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Haemolysis Assay for Biomaterials

Kit Manual

www.HaemoScan.com

Product number: K003
Version: 02-11-2026

Summary

Haemolytic activity must be evaluated for blood-contacting medical devices. Erythrocyte lysis may be induced by direct contact, leachables, toxins, metal ions, surface charge or any other cause. This assay is based on direct contact between the biomaterial and an erythrocyte suspension, and on the release of haemoglobin, which is measured spectrophotometrically. The method is suitable for evaluating the haemocompatibility of (bio)materials and medical devices in accordance with ISO 10993-4.

Introduction

Interactions between blood and biomaterials may induce erythrocyte lysis. Particularly during prolonged contact, or during contact of blood with large surfaces, this process (haemolysis) may cause adverse events in vivo, owing to anaemia and the release of iron and other bioactive substances. Optimal conditions for performing a haemolysis assay have been described: slightly diluted human blood at a blood-to-material ratio of 1 mL/cm^2 in a rotating chamber is recommended.

Principle of the Test

An erythrocyte suspension is incubated with the test material for 24 hours at 37 °C under rotation. After incubation, samples are centrifuged to obtain a supernatant containing free haemoglobin, and the haemoglobin concentration is measured spectrophotometrically. Test samples are compared with reference materials; reference materials with high and low haemolytic activity are included in the kit. It is recommended to include at least two reference materials in each analysis.

The results of the test materials relative to those of the reference materials may be used to evaluate haemolytic activity. Note that pass/fail criteria are based on a threshold of 2% haemolysis; the total haemoglobin concentration of the erythrocyte suspension must therefore also be determined.

The kit is designed to determine the haemolytic activity of small biomaterial samples. Larger samples can also be used, provided that the ratio between the volume of erythrocyte suspension and the size of the material is maintained.

Precautions

- Store the kit, or at least the erythrocytes, between −80 °C and −20 °C, as cold as possible. The shelf life of 6 months is based on storage at −80 °C.
- Once thawed, the erythrocytes must not be refrozen but should be kept at 2–8 °C. The other reagents can be refrozen.
- The kit is intended for research use only. It is not intended for diagnostic or therapeutic procedures.
- Do not use the kit beyond its expiry date.
- Do not combine reagents from Haemolysis Assay kits with different lot numbers.
- The erythrocytes and the Haemoglobin standard are of human origin. Each donation was tested and found negative for anti-HIV-1/2, HBsAg, anti-HCV, syphilis, HIV-1 RNA, HBV DNA and HCV DNA. As no test method can completely exclude the presence of infectious agents, handle these components as potentially infectious material (biosafety level 2).
- The RBS cleaning solution is supplied as a concentrate and is corrosive: it causes severe skin burns and eye damage and may be corrosive to metals. Wear tightly fitting safety goggles, face protection and nitrile gloves when handling the undiluted solution.
- Dispose of the kit components, samples and contaminated materials as biological (potentially infectious) waste, e.g. by autoclaving or incineration, 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.
- Use disposable pipette tips throughout the procedure to avoid contamination of reagents.

Contents of the Kit

• Erythrocyte concentrate (human)	3 mL	3 tubes
• Dilution buffer I	10 mL	1 bottle
• Dilution buffer II	10 mL	1 bottle
• Dilution buffer III	100 mL	1 bottle
• Lysis fluid	8 mL	1 vial
• Haemoglobin standard (human), 10 mg/mL	0.5 mL	1 vial
• Assay buffer	10 mL	1 bottle
• Reference 1, silicone elastomer (SE)	1.5 cm^2	4 pieces
• Reference 2, nitrile rubber (Buna-N)	1.5 cm^2	4 pieces
Reference 3, titanium (Ti)	1.5 cm^2	4 pieces

- RBS cleaning solution 2 mL 1 vial
  (RBS 35 concentrate, Carl Roth)

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
- Water bath at 37 °C
- End-over-end rotator (10–20 rpm)
- Microplate reader capable of measuring at 380, 415 and 450 nm
- 96-well flat-bottom microplate and plate shaker
- Tweezers
- Microcentrifuge and 1.5 mL microcentrifuge tubes
- Optional, for cleaning of test samples: sonicator
- Ultrapure water
- Ethanol (70%)

Test Procedure

Preparation of Materials for Testing

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

1. Dilute the RBS concentrate 50-fold in ultrapure water (2% RBS).
2. Sonicate the biomaterial for 15 minutes in the 2% RBS solution and wash three times with ultrapure water.
3. Sonicate the biomaterial for 5 minutes in 70% ethanol and wash three times with ultrapure water.
4. Allow the material to air-dry.

