An automated screening technique for the detection of sickle-cell haemoglobin

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SYNOPSIS An automated technique is described which is capable of detecting sickle-cell haemoglobin and differentiating the sickle-cell trait from sickle-cell anaemia. The method is based upon the Itano solubility test and utilizes Technicon equipment.

There is an increasing demand for rapid techniques capable of recognizing sickle-cell haemoglobin. The needs of the clinician, anthropologist, and, more recently, the genetic counsellor are already established. However, three other sections of the community may in the future also require testing.

The recently reported deaths of four soldiers with the sickle-cell trait after arduous training at 4 060 feet (Jones, Binder, and Donowho, 1970) suggests that all recruits should be screened at the time of enlistment. During a survey of 1 000 negro military recruits (Binder and Jones, 1970) 73 were found to have the sickle-cell trait, one had sickle-cell haemoglobin C disease, and one had sickle-cell thalassaemia.

Passengers with sickle-cell haemoglobin C disease are particularly liable to sickling crises when travelling by air. In 1961 the Aerospace Medical Association suggested that such people should not fly. Despite this recommendation, sickling crises still occur in passengers on scheduled pressurized flights and these have proved distressing not only to the patient but also to the crew and the other passengers who witness them. If these situations are to be avoided it appears necessary to screen all negro passengers before flight (Green, Huntsman, and Serjeant, 1971).

Following a fatality during an exchange transfusion of an infant, the donor being a sickle-cell trait carrier (Veiga and Vaithianathan, 1963), it may be argued that all blood donors should be routinely screened for sickle-cell haemoglobin. In practice, sickling-positive blood donors would be likely to have either the sickle-cell trait or sickle-cell haemoglobin C disease; cases of sickle-cell anaemia and sickle-cell thalassaemia would probably be rejected because of low haemoglobin levels. It might prove administratively more convenient for the blood bank

to screen for the presence of sickle-cell haemoglobin in all donors rather than relying upon a technician to reject a blood bottle at the time of cross-matching for neonatal transfusion.

Four techniques, which are not dependent on electrophoretic separation and which utilize whole blood, are available for the screening of patients for sickle-cell haemoglobin.

1 The sickle-cell test (Daland and Castle, 1948) is cheap but false positive and negative results are not uncommon (Schneider, Alperin, and Lehmann, 1967).

2 Differential elution of normal adult haemoglobin from sickle-cell haemoglobin in blood films washed in 2.48 M phosphate buffer (Yakulis and Heller, 1964).

3 The Sickledex test (Ortho) is a proprietary preparation, which detects sickle-cell haemoglobin by precipitation. This test is rapid and simple to perform. It appears to be reliable (Diggs, Schorr, Ascari, and Reiss, 1968; Loh, 1968; Ballard, Radcliff, Sakhadeo, and Schorr, 1970; Canning and Huntsman, 1970) but it is expensive and may be prohibitively so in large numbers of examinations are required. Recently, an automated technique has been described which uses Sickledex reagent. The sickle-cell haemoglobin is detected by the impedance of light transmission in the turbid sample containing the precipitated haemoglobin (Henry, Nalbandian, Nichols, Wolf, Camp, and Conte, 1970).

4 Non-proprietary tests based upon the Itano solubility test are also available (Huntsman, Barclay, Canning, and Yawson, 1970; French, 1971; Raper, 1971). The automated test described is based upon the manual technique of Huntsman *et al.* (1970). This test detects and distinguishes between the sickle-cell trait and sickle-cell anaemia.

Material and Methods

SAMPLES

Analyses were performed on sequestered venous

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blood. To avoid partial clotting, it is essential to ensure that all samples are well mixed at the time of collection.

REAGENTS

1 Stock buffer to precipitate sickle-cell haemoglobin

Potassium dihydrogen orthophosphate¹ .. 540 g
Dipotassium hydrogen orthophosphate¹ .. 952 g
White saponin 40 g
Distilled water to 4l

Sodium dithionite (1% w/v) is added, before each day's work, to sufficient buffer solution.

2 Stock total haemoglobin control solution

White saponin 40 g
Distilled water to 4l

Sodium dithionite (1% w/v) should again be freshly added each day. Stock solutions 1 and 2 should be refrigerated.

3 0.85% W/V Sodium chloride in distilled water

4 Whatman filter paper reels

Grade 52, width 2.5 cm

APPARATUS

This is illustrated in Figure 1. It consists of a Technicon sampler II with mixer/stirrer attachment

¹These chemicals must be anhydrous. Unless carefully stoppered a used reagent bottle may contain partially hydrated salts.

and 40 samples per hour, one to six samples to buffer wash cam, and the Technicon proportioning pump I which was used for the described work, but a mark II pump would allow for easier arrangement of pump tubes. Helper springs are required under the mark I pump platten.

Technicon continuous filter

A simple modification (Fig. 2) of the standard filtering unit was prepared in which a 5 cm × 6 cm Perspex plate, drilled centrally with a 3 mm hole, was glued on top of the filter block, so that the paper travelled along the 6 cm length of the Perspex. The leading edge was rounded to allow smooth passage of the paper, which moves at a speed of 5 cm/min. The mixer assembly was removed, the blood/buffer mixture being debubbled and then allowed to drop from a height of 0.5 cm to 1 cm onto the filter paper at a position 1 to 1.5 cm behind the leading edge of the Perspex support. With this modification, the filter paper became wetted over its entire leading edge immediately before its passage over the Perspex plate. The modification prevents the appearance of numerous air bubbles in the filtrate.

