INSTRUCTIONS FOR USE

WIESLAB® Complement system

Classical pathway

Qualitative and Semi-Quantitative test

Enzyme immunoassay for assessment of
Complement functional activity

Break apart microtitration strips (12x8) 96 wells
Store the kit at +2-8° C
Store the positive and activity control at -20° C

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December 2018

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

REF **COMPL CP310 RUO**

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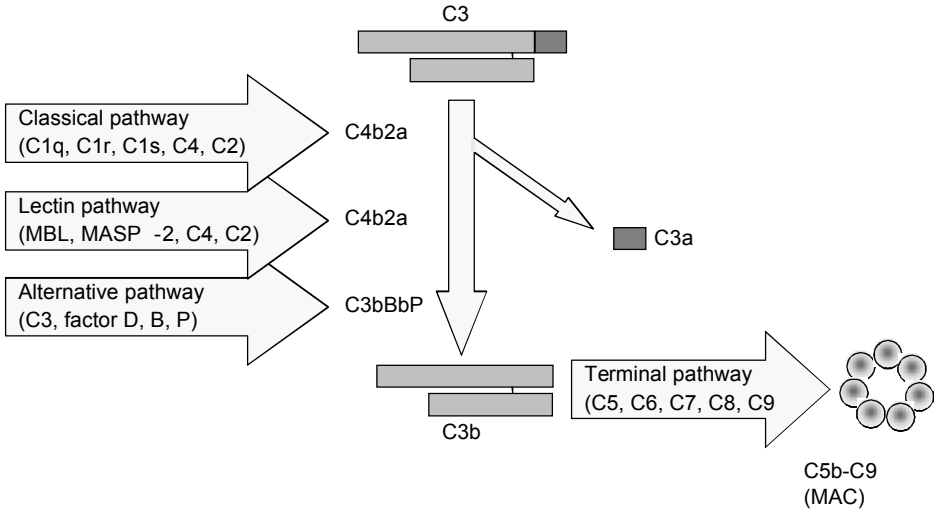
PURPOSE OF RESEARCH PRODUCT

The Wieslab® Complement system Classical pathway is an enzyme immunoassay for the qualitative and/or semi-quantitative determination of functional classical complement pathway in human serum, the result shall not be used for clinical diagnosis or patient management.

Summary and explanation

The complement system plays an essential role in chronic, autoimmune and infectious disease. There are three pathways of complement activation (fig. 1), namely the classical, the alternative and the lectin pathway.



Impaired complement activity causes humans to become susceptible to repetitive fulminant or severe infections and may contribute to development of autoimmune disease. Inappropriate activation of complement contributes to chronic inflammation and tissue injury.

In vitro activation of the complement sequence leads to the consumption of complement components which, in turn, leads to a decrease in their concentration. Thus, the determination of complement proteins or complement activity is used to indicate whether the complement system has been activated by an immunologic and/or pathogenic mechanism. Both functional and immunochemical complement measurements are used to evaluate subjects when a complement-activating disease is suspected or an inherited deficiency is possible. The level of complement activity evaluated by functional assays such as Wieslab® Complement kit takes into account the rate of synthesis, degradation, and consumption of the components and provides a measure of the integrity of the pathways as opposed to immunochemical methods which specifically measure the concentration of various complement components.

PRINCIPLE OF THE WIESLAB® COMPLEMENT CLASSICAL PATHWAY ASSAY

The Wieslab® Complement Classical pathway assay combines principles of the hemolytic assay for complement activation with the use of labelled antibodies specific for neoantigen produced as a result of complement activation. The amount of neoantigen generated is proportional to the functional activity of complement pathways.

In the Complement CP kit, the wells of the microtitre strips are coated with specific activators of the classical pathway. This in combination with sample dilution buffer composition and serum dilution level ensure that only the Classical pathway is activated.

During the incubation of the diluted serum in the wells, complement is activated by the specific coating. The wells are then washed and the amount of C5b-9 complex formed on the plate surface is detected

with a specific alkaline phosphatase labelled antibody to the C5b-9 neoantigen formed during MAC (Membrane Attack Complex) formation.

