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Erythrocyte Fragility

(osmotic)

Product information for testing erythrocyte fragility under conditions of osmotic stress.

Principle

This tests determines the resistance of red blood cells (RBCs) to the hemolytic effect of hypotonic solutions.

The diagnostically relevant range is 0.30…0.70 % NaCl.

Reagents

Risks and Safety

Please observe the necessary precautions for use of laboratory reagents and body fluids. Applications should be performed by expert personnel only. Follow the national and laboratory internal guidelines for work safety and infection control. Wear suitable protective clothing and disposable gloves while handling.

It is important to ensure effective protection against infection according to laboratory guidelines.

Use a capillary holder for volume capillaries.

SDS ID International
www.sds-id.com

For additional and general safety information please see details on the label and the corresponding Safety Data Sheet (SDS).

Download by QR code or link: www.sds-id.com/100136-9

Contents / Main Components

004031-... Cont. NaCl 318.0 ± 0.5 mosm/kg H_2O

004031-... Erythrocyte Fragility Stock Solution

004031-0500 1× 500 mL Erythrocyte Fragility Stock Solution

Additionally required or recommended materials

099920-0001 * Capillary holder

\+ 5 mL Disposable syringe with cannula

009101-0100.10.R * + Sodium citrate solution 0,11 mol/L, unbuffered anticoagulant *

\+ Blood collection tubes with 0,11 mol/L tri-sodium citrate and dilution 1 : 5.; unbuffered

\+ Pipettes or Dilutor, transparent tubes with volume for 1 mL of the same size in a series.

* Available from Bioanalytic GmbH.

Sample Material

Citrate blood 1 : 5 (1 mL tri-sodium citrate 0,11 mol/L + 4 mL vein blood) *2), *3) or equivalent blood collection tubes with the same dilution ratio.

Do not use buffered tri-sodium citrate as this may alter osmolality.

A fresh blood sample is required (max. 2 hours).

Blood collection

Please observe the applicable legal and insurance regulations as well as guidelines from medical associations etc.

Fill a disposable 5 mL syringe with 1 mL Sodium citrate solution 0,11 mol/L, unbuffered anticoagulant. Then aspirate 4 mL of venous blood with a new sterile needle. Aspirate approx. 0.5 mL air and remove the needle. Mix the contents of the syringe carefully. Transfer the citrate blood into an empty, closeable sample tube.

Alternatively, proceed with a citrate blood collection tube for dilution 1 : 5.

Reference Ranges

Normal range [1]:
Incipient hemolysis at ..... 0.46 ... 0.42 % NaCl. Indicated by yellow supernatant – do not disturb (no shaking)!
Complete hemolysis at ..... 0.34 ... 0.30 % NaCl Shaking does not result in any turbidity caused by dispersed erythrocytes

Procedure

Use reagent at room temperature (20 ... 25 °C).

Preparing the vials

Prepare a dilution series with a concentration interval of 0.02 %.

For this, arrange 21 tubes in a row first. Then use a 1 mL pipette to load tubes from the outside-in in a pairwise mirrored sequence with exactly 1 mL reagent as follows:

- Pipet 0.30 mL into the 1st tube and the remaining 0.70 mL into the 21st tube.
- Re-fill the pipette and load 0.32 mL into the 2nd tube and the remaining 0.68 mL into the 20th tube. Proceed until all tubes are filled as outlined in the table below.
- Now fill all tubes to 1 mL with destilled water (e.g. Bioanalytic REF 005100) in the opposite way by adding 0.70 mL Aqua p.a, to 0.30 mL reagent etc.
- Then close all tubes and mix sufficiently to obtain a uniform osmolality (else the results will be distorted!).

The test series is now ready for use.

Note:

Preparing the test series is easier with a programmable dilutor system. Once programmed, it automatically creates the dilution series using distilled water and a 1 % NaCl solution.

Attention! Simultaneous dilution with blood together with preparing the dilution series leads to incorrect results as osmotic homogeneity of the dilution solution is not achieved.

