MICROGEN BIOPRODUCTS

CE

REF M96-50 S-TEST KIT

50

IVD

INTENDED USE

Microgen Bioproducts S-Test Kit is intended for in vitro screening of human blood samples to detect the presence of haemoglobin S which is associated with sickle cell trait. As the product is a screening test, all positive results should be confirmed by haemoglobin electrophoresis. The product is intended for professional use only.

PRINCIPLE OF THE TEST

The test is based upon the relative insolubility of haemoglobin S in a buffer containing a reducing agent[1]. The kit contains two reagents, a dry powder and a buffer solution which are mixed together prior to use. The blood sample is added to the reconstituted solution, mixed and allowed to stand. The reagent mixture lyses the red blood cells and separates the haemoglobin chains. Haemoglobin S is not soluble in the reagent whereas normal haemoglobin (Hb-A) will dissolve. The presence of Hb-S in the blood sample is determined by assessing the turbidity of the resultant solution.
A variation of the method involving centrifugation of the sample and reagent mixture can be used to distinguish between homozygous and heterozygous sickle cell traits, and to aid the interpretation of equivocal results.

KIT PRESENTATION

CONT			
REAG	A	M96a	5 x 20mL

Phosphate Buffer Solution

REAG	B	M96b	5 x 2.2g

Powder reagent containing saponin and sodium dithionite
(Refer to Safety Precaution 3 below)
Viewing Rack
Instructions for Use

Materials and Equipment required but not provided

- Positive and negative control samples
- Pipette with disposable tips to deliver 20µL
- Pipette to deliver 2mL
- Clear plastic or glass test tubes (75 x 12mm)
- Timer
- Centrifuge capable of spinning tubes at 1200g (for spin technique only)
- Latex gloves, safety glasses and other appropriate protective garments
- Biohazardous waste containers

WARNINGS AND PRECAUTIONS

Safety

1. The reagents supplied in this kit are for *in vitro* diagnostic use only.
2. All human blood samples should be treated as potentially infectious.
3. Reagent B (M96b) contains saponin and sodium dithionite which are harmful by dust inhalation/ingestion, and are irritants to skin and eyes. Take appropriate precautions when handling both the dry powder and the reconstituted reagent.
4. If any reagent comes into contact with skin or mucous membranes, wash with copious amounts of water.
5. Waste reagents and materials should be decontaminated and discarded according to national guidelines. Decontamination of infectious material can be achieved with sodium hypochlorite at a final concentration of 3% for 30minutes. Liquid waste containing acid must be neutralised before treatment.

Procedural

1. The kit should be used in accordance with these Instructions for Use.
2. Ensure that all reagents have reached room temperature (18-25°C) before use.
3. Do not mix reagents from different batches.
4. Do not cross-contaminate reagents.
5. Positive and negative controls (not supplied) should always be run in parallel with test samples to confirm that the test system is functioning correctly.

STORAGE AND SHELF LIFE

Kit components:
All unopened components can be used until the date printed on the outer carton label provided they are stored at 2-8°C. Following reconstitution, Reagent B can be used repeatedly for up to 14 days post-reconstitution provided that it is stored at 2-8°C and that reasonable precautions are taken to avoid high levels of microbial contamination.

Specimens:
Venous blood (2mL) should be collected into EDTA anti-coagulant and mixed gently. Samples can be stored for up to 14 days at 2-8°C.

RECONSTITUTION OF REAGENT

Add one vial of Reagent B to one bottle of Reagent A – care should be taken to transfer all the powder. Shake vigorously for 10 seconds, then place on a roller mixer until dissolved. It is useful to record the date of reconstitution on the bottle label; use within 14 days of reconstitution.

TEST PROCEDURE - SCREENING TEST

1. Ensure that all reagents are at room temperature (18-25°C) and mixed thoroughly.
2. Always run positive and negative controls to confirm the test system is functioning correctly.
3. Add 20µL blood sample to 2mL reconstituted reagent in a plastic or glass test tube (75 x 12mm) and mix thoroughly.
4. **Note:** If the haemoglobin content of the sample is less than 6g/decilitre, a larger volume of sample should be added. This however will result in an increase of the plasma protein concentration in the tube, which may generate turbidity and interference (see Limitations in Use point 5 below). For this reason, when the sample volume is increased, the red blood cells should be washed with 0.9% sodium chloride solution to remove excess protein prior to testing.
5. Allow mixture to stand for 3-5 minutes and examine for **turbidity** using the viewing rack provided.

INTERPRETATION - SCREENING TEST

1. A **turbid** reaction denotes a **positive** result – i.e. the lines on the viewing rack cannot be seen when viewed through the test tube.
2. A **clear** reaction denotes a **negative** result – i.e. the lines on the viewing rack are clearly visible when viewed through the test tube.