After a further washing step, detection of specific antibodies is obtained by incubation with alkaline phosphatase substrate solution. The amount of complement activation correlates with the colour intensity and is measured in terms of absorbance (optical density (OD).

Warnings and precautions

- ***For Research Use Only. Not for use in diagnostic procedures.***
- The human serum components used in the preparation of the controls in the kit have been tested for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen by FDA approved methods and found negative. Because no test methods can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
- The Centers for Disease Control and Prevention and National Institutes of Health recommended that potentially infectious agents be handled at the Biosafety Level 2.
- All solutions contain ProClin 300 as a preservative. Never pipette by mouth or allow reagents or samples to come into contact with skin. Reagents containing ProClin may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.
- Safety data sheet for all hazardous components contained in this kit is available on request from Svar Life Science.

BUF	WASH	30X
DIL		
CONJ		

CONTROL	-
SUBS	pNPP

Warning

Contains ProClin 300:
Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC no. 220-239-6] (3:1)

H317:	May cause an allergic skin reaction.
P264:	Wash hands thoroughly after handling.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P302+352:	IF ON SKIN: Wash with plenty of soap and water.
P333+313:	If skin irritation or rash occurs: Get medical advice/attention.

Specimen collection

Blood samples are to be collected using aseptic venipuncture technique and serum obtained using standard procedures. A minimum of 5 mL of whole blood is recommended. Allow blood to clot in serum tubes, for 60-65 minutes at room temperature (20-25° C). Centrifuge blood samples and transfer cell-free serum to a clean tube. **Sera must be properly handled to prevent *in vitro* complement activation.** Sera should be frozen at -70° C or lower in tightly sealed tubes for extended storage or for transport on dry ice. Samples should not be frozen and thawed more than once.

Do not use sera which are icteric, lipemic and hemolyzed. Heat-inactivated sera cannot be used. Plasma cannot be used. The CLSI provides recommendations for storing blood specimens (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18A, 1990).

Kit components and storage of reagents

- One frame with blue coloured break-apart wells (12x8) coated with human IgM, sealed in a foil pack with a desiccation sachet.
- 2 x 35 mL Diluent CP (Dil CP), labelled blue.
- 13 mL conjugate containing alkaline phosphatase-labelled antibodies to C5b-9 (blue colour).
- 13 mL Substrate solution ready to use.
- 30 mL wash solution 30x concentrated.
- 0.2 mL negative control (NC) containing human serum (to be diluted as for a subject serum sample).
- Lyophilized positive control (PC) containing freezed dried human serum, to be reconstituted in 0.2 mL distilled water, see “Reconstitution of positive control”, below.
- Lyophilized activity control (AC) for semi-quantitative application, containing freezed dried human serum (different origin than PC), see “Reconstitution of activity control” under procedure for semi-quantitative application.

The positive control and the activity control should be stored at -20° C upon arrival.

Please note: The reconstitution volume for AC is indicated in Certificate of Analysis (CoA) (XXX µl) and on AC label.
All reagents in the kit are ready to use except wash solution and controls. The reagents should be stored at 2-8° C except the positive control and the activity control. The reconstituted positive control and activity control should be stored at –70° C and may be thawed once.

Materials or equipment required but not provided

- Microplate reader with filter 405 nm.
- Precision pipettes with disposable tips.
- Washer for strips, absorbent tissue, tubes and a timer.

PROCEDURE – QUALITATIVE APPLICATION
Remove only the number of wells needed for testing, resealing the aluminium package carefully. Let all solutions equilibrate to room temperature (20-25° C) before analysis. Do not mix reagents between lots.

Preparation of washing solution
In case salt crystals are observed in the vial with concentrated wash solution, place the vial at 37°C water bath until the crystals have dissolved before dilution of wash solution.
Dilute 30 mL of the 30x concentrated wash solution in 870 mL distilled water. When stored at 2-8° C, the diluted wash solution is stable until the date of expiration of the kit.

