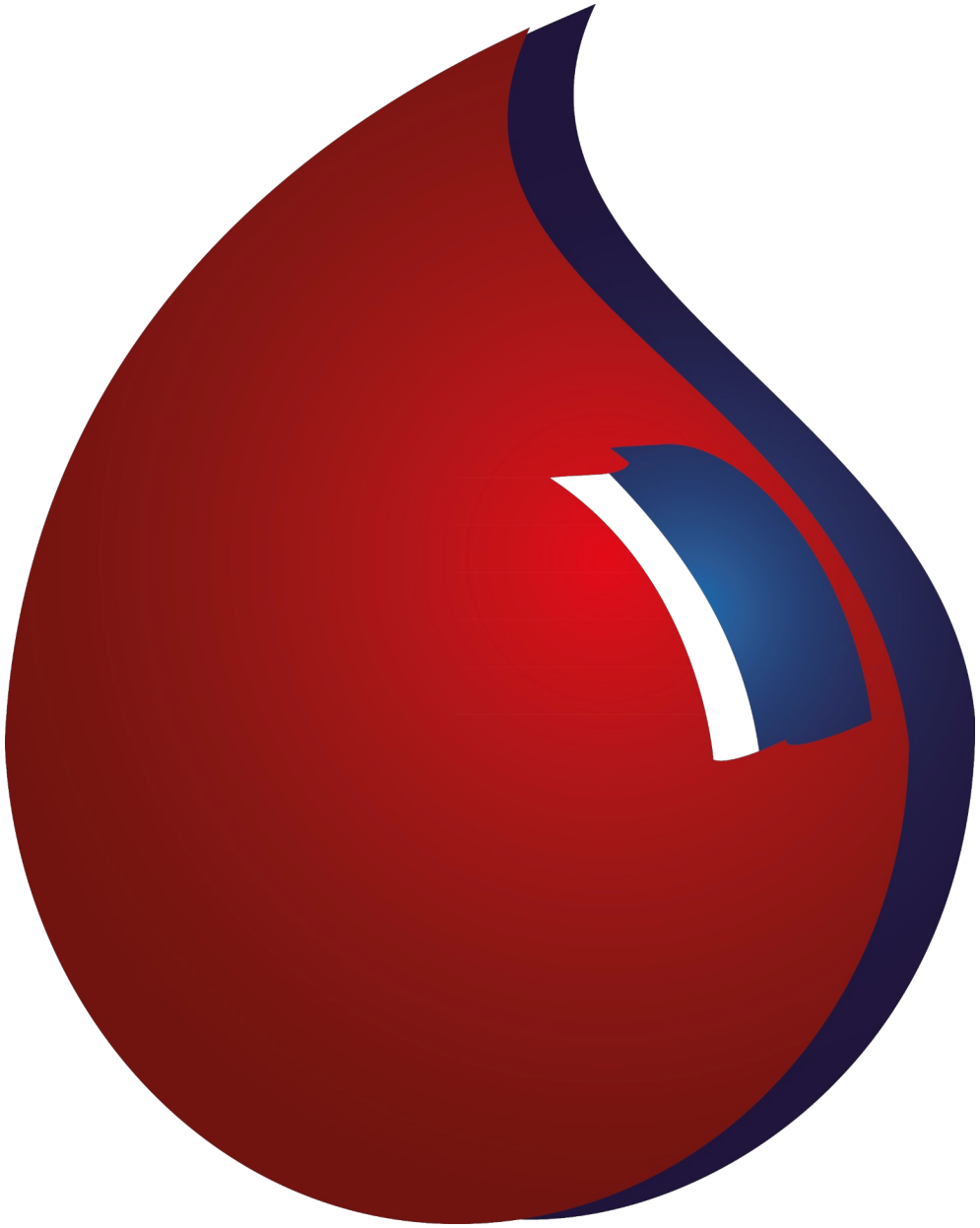

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Complement Convertase Assay

Kit Manual

www.HaemoScan.com

Product number: K005
Version: 02-11-2026

Summary

Measurement of complement convertase activity is a sensitive and specific method for determining complement activation by biomaterials. During incubation with plasma, complement factors can bind to the surface of the material, followed by the formation of complement convertase complexes. After washing to remove unbound complement proteins, the biomaterials are incubated in a medium containing a specific chromogenic substrate. Cleavage of the substrate is quantified by measuring the optical density at 405 nm. Reference materials are included in the kit, and a blank is included in the procedure.
This method can be used to evaluate the haemocompatibility of biomaterials and medical devices in accordance with ISO 10993-4.