Technicon colorimeters

Two, fitted with 550 nm filters and 15 mm tubular flow cells.

Also required are a two-pen recorder and a 2.5 K ohm linear potentiometer (variable resistance).

Fig. 1 Flow diagram for the detection of sickle-cell haemoglobin (see apparatus and manifold).

Fig. 2 Adaptation to continuous filter unit (see Apparatus).

MANIFOLD

The flow diagram is shown in Fig. 1, standard tygon pump tubes being used. The polypropylene sample tubes should be left as short as possible but their lengths can be finely adjusted to superimpose the haemoglobin peaks obtained from each sample. The filtrate line should also be left as short as possible.

After the passage of two samples, the wash characteristics of the system will be established. If, in order to identify the samples, a saline wash cup is periodically inserted, this should be followed by two normal samples before the run is recommenced.

The peak heights should be adjusted by selection of suitably sized pump tubes so that the total haemoglobin peak has equal or slightly greater absorbance than the peak obtained from the filtrate. A fine adjustment can be subsequently made by the insertion of a 2.5 K ohm linear potentiometer connected as a variable resistance between the filtrate colorimeter and recorder input (Annan and Fisher, 1971). If the potentiometer is initially set at zero, after the wash characteristics have been established the filtrate peak can, if necessary, be raised by increasing the resistance.

Results

The following samples have been examined by the automated technique:

Sample	No.	Sample	No.
Normal	131	Sickle-cell thalassaemia	1
Sickle-cell trait	29	Sickle-cell haemoglobin C disease	4
Sickle-cell anaemia	4	Haemoglobin C trait	4
Sickle-cell anaemia with high Hb F	3	Haemoglobin D trait	1

All these samples were also examined by the sickling test (Daland and Castle, 1948) and by paper electrophoresis (Cradock-Watson, Fenton, and Lehmann, 1959). Any sample in which sickle-cell haemoglobin was detected by either of the above two methods or the described automated technique was in addition subjected to agar gel electrophoresis (Robinson, Robson, Harrison, and Zuelzer, 1957).

In three cases the results of the orthodox sickling test and the new automated test were discrepant. Subsequent investigation showed that the sickling test had been falsely reported as positive in two cases and negative in one.

Because of the different wash characteristics of the two systems, it is not possible to equate quantitatively the two peak heights. Figure 3 shows that the total haemoglobin peaks will return almost to zero between each peak. An indication, but not an accurate assessment, of the patient's haemoglobin level is possible. In contrast, because of the poorer wash characteristics of the non-sickle haemoglobin (filtrate) system, this peak height is to some extent influenced by the amount of the non-sickle haemoglobin in the preceding specimen. For example, one would expect the filtrate peak height of the specimen following a sample of sickle-cell anaemia to be somewhat reduced (peaks 12 and 13 in Fig. 3). For a similar reason, a preceding high filtrate peak will tend to elevate the following filtrate peak (peaks 15 and 16 in Fig. 3). This phenomenon could be reduced by introducing a buffer-filled cup between each sample. However, simple observation of a 40-sample an hour run should prove adequate to differentiate normal samples from the sickle-cell trait and sickle-cell anaemia.

Discussion

Apart from the capital expenditure (£3 850) the running costs are negligible (approximately 200 tests per £1 including disposable AutoAnalyzer cups).

The automated test suffers from the same disadvantages as the manual technique upon which it is based. It fails to distinguish between sickle-cell trait and sickle-cell haemoglobin C disease (peaks 6 and 8, Fig. 3) and it also fails to distinguish the unusual benign cases of sickle-cell anaemia with high foetal haemoglobin from the sickle-cell trait. All samples where sickle-cell haemoglobin is detected should therefore be subsequently submitted to electrophoresis.

Whilst the test appears capable of detecting and distinguishing between sickle-cell anaemia and the sickle-cell trait in samples adjusted to as low as 5 g% Hb level (peaks 18 and 20, Fig. 3) the distinction becomes progressively more difficult as the

Fig. 3 A tracing of the patterns obtained in the analyzer on a number of selected samples.

———— Tracing of the filtrate after precipitation of the sickle-cell haemoglobin.

----- Tracing of the total reduced haemoglobin.

1 normal (15 g/100 ml), 2 normal (15 g/100 ml), 3 normal (10 g/100 ml), 4 normal (5 g/100 ml), 5 normal (15 g/100 ml), sickle-cell trait, 7 normal (15 g/100 ml), 8 sickle-cell haemoglobin C disease, 9 normal (15 g/100 ml), 10 sickle-cell thalassaemia, 11 normal (15 g/100 ml), 12 sickle-cell anaemia, 13 normal (15 g/100 ml), 14 normal (15 g/100 ml), 15 normal (15 g/100 ml), 16 normal (5 g/100 ml), 17 normal (15 g/100 ml), 18 sickle-cell trait (5 g/100 ml), 19 normal (15 g/100 ml), 20 sickle-cell anaemia (5 g/100 ml), 21 normal (15 g/100 ml).

level of haemoglobin falls. In order to avoid false negative results it appears desirable that all anaemic samples (below 10 g%) should be retested after the haemoglobin level has been recorded. The second test is carried out with the packed cell volume adjusted to approximately 50%. This is achieved by pipetting off the excess plasma in the sedimented AutoAnalyzer cup. A clear distinction between the sickle-cell trait and sickle-cell anaemia will then be possible.

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