EVALUATION OF A LIPOSOME-BASED TURBIDIMETRIC ASSAY FOR MEASUREMENT OF TOTAL COMPLEMENT ACTIVITY

Anu S. Maharjan[1], Jeffrey Basker[2], Benjamin Hansen[2], Tanya Wilcox[2], Thomas B. Martins[1,3], Igor Pavlov[3], Michael Bandhauer[3], and Lisa K. Peterson[1,3].

[1]Department of Pathology, University of Utah; [2]ARUP Laboratories; and [3]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT 84108.

33rd AMLI Annual meeting, August 2020, Virtual

INTRODUCTION AND OBJECTIVE

Assessment of total complement activity, frequently referred to as CH50, is a screening test for complement deficiencies. Initially this testing was performed using a hemolytic assay. However, this method was considered complex and labor intensive, especially for a high-volume reference laboratory. Previously, we validated an CH50 ELISA that showed good correlation with the hemolytic assay. As demand for this testing has grown, the incubation temperature requirements present an obstacle for automation to accommodate increasing test volumes. The purpose of this study is to evaluate the performance of a fully automated liposome-based turbidimetric assay for the detection of CH50.

MATERIALS AND METHODS

ELISA CH50 Method	Liposome CH50 Method
Kit form Diasorin performed manually	Kit from The Binding Site for use on the Optilite analyzer
MAC formation creates neoantigen which is bound by an HRP-conjugated antibody that reacts with a chromogen that is detected spectrophotometrically	MAC formation lyses liposomes releasing G6PDH which reacts with NAD+G6P in the assay mixture forming NADH that is detected spectrophotometrically.
Normal range: 60 - 144 CAE Units	PI Normal range: 41.68 – 95.06 U/ml New Normal range: 38.7 – 89.9 U/ml

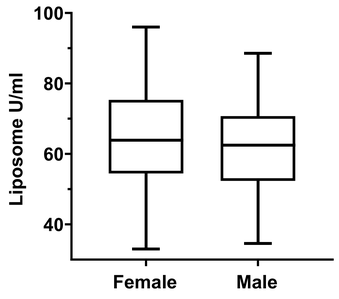
Two hundred twenty-three (n=223) serum samples were used in this evaluation. These included sera from 103 patients previously tested for CH50 by ELISA and 120 controls ("self-reported" healthy individuals)

Preliminary testing failed to confirm the normal range from the package insert, so a new reference interval was established using the 120 controls.

The performance of the CH50 liposome assay was compared to results for the 103 serum samples previously tested by ELISA based on ranges established during validation testing (<38.7 U/mL=low, 38.7-89.9 U/mL=normal, >89.9 U/mL=high). Linearity, precision, interference, and stability were also evaluated.

RESULTS

CH50 liposome reference range was established using serum from 120 self-proclaimed healthy controls



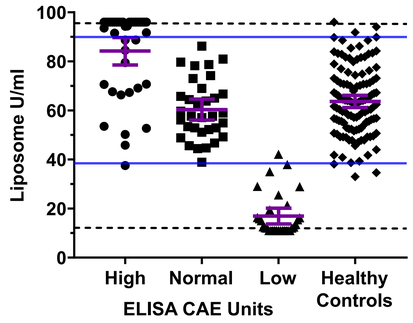
	All	Female	Male
2.5%	38.7	39.9	38.5
97.5%	89.9	86.0	92.5

	All	Female	Male
LL 90%CI	34.6	33.0	34.6
UL 90%CI	41.8	46.6	45.0
Lower	38.7	38.5	39.9
Upper	89.9	92.5	86.0
LUpp 90%CI	86.3	86.3	82.4
UUpp 90%CI	94.1	96.0	88.6

CH50 was measured in 69 females and 51 males to establish reference intervals for the liposome-based turbidimetry assay. No significant difference was observed between males and females. Thus, a single range will be used.

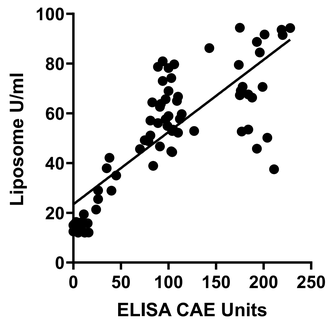
CH50 liposome results show good overall agreement with the predicate assay although challenges with high results were observed

ELISA Result Range	Category	Number	% Agreement
<60 CAE Units	Low	31	96.8%
60-144 CAE Units	Normal	33	100.0%
>144 CAE Units	High	39	61.5%
Overall	All	103	85%



Comparison of liposome CH50 results with those of the ELISA predicate assay using the reference intervals established during the validation [<38.7 U/ml = low (lower blue line), 38.7-89.9 U/ml = normal, >89.9 U/ml = high (upper blue line)] and those from the original package insert for the predicate assay. The analytic measurement range of the liposome assay is indicated by the dashed lines.

CH50 liposome results show poor quantitative correlation with the predicate assay



Quantitative Comparison Summary	
Correlation Coefficient, R^2	0.6466
Slope	0.2904
Intercept	23.43

Quantitative comparison between liposome CH50 results and ELISA CH50 results. 23 results fell above or below the reference range of the liposome assay and were excluded.

Acceptable results were obtained for all performance characteristics except for samples that had been frozen and thawed

	Parameters	Low	Normal	High
		Mean CV (Range)	Mean CV (Range)	Mean CV (Range)
Precision	Within Run	1.1% (0.9-1.3%)	1.2% (1.1-1.2%)	2.5% (2.2-2.8%)
	Between Run	9.7% (7.1-12.2%)	5.4% (2.0-8.8%)	8.0% (7.7-8.2%)
		%Difference	%Difference	%Difference
Interference	Hemoglobin	0.0%	4.4%	9%
	Triglycerides	7.7%	14.6%	12.7%
	Bilirubin	2.2%	7.8%	2.9%
Stability	30 days -20ºC	4.2% (3.5-4.8%)	2.5% (2.2-2.7%)	14.9% (14.9%*)
	1 Freeze/Thaw	0.9% (0.9-0.9%)	2.8% (1.7-3.8%)	23.4% (23.4%*)
	2 Freeze/Thaw	1.1% (0.9-1.3%)	3.6% (3.3-3.9%)	27.9% (27.9%*)
	3 Freeze/Thaw	3.8% (3.3-4.3%)	10.0% (6.4-13.6%)	29.4% (29.4%*)

Data shown in the table above are the mean variation and range of 2 specimens at each result level for precision and stability. The first result from each of 3 runs was used to evaluate between run precision. Hemoglobin concentrations up to 500 mg/dL, triglycerides up to 250 mg/dL, and free bilirubin up to 20 mg/dL had no significant influence on results. *One of the specimens with high results had some values greater than the analytical measurement range, so that value was excluded from the calculation of the mean and range.

DISCUSSION AND CONCLUSIONS

- Validation of the liposome-based turbidimetric assay required establishment of our own reference intervals
- CH50 liposome results show good overall agreement with the predicate assay although challenges with high results were observed. This may be due to the compressed analytic measurement range of the turbidimetric assay, which focuses on results within the normal and low range as it is intended as a screen for complement deficiency.
- Liposome CH50 measurements demonstrated excellent precision
- Liposome CH50 results were unaffected by interfering substances such as, bilirubin, triglyceride, or hemoglobin at concentrations specified by the manufacturer.
- Similar results can be obtained using freshly collected frozen specimens and those stored at -20 C for up to 30 days. However, freeze/thaw cycles should be avoided.
- The automated liposome-based turbidimetric assay is acceptable for the detection of CH50 in the clinical laboratory.