Introduction

Medical devices that come into contact with blood may activate the host defence mechanisms of the blood through a foreign-body reaction. One of the potential effects is activation of the complement system, predominantly via the alternative pathway. In view of the wide-ranging biological effects of the complement system, the consequences of uncontrolled complement activation can be severe. Continued activation of the complement system attracts and activates leucocytes, which release lysosomal enzymes and reactive oxygen species that may in turn damage healthy tissue.

Under normal conditions, the complement system is tightly regulated to protect host tissue. The cascade is intrinsically limited by the instability of the enzymes (convertases) that are formed: once a component has been activated, it decays unless it rapidly binds to its substrate. During the use of medical devices, however, these regulatory mechanisms are often inadequate because of the foreign nature of the device surface. Testing the complement convertase activity generated by materials used in medical devices is therefore needed to select materials with the lowest possible complement activation.

This method was developed for in vitro testing of complement activation by materials, based on the formation of convertases on the material surface and the conversion of a chromogenic substrate by these convertases (Complement Convertase Assay, CCA). Because the C5 convertase is the most biologically relevant convertase, the kit contains a substrate with an amino acid sequence similar to the C5 cleavage site, at which C5a is released.

Principle of the Test

A test material of interest (ideally with a known surface area) is incubated with plasma. During incubation, complement factors can bind to the surface of the material, followed by the formation of complement convertase complexes. After incubation, the samples are washed and analysed for complement convertase activity with a convertase-specific chromogenic substrate. The rate of colour development depends on the amount of convertase generated on the surface of the biomaterial.

Precautions

- The kit is intended for research use only. It is not intended for diagnostic or therapeutic procedures.
- Store the kit at −20 °C.
- Do not use the kit beyond its expiry date.
- Do not combine reagents from CCA kits with different lot numbers.
- The CCA plasma is of porcine origin, obtained from animals that have been inspected and approved for human consumption. Nevertheless, handle all components of biological origin as potentially infectious material during use and disposal.
- Dispose of the kit components and contaminated materials in accordance with local and national regulations. For further information, please refer to the Safety Data Sheet (SDS), available at www.haemoscan.com.
- Wear disposable nitrile gloves, a laboratory coat and safety glasses. Do not pipette by mouth.
- Avoid skin contact with the Substrate: its solvent, DMSO, readily penetrates the skin and may carry dissolved substances into the body.
- Use disposable pipette tips throughout the procedure to avoid contamination of reagents.

Contents of the Kit

• CCA plasma, freeze-dried (porcine plasma)	20 mL	2 bottles
• Wash buffer, 10× concentrate (borate–NaCl solution)	32 mL	1 bottle
• Substrate, convertase-specific chromogenic substrate in DMSO	580 µL	2 vials
• Colouring medium (Tris–borate solution)	32 mL	1 bottle
• Reference 1, low-density	1.0 cm^2	5 pieces

polyethylene (LDPE)		
• Reference 2, polydimethyl-siloxane (PDMS)	1.4 cm^2	5 pieces
• Reference 3, medical-grade steel (MS)	1.2 cm^2	5 pieces

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
- Timer
- 96-well microplate
- Microplate reader capable of measuring at 405 nm
- Tweezers
- Vortex mixer
- Microcentrifuge tubes (0.5 mL and 1.5 mL)
- NaCl solution, 0.9%
- Optional, for cleaning of test samples: RBS 35 (Carl Roth) or a comparable laboratory detergent
- Sonicator
- Ultrapure water
- Ethanol (70%)

Test Procedure

Preparation of Materials for Testing

The CCA can be used for coated or uncoated biomaterials. It is recommended that clean samples are tested. The following procedure is recommended for cleaning biomaterials:

1. Sonicate the biomaterial for 15 minutes in 2% RBS and wash three times with ultrapure water.
2. Incubate the biomaterial for 5 minutes in 70% ethanol and wash three times with ultrapure water.
3. Allow the material to air-dry.

Notes:

- Certain materials may be affected by RBS, sonication and/or ethanol. This cleaning procedure is therefore a recommendation only; each user should determine the optimal procedure for their own materials.

- Cleaned materials should always be handled with tweezers.
- The reference materials provided in the kit are clean and ready for use.

Reagent Preparation

Determine the required volumes of reagents and the number of microplate strips.

