



COMPANY WITH
QUALITY MANAGEMENT SYSTEM
CERTIFIED BY DNV GL
= ISO 9001 =

Complement CH50 Assay

Kit Insert

www.HaemoSCAN.com

Version: November 2016

Summary

Complement hemolytic activity is a functional test of the classical and alternative pathway of complement in plasma or serum. The classical pathway method (CH50) is based on lysis of sensitized sheep erythrocytes in the presence of Ca^{++} and Mg^{++} . This method is suited to evaluate the haemocompatibility of biomaterials and medical devices according to the international standard ISO 10993-4:2002 after blood, plasma, or serum contact with biomaterials and to assess the effects of pharmaceuticals on inhibition or consumption of complement components.

Introduction

Interactions between blood and biomaterials may activate the complement system. Particularly during prolonged contact or during contact of blood with large surfaces, this may induce adverse events, due to generation of an inflammatory reaction and loss of host defense mechanism. Similarly, pharmaceuticals or their carriers may affect the complement system. This results in consumption of complement proteins, which reduces the CH50 or AP50 level (i.e. the dilution of serum to obtain 50% lysis of erythrocytes).

Principle of the Test

An erythrocyte suspension is incubated for 30 minutes with serial diluted serum or plasma at 37 °C.

After incubation samples are centrifuged to obtain supernatant, containing free hemoglobin. The hemoglobin concentration is measured by means of a spectrophotometer.

Positive reference is total lysis induced by lysis fluid, negative reference is obtained after incubation with buffer.

The kit is designed to determine hemolytic activity of small samples (50 μL or less). And can be performed in a 96 well microtiter plate. The same template can also be used with microcentrifuge tubes.

Precautions

- The kit is intended for research use only.
- The kit should not be used beyond its expiry date.
- Do not combine reagents from Complement CH50 kits with different lot numbers.

- The erythrocytes are of ovine origin and these animals have been tested and approved for consumption.
- Chemicals and reagents have to be treated as hazardous waste according to biohazard safety guidelines or regulations.
- Wear disposable (latex) gloves when handling specimens and reagents.
- Never pipette by mouth and avoid contact of skin and mucous membranes
- Use disposable pipette tips throughout the procedure to avoid contamination of reagents.

Contents of the Kit

- | | | |
|---|--------|----------|
| • Erythrocyte concentrate | 1,1 mL | 1 tubes |
| • Dilution Buffer | 100 mL | 1 bottle |
| • Lysis fluid | 3,5 mL | 1 bottle |
| • Stop Solution | 100 mL | 1 bottle |
| • Reference 1, low complement plasma | | |
| • Reference 2, normal complement plasma | | |

Additional Materials and Equipment

The following materials and equipment are required but are not provided with the kit:

- (Calibrated) adjustable pipettes with disposable tips.
- Incubator at 37 °C.
- Spectrophotometer capable of measuring at 415 nm.
- Micro-centrifuge + vials (1,5 mL) or centrifuge for microtiter plates.

Test Procedure

Reagent Preparation

- **Erythrocyte suspension:** Add slowly, while mixing, the erythrocyte suspension to 5 mL Dilution Buffer. Mix gently by end-over-end tumbling of the tube. Centrifuge the tube, without cap, for 10 minutes at 400xg. Remove the supernatant. Repeat this procedure if the OD415 of the supernatant is >0,500. Resuspend the pellet in 40 mL Dilution buffer, this is sufficient for 7 microtiter plates. Dilute 25 µL of erythrocyte suspension in 75 µL lysis fluid, this should give an OD415 between 0,800 and 0,900, when measured in a microtiter plate in a spectrophotometer. Prewarm the suspension at 37°C just before use.

- **Test samples:** Human plasma is used in dilutions of 4, 8, 16, 32, 64 and 128 times, made by serial dilution in a round bottom plate, resulting in 50 µL per well. The positive control is lysis fluid instead of plasma, the negative control is dilution buffer instead of plasma.
- **Reference plasma:** Both reference plasma samples are reconstituted with 250 µL distilled water and are also used in dilutions ranging from 4 to 128 times.

Assay Procedure

1. Pipette test samples or reference dilutions and controls in a round bottom plate (see plate format example).
2. Add 50 µL of the erythrocyte suspension to each well.
3. Cover the plate with a microplate sheet.
4. Incubate for 30 minutes in an incubator at 37 °C.
5. Pipette 100 µL stop solution to all wells.
6. Centrifuge the plate (or microcentrifuge tubes) at 400xg for 10 minutes.
7. Transfer 100 µL of supernatant to a well of a flat bottom microtiter plate.
8. Measure OD 415 nm.

Calculations

1. Correct, for all test and reference materials, for the OD415 of the negative control.
2. Calculate the amount of haemolysis for each sample and dilution.
3. Plot for each sample the haemolysis (%) on the x-axis and sample concentration (%) on the y-axis.
4. Fit a linear curve ($y=ax+b$) through the data points.
5. Calculate $CH50 = a * 0,5 + b$.

Upon request an example calculation spreadsheet is available.

Characteristics

	1	2	3	4	5	6	7	8	9	10	11	12
A	1 4x	1 8x	1 16x	1 32x	1 64x	1 128x	9 4x	9 8x	9 16x	9 32x	9 64x	9 128x
B	2 4x	2 8x	2 16x	2 32x	2 64x	2 128x	10 4x	10 8x	10 16x	10 32x	10 64x	10 128x
C	3 4x	3 8x	3 16x	3 32x	3 64x	3 128x	11 4x	11 8x	11 16x	11 32x	11 64x	11 128x
D	4 4x	4 8x	4 16x	4 32x	4 64x	4 128x	12 4x	12 8x	12 16x	12 32x	12 64x	12 128x
E	5 4x	5 8x	5 16x	5 32x	5 64x	5 128x	13 4x	13 8x	13 16x	13 32x	13 64x	13 128x
F	6 4x	6 8x	6 16x	6 32x	6 64x	6 128x	14 4x	14 8x	14 16x	14 32x	14 64x	14 128x
G	7 4x	7 8x	7 16x	7 32x	7 64x	7 128x	15 4x	15 8x	15 16x	15 32x	15 64x	15 128x
H	8 4x	8 8x	8 16x	8 32x	8 64x	8 128x	neg con trol	neg con trol	neg con trol	pos con trol	pos con trol	pos con trol

Figure 1. Suggested 96-well template for the CH50 assay.