University of Colorado Anschutz Medical Campus

ICW Poster 053

Comparison of a Laboratory-Developed Test with a CE Marked Commercial Kit for the Analysis of Complement Function and Inhibition.

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Abstract

We conducted a comparison of two methodologies for the testing of complement function: one a hemolytic laboratory-developed assay and a second method that is approved and CE marked in Europe. Specifically, the classic CH50 and AH50 hemolytic assay was compared with the Wieslab CP and AP assay. While both classes of assays are designed to measure the presence and functionality of the complement system, the two methods differ dramatically. The historic hemolytic method utilizes sensitized red blood cells as a target for lysis by the membrane attack complex of complement, with the release of hemoglobin being the end readout. The CE marked method is plate-based and similar to an ELISA type assay. The completed testing included the comparison of results from testing specimens for 80 normal humans. The precision and linearity of all four assays were determined and compared. Further, we investigated the sensitivity and lower limit of quantitation for both assay types. The comparison of the values for the 80 normals showed weak, but present, correlation between the two methods. The alternative pathway test demonstrated slightly better correlation between the two methodologies, however the plate-based assay was modified from the manufacturer's protocol. The intra-assay variability was largely comparable across all assays tested and demonstrated a percent CV below 10% in all cases. An ability to reliably measure low levels of function is important for following patients on therapeutic complement inhibition. When testing the level of function at the level of 20% of normal, or below, more differences were seen . The classical pathway plate-based assays demonstrated lower coefficients of variance, but the hemolytic assay demonstrated a low limit of detection. For the alternative pathway function tests of the plate-based assay it is important to consider the method of calculation of values when looking at the limit of quantitation. The differences in methodology lead to differences in the measured values, therefore interchanging the results of hemolytic style and plate-based complement function assays should be approached with caution. These data indicate that picking a method for functional analysis at the lower levels of remaining function requires a balance between less inter-assay variability and a lower level of detection.

Background:

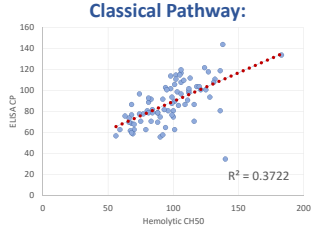
Hemolytic lysis was the original method for testing functional analysis, and while this method is still utilized it does present some issues for the standard laboratory. Specifically, hemolytic assays require a constant source of fresh animal red blood cells that, in turn, need to be prepared with consistency.

There have therefore been other methods developed. One of these is an ELISA style assay marketed by Eurodiagnostica. The classical pathway (CP) version of the Wieslab Assays has also been CE marked for clinical testing use in the EU.

We conducted optimization and validation of both the Hemolytic and Wieslab assays. Presented is the results of the validations for comparison of the performance. Not addressed are some of the operational considerations.

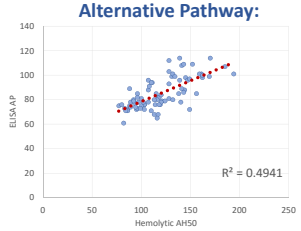
Comparison of Measurements

Classical Pathway:



Only 4% discordance on diagnostic category

Alternative Pathway:

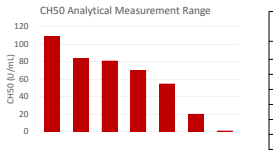


Only 6% discordance on diagnostic category

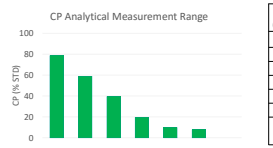
80 Normals (40 males & 40 Females) were measured by both methods

Dilution Sensitivity Testing

Classical Pathway Tests



CH50 (U/mL)
109
84
81
70
55
20
1



CP (% STD)
78.5
58.9
39.3
19.6
9.8
7.8
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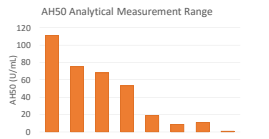
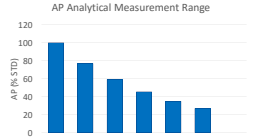
Alternative Pathway Tests