Prepare reagents as follows:

- **CCA plasma:** Reconstitute the freeze-dried plasma with 20 mL of ultrapure water and keep at room temperature until use. Reconstituted plasma must be used within 4 hours and cannot be frozen. Do not expose the plasma to 37 °C unnecessarily at this stage.
- **Wash buffer:** Dilute the Wash buffer 10× concentrate 10-fold with ultrapure water. Diluted Wash buffer can be stored for 48 hours at 2–8 °C. After thawing, the Wash buffer 10× concentrate can be refrozen for later use.
- **Substrate solution:** Add 14 mL of Colouring medium to one vial of Substrate. Prepare freshly before use; this solution cannot be stored for longer than 1 day. Keep at room temperature until use.

Assay Procedure

Notes in advance:

- Never use glass pipettes or glass tubes, since glass may substantially activate the plasma.
- Select 2 or 3 reference materials with which the activities of the test materials can be compared.
- The reference materials provided in the kit have been cleaned according to the protocol described above and are ready for use.
- The volumes given in this procedure are based on pieces of material of 1.0 × 0.5 cm (two-sided surface area of approximately 1 cm^2). Test materials should be prepared in pieces of approximately the same size.

1. Using tweezers, place the selected reference and (cleaned) test materials in 1.5 mL microcentrifuge tubes. Wedge the materials vertically between the walls of the tubes to prevent them from floating.
2. Place Reference 1 in an identical tube to serve as the blank.
3. Add 650 µL of CCA plasma to each tube containing test or reference material. Ensure that the biomaterial is completely immersed in the plasma.
4. Add 650 µL of 0.9% NaCl to the blank tube(s).

5. Incubate the tubes for 15 minutes at room temperature.
6. During this incubation, prepare an identical number of 1.5 mL microcentrifuge tubes, each containing 1.5 mL of diluted Wash buffer.
7. After the 15-minute incubation, add 1 mL of diluted Wash buffer to the tubes containing the specimens. Remove the specimens with tweezers, blot them briefly on a tissue or filter paper and transfer them to the tubes containing 1.5 mL of Wash buffer. Do not mix or vortex.
8. Remove the specimens as before and transfer them to 0.5 mL tubes. Add 0.5 mL of Substrate solution to each tube.
9. Incubate for up to 24 hours at room temperature. The rate of colour development depends on the amount of convertase generated on the surface of the biomaterial and is linear up to an OD_{405} of 0.75.
10. Transfer 225 µL from each tube to a 96-well microplate. Table 1 can be used as a template.
11. Read the optical density at 405 nm (OD_{405}) within 30 minutes.

Calculations

1. Correct the measured OD_{405} values for the mean blank result (BLK).
2. Calculate the CCA result of the test and reference materials, expressed as OD_{405}/24 h/cm^2, by dividing the blank-corrected OD_{405} by the incubation time (in units of 24 h) and by the surface area of the specimen (in cm^2).

Assay Criteria

- The result of the blank (BLK) should be $OD_{405} \leq 0.075$.
- All measured OD_{405} values should be ≤ 0.75.
- Recommended target ranges for the reference materials are given in the Certificate of Analysis.

Table 1. Suggested 96-well plate layout for the CCA (in duplicate). BLK: blank; REF: reference material; SPL: test sample.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	BLK	SPL 5	SPL 5	SPL 13	SPL 13						
B	REF 1	REF 1	SPL 6	SPL 6	SPL 14	SPL 14						
C	REF 2	REF 2	SPL 7	SPL 7	SPL 15	SPL 15						
D	REF 3	REF 3	SPL 8	SPL 8	SPL 16	SPL 16						
E	SPL 1	SPL 1	SPL 9	SPL 9	SPL 17	SPL 17						
F	SPL 2	SPL 2	SPL 10	SPL 10	SPL 18	SPL 18						
G	SPL 3	SPL 3	SPL 11	SPL 11	SPL 19	SPL 19						
H	SPL 4	SPL 4	SPL 12	SPL 12	SPL 20	SPL 20						

Interpretation of Results

CCA results are classified as follows:

INACTIVE:	OD_{405}/24 h/cm^2	≤ 0.06
LOW:	0.06 < OD_{405}/24 h/cm^2	≤ 0.20
MEDIUM:	0.20 < OD_{405}/24 h/cm^2	≤ 0.60
HIGH:	OD_{405}/24 h/cm^2	> 0.60