Reconstitution of positive control
Gently tap down all lyophilized material to the bottom of the vial and remove the cap. Immediately add 200 µL of distilled water directly to the lyophilized material. Replace the cap. Allow the vial to stand on ice for 5 minutes and then gently shake or vortex occasionaly until completely dissolved. Dilute the reconstituted control in the same way as a subject serum sample. The reconstituted positive control can be stored for up to 4 hours prior to use if kept at 2-8° C or on ice. It should be stored at –70° C and may be thawed once.

Serum
Partially thaw frozen sera by briefly placing in a 37° C water bath with gentle mixing. After partially thawing immediately place the tubes in an ice bath and leave on ice until completely thawed. Mix briefly on a vortex mixer.

Dilution of serum
Dilute the serum 1/101 with Diluent CP, blue label, (500 µL Diluent + 5 µL serum) and mix thoroughly but gently on a vortex. The diluted serum can be left at room temperature for a maximum of 60 minutes before analysis.

Incubation of samples
Pipet 100 µL/well in duplicate of Diluent (Dil) as a blank, positive control (PC), negative control (NC) and diluted subject's serum (P) according to the diagram below. Incubate for 60-70 minutes at +37º C with lid.

Classical Pathway

	1	2	3
A	Dil CP	P2	
B	Dil CP	P2	
C	PC	etc	
D	PC		
E	NC		
F	NC		
G	P1		
H	P1		

After serum incubation
Empty the wells and wash 3 times with 300 µL washing solution, filling and emptying the wells each time. After the last wash, empty the wells by tapping the strip on an absorbent tissue.

Adding conjugate
Add 100 µL conjugate to each well. Incubate for 30 minutes at room temperature (+20-25° C).

After conjugate incubation
Wash 3 times as before.

Adding substrate solution
Add 100 µL substrate solution to each well, incubate for 30 minutes at room temperature (+20-25° C). Read the absorbance at 405 nm on a microplate reader. (5 mM EDTA can be used as stop solution, 100 µL/well. Read the absorbance of the wells within 60 minutes.)

Calculation of result
Subtract the absorbance of the Blank (Diluent) from the absorbances of the NC, PC and the samples. The absorbance of the positive control should be >1.0 and the negative control absorbance < 0.2 after substraction of the Bank.

Calculate the mean OD405nm values for the sample, PC and NC and calculate the % complement activity as follows: (Sample-NC)/(PC-NC)x100. The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cut-off. It is recommended that each laboratory establish its own reference level and cut-off value for deficiencies.

If any of the controls are not within their respective range, the test should be considered as invalid and repeated**.**

Quality control
CoA included in this kit is lot specific and is to be used to verify results obtained by your laboratory. The results indicated on CoA are to be used as a guideline only. The results obtained by your laboratory may differ.

Limitations
The individual subject's complement level can not be used as a measure of disease severity, as it may vary from subject to subject. Thus, it is difficult to obtain an absolute standardization of results.
The test should not be relied upon as the sole basis of decisions on clinical therapy, but should be used in combination with clinical symptoms and the results of other available tests. Therapy should not be started on basis of the complement assay result. Initiation or changes in treatment should not be based on changes in complement levels alone, but rather on careful clinical observation.

Expected results
When decreased levels of complement components or complement function are found, a deficiency or an ongoing, immunologic process, leading to increased breakdown of components and depression of complement levels is considered by clinicians.

- The normal distribution within 2SD has, for the qualitative assay, been determined to be 69-129% of the positive control, see table 1. Results within this range indicate a normal functionality of the classical pathway. It is recommended that each laboratory confirms or establishes own reference range for the population they serve.
- A value below the 69-129% range indicates either increased activation, resulting in consumption of the classical complement pathway capacity, or a genetically determined low activity.
- Values below 5% strongly suggest a complete deficiency either caused by excessive activation or an inherited deficiency in the classical pathway. To establish which complement factor(s) causes the lowered activity further analysis of complement proteins is needed.
- A negative result i.e. suspected deficiency, should always be verified by testing a new, carefully handled sample to ensure that no in vitro complement activation has taken place.
- Increased complement levels are usually a nonspecific expression of an acute phase response.