TEST PROCEDURE – SPIN METHOD

This method can be used to differentiate between heterozygous Hb-S and homozygous Hb-S and to clarify equivocal results obtained by the screening test.

1. Ensure that all reagents are at room temperature (18-25°C) and mixed thoroughly.
2. Always run positive and negative controls to confirm the test system is functioning correctly.
3. Add 20μL blood sample to 2mL reconstituted reagent in a disposable plastic test tube (75 x 12mm) and mix thoroughly.
4. Allow mixture to stand for 3 minutes.
5. Centrifuge at 1200g for 5 minutes. Allow the centrifuge to stop **without** braking.

INTERPRETATION – SPIN METHOD

1. Red precipitate on top of pink solution – **Heterozygous Hb-S**
2. Red precipitate on top of yellow solution – **Homozygous Hb-S**
3. Nil or slight grey precipitate on top of dark red solution – Hb-A

LIMITATIONS OF USE

1. Results from the S-Test Kit should always be interpreted in the context of all available clinical and laboratory data.
2. As the test is a screening method, all positive results should be confirmed by standard haemoglobin electrophoresis.
3. Results obtained from neonates and young infants may be unreliable because of the low percentage of Hb-S and the high percentage of Hb-F.
4. Very low levels of Hb-S may not be detected by the S-Test Kit. A presumptive identification of Hb-S can be made if the variant haemoglobin has the electrophoretic mobility of Hb-S at both acid and alkaline pH.
5. Abnormally elevated plasma protein levels (such as those occurring in patients with myeloma or gross anaemia) may cause false positive results. If this is suspected, the red blood cells should be washed with 0.9% sodium chloride solution to remove excess protein prior to testing.
6. Specimens should be tested as soon as possible after drawing from the patient. Haemolysed or contaminated samples may give erroneous results.

PERFORMANCE CHARACTERISTICS

The analytical sensitivity of S-Test Kit has been independently evaluated by a leading UK haematology laboratory using 85 patient samples with a range of Hb-S concentration of 4 – 98%. S-Test Kit was compared with three other commercially available tests based on sickle solubility, the in-house solubility test used by the trialists, and a commercial test based on a monoclonal antibody to an epitope on haemoglobin-S.

Sample		Test					
% HbS	No	S-Test Kit	Solub. Test 1	Solub. Test 2	Solub. Test 3	In-house Test	Mab Test
>42	33	+ 33	+ 33	+ 33	+ 33	+ 33	+ 33
19-42	33	+ 32 E/+ 1	+ 32 E/+ 1	+ 32 E/+ 1	+ 32 E/+ 1	+ 29 E/+ 4	+ 33
10-18	6	+ 2 E/+ 4 E/E 0 - 0	+ 4 E/+ 2 E/E 0 - 0	+ 2 E/+ 3 E/E 0 - 1	+ 3 E/+ 1 E/E 2 - 0	+ 1 E/+ 1 E/E 2 - 2	+ 5 E/- 1
4-9	13	+ 0 E/+ 1 E/E 2 - 10	+ 1 E/+ 1 E/E 1 - 10	+ 1 E/+ 1 E/E 2 - 9	+ 0 E/+ 1 E/E 1 - 11	+ 0 E/+ 0 E/E 1 - 12	+ 3 E 5 E/- 2 - 3

Key:

Solubility Tests:	+	Positive
	E/+	Equivocal turbidity, positive using centrifugation method
	E/E	Equivocal turbidity, equivocal using centrifugation method
	-	Negative
Mab Test:	+	Positive
	E	Equivocal
	E/-	Equivocal, repeat test negative
	-	Negative

Reliable results can be obtained with S-Test down to approximately 10% Hb-S. At low levels of Hb-S (10-20%), the turbidity result may be equivocal and require confirmation by the centrifugation technique. Below 10% Hb-S, results of all solubility tests including S-Test are likely to be equivocal or negative. However, this level of sensitivity is sufficient for the diagnosis of cases of sickle cell trait with a low percentage of haemoglobin-S due to coexisting α thalassaemia.

REPRODUCIBILITY

Intra-batch reproducibility – This has been evaluated by testing the sensitivity of one batch of kits for haemoglobin-S in conjunction with varying concentrations of total haemoglobin. The tests were carried out on six different occasions using 3 operators. Test sensitivity was constant across all the assay occasions.

Inter-batch reproducibility – Six batches of S-Test Kit were tested for haemoglobin-S sensitivity over a range of total haemoglobin concentrations. Batch to batch variation was minimal such that no differences in sensitivity could be seen.

BIBLIOGRAPHY

1. Itano H A, (1953) Solubility of naturally occurring mixtures of human haemoglobins, Arch Biochem Biophys, 47, 148-159

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