DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

New methylene blue technical information
Technical card code 09-260
Product code 09-260
Stability of product properly conserved 24 month.
Pack 500 ml or on request

Produce in Italy by:
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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 |

## Principe's

New methylene blue stain is intended for the identification of reticulocytes in blood films. New methylene blue stain is for "In Vitro Diagnostic Use."
In 1949, Brecher introduced the new methylene blue method for identification of reticulocytes based on precipitation of ribosomal RNA by the cationic dye. This has now replaced other methods and is the recognized procedure for the quantisation of reticulocytes in peripheral blood.
DDKItalia provides a stable solution for the staining of reticulocytes in blood films. The blood and reticulocyte staining solution are mixed and incubated briefly at room temperature. Wedge or spun smears are made on microscope slides, air dried and evaluated under oil immersion on a light microscope. A red blood cell scoring positive would be observed containing two or more blue-stained granules.

## Method

Collection:
Blood may be collected in standard clinical laboratory evacuated tubes. All routine anticoagulants are acceptable (e.g., heparin, citrate and oxalate). The tripotassium salt of ethylenediamine tetraacetic acid (K3 EDTA) is the anticoagulant of choice. If procedure is not carried out within 2–3 hours of collection, store blood at 4°C. Blood should be warmed to room temperature and mixed thoroughly prior to staining. Blood over 24-hours old is not recommended for use.

## Notes:

It is recommended that blood smears prepared from healthy donors be processed along with patient samples as normal controls.
A small amount of precipitate may form in the Reticulocyte Stain. If precipitate is noticed, filter through laboratory grade filter paper.
The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

## Procedure:

1. Add three drops of thoroughly mixed blood and two drops of new methylene blue stain, to a glass tube and mix well.
Let stand 10 minutes at room temperature (18–26°C).
2. Prepare a conventional wedge or spun smear and air dry at least 15 minutes.
Counterstaining is not recommended.
3. Coverslip and examine microscopically.

Stained blood films are evaluated subjectively for the presence or absence of reticulocytes. A reticulocyte is considered any red blood cell containing two or more blue-stained particles. Using a 100x oil immersion objective and a 10x ocular, randomly pick areas of the film where red cells are close to each other but do not touch or overlap. Count 1000 red blood cells including reticulocytes. The proportion of reticulocytes may be calculated as:

$$\text{Reticulocyte Count (\%)} = \frac{\text{Total Number of Reticulocytes}}{10}$$

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Normal adult values at sea level are 1.0 ± 0.5%.2
Due to variability in the hematocrit, it may be necessary to correct the observed reticulocyte count to a normal hematocrit of 45%:
Corrected Reticulocyte Count (%) =

$$\text{Observed Count} \times \frac{\text{Measured hematocrit (\%)}}{45\%}$$

In the normal non-anemic patient, reticulocytes are found in circulating blood on the fourth day of maturation following three days of maturation within the marrow. Factors increasing erythropoiesis may shorten bone marrow maturation time while lengthening blood maturation time. This shift results in large numbers of "shift" reticulocytes circulating in the blood and these should not be considered when quantitating reticulocytes as a reflection of red blood cell production. Shift cells may be detected by a Wright-stained film and a reticulocyte production index established on the corrected reticulocyte count and hematocrit:

$$\text{Reticulocyte production index} = \frac{\text{corrected reticulocyte count (\%)}}{\text{expected maturation time (days)}}$$

With expected maturation time as follows:

| Days | Hematocrit |
|---|---|
| 1 | 45% |
| 1.5 | 35% |
| 2 | 25% |
| 3 | 15% |

A reticulocyte production index greater than or equal to 3 is considered normal, while an index of less than 2 is below normal.

* 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.

* Conservation. The staining solution should be stored at a temperature between +15°C to 20°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