The Wieslab® Complement system Classical Pathway can be helpful for detection of complement deficiencies related to the Classical Pathways as shown in the table below: A more complete and in-depth functional assessment of all three complement pathways may be achieved using Wieslab® Complement system Screen.

Classical pathway	MBL pathway	Alternative pathway	Possible deficiency
Positive	Positive	Positive	None
Negative	Positive	Positive	C1q, C1r, C1s
Positive	Positive	Negative	Properdin, Factor B,D
Positive	Negative	Positive	MBL, MASP2
Negative	Negative	Negative	C3, C5,C6,C7,C8,C9
Negative	Negative	Positive	C4, C2 or combination

Performance characteristics
120 sera from blood donors were tested and the normal reference range was calculated. The values were expressed in % of the positive control. See Figure 1 and Table 1. No blood donor was below 40 %.

Figure 1.

CP Qualitative application

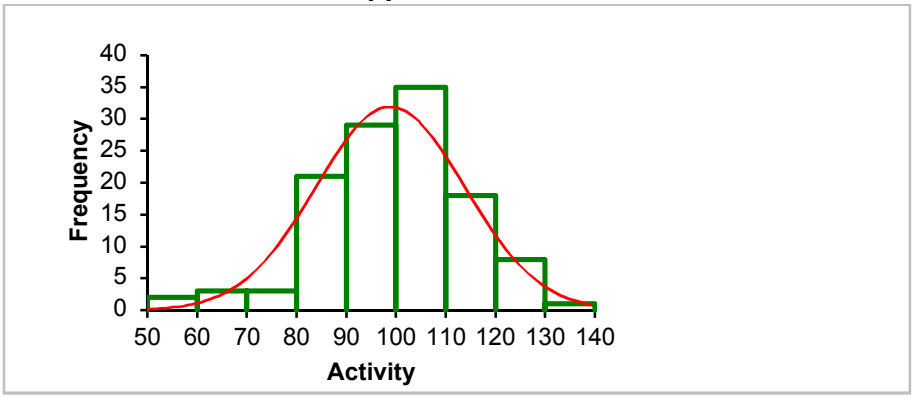


Table 1.

	n	Mean (%)	±2SD (%)	Median (%)
Classical pathway	120	99	69-129*	100

*)This is a statistical calculation and will not guarantee a true cut-off.
It's recommended that each laboratory establish its own reference level and cut-off value for suspected deficiency.

Table 2.
Sera with known complement deficiencies were tested in the assay and the following results were obtained. All deficient sera were detected in the assay and gave values below 5 %**).

Deficiency	C2	C3	C4	C5	C7	C8
Number of subjects	5	1	1	1	2	2
Number of deficient sera detected	5	1	1	1	2	2

**) See "M.A. Seelen et. al, Functional analysis of the classical, alternative and MBL pathways of the complement system: standardization and validation of a simple ELISA. J Immunol Meth 2005, 296, 187-198", for extended tests of deficient subject samples tested with qualitative application

Depletion	C1q	C3	C4	C5	C7
Number of depleted sera	2	1	1	1	1
Number of detected depletion	2	1	1	1	1

Table 3. Inter-assay precision for qualitative application was determined by testing three samples in duplicate. Results were obtained for six different runs.

	CP P1	CP P2	CP P3
Mean value %	98	92	21
SD	4.3	3.9	1.7
CV%	4	4	8

Table 4. Intra-assay precision for qualitative application was determined by testing one sample in 40 wells.

Assay	Mean value %	SD	CV %
CP	85	2.9	3

PROCEDURE – SEMI-QUANTITATIVE APPLICATION

The semi-quantitative application differ from the qualitative in that a calibration curve is made by diluting the kit PC giving a calibration curve with 100, 75, 50, 25 and 12.5% activity. Remove only the number of wells needed for testing, resealing the aluminium package carefully. Let all solutions equilibrate to room temperature (20-25° C) before analysis. Do not mix reagents between lots.

