VALIDATION OF THE
WIESLAB® COMPLEMENT SYSTEM SCREEN ASSAY
AND COMPARISON WITH A HAEMOLYTIC BASED METHOD

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BACKGROUND

Assessment of complement activity is essential in the diagnosis of many diseases. Jeroen Bosch Ziekenhuis (JBZ) in Hertogenbosch, The Netherlands, was using a haemolytic based test for assessment of the classical and alternative complement pathways. However, these tests were associated with some drawbacks such as:

- Need for large sample volumes is a problem especially with paediatric patients
- The test is labour intensive
- The haemolytic test system is difficult to standardise and is associated with stability/quality issues regarding the erythrocytes used in the assay

The scope of this study was to investigate whether the WIESLAB® Complement System Screen (Euro Diagnostica AB, Sweden, product code COMPL300) may be an alternative to the haemolytic based test for assessment of complement activity.

INTRODUCTION

The complement system is a key component of the innate immune system and acts through three different pathways called classical and alternative pathway, respectively. The activity of each pathway is triggered by different mechanisms/components.

Historically, complement was not perceived to play a major role in human disease. However, over the last decades a shift in perception has occurred and complement is now regarded as an important component in the progression of a number of pathological conditions and also in clinical situations such as transplantation. Another effect of this awareness is the appearance of drugs for the manipulation of complement activity, e.g. Soliris (Alexion Inc) (2,3). As a consequence of this boost in interest and awareness of the significance of complement regulation, an increased demand for laboratory testing of relevant complement components and, in particular, functional assessment has grown substantially.

Haemolytic Assays

Although described in numerous modifications, haemolytic assays are still based on protocols first described by Mayer (4) and Rapp and Borsos (5) in the sixties and seventies. The sample to be analysed is incubated with antibody-sensitised sheep erythrocytes at a defined temperature. Haemolytic assays are performed in test tubes or in agarose gel plates. Usually the results are expressed as reciprocal dilutions of the sample required to yield 50 or 100% lysis (CH50 or CH100, respectively). Detection of low or absent haemolytic activity in CH50 and/or AH50 suggests further complement analysis.

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WIESLAB® Complement System Screen ELISA

An alternative test format for assessment of complement function is given by the WIESLAB® Complement System Screen ELISA. This assay allows for testing of both classical, alternative and lectin (MBL) pathways. The assay is CE-marked and is based on the principle of measuring a neo-epitope generated by the formation of the membrane attack complex (MAC). The amount of formed MAC (neo-epitope) reflects the activity of the complement cascade. The result is expressed semi-quantitatively using the ratio in optical density between a positive control and the sample. A detailed account of the design and performance of the test can be found in the papers by Seelen et al (6) and Roos and Wieslander (7).

Design of the study

The validation focused on the following quality parameters:

- Reproducibility inter-assay (run-to-run) with reduced and normal sample
- Reproducibility intra-assay
- Correlation between WIESLAB® Complement System Screen ELISA and the in-house assays
- Check reference values

Patient material

- Patient serum samples that have been stored and handled accordingly prior to analysis
- Selection of SKML (Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek) ring trial serum samples

The conclusion drawn from the present validation study is that the WIESLAB® Complement System Screen can replace the in-house haemolytic assay and will further strengthen and improve complement functional assessment in different clinical situations.

RESULTS

Accuracy

The accuracy was assessed using six samples obtained from the SKML survey "Complement factors."

Predefined standard accuracy:
Correlation Study: <20% deviation SKML samples.
Concordance > 90%.

	Consensus SKML			Wieslab ELISA		
Sample	**CP**	**AP**	**MBL**	**CP**	**AP**	**MBL**
SKML A	104	98	normal	90	111	34
SKML B	102	57	increased	104	46	121
SKML B	100	102	decreased	90	88	0
SKML D	1,9	96	decreased	2	109	0
SKML E	104	100	normal	90	121	90
SKML F	48	3,8	normal	43	1	23

Values expressed in %. Good agreement in results were seen between SKML consensus and results obtained by using the Wieslab ELISA.

Intra-assay reproducibility

Results mean CVs between duplicates:

- Classical pathway (CP): <7% over the entire measuring range
- Alternative pathway (AP): <10% over the entire measuring range
- Lectin (MBL) pathway (MP): < 5% over the entire measuring range

Results in patients pooled material run reproducibility (n = 8)

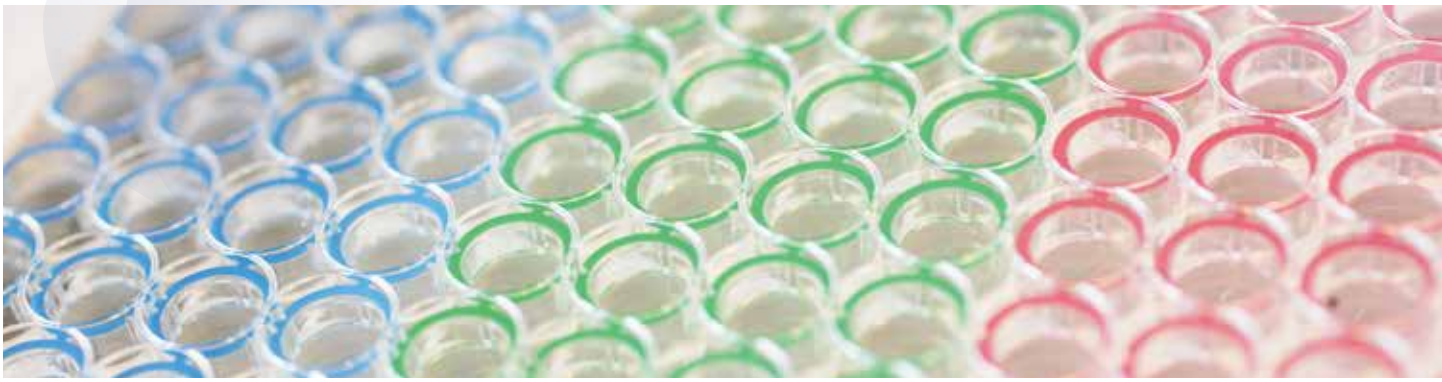
- Classical pathway (CP): 2.8% CV at level 102%
- Alternative pathway (AP): 3.0% CV at level 100%
- Lectin (MBL) pathway (MP): 4.9% CV at level 83%

