

# Kleihauer

## For In-Vitro Diagnostics

## For Professional Use Only

Kleihauer test kit is used to detect foetal red cells in mother's blood.

### MODE OF ACTION

Hb F, the main Hb component in foetal blood, is present at levels of 65%-95% at birth and usually drops to less than 2% by 6-12 months of age. Thus the majority of foetal cells contain Hb F, allowing them to be differentiated from maternal cells in the mother's circulation.

An acid elution cytochemical method was introduced by Kleihauer et al. in 1957. It is a sensitive procedure which identifies individual cells containing Hb F even when few are present and their detection in blood allows the magnitude of blood loss from the foetus into the maternal circulation to be assessed.

The identification of cells containing Hb F depends upon the fact that they resist acid-elution to a greater extent than do normal adult cells; thus with this method foetal cells appear as isolated darkly-stained cells amongst a background of pale-staining ghost cells.

The occasional cells which stain to an intermediate degree are less easy to evaluate, some may be reticulocytes as these also resist acid-elution to some extent.

### WARNINGS AND PRECAUTIONS

Kleihauer A,B & D contain ethanol and are highly flammable. Keep away from flame and ignition sources.

Kleihauer C is irritating and corrosive

Use suitable personal protective equipment when handling these products. Consult SDS and packaging labels before use.

### INGREDIENTS

#### KleihauerA

Chemical	CAS	Conc.
Ethanol	64-17-5	<80%
Water	7732-18-5	Balance

#### KleihauerB

Chemical	CAS	Conc.
Haematoxylin	517-28-2	<1%
Ethanol	64-17-5	Balance

#### KleihauerC

Chemical	CAS	Conc.
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Iron(III) Chloride	7705-08-0	<3%
Hydrochloric Acid	7647-01-0	<1%
Water	7732-18-5	Balance

#### KleihauerD

Chemical	CAS	Conc.
Eosin Y	17372-87-1	
Water	7732-18-5	Balance

### SPECIFICATIONS

#### KleihauerA

Appearance Clear, colourless liquid with alcoholic odour  
Density @20C 0.850-0.860 g/mL

#### KleihauerB

Appearance Clear reddish brown solution with alcoholic odour  
Density @ 20C 0.790 – 0.800 g/mL

#### KleihauerC

Appearance Clear yellowish solution  
Density @ 20C 1.00 – 1.02 g/mL  
FeCl<sub>3</sub> 2.2 – 2.5% w/v  
pH @ 20C <2

#### KleihauerD

Appearance Reddish Orange Liquid  
Density @ 20C About 1.00 g/mL  
Absorbance (λ max) 517nm

### STABILITY

All Kleihauer kit components are stable for 12 months when stored at room temperature in sealed bottles away from heat and light.

### SAMPLE PREPARATION

4 mL of venous maternal blood in EDTA. Sample should be collected 1 hour post partum, or no later than 72 hours post partum.

### TECHNICAL PROCEDURE

#### Reagents

**Fixative** 80% Ethanol (KleihauerA).

**Elution reagent** Freshly prepared from stock solutions A and B.

(2.5ml solution A + 0.5ml solution B )

#### **Stock A Solution**

0.75% Alcoholic Haematoxylin (KleihauerB)

#### **Stock B Solution**

Ferric Chloride HCL solution (KleihauerC)

#### **Counterstain**

0.5% Aqueous Eosin (KleihauerD)

### QUALITY CONTROL

Control slides must be prepared to ensure the staining protocol adequately differentiates between adult and foetal cells, and to standardise the counting of foetal cells. All controls are prepared freshly on a weekly basis.



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**POSITIVE CONTROL**

1. Select a fresh EDTA cord blood specimen that is Direct Coombs Test (DCT) Negative. (The red cell count should be approximately 4.40).
2. Select an adult male, EDTA specimen that is ABO compatible with the cord blood. Ensure that there is at least 2mls of blood. The red cell count should be between 4.00 to 4.40.
3. Add 5µl of cord blood to 2mls of adult blood. Mix well.
4. Then dilute this suspension 1:1 with saline, i.e. 10 drops suspension with 10 drops of saline, into a separate tube.
5. The positive control is equivalent to 0.2% to 0.25% foetal cells which is approximately equivalent to a 6ml foetal bleed.

This method is based on the following formula:

$$A = \text{Adult RCC}$$

$$B = \text{No. of adult cells in 2mls} = \frac{A \times 2}{1000}$$

$$C = \% \text{ fetal cells required (0.25\%)}$$

Hence No. of foetal cells required to be added

$$\frac{C \times B}{100}$$

Convert the number of cells to a volume, i.e. volume of foetal cells to be added in mls

$$= \frac{\text{No. of foetal cells (as above)}}{\text{foetal RCC}} \times 1000$$

Source: 2002 RCPA QAP Recommendation.

**NEGATIVE CONTROL**

10 drops of adult **male** blood + 10 drops saline

**METHOD**

1. Dilute a well mixed patient test sample/s 50/50 with saline (3 drops cells + 3 drops saline) and spread thin films using small drop of dilution and label.
2. Make fresh films for POSITIVE and NEGATIVE controls, label and date.
3. Allow to dry for 5 minutes – DO NOT LEAVE FOR > 30 MINS before fixing.
4. Fix in **80%** Ethanol for 45 seconds.
5. Rinse **well** in tap water and dry with hairdryer or allow to air dry.
6. Prepare a fresh working solution of elution reagent (2.5ml solution A + 0.5ml solution B) and Elute (stain) for 20 seconds.
7. Immediately wash in tap water and allow to air dry at room temperature.
8. Counterstain with Aqueous Eosin for 1 minute.
9. Wash, dry, coverslip and examine slide.

**RESULTS**

Red Cells that contain Hb A appear as ghost cells. Red cells that contain Hb F stain bright pink.

**TECHNICAL COMMENTS**

**FALSE POSITIVES**

Pregnant women can produce HbF themselves around mid pregnancy. This can make the interpretation of their results difficult. Similarly, women with thalassaemia may produce HbF cells. It is felt that HbF cells produced by the woman herself are more likely to be of irregular staining intensity and that genuine HbF cells of foetal origin are dark red and of similar staining intensity. Reticulocytes will also give an intermediate staining appearance

**FALSE NEGATIVES**

If the elution reaction proceeds beyond 15-20 seconds, HbF will start to elute. This time is critical.

**REFERENCES**

1. *Dacie, Sir John V & Lewis, S.M, Practical Haematology 11<sup>th</sup> Edition. Churchill Livingstone, New York USA, 2012*

**ORDERING INFORMATION**

Code	Product Name	Size
FNNKLEIHAUER	Kleihauer Kit	500mL
FNNKLEIHAUER5	Kleihauer Kit	5L