Gefäß #		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		Tube #
NaCl [%]		0,30	0,32	0,34	0,36	0,38	0,40	0,42	0,44	0,46	0,48	0,50	0,52	0,54	0,56	0,58	0,60	0,62	0,64	0,66	0,68	0,70		NaCl [%]
Aqua dest.		0,70	0,68	0,66	0,64	0,62	0,60	0,58	0,56	0,54	0,52	0,50	0,48	0,46	0,44	0,42	0,40	0,38	0,36	0,34	0,32	0,30		Distilled water
mmol/l		51,3	54,8	58,2	61,6	65,0	68,4	71,9	75,3	78,7	82,1	85,6	89,0	92,4	95,8	99,2	102,7	106,1	109,5	112,9	116,4	119,8		mmol/l
mosm/kg	(1)	97,2	103,6	109,9	116,2	122,7	128,9	135,3	141,6	147,9	154,2	160,5	166,7	173,1	179,3	185,6	191,9	198,1	204,4	210,7	216,9	223,2	(1)	mosm/kg
mosm/kg	(2)	98,0	104,4	110,4	117,0	123,2	129,4	136,6	142,4	148,8	154,8	161,2	167,2	173,6	180,6	186,4	192,8	199,8	206,0	211,8	216,8		(2)	mosm/kg
	(1) = theoretische Osmolalität (berechnet) • theoretical osmolality (calculated)																							
	(2) = typische gemessene Osmolalität (Gefrierpunktserniedrigung) • typically measured osmolality (freezing point reduction)																							

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Dilution of blood

- Sufficiently resuspend blood cells in the collection tube prior to use (e.g. using a roller mixer).
- Pipet 25 µL citrate blood into each tube in the test series. Ideally, use a dispenser that allows serial dispensing of 25 µL volumes.
- Close the tubes.
- Mix by shaking the tube between thumb and index finger or use a suitable mixer.
- Incubate at room temperature for 1 hour.
- Briefly mix the tubes again (shake 2×).
- Incubate at room temperature in a vibration- free spot (e.g. no centrifuge or printer on the same table/desk)
- Record the result after 2 to 6 (or 12) hours (depending on the protocol in the literature) *1).

Visual scoring

Without centrifugation

After 2 to 6 (or 12) hours of absolutely vibration-free incubation, score the tubes visually *1).

With centrifugation

If analysis is done after centrifugation, vibration-free incubation is irrelevant. Spin the tubes for 1 min at 10000 rpm or 10 min at 4000 rpm in a standard hematology centrifuge.

Spectrophotometric analysis

For photometric analysis, preparing a dilution series with a programmable dilutor is recommended.

After incubation, spin the tubes for 1 min at 10000 rpm or 10 min at 4000 rpm. Proceed to measure the extinction of the clear supernatant photometrically.

Wavelength: ..... 540 nm
Optical path length: ..... 10 mm
Blank: ..... freshly distilled water

You can enter the results into an Excel spreadsheet we have prepared. Download it from our website or request it by E-Mail from support@bioanalytic.de.

Interpretation

Record these results:

Minimum Resistance
= Concentration step at which the RBCs start hemolyzing
= minimum hemolysis

Maximum Resistance
= first tube that contains RBCs not yet hemolyzed
= concentration step at which all RBCs are hemolyzed +1.
= maximum hemolysis +1

Reduced osmotic resistance:

Typical e.g. for spherocytosis (spherical RBCs), acquired haemolytic anaemia, benzene poisoning.

Increased osmotic resistance:

Typical e.g. for thalassemia and hypochromic iron deficiency anemias (RBCs with reduced hemoglobin levels take up increased amounts of water before their membranes burst). Juvenile elastic reticulocytes show increased osmotic resistance, too.

Increased resistance interval:

Characteristically conspicuous in e.g. thalassemias.

Please note:

A normal osmotic resistance does not rule out hemolytic anemia. Reduced osmotic resistance is not specific for hereditary spherocytosis.

Error Identification

The order of the tubes must always yield an ascending series (resistant cells) and a descending series (hemolysis). Odd, unexpected read-outs usually result from errors and must not be recorded. In this case, you should repeat the test series.

Number the tubes to avoid accidentally swapping them.

Information

Periodically check for updates of this product information on our website.

Classifications

Not for human diagnostics.

Support / Information service

For methodological and technical support, please contact us by E-Mail at support@bioanalytic.de.

Periodically check for updates of this product information on our website.

Waste Management

Please observe your national laws and regulations.

Used and expired solutions must be disposed of in accordance with your local regulations.

Inside the EU, national regulations apply that are based on the current, amended version of Council directive 67/548/EEG on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

Decontaminated packaging can disposed of as household waste or recycled, unless otherwise specified.

Literature & Footnotes

Legends for the graphic symbols and tags used follow relevant norms or are available on our internet pages.

[1] Kompendium der praktischen Hämatologie; G. Zeile et al; 2. Aufl. 1983; Git-Verlag Ernst Giebeler, Darmstadt, Germany.

*1) Values differ with the reference source.