

## SHEPARD'S STAIN KIT (MODIFIED KLEIHAUER)

IVD

Catalogue numbers  
610360 / 610920 / 610940

### INTENDED USE

The Clin-Tech Shepard's Stain Kit (Modified Kleihauer) can be used for the screening and quantification of feto-maternal haemorrhage (FMH). The results obtained should be considered as provisional: significant quantities of FMH should be confirmed by an alternative method (e.g. by flow cytometry).

### PRINCIPLE OF THE METHOD

Foetal haemoglobin (Hb F) is less soluble in an acidic environment than adult haemoglobin (Hb A). A technique introduced by Kleihauer<sup>3</sup> and modified by others<sup>5,6</sup> exploits this difference in which a blood film is prepared and after fixation in alcohol, is concurrently acidified and stained with haematoxylin. Next, solubilised Hb A present in adult cells is eluted in an aqueous rinse step, and in the final step, Hb F contained in foetal cells are stained by eosin. In this way foetal red blood cells (stained red by eosin) can be distinguished from white cells (stained grey-purple by haematoxylin) and adult red blood cells (unstained, empty ghost cells). Accurate quantification is essential to determine the amount of FMH<sup>1</sup>.

### KIT PRESENTATION

Reagents	Two-part stain 610360	Complete kit 610920	Complete kit 610940
<b>Solution 1</b> (Alcoholic Haematoxylin)	500 mL	500 mL	500 mL
<b>Solution 2</b> (Acidified Ferric Chloride)	250 mL	250 mL	250 mL
<b>Fixative/Diluent</b>	not included	1000 mL	1000 mL
<b>Eosin Y 1%</b>	not included	1000 mL	not included
<b>Counterstain*</b>			
<b>Erythrosine B 0.1%</b>	not included	not included	1000 mL
<b>Counterstain</b>			

### PROTOCOL

- 1) Prepare the Working Solution by mixing together:
  - 2 parts **Solution 1**,
  - 1 part **Solution 2**
  - 1 part **Fixative/Diluent**
- 2) Prepare a fresh, air-dried film on a clean microscope slide from blood collected in EDTA and diluted 1 in 2 to 1 in 3 with normal saline.
- 3) Fix films in Fixative/Diluent for 5 minutes at room temperature.
- 4) Dry thoroughly by standing in a vertical position for about 10 min.
- 5) Place in Working Solution for 20 seconds.
- 6) Rinse in purified water.
- 7) Stain with Eosin (or Erythrosine) Counterstain for up to 3 minutes.
- 8) Rinse and air dry (prepare a temporary mount if examining with an immersion oil objective).
- 9) Examine using the x10 objective and x10 eyepiece for screening and, if required, using the x40 objective for quantitation of FMH.
- 10) It is essential to follow current local or national guidelines when interpreting the results. For the UK see Austin et al (1999)<sup>1</sup>.

### EXPECTED RESULTS

- Foetal red cells containing Hb F stain red
- Maternal (adult) red cells containing Hb A are completely eluted, leaving 'ghost' cells.
- White cells stain greyish-purple.
- See also Lewis et al (ref 4) and Carpanen et al (ref 2).

### NOTES

- The use of EDTA anticoagulated blood is preferable. If necessary, specimens up to 48 hours old may be used
- It is essential that the working solution is prepared correctly.
- The Working Solution is stable for up to six weeks at room temperature, and may be filtered if a precipitate forms.
- Protocol timing may be varied to suit individual preference.
- Positive and Negative controls should be used with each batch of investigations: -

**NEGATIVE** control slides are prepared from normal adult blood specimens diluted; in saline.

**POSITIVE** control slides are prepared from 1 part cord blood mixed with 10 to 20 parts autologous adult blood diluted in saline.

### REFERENCES

1. Austin E, Bates S, de Silva M et al (working party of the British Committee for Standards in Haematology, Transfusion Taskforce). Guidelines for the Estimation of Feto-maternal Haemorrhage. British Society for Haematology; London. 2009.
2. Carpanen E, Parker Williams J. Kits for feto-maternal haemorrhage screening and quantitation. MHRA Evaluation Report 05053. HMSO 2005.
3. Kleihauer E, Braun H & Betke K (1957) Demonstration von fetalem Hämoglobin in den Erythrocyten eines Blutausstrichs [Demonstration of fetal hemoglobin in erythrocytes of a blood smear]. Klin.Wschr. 1957; 35: 637.
4. Lewis SM, Bain BJ, Bates I. Dacie and Lewis practical haematology, 10th edition. Churchill Livingstone Elsevier 2006.
5. Nierhaus K & Betke K. Eine vereinfachte modifikation der sauren Elution die cytologische Darstellung vom fetalem Haemoglobin [A simplified modification of the acid elution for the cytological demonstration of fetal hemoglobin]. Klin.Wschr. 1968; 46: 47.
6. Wallet LHB & Baxter Robinson J. Human haemoglobin variants and their identification. Laboratory Aids Series. 1968. Butterworths: London.