Notes:

- Cleaned materials should always be handled with clean tweezers.
- This cleaning procedure is a recommendation only; each user should determine the optimal procedure for their own materials. The procedure may damage (biological) coatings.
- The reference materials provided in the kit are clean and ready for use.
- It is recommended to test the (reference) materials in duplicate.

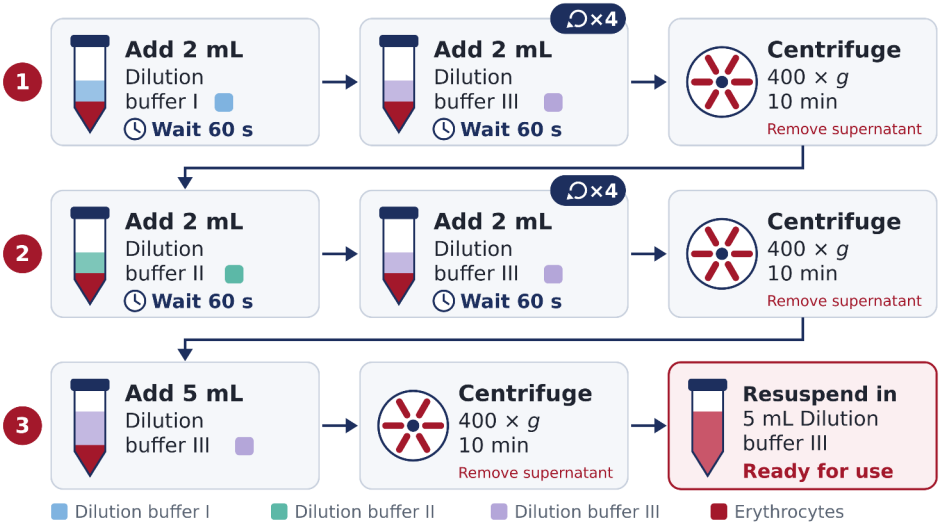
Reagent Preparation

Select the required reagents and number of tubes. Keep spare tubes of erythrocytes between −80 °C and −20 °C. The volumes given in the procedure are based on biomaterial pieces of 0.5 × 1.5 cm (two-sided surface area approximately 1.5 cm^2).

Allow all reagents to reach room temperature. Thaw the Erythrocyte concentrate in a water bath at 37 °C.

Prepare reagents as follows:

- **Erythrocyte suspension:** Always add the Dilution buffers slowly to the erythrocytes. Before each waiting step, mix the suspension gently by end-over-end inversion of the tube.



The erythrocyte suspension is now ready for use. The quality of the final suspension can be checked by centrifuging 0.5 mL in a microcentrifuge tube; the supernatant should be almost colourless. Each washed tube of erythrocytes is sufficient for 10 biomaterial samples.

- **Test samples:** Samples are preferably placed in 1.5 mL microcentrifuge tubes.
- **Haemoglobin calibrators:** Use the Haemoglobin standard undiluted as the highest calibrator and prepare a two-fold serial dilution in Lysis fluid in separate tubes, according to Table 1.

Table 1. Preparation of haemoglobin calibrators.

		Haemoglobin (mg/mL)
CAL1	350 µL Haemoglobin standard	10
CAL2	150 µL CAL1 + 150 µL Lysis fluid	5
CAL3	150 µL CAL2 + 150 µL Lysis fluid	2.5
CAL4	150 µL CAL3 + 150 µL Lysis fluid	1.25
CAL5	150 µL CAL4 + 150 µL Lysis fluid	0.63
CAL6	150 µL CAL5 + 150 µL Lysis fluid	0.31
CAL7	150 µL CAL6 + 150 µL Lysis fluid	0.16
BLK	150 µL Lysis fluid	0

Assay Procedure

Notes in advance:

- 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 0.5 × 1.5 cm (two-sided surface area approximately 1.5 cm^2). Larger materials require a larger volume. Test materials should be prepared in pieces of approximately the same size. A correction for surface area is made in the final calculations.
- Always avoid vigorous contact with air and the formation of bubbles, since these may increase haemolysis.