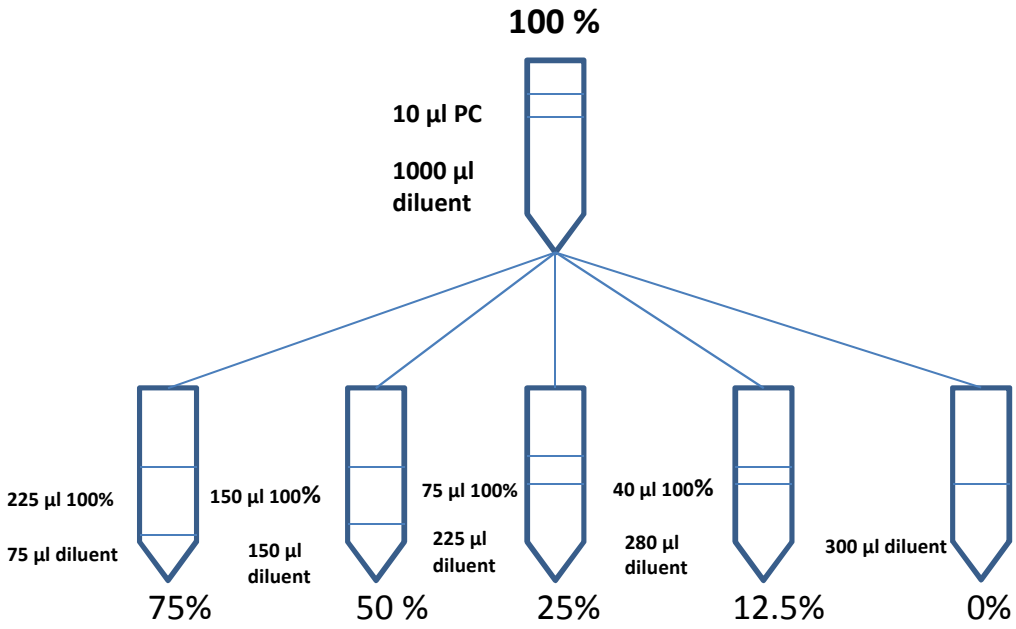
Preparation of washing solution

In case salt crystals are observed in the vial with concentrated wash solution, place the vial at 37°C water bath until the crystals have dissolved before dilution of wash solution.
Dilute 30 mL of the 30x concentrated wash solution in 870 mL distilled water. When stored at 2-8° C, the diluted wash solution is stable until the date of expiration of the kit.

Reconstitution of positive control and dilution for use as calibrator

Gently tap down all lyophilized material to the bottom of the vial and remove the cap. Immediately add 200 µL of distilled water directly to the lyophilized material. Reattach the cap. Allow the vial to stand on ice for 5 minutes and then gently shake or vortex occasionaly until completely dissolved. The reconstituted positive control can be stored for up to 4 hours prior to use if kept at 2-8° C or on ice. It can be frozen at –70° C and thawed once.

For dilution of reconstituted positive control to calibrators please see picture below.



The calibrator can be left at RT up to 1 h before use. The calibrator must be prepared fresh and cannot be stored at -20 °C after dilution for later usage.

Reconstitution of activity control (AC)
Gently tap down all lyophilized material to the bottom of the vial and remove the cap. Add immediately the volume distilled water indicated in the CoA /AC label directly to the lyophilized material. Reattach the cap. Allow the vial to stay on ice for 5 minutes and then shake or vortex gently until complete dissolution. Dilute the reconstituted control in the same way as a subject serum sample. The reconstituted activity control can be stored for up to 4 hours prior to use if kept at 2-8° C or on ice. It can be stored at –70° C and thawed once.

Serum
Partially thaw frozen sera by briefly placing in a 37° C water bath with gentle mixing. After partially thawing immediately place the tubes in an ice bath and leave on ice until completely thawed. Briefly mix on a vortex mixer.

