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EryFragility-TIC

Erythrocyte Fragility (osmotic)

Product information for determination of erythrocyte fragility induced by 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 to 0.70 % NaCl, approx. 95 to 220 mosm/kg.

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

Main Components

004031-... Cont. NaCl Dilution 0,30 ... 0,68 %
Osmolality see table; stabilized.

004031-6005 KIT EryFragility-TIC® plus • Single test with capillaries

1. 5× 20 pcs EryFragility-TIC®

ETE020 2. 100× 20 µL End-to-end volume capillaries

Do not use other capillaries that are not intended for this TIC test kit.

Different coatings may result in incorrect results.

Additionally required or recommended materials

099920-0001 * Capillary holder *
\+ 5 mL Disposable syringe with cannula
009101-0100 * \+ 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

A fresh blood sampling is required (within 2 hours).

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 anticoagulant (see sample material). 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 blood into an empty, closeable sample tube.

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

General steps

Use reagent at room temperature (20 ... 25 °C). The kit contains 5 dilution series of 20 reaction tubes each.

- Place the box in front of you on the table so that you can read the label. Carefully cut the label along the marked line. Use e. g. a letter opener or a suitable knife. Open the box cover.
- Open the styrofoam cover inside the box carefully. Don't mix up any tubes. The box contains 5 double lines of 20 tubes each, sorted from green (left side below) to red (right side above) containing solutions of different concentration.

The green tube marks the starting point of each dilution series and contains the lowest concentration = lowest osmolality. The red tube is the last vial of the dilution row and contains the highest concentration = highest osmolality. Between them are 18 unmarked reaction tubes with increasing concentrations (see table).

Attention!

We strongly recommend numbering each double row of 20 tubes on the top before use. Proceed from green (= 1 left) to red (= 20 right) using a suitable indelible marking pen. Ideally, mark all tubes directly after first opening the kit pack.

Table:

Gefäß #		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		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		NaCl [%]
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		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	(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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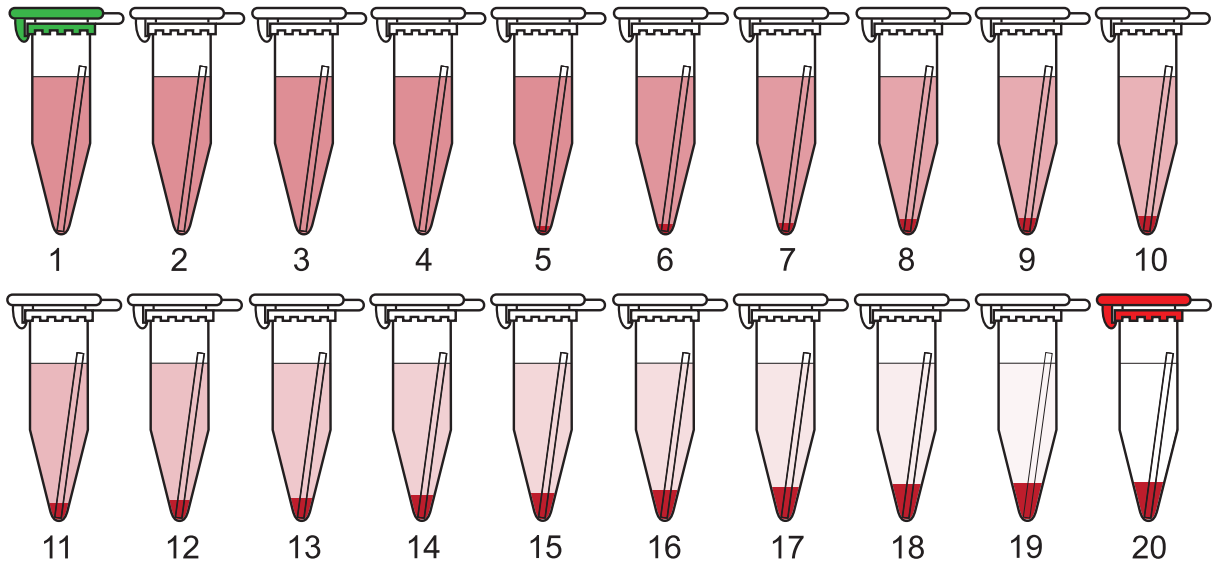

Example of dilution series according to the table with patient sample:

Tube 1 to 4 show complete hemolysis. Tube 5 with a low fraction of non-lysed erythrocytes = Maximum resistance. Tube 5 to 19 (= resistance interval) with decreasing hemolysis and increasing resistance. Tube 19 = minimum resistance. Tube 20 shows no hemolysis. Note: The depicted level of non-lysed cells in the tube is not true to scale.

Procedure

- Mix citrate blood sufficiently before use.
- Open reaction tube #1 (green) to #20 (red) without spilling any solution.
- Fill a 20 µL capillary with citrate blood by using the capillary holder.
- Place the entire capillary into reaction tube #1 (green).
- Proceed in the same way for tube #2 to #20 (red).
- Close tube #1 to #20.
- Mix tube #1 to #20 by vigorously shaking between thumb and index finger or by using a rotation mixer until all blood is removed from capillary.
- Incubate at room temperature for 1 hour.
- Briefly mix the vial once 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 or in accordance with your literature [*1)].

Alternative Procedure

Instead of using a 20 µL capillary, you may also use an automatic pipette with the same volume. Carefully flush pipette tip with the solution in the tube. Use a new pipette tip for each tube.

Visual Determination

Without Centrifugation

To facilitate visual assessment, we recommend arranging the numbered vials in a transparent Plexiglas rack before preparation with blood..

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.

Spectrophotometric Analysis

After incubation, spin the tubes for < 3 minutes at 10000 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 www.bioanalytic.de 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
= 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:

Conspicuously characteristic for 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).

Unexpected readouts usually result from errors and must not be recorded. In this case, you should repeat the test series.

Numbering the tubes avoids accidentally swapping them.

Notes

This product information exclusively relates to the product described in this leaflet. In particular, this product information cannot be applied to similar reagents from other manufacturers.

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

Classifications

Not for human diagnostics.

Instruction for Use

For professional use only.

To avoid errors, the use of qualified personnel is carried out. National guidelines for work safety and quality assurance must be followed.

The used equipment must comply with the state of technology and the laboratory requirements.

All samples and used tubes/vials must be marked clearly identifiable to exclude any confusion.

Protection against infection

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

Laboratory personnel working with human samples should at a minimum be immunized against Hepatitis B (HBV).

Support / Infoservice

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

Feedback

Information from users can be reported to support@bioanalytic.de.

Suggestions for further developments will be considered.

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 between reference sources.