Technical method

An integrated two-test automated syphilis screening system

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The Technicon Auto Analyzer (A411) has been utilised in this laboratory for the past four years to screen specimens for syphilis serology, using the automated reagin test (ART) and the automated Reiter protein complement fixation test (ARPCFT). These two tests were performed separately with two sets of equipment as previously described (Macfarlane *et al.*, 1976; Macfarlane and Elias-Jones, 1977). It soon became evident that an increase in the sample speed of the ARPCFT from 80 samples/hour to 100 samples/hour was desirable as this would increase throughput and bring the system into line with the ART, which samples at 100/hour, thus allowing the two tests to be integrated. Increased throughput would increase the workload involved in the preparation of specimens for testing, a process that was becoming more time-consuming and labour-intensive than the tests themselves. An attempt was therefore made to reduce the amount of preparation required before testing and to integrate the ART and ARPCFT.

Preparation of samples for testing

Clinicians were persuaded to use Monovettes (Sarstedt) for collection of blood samples. This allowed the syringe barrel to be used as a transport tube for the sample. On arrival at the laboratory the caps were removed from the Monovettes, and a constant level device was fitted to the top of each tube. This consists of a circular flanged cap with a centrally located hole to allow passage of the sampler probe. Suspended from the cap and hanging down into the Monovette is a small sample cup which tapers to one side (Fig. 1). These devices were originally designed for use on the Searle Autotape System, which is used for brucellosis testing at the Central Veterinary Laboratories in Weybridge. Once the tubes had been fitted with the constant level device they were stored at 4°C to allow clotting to continue and RBCs to settle to the base of the tube. After a suitable time interval the samples were

removed from the refrigerator, a disposable shaft from the Monovette was refitted, and gentle pressure was exerted to displace serum upwards into the constant level device. The samples were then loaded in a predetermined order on to the sample tray fitted with the appropriate adaptors, and stored at 4°C until required for testing. It was originally proposed to fill the constant level devices by inverting the sample tube. This system has been used with Vacutainer tubes to fill large numbers of sample cups 'en bloc' and works satisfactorily, provided the constant level devices are pretreated with a 0.4% solution of nonidet (personal communication,

Fig. 1 Constant level device fitted to Monovette.

Fig. 2 Flow diagram for combined ART/ARPCFT screening test. Rates in millilitres per minute.

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Mr. Gower, Central Veterinary Laboratories, Weybridge). The narrower bore of the 5ml Monovette prevents adequate filling of the constant level device when the tube is inverted.

As it was intended to sample directly from the clotted blood specimens it was not possible to inactivate the sera by placing the tubes in a 56°C waterbath for 30 minutes. This problem was overcome by passing the sample through a 1 minute delay coil in a 60°C oil bath (Fig. 2).

Integrated ART/ARPCFT test system

When the ARPCFT system was originally devised the maximum sampling speed that would permit clear sample identification and minimum carryover between samples was 80/hour. Early experience with ARPCFT indicated that the complex reaction system of six glass coils with several joints and bends was a major source of 'noise' in the recorder trace. Although this was minimal at 80 samples/hour, it became a major problem at higher speeds. Experiments with a shorter coil system had shown that complete haemolysis could be achieved in 10 minutes at 37°C with one 32-turn mixing coil as opposed to the 22 minutes and three reaction coils used initially. A simplified system was therefore designed consisting of one 32-turn coil for the antigen/antibody reaction, one 32-turn coil for the RBC/haemolysis reaction, and one 10-turn coil to mix the diluting buffer (Fig. 2). This short set of reaction coils was tested with a number of known positive and negative sera and gave excellent results. The sample speed was therefore increased from 80 to 100/hour. This was achieved without any evidence of 'noise' in the recorder trace.

The ability to achieve a common sampling speed for both tests allowed the two systems to be integrated using one pump and one sampler (Fig. 3). This released one sampler and one pump, which were exchanged for a digital printer to reduce the amount of work involved in reading and recording results. A flow diagram of the integrated system is

Fig. 3 Arrangement of modules for combined ART/ARPCFT screening test.