Dilution of serum and the activity control
Dilute the serum 1/101 with Diluent CP, blue label, (500 µL Diluent + 5 µL serum) and mix thoroughly but gently on a vortex. The diluted serum and activity control can be left at room temperature for maximum 60 minutes before analysis.

Incubation of samples
Pipet 100 µL/well in duplicate of calibrator (100%-0%), negative control (NC), and activity control (AC) and diluted subject's serum (P) according to the diagram. Incubate for 60-70 minutes at +37º C with lid.

Classical Pathway

	1	2	3
A	100 %	12.5 %	P1
B	100 %	12.5 %	P1
C	75 %	0 %	P2
D	75 %	0%	P2
E	50 %	NC	etc
F	50 %	NC	
G	25 %	AC	
H	25 %	AC	

After serum incubation
Empty the wells and wash 3 times with 300 µL washing solution, filling and emptying the wells each time. After the last wash, empty the wells by tapping the strip on an absorbent tissue.

Adding conjugate
Add 100 µL conjugate to each well. Incubate for 30 minutes at room temperature (+20-25° C).

After conjugate incubation
Wash 3 times as before.

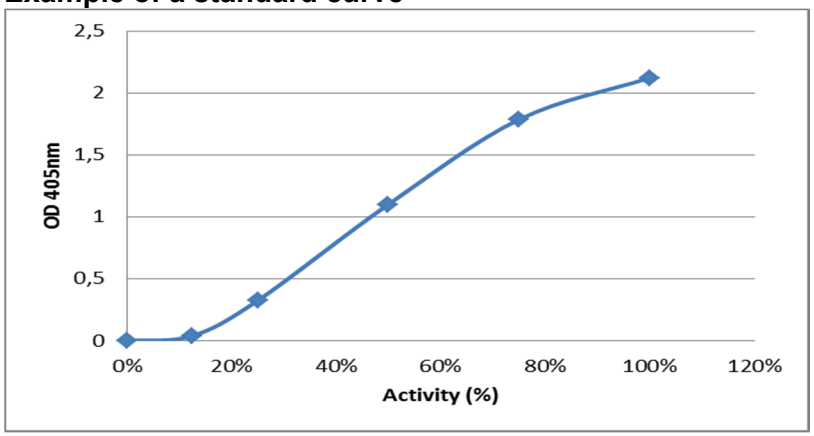
Adding substrate solution
Add 100 µL substrate solution to each well, incubate for 30 minutes at room temperature (+20-25° C). Read the absorbance at 405 nm on a microplate reader. (5 mM EDTA can be used as stop solution, 100 µL/well. Read the absorbance of the wells within 60 minutes.)

Calculation of result
Curve fit 4-parameter logistic (Marquardt) is recommended. Subtract the absorbance of the 0 % - calibrator from all OD values.

The absorbance of Calibrator 100% should be > 1.0, NC absorbance < 0.2.and AC activity >30%.
In cases where the obtained sample values are higher than the highest Calibrator 100%, the samples can be diluted 1/201 and retested.
Please note that the obtained activity value in this case should be adjusted according to applied sample dilution.
If any of controls are not within their respective ranges, the test should be considered as invalid and then repeated.
It is recommended that each laboratory establish its own reference level and cut-off value for deficiencies.

Quality Control
CoA included in the kit, is lot specific, and is to be used to verify results obtained by our laboratory. The results indicated on CoA are to be used as a guideline only. The results obtained by your laboratory may differ.

Example of a standard curve



Please Note: The figure above shows an example of a semi-quantitative standard curve and should not be used for actual subject sample interpretation.

Limitations
The individual subject's complement level cannot be used as a measure of disease severity, as it may vary from subject to subject. Thus, it is difficult to obtain an absolute standardization of results.
The test should not be relied upon as the sole basis of decisions on clinical therapy, but should be used in combination with clinical symptoms and the results of other available tests. Therapy should not be started on basis of the complement assay result. Initiation or changes in treatment should not be based on changes in complement levels alone, but rather on careful clinical observation.

