CV at ~ 19.0 IU/mL.
- Method comparison showed acceptable correlation to a competitive assay, with slopes between 0.95 – 0.99 and intercepts between 0.19 – 5.59.
- On-board stability was demonstrated up to 29 days and calibration stability up to 15 days.
- There was no significant bilirubin, Intralipid and hemoglobin interference up to 60, 1000 and 1000 mg/dL, respectively.

References

- World Health Organization 3rd International Standard for human serum Immunoglobulin E, 11/234
- Clinical Laboratory Standards Institute, www.clsi.org

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