Fig. 4 Recorder trace from ARPCFT at 120 samples/h with regeneration showing one weak positive (no. 9), three strong positives (nos 5, 12, and 15), and 12 negative samples.

shown in Figure 2. Encouraged by the success at 100 samples/hour, the sampling speed was further increased to 120 samples/hour. A clear recorder trace free of 'noise' was obtained at this speed (Fig. 4). The reduction of 'noise' in the recorder trace had two additional advantages: it permitted testing with low levels of complement, thus increasing the sensitivity of the test, and allowed the inclusion of a curve regenerator (Walker *et al.*, 1972) to reduce the carryover effect from strong positives (Fig. 4).

Conclusion

The introduction of the Monovette system and in line serum inactivation has resulted in a considerable reduction in the workload involved in the preparation of specimens for testing (Table 1). It is possible that for large numbers of samples the Vacutainer system would have the additional advantage of allowing rapid filling of the sample cups on the constant level devices by simultaneous inversion of large numbers of tubes. There is,

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Table 1 Collection and preparation of samples for autoanalyser

Previous system	Modified system
1 Collect blood sample from patient in syringe	1 Collect blood sample from patient in Monovette
2 Transfer blood to tube and label same	2 Label and dispatch to lab
3 Dispatch sample to lab	3 Remove cap and fit constant level device
4 Centrifuge sample	4 Store at 4°C to clot
5 Remove serum from sample and transfer to analyser cup	5 Attach shaft, press piston, and fill sample cup with serum
6 Label analyser cup and cap	6 Place Monovettes on sample tray for testing
7 Store samples in rack at 4°C	
8 Inactivate at 56°C for 30 min in waterbath	
9 Store samples at 4°C overnight	
10 Inactivate at 56°C for 30 min before testing	
11 Transfer cups to sample trays, remove and discard caps	

however, the danger that this procedure could lead to the introduction of an unacceptable number of red blood cells into the sample cup.

The combined ART/ARPCFT test system operating at 120 samples/hour has resulted in a 50% increase in capacity with a corresponding reduction in the volumes of expensive reagents required. The time required to complete the complement fixation test has been halved from 50 to 25 minutes, and reduced noise levels in the recorder trace have increased the sensitivity of the test (Table 2) and permitted the use of a curve regenerator to reduce carryover effect. These improvements were achieved

Table 2 Automated serology results (AU) on 12 Reiter control sera

Old method	Integrated system
190	610
85	310
61	240
210	685
516	1 501
986	2 937
5 162	13 101
7 963	18 745
3 974	11 169
1 773	5 184
12 943	24 588
22 500	34 639

using less equipment than was necessary for the original separate tests.

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Letters to the Editor

Legionnaires' disease

We have recently carried out a retrospective study for antibodies to *Legionella pneumophila* in sera submitted to this laboratory during 1978. Sera from patients with pneumonia or respiratory disease, for which no specific aetiology had been found in the routine bacterial and viral

screens, were studied. *Legionella pneumophila* (Pontiac strain), grown in yolk sac and then formalised, was used as antigen in an indirect immunofluorescence test (McDade *et al.*, 1977). A fourfold rise in titre to at least 1:64 or a single titre of $\geq 1:256$ with a relevant clinical history are presently considered to be diagnostic in this test (Taylor *et al.*, 1979).

We examined a total of 390 sera from 340 patients and found five patients with titres of $\geq 1:16$ (1.5%) (Table).

Only one patient met the appropriate serological criteria with a titre of 1:256 in a sample taken in June 1978, falling to 1:128 nine months later. This 61-year-old man had pneumonia in the right lung, involving the upper and lower lobes. In addition, he had cerebellar ataxia with slurred speech and a right hemiparesis, although his lung and brain conditions are thought to be aetiologically unrelated.

A second male patient, aged 65 years, working as a photographer in the printing

Examination of sera by an IFAT for *Legionella pneumophila*

IFAT (reciprocal of titre)	<8	8	16	32	64	128	256	512
No. of sera	373	8	3	2	0	2	2	0