Expected results
When decreased levels of complement components or complement function are found, a deficiency or an ongoing, immunologic process, leading to increased breakdown of components and depression of complement levels is considered by clinicians.

- The normal distribution within 2SD has, for the semi-quantitative assay, been determined to be 66-113% of the positive control, see table 5. Results within this range indicate a normal functionality of the classical pathway. It is recommended that each laboratory confirms or establishes own reference ranges for the population they serve.
- A value below the 66-113% range indicates either increased activation, resulting in consumption of the classical complement pathway capacity, or a genetically determined low activity.
- Values below 15% strongly suggest a complete deficiency either caused by excessive activation or an inherited deficiency in the classical pathway. To establish which complement factor(s) causes the lowered activity further analysis of complement proteins is needed.

- A negative result i.e. suspected deficiency, should always be verified by testing a new, carefully handled sample to ensure that no in vitro complement activation has taken place.
- Increased complement levels are usually a nonspecific expression of an acute phase response.

The Wieslab® Complement system Classical Pathway can be helpful for detection of complement deficiencies related to the Classical Pathways as shown in the table below. A more complete and in-depth functional assessment of all three complement pathways may be achieved using Wieslab® Complement system Screen.

Classical pathway	MBL pathway	Alternative pathway	Possible deficiency
Positive	Positive	Positive	None
Negative	Positive	Positive	C1q, C1r, C1s
Positive	Positive	Negative	Properdin, Factor B,D
Positive	Negative	Positive	MBL, MASP2
Negative	Negative	Negative	C3, C5,C6,C7,C8,C9
Negative	Negative	Positive	C4, C2 or combination

Performance characteristics
120 sera from blood donors were tested and the normal reference range was calculated. See Figure 2 and Table 3. No blood donor was below 40 %.

Calibrator measurement range: 12.5% - 100 %
Limit of Detection (LOD) = 8%

Figure 2. CP semi-quantitative

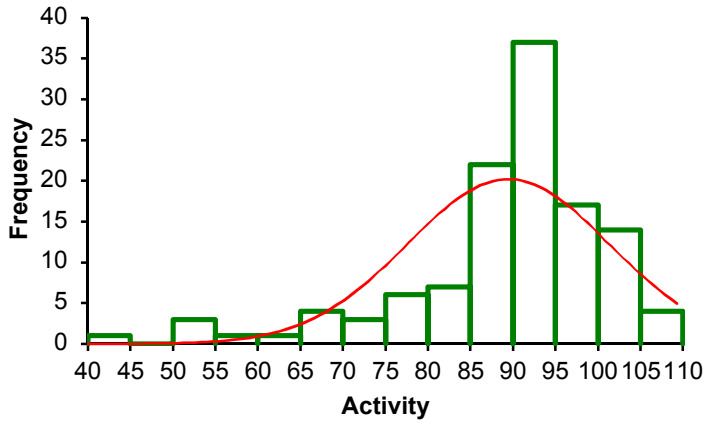


Table 5.

	n	Mean (%)	±2SD (%)	Median (%)
Semi-quantitative application	120	89	66-113*	92

This is a statistic calculation and will not guarantee a true cut-off. It is recommended that each laboratory establish its own reference level and cut-off value for deficiency.
*) Including samples diluted 1/201 to end up on the curve

Table 6.
Sera with known complement deficiencies and specific complement factor depleted sera were tested in the assay. All deficient/depleted sera were low in the assay and gave values below 15 %.

Deficiency	C2	C3	C4	C5	C7	C8
Number of subjects	5	1	1	1	2	2
Number of deficient sera detected	5	1	1	1	2	2

Depletion	C1q	C3	C4	C5	C7
Number of depleted sera	2	1	1	1	1
Number of detected depletion	2	1	1	1	1

Table 7. Inter-assay precision for semi-quantitative application was determined by testing seven samples in eight replicates at three different occasions.

