DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Blue cresyl brilliant technical information
Technical card code 09-121
Product code 09-121
Stability of product properly conserved at 15-25°C 24 month.
Pack 500-200 ml or on request

Producers supplier
DDKItalia S.r.l.
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in case of emergency UE number	☎	112
in case of emergency UK number	☎	999
en cas d'urgence Suisse number	☎	145

Principle

Method for the visualization of erythrocyte regeneration by counting of reticulocytes. Measurement of substantial granulofilamentosa (ribonucleoproteins) with fresh, non-fixed, young erythrocytes (supravital staining).

Four stages of substantia granulofilamentosa maturation can be distinguished depending on the stage of reticulocytes development: coiled skein (I), incomplete network (II), complete network (III) and granular form (IV). In peripheral blood the development stages III and IV are found most commonly.
When stained with brilliant cresyl blue they display a bluish black network or bluish black dots.

Sample material

Venous blood, in exceptional cases capillary blood

Tests in series

Prepare thin smears of brilliant cresyl blue solution on microscope slides using a glass rod. Air-dried slides prepared in this way can be stored for 2-3 weeks. For reticulocytes, counts smear a small drop of blood quickly over the stain layer, and immediately place the still wet preparation in a moist chamber (Petri dish with damp filter paper). Leave for 5-10 min and then allow drying in air.

Procedure

Counting under the microscope
Count the reticulocytes per 1000 erythrocytes with oil immersion under the microscope following a meandering pattern. In order to avoid confusion when counting it is advisable to place a reticulocyte-counting grid subdivided into small squares (or a square paper diaphragm) in one of the two eye pieces. In peripheral blood, the development stages III and IV are found most commonly. When stained with brilliant cresyl blue they display a dark blue network and dark blue dots.

Result

The reticulocyte count is expressed in relation to 1000 counted erythrocytes (i.e. as °/00). If the erythrocyte count is low, then the absolute reticulocyte count/µl is used.

Calculation

$$\text{Reticulocyte count} = \frac{E/\mu l \times R\ (°/00)}{1000} \quad [\text{cells}/\mu l]$$

E = erythrocyte count
R = reticulocyte count

Normal range

	°/00	reticulocyte count/µl
Adults	5 - 15	25,000 – 75,000
Newborn babies	20 - 60	100,000 – 300,000

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Notes.
Distilled water or tap water can be used for rinsing and hydration. Always check the pH of your tap water and the levels of chlorine before proceeding with any type of biological tissue, and stain.

* Technical's note: staining time vary according to age, types of solutions, thickness of sections, et. When Gill (code 09-178) modified solution is used, get the best result, staining time (maximum 1-5 minutes), for best change in color, wash quickly in tap water, and then in Scott acidulated solution, (code 00-136). For sections fixed in Bouin, we recommend the use of haematoxylin modified acid AB (code 09-183).

** Please note the alcoholic loses eosin stain with the use, of the days are stretched over time colouring. If you are using purified eosin, check the time, and possibly diluted in ethyl alcohol 96 ° C, if the cytoplasmic staining was too strong. Before use, filter the following solutions; alcoholic eosin, eosin phloxine; Harris haematoxylin, Gill's haematoxylin. The acidified aqueous solution of eosin is prepared by slowly adding glacial acetic acid.

* Follow normal precautions for laboratory reagents. Dispose of waste according to regulations at the local, regional or national level. Refer to Data Sheet Material Safety Data for updated information on risks, hazards and safety associated with the use of these products.

* Risk and Safety Statements outside the EU.
The eosin solution in alcohol is flammable and harmful. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible).

* Target organs: eyes and nerves.
Eosin in aqueous solution. Caution: substance not yet fully tested. Avoid contact and inhalation of the solution of Harris haematoxylin. Organs: heart and nerves. Solutions based hemallum are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing. Organs affected: liver and kidneys. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible).

* Risk and Safety Statements (U.E.)
The eosin solution in alcohol is highly flammable and harmful. Highly flammable. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible). Eosin in aqueous solution. Caution: Substance not yet fully tested. Solution of haemalum. Do not breathe vapors. Avoid contact with skin and eyes. Gill haematoxylin Solutions are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing.

* The microscope used should meet the requirements of a medical diagnostic laboratory. Carefully follow the instructions for the fixative. If an automated tool was used for staining, follow the instructions of the equipment and software. Remove surplus immersion oil before storing. Sample preparation. All samples must be treated according to the technology. All samples must be marked so as to be easily identified. Tools should be used for sampling and sample preparation, which must be observed strictly to manufacturer's instructions about the application and instructions.

* Diagnostics
The diagnosis should be performed only by authorized and trained persons.
Valid nomenclatures must be used. Further tests must be selected and implemented according to recognized methods.

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* Conservation. The staining solution should be stored at a temperature between +15 ° C to +25 ° C, the dye at +5 ° C to +30 ° C. store at 4-6 °C all kit containing silver solutions and Schiff reagents. The solution and dyes must be used before the expiration date. Stability. After first opening the bottle, the dye solution and the dyes are stable until the expiration date when stored at the temperature requested. Always keep the bottles tightly closed.

* Instructions for use
To avoid errors, the staining process must be performed by qualified personnel. For professional use only. Must observe the National guidelines for work safety and quality assurance. Microscopes are used according to the standard. Protection against infection. Must be taken with laboratory guidelines for the protection against infection.

* Instructions for disposal
The solutions used and those have expired must be disposed of as special waste according to local regulations regarding disposal of waste.

Endnotes
1 The timing suggested in the leaflet are approximate and may vary according to your specific needs. If they are used intensively, for staining solutions may lose their dyes, so it is necessary to extend the time of staining solutions, or replace with new products.
2. Include positive control slides in each session.
3. Some hydraulic systems deliver acidic water, unsuitable for use for the part of the procedure for the blue coloration. If tap water is acidic, instead using a dilute alkaline solution, for example, water buffered by Scott.
4. The presence of purple or red-brown nuclei a blue color indicates unsatisfactory.
5. If you over-eosin staining, nuclear staining may be masked. If done correctly, with eosin staining shows a three-tone effect. To increase the differentiation of eosin, extend the time of immersion in alcohol, or use a first alcohol with a higher water content. You can adjust the times of immersion in alcohol to obtain an adequate eosin staining.
6. We do not recommend the addition of stock solution in the working solutions of haematoxylin and eosin.
7. Avoid excessive drag (carryover) of water solutions in alcoholic eosin.
8. The data generated by this procedure are to be used only to support the diagnosis and should be evaluated in conjunction with other tests and diagnostic data