AH50 (U/mL)
111
75
68
53
19
9
11
1



AP (% STD)
100
76.9
59.2
45.5
35
26.9
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Intra-Assay & Inter-Assay Precision

Assay Precision, %CV			
Pathway	Level	Intra-Assay	Inter-Assay
CH50 Hemolytic	High	7.8%	NT
	Med	1.6%	6.0%
	Low	8.1%	NT
CP ELISA	High	6.2%	10.1%
	Med	2.6%	15.1%
	Low	2.2%	15.6%

Assay Precision, %CV			
Pathway	Level	Intra-Assay	Inter-Assay
AH50 Hemolytic	High	4.6%	NT
	Med	3.7%	6.0%
	Low	5.9%	NT
AP ELISA	High	6.0%	11.4%
	Med	1.8%	6.2%
	Low	1.7%	8.3%

A minimum of 15 repeat measurements were made by a minimum of two technologists. For inter-assay, measurements were made over at least 2 months.

NT, Not Tested Due to Run Size

Additional Sensitivity & Reproducibility Testing

CH50 Repeat Testing			
Specimen	Mean (U/mL)	St Dev	%CV
HC02	99.8	7.4	7%
HL02	49.1	3.6	7%
HVL02	30.3	2.4	8%
HXL02	14.2	3.9	28%
HLA1	8.1	2.5	31%

Wieslab CP Repeat Testing			
Specimen	Mean (% Std)	St Dev	%CV
HC02	85.8	16.5	19%
HL02	44.6	2.8	6%
HVL02	29.0	1.4	5%
HXL02	19.3	0.7	4%
HLA1	15.1	1.1	7%

This testing was performed by preparing five specimens of descending classical pathway function and then aliquoting the specimens into signal use volumes. Therefore each test could be performed on first thaw. The specimens were then tested a minimum of eight times over at least fives days. The resulting values are presented. The specimens were based on residual clinical specimens.

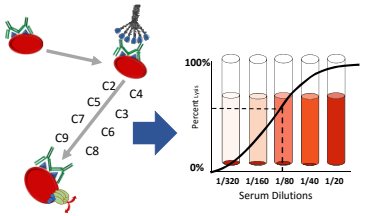
Exsera Methods & Operational Considerations:

The Hemolytic assays were based on the method of Kabat and Mayer (1961) and run as a Laboratory Developed Test (LDT). Fresh red blood cells were sourced locally. The sheep RBC were from one screened sheep and the rabbit RBC were from pooled rabbits. The sheep RBC were sensitized in the laboratory with a standardized and tested amount of antibody.

The Wieslab assays were run largely per manufacturer protocols but adjustment were made within Exsera. Most notably the AP assay was adjusted to utilize a Exsera created and characterized standard curve. This was done in part to better comply with regulatory requirements in the USA.

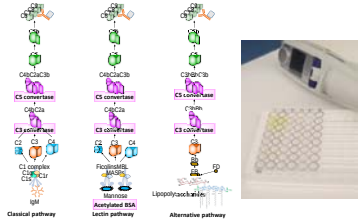
Operationally , it is important to note that while the hemolytic assay affords a five point dilution of each specimen to account for the non-linear nature of the reaction, this limits the run size to eight. This limit was in large part because we run the analysis in test tubes. The Wieslab assays standard curves were fit with a 4-parameter curve and were run in 96 well plates. This methods allows for greater throughput of specimens.

Principle of the Hemolytic Function Assay



This assay is a laboratory developed test

Principle of the Wieslab Function Assay



The CP Assay is CE Marked by RUO in USA

Conclusions:

These two methods for measuring complement function performed comparably for assay precision. The hemolytic versions did demonstrate lower limits of detection. There was discordance between the two methods at the raw data level. However, this resulted in only a 4% and 6% discordance in the diagnostic category.

The assays compared reasonably well but each method appears to have its own advantages. The Wieslab assays have tighter supply chain and this is reflected in tighter consistency. The Hemolytic CH50 may have a lower limit of detection, but counters this with a method that may be more difficult for a laboratory that does not run these assays regularly.

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