

**USE**

Demonstration of HbF in blood smears.

**PRINCIPAL OF TEST**

Fetal haemoglobin (HbF) resists acid elution whereas adult haemoglobin (HbA) does not. Blood smears are treated with acid buffer, during this process HbA is eluted from erythrocytes whereas HbF is not. Subsequent staining reveals erythrocytes containing HbF as coloured red. Erythrocytes containing HbA appear as 'ghost' cells (only outer cell membrane visible).

**REAGENTS**

- 1 - HbF Fixative 4 x 30ml
- 2 - Citric-phosphate buffer concentrate pH 3.20-3.30 100ml.
- 3 - Haematoxylin reagent 2 x 50ml
- 4 - Erythrosine Counterstain x 50ml

Reagent components in this kit are for 'In-vitro Diagnostic use'. Standard precautions in the handling of laboratory reagents should be followed.

**STORAGE AND STABILITY**

- 1 - Store citric-phosphate buffer in refrigerator [2-4DC]. Discard if microbial growth occurs before expiry date and contact us for replacement.
- 2 - Store remaining components at room temperature in dark or subdued light .

NOTE: On usage Haematoxylin Reagent will degrade over time. Discard when acceptable staining time exceeds 6 minutes. Sufficient reagent is provided with the kit [2 x 50mls]. Should this prove not to be the case during the lifetime of the product please contact us for additional reagent free of charge.

**ADDITIONAL REAGENTS REQUIRED**

Deionised water

**SPECIMEN COLLECTION & STORAGE**

All blood derivatives should be considered potentially infectious. Take appropriate precautions.

Capillary blood or venous anti-coagulated (EDTA or Oxalate) blood may be used. Samples of venous blood – EDTA or oxalate mixtures should be refrigerated and tested promptly, within 24 hours,

Using capillary blood – prepare smears from the whole blood, test within 24 hours.

Due to the high content of HbF, samples from newborn may require dilution with 0.85% saline.

**REAGENT PREPARATION & SET UP**

- 1 - fixative: Coplin jar or smaller container ( eg plastic slide mailer) filled with HbF Fixative.
- 2 - Rinse: beaker x 200ml Deionised water
- 3 - Elution reagent: Dilute 5ml of citric-phosphate buffer concentrate to 50ml with Deionised water. Transfer to Coplin jar. Keeps for up to 14 days if refrigerated (2-4DC) but discard after use.
- 4 - Rinse: beaker x 200ml Deionised water.
- 5 - Copin jar containing Haematoxylin Reagent. Return reagent to bottle after use.
- 6 - Rinse: beaker containing 400ml TAPWATER.
- 7 - Copin jar containing Erythrosine Counterstain. Return reagent to bottle after use.
- 8 - Final Rinse: beaker 400ml Deionised water.

**TECHNIQUE**

- 1 - Prepare smears on labelled slides (stained smear slide is difficult to see), air dry for 10-15 minutes.
- 2 - Fix smears in fixative for 5 minutes. Rinse in Deionised water and air dry.
- 3 - Warm Elution reagent in Coplin jar to 37DC and incubate smears for 6 minutes, agitating the slides occasionally throughout. After incubation, rinse in deionised water and air dry.
- 4 - Stain in Haematoxylin Reagent for 3-4 minutes, rinse in TAPWATER for 10 seconds. Shake off excess water.
- 5 - Stain in Erythrosine Counterstain for 3-4 minutes. Rinse in Deionised water and air dry.
- 6 - Examine using light microscopy under high (x700) dry magnification. Alternatively place coverslip on slide and examine under oil immersion. Do not apply oil directly to smear, observation is difficult under oil applied this way.

## QUALITY CONTROL

A known normal sample (HbA) and sample for an infant or prepared from 1 drop normal HbA and 1 drop cordblood (being ABD Blood group compatible) should be run at the same time as quality/performance controls.

## EXPECTED OBSERVATIONS

HbF containing cells	-	Red
Normal adult cells	-	Very pale pink 'ghost' cells (only outer cell membrane visible)
Leukocytes	-	Blue nucleus, red cytoplasm
Platelets	-	Red-Pink

## REPORTING

Oski and Naiman \* reported the following methods for estimating HbF containing erythrocytes is maternal blood.

- 1 - Count erythrocyte total in 5 fields and calculate the average per field.
- 2 - In 30 fields count the number of deeply stained HbF containing erythrocytes and calculate the average number per field.
- 3 - Calculate percentage of HbF present using this formula.  
AV.No. at HbF containing erythrocyte per field x 100 = %HbF present.  
AV No. at erythrocyte total per field

There are several other methods of estimating % age HbF present. The laboratory should establish its own procedures.

\*Oski,Naiman : Haematologic problems in the Newborn.  
2<sup>nd</sup> Edition. Saunders 1972 p62-63.

## NORMAL RANGES

AGE	%HbF
Birth	50-90
<2 years	0-4
>2 years	0-2

## HEALTH & SAFETY

HbF Fixative contains Ethanol and is FLAMMABLE. Keep away from sources of ignition. Do not consume these reagents.

All other kit components contain organic compounds/dyestuffs that are potentially harmful in large amounts or irritant by ingestion, or contact with skin & eyes. Wear appropriate safety gear and take appropriate precautions for the handling of laboratory chemicals. If any reagent comes into contact with the eyes, rinse with eye bath for minimum 5 minutes. If any soreness persists then seek medical attention. If any reagent comes into contact with skin wash well with soap and water. If ingested or you feel unwell seek immediate medical attention and show this leaflet.

## SPILLAGE & WASTE DISPOSAL

Mop up any reagent spillage with cloth and rinse under tap water to drain. Run any waste reagents to Public Sewer diluting greatly with water.

GCC Diagnostics guarantees that the highest quality reagents are supplied with this product and that the product conforms to the

Information contained in this leaflet.

The user should however, determine the suitability of this product for their particular use

.If you wish to report any findings to us or if you require help or further information on the use of this product please contact us.

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