DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Fetal hemoglobin technical information
Technical card code 12-140
Product code 12-140
Stability of product properly conserved at 4°C 12 month.
Pack 1 kit 100 test or on request

Produce in Italy by
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Intended use

Determination of fetal hemoglobin in blood smears. Fetal hemoglobin stain reagents are for "In Vitro Diagnostic Use." As early as 1864, Korber recognized that the hemoglobin of the fetus was more resistant to alkali denaturation than that of the adult. Advances in techniques for protein isolation and characterization led to the discovery that there are several distinguishing properties that make it possible to differentiate fetal from adult hemoglobin. Among these is the resistance of fetal hemoglobin (hemoglobin F) to acid elution. When blood smears are immersed in acid buffer, for example, adult hemoglobin is eluted from the erythrocytes, whereas fetal hemoglobin is not. If blood smears are treated in this manner and subsequently stained, erythrocytes having hemoglobin F will take up the stain, while those containing only adult hemoglobin appear as "ghosts".
The slide technique for demonstrating fetal hemoglobin in terms of its resistance to acid elution was originally proposed by Kleihauer, and later modified by Shepard. This procedure represents a further improvement in this approach as described by Oski and Naiman.
Fetal hemoglobin estimations are sometimes made to determine possible hemorrhage in the newborn, particularly in cases where there are signs of rectal bleeding. Hemoglobin F assay is also applied to adults as an aid in diagnosing certain types of anemia. For example, from 10-90% fetal hemoglobin is encountered in patients with thalassemia major. Moreover, small increases of fetal blood pigment is usually observed in patients with sickle cell disease.
It is becoming increasingly common in cases of Rh incompatibility to suppress immune reactions to red blood cells entering maternal circulation from the fetus. The amount of specific gamma globulin, containing anti Rh(D) to be administered, is calculated by assessing the magnitude of fetal-maternal hemorrhage. According to described technique, blood smears, which have been properly dried and fixed, are immersed in a citrate buffer pH 3.3 at 37°C. Adult hemoglobin A (HbA) dissolves out of the cells, whereas fetal hemoglobin (HbF) which is acid resistant, remains intracellularand can be stained for microscopic examination.

Procedure:

1. Citrate phosphate buffer solution should be warmed to 37°C in a Coplin jar or staining dish.
2. Using clean, labelled microscope slides, make thin blood smears. Prepare control slides using positive HbF blood (cord-blood) and normal adult blood. Air dry approximately 10 minutes.
3. Fix slides by immersing in ethanol fixative, for 5 minutes, rinse thoroughly with tap water and air dry.
4. Immerse test and control slides in pre-warmed citrate phosphate buffer solution at 37°C for 5 minutes. Agitate after 1 and 3 minutes of immersion. Degree of agitation may be varied to achieve most desirable results. Rinse thoroughly with distilled water and air dry completely to avoid staining artefacts.
5. Stain the slides for 3 minutes in acid haematoxylin solution. Rinse slides with distilled water and shake off excess water.
6. Counterstains slides for 4 minutes in eosin solution. Rinse thoroughly with distilled water and air dry.
7. Place dry coverslip on slide and examine using oil immersion (1000X). The absence of HbF is evident by the presence of ghost cells while retained HbF causes cells to appear bright red. Do not apply oil directly to slide.

Note: The 400X magnification may be used, but the resulting larger field may be more difficult to count.

Result

The proportion of erythrocytes containing fetal hemoglobin may be estimated several ways. When studying maternal blood for evidence of HbF-containing cells, Oski and Naiman4 recommended the following:
1. Count total number of erythrocytes in 5 fields and determine the average number per field.

2. Then, count the number of deeply stained HbF-containing erythrocytes in about 30 fields and determine the average number per field.
3. Calculate percentage of HbF-containing erythrocytes on the basis of the total number of erythrocytes per field.
Results are reported as the percent HbF present.
Sensitivity studies:
According to Oski and Naiman this method is capable of detecting as little as 0.1 ml of fetal blood in maternal circulation.
Reproducibility studies:
Using a series of fresh blood specimens, replicate slides were prepared from each and treated with several different lots of stain on separate occasions. Microscopic examination revealed essentially identical results with each blood sample.
Correlation studies:
Mixtures of cord blood and compatible adult blood were prepared to yield specimens with HbF concentrations ranging from 26–66%.6 The blood mixtures were examined by the described technique and assayed chemically by an alkali denaturation method.10 The percent HbF values showed an average difference of about 7% between methods.

Notes:

For quality control purposes, it is recommended that blood from a normal adult (HbA) and from a newborn or infant (HbF) be included in each series of tests. Barr and Shafer report that fixed positive control slides from cord blood in EDTA can be preserved up to 1 year at –20°C, in a sealed cardboard box. However, EDTA negative controls do not elute completely after being stored for more than 2 months at -20°C. These investigators suggest preparing positive and negative smears on the same slide; thus, providing clear and rapid contrasts as reference in reading test slides.

Normal Ranges

Age	Fetal Hemoglobin (%)
At Birth	50-90
<2 years	0–4
>2 years	0–2

Excessive values are observed in:
Aplastic anemia
Erythremic myelosis9
Hemoglobin H disease9
Hereditary persistence of hemoglobin F
Hereditary spherocytic anemia9
Thalassemia major (40–90% fetal hemoglobin)
Thalassemia minor (5–10% fetal hemoglobin)
Sickle cell anemia
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.

References
1. Korber E: Cited by H Bischoff. Inaugural Dissertations. Dorpat Z

Reagent

Citrate phosphate buffer solution	30 ml (10*)
Acid haematoxylin	2x30 ml
Eosin b solution	2x30 ml

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

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* 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 hemallum. Do not breathe vapours.
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.

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* 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