	1	2	3	4	5	6	7
Mean value							
%	71	73	69	72	25	35	31
SD	9	9	6	11	1	2	2
CV%	13%	13%	9%	15%	4%	5%	5%

Table 8. Intra-assay precision for semi-quantitative application was determined by testing seven different samples in eight replicates at one occasion.

	1	2	3	4	5	6	7
Mean value							
%	79	76	72	83	24	34	30
SD	10	11	6	9	0	1	1
CV%	13%	15%	8%	10%	2%	4%	2%

Table 9. Batch to batch variation semi-quantitative application was determined by testing seven samples in duplicate on three different batches by three different persons.

Sample	1	2	3	4	5	6	7
Mean value							
(%)	8	73	85	24	37	74	78
SD	0,80	14,55	12,50	1,32	1,91	7,46	6,08
%CV	10%	20%	15%	5%	5%	10%	8%

Table 10.
Dilution recovery was determined by testing five serial dilutions for three different samples.

Sample	Dilution	Mean Measured Activity (%)	Theoretical Activity (%)	Dilution Corrected % Recovery
1	1/200	55	55	100
	1/400	32	28	114
	1/800	17	14	121
	1/1600	0	7	0
Sample	**Dilution**	**Mean Measured Activity (%)**	**Theoretical Activity (%)**	**Dilution Corrected % Recovery**
2	1/200	46	46	100
	1/400	25	23	109
	1/800	13	12	108
	1/1600	6	6	100
Sample	**Dilution**	**Mean Measured Activity (%)**	**Theoretical Activity (%)**	**Dilution Corrected % Recovery**
3	1/100	84	84	100
	1/200	37	42	88
	1/400	21	21	100
	1/800	11	11	100
	1/1600	7	6	117

TROUBLESHOOTING

Problem	Possible causes	Solution
Control values out of range	Incorrect temperature, timing or pipetting, reagents are not mixed	Check that the time and temperature were correct. Repeat test.
	Cross contamination of controls	Pipette carefully.
	Optical pathway is not clean.	Check for the dirt or air-bubbles in the wells. Wipe plate bottom and reread.
	Controls (positive and/or activity controls) are not correctly reconstituted. Improper dilution of calibrator.	Check the controls, dissolve a new. Check the preparation and make a new dilution.
All test results negative	One or more reagents are not added, or added in wrong sequence.	Recheck procedure. Check for unused reagents. Repeat test.
	Antigen coated plate is inactive.	Check for obvious moisture in unused wells. Wipe plate bottom and reread.
	Serum inactive.	Dilute new samples.
All test results yellow.	Contaminated buffers or reagents.	Check all solutions for turbidity.
	Washing solution is contaminated.	Use clean container. Check the quality of water used for preparation of solution.
	Improper dilution of serum.	Repeat test.

Poor precision.	Pipette delivery CV >5% or samples not mixed.	Check the calibration of pipette. Use reproducible technique. Avoid air bubbles in pipette tip.
	Serum or reagents are not mixed sufficiently or not equilibrated to room temperature.	Mix all reagents gently but thoroughly and equilibrate to room temperature.
	Reagent addition is taking too long time, inconsistency in timing intervals.	Develop consistent uniform technique and use multi-tip device or auto-dispenser to decrease time.
	Optical pathway not clean.	Check for air bubbles in the wells. Wipe plate bottom and reread.
	Washing not consistent, trapped bubbles, washing solution left in the wells.	Check that all wells are filled and aspirated uniformly. Dispense liquid above level of reagent in the well. After last wash, empty the wells by tapping the strip on an absorbent tissue.

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EXPLANATION OF SYMBOLS.

	Batch code.
	Catalogue number.
	Use-by date.
	Temperature limit.
	Biological risk.
	Consult instructions for use.
	Warning.
	Manufacturer.
	Contains sufficient for 96 tests.

Ag	Antigen.
DIL	Diluent.
CONJ	Conjugate
BUF WASH 30X	Wash solution 30x conc.
SUBS pNPP	Substrate pNPP.
CONTROL -	Negative control.
CONTROL + LYO	Lyophilized positive control.
CONTROL AC LYO	Lyophilized activity control.

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