1. Using tweezers, place the selected reference and (cleaned) test materials in microcentrifuge tubes. Wedge the materials vertically between the walls of the tubes to prevent them from floating. Use tubes without material as the negative control (CTRL).
2. Add 0.5 mL of erythrocyte suspension to each tube (test material, reference material or negative control). Ensure that the biomaterial is completely immersed in the suspension.
3. To determine the total haemoglobin concentration, add 10 µL of erythrocyte suspension to 990 µL of Lysis fluid (100-fold dilution).
4. Incubate the tubes for 24 hours at 37 °C with end-over-end rotation (10–20 rpm).
5. Centrifuge the tubes for 1 minute at high speed (> 4000 × g).
6. Pipette 20 µL of each supernatant, of the total haemoglobin sample and of each calibrator into a well of a flat-bottom microplate.
7. Add 180 µL of Assay buffer to each well.
8. Mix on a plate shaker.
9. Measure the OD at 415, 450 and 380 nm (Harboe method), or at 415 nm only if the other filters are not available. The corrected optical density according to the Harboe method is: $OD^* = 2 \times OD_{415} - (OD_{380} + OD_{450})$.

Calculations*

1. Plot the corrected OD* against the haemoglobin concentrations of the calibrators. The calibration curve should be a straight line (OD* = A × [Hb] + B).
2. Calculate the haemoglobin concentration of all samples from the calibration curve and subtract the haemoglobin concentration of the negative control from those of the test and reference materials.
3. Calculate the total haemoglobin concentration of the erythrocyte suspension by multiplying the value measured in the 100-fold dilution (step 3 of the assay procedure) by 100.
4. Calculate the percentage haemolysis, % haemolysis = ($[Hb]_{sample}$ − $[Hb]_{CTRL}$) / $[Hb]_{total}$ × 100%, and divide it by the surface area of the specimen to express the result in %/cm^2.

Assay Criteria

- The correlation coefficient of the calibration curve should be ≥ 0.98.
- The haemoglobin concentration of the negative control should be ≤ 20% of the total haemoglobin concentration.
- The result of the negative control should be lower than those of the haemolytic reference materials.

Example Results

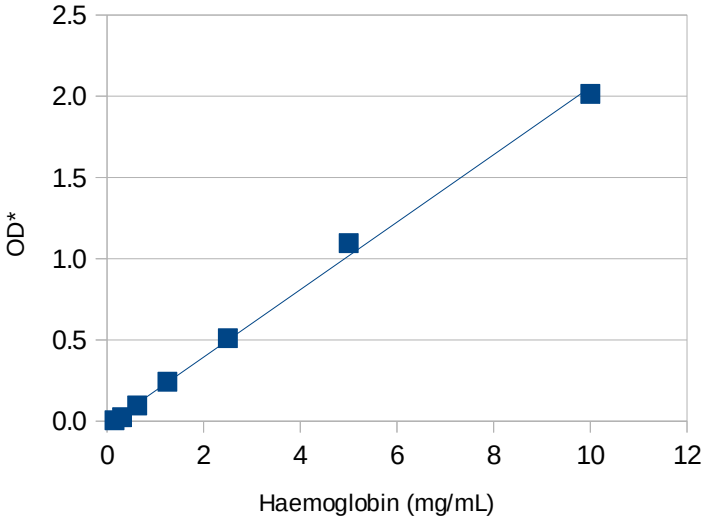


Figure 1. Example of a haemoglobin calibration curve.

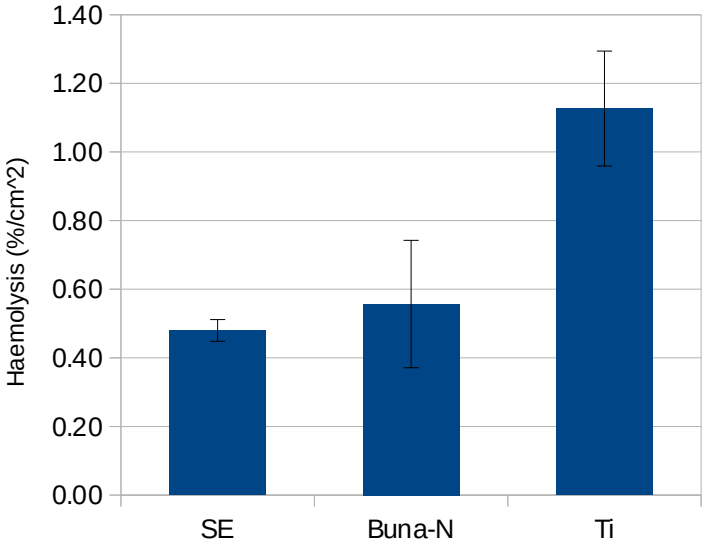


Figure 2. Example of haemolysis by the reference materials, corrected for the negative control.