Inter-assay reproducibility

- The negative control was determined 5 days in duplicate.
- A patient serum pool was determined 3 days in duplicate.

Sample	**N**	**SD**	**CV %**	**Level %/OD**
CP pool	3	12	13	92
AP pool	3	6	6	104
MP pool	3	6	8	79
CP QC low	5	7.7	6	0.121
AP QC low	5	4.6	4	0.109
MP QC low	5	6.5	6	0.116

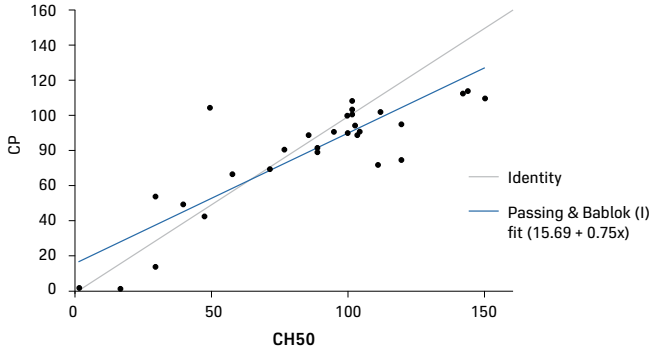
Predetermined standard between run reproducibility CV %: <15%
Meeting local laboratory quality requirements

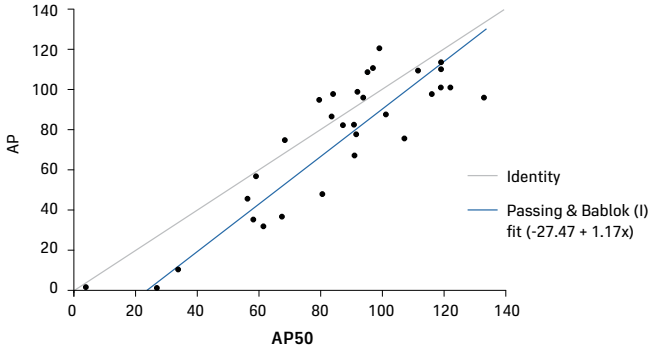


Correlation

Technical correlation (Haemolytic assay/ELISA):
CP: The correlation study was conducted with 31 patients, including six samples from SKML ring trial
AP: The correlation study was conducted with 30 patients, including six samples from SKML ring trial
MP: New test; was analysed with six samples from SKML ring trial

Scatter Plot with Passing & Bablok Fit

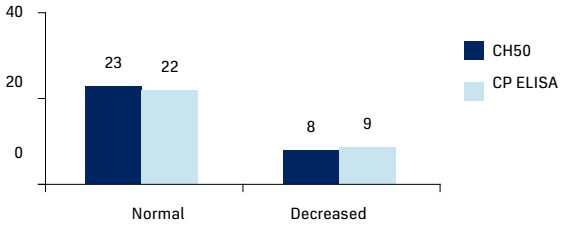




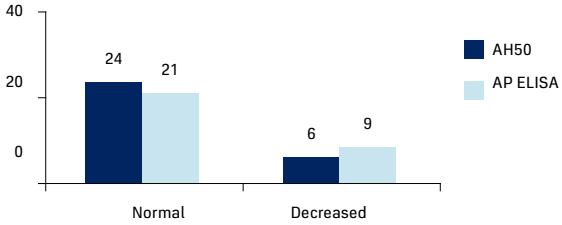
The correlation CH50 vs Elisa CP: y = 11 + 0.75 x.
The correlation AP50 vs Elisa AP: y = -27 + x 1:17
All JBZ MP and MP SKML samples showed valid results

Clinical Correlation

Clinical correlation CH50 and CP ELISA (31 patients)



Clinical correlation AH50 and AP ELISA (30 patients)



The overall correlation for both pathways was acceptable. The few discrepant results were values close to the borderline and did not generate different clinical interpretation. All strongly reduced samples that could be associated with genetic primary deficiency were detected in both tests (13 samples in total).

CONCLUSION

It is well known that the ELISA format offers superior ease of handling, increased objectivity in interpretation, faster turn-around time and increased stability and reagent quality as compared to haemolotyc assays. Moreover, the ELISA is suitable for automation which further adds to the ease of use.

The Wieslab ELISA correlated well with the haemolytic assay that was presently in use at JBZ regarding overall performance and specifications. The conclusion drawn from the present validation study is that the Wieslab ELISA can replace the in-house haemolytic assay and will further strengthen and improve complement functional assessment in different clinical situations.

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