

Kleihauer Mod. Hb F Kit

(For the detection and demonstration of foetal haemoglobin.)

Overview:-

The identification of cells containing Haemoglobin F (Hb-F) foetal haemoglobin, is dependent upon their ability to resist acid-elution to a greater extent than normal erythrocytes; as a result they appear as deeply stained cells amongst unstained "Ghost" or normal cells which have been eluted of their cytoplasmic contents. Other erythrocyte forms may also exhibit increased resistance to acid-elution, such as reticulocytes and nucleated RBC's; in the experienced observer this should present little difficulty although this should always be considered if clinically indicated.

The first successful cytochemical method for the demonstration of individual cells containing Haemoglobin-F was introduced by Kleihauer, Braun and Betke¹, and utilised a pH system of 3.2. While the original method is sensitive and reliable, the modification of Nierhaus and Betke² utilises a pH system of 1.5 and is less prone to some of the problems encountered as described earlier, as well as staining nuclear elements viz. Lymphocytes, neutrophils and platelets etc. The Nierhaus et al.

¹ Kleihauer, E., Braun, H., and Betke, K. (1957). "Demonstration von fetalem haemoglobin in den Erythrocyten eines Blutaussstrichs". *Klin. Wschr.*, **35**, 637.

modification forms the basis of this kit; which contains all the necessary reagents, pre-formulated and pre-measured to allow ease of use with minimal preparation.

Materials supplied:-

- Fixative solution:- 80% Ethanol 1x500ml.
- Kleihauer Elution Solution "A" (AKA.20ML) 0.75 Alcoholic Haematoxylin Certified 21x20ml.
- Kleihauer Elution Solution "B" (AKB.4ML) Acidified Ferric Chloride 2.4% Aq.21x4ml.
- Eosin counterstain;- 0.5% Buffered Aq. Eosin. 1x500ml.

User Materials:-

- Two (2) coplin Jars with lid (We recommend glass Wheaton jars with screw lids to minimise evaporation.)
- Drying rack.
- Water rinse bath with running water.

Requirements:-

We use and recommend Fetro control sets.

Positive Control:- An artificial spiked positive control may be made by mixing 1 part of normal cord blood with 5-6 parts of normal adult blood.

² Nierhaus, K., and Betke, K. (1968). "Eine vereinfachte Modifikation der sauren Elution für die cytologische Darstellung von fetalem Haemoglobin". *Klin Wschr.*, **46**, 47.

EDTA samples are preferred, with the cord blood being less than 6-8 hours old and correctly stored. Mix well and make thin smears.

Negative Control:- Make thin smears from the same adult blood used for the positive control.

Patient/Test:- make thin smears from a fresh EDTA sample.

Method:-

1. Smears freshly prepared are allowed to thoroughly dry without the aid of heat.
2. Fix smears in Coplin Jar of 80% Ethanol (Fixative) for 5 minutes.
3. Prepare elution solution by decanting contents of Elution Solution B (smaller 4ml vial) into larger Elution A vial (20ml vial), cap and mix by inversion.
4. Immerse slides singly or two slides back to back into Working Elution Solution for 30 - 60 secs. Raise and lower the slides in the solution every 5-10 secs to facilitate surface contact and elution.
5. Remove slides and rinse in running water for 2-5 minutes.
6. Immerse slides in coplin jar of Eosin counterstain for 1-2 minutes.

7. Rinse slides in water and allow to drain and dry without heat.
8. Coverslip slides and examine under x20 and x40 high dry, or use oil immersion.

Results:-

Foetal cells stain bright red and adult ghost cells stain very pale pink or not at all. Nuclear material, including neutrophils, lymphocytes and platelets stain bluish grey/black.

Additional Method Notes:-

- If desired at step 4) earlier, users may elect to flood slides with elution reagent instead; multiple rinsing may be required to effectively elute the sample and the time required will increase to at least 1 minute, otherwise elution may be patchy.
- The user is warned against using heat to dry slides as there may be heat fixation as well which will block acid elution.
- Aged dry slides are also resistant to acid elution, do not use old smears, they should be as fresh as possible.
- Working Elution Solutions once prepared are stable for at least 24 hours and may be used repeatedly during this time. If kept longer the solution will require filtration before use. Do not keep for more than 48 hours.

Re-order Separate Bulk Reagents:-

- Kleihauer Elution Solution "A" 2.5 Litre. (Code: AKA.2.5L)
- Kleihauer Elution Solution "B" 500ml. (Code: AKA.500ML)
- Kleihauer Eosin Counterstain 500ml (Code: AKE.500ML)

Special Notes for Bulk Reagent users:-

Technical staff are referred to Methodology as fully detailed in Dacie & Lewis "Practical Haematology" from the 4th to the current Edition; in which four (4) parts of Kleihauer Reagent A is mixed with one (1) part of Kleihauer Reagent B as described in step 3. earlier.

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