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Internal quality control in serological tests for syphilis

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SUMMARY The importance of syphilis serological tests demands that laboratory reports are reliable. Internal quality control applied to the organisation of a syphilis serology service improves laboratory bench performance and reporting. Described here are internal quality control procedures of a department that serves a genitourinary medicine clinic and conducts 70 000 tests a year to investigate for syphilis.

Introduction

The history of testing serum for lipoidal and treponemal antibodies is well documented. Wassermann *et al* applied the complement fixation reaction to investigating syphilis.[1] Thereafter numerous serological tests have been described with the objectives of improved specificity and sensitivity.

Diagnostic confirmation of early clinical syphilis is by the recognition of viable *Treponema pallidum* from suitable lesions using dark field microscopy. In recent years several workers including Daniels and Ferneyhough have proposed using a direct fluorescent antibody staining technique as an alternative method.[2] The clinician will request serological tests to support positive microscopical results. Microscopy sometimes gives negative results when the clinical history of the patient indicates early syphilis: it is then that serological tests are essential to confirm diagnoses based on the clinician's experience. A battery of serological tests provides an antibody profile suggesting active or past treponemal infection. Problem results can be checked by using the facilities of a reference laboratory. Untreated and treated disease usually follows predictable serological patterns with individual variations. The choice of tests depends on local policy. I consider three such tests: the Venereal Disease Research Laboratory (VDRL) test, the *Treponema pallidum* haemagglutination assay (TPHA), and the fluorescent treponemal antibody absorbed (FTA-ABS) test.

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IMPLICATION OF INTERNAL QUALITY CONTROL

Table I lists the procedures undertaken in the organisation of a syphilis serology service. At any stage from (a) to (i) an error can occur, and these errors may be multiplied. To prevent mistakes it is

TABLE I *Organisation of syphilis serology service*

(a)	Sources and types of requests	
(b)	Reception of specimen	
	Sorting; clerking in; preparation; storage; selection of tests	
(c)	Daily organisation	
	Work sheets; timing and sequence of operations	
(d)	VDRL method	(i) Reagents
		(ii) Dilutions of serum
		(iii) Conduct of tests
(e)	TPHA method	(i) Reading individual results
		(ii) Batch testing
(f)	FTA-ABS	
(g)	Recording of results	
	Individual tests	
	Batch testing	
(h)	Reporting and surveillance of results	
	Individual test forms	
	Procedures for scrutiny of results	
	Reporting results to clinics	
	Monthly and quarterly returns	
(i)	Retrieval of results	
	Individual tests	
	Batches and categories	
(j)	Quality control	
	Use of reference reagents	
	Quality control procedures	
	Participation in external quality assurance	
(k)	Repeats and referrals	
	Indications for repeating tests on single specimens	
	Indications for requesting repeat specimens	
	Indications for referring patients	
	Indications for referring serum sample to reference laboratory	
(l)	Organisational and financial	
	Handling, storage, clerking in	
	Performance of tests	
	Reporting, sorting, and retrieval of results	
	Weighting of individual tests	
	Anticipated workload	
	Anticipated costs	

obviously necessary that an internal quality control scheme should be implemented. Examples of factors affecting laboratory results are: mislabelled blood specimens; contaminated serum; clerical errors; errors of transfer of liquid; errors of technique; inadequate evaluation of reagents; insufficient reagent controls; unofficial modification of protocols; inadequate performance of equipment; lack of understanding of laboratory routine; insufficient supervision; and poor scrutiny of results.

Although the onus is on individual laboratories to introduce their own internal quality control procedures, no active encouragement in this field has been given to laboratories undertaking syphilis serological tests through the agency of the external quality assurance (EQA) scheme of the United Kingdom. Participation in an external quality assurance scheme provides retrospective comparison of laboratory performance against a target value or a consensus of results. A laboratory participating in external quality assurance without its own internal quality control, however, may be under an illusion regarding its own routine performance because it may set up special procedures for external quality assurance samples. Internal quality control gives information on routine laboratory performance at the time of testing. It acts as an early warning system to enable errors, malfunctions of equipment, or substandard reagents to be identified and corrected. Scrutinising data on patients can detect mistakes of sampling, collecting, handling, and clerking that external quality assurance schemes do not disclose. Improved performance derived from internal quality control will benefit the management of patients.

Implementing internal quality control is not difficult but requires discipline from the laboratory staff in respect of monitoring and recording specific data. It also costs time; an estimated 12% of a specific work load is taken up by internal quality control, and laboratories should include this as a separate item of expenditure in their financial budgets.

Materials and methods

REFERENCE SERUM

International biological standards are used to compare similar materials under the same test conditions. In referring to their use it is necessary to identify the status of the material in the categories of the World Health Organisation (WHO) nomenclature of standards.[3] The WHO reference serum (ref 3-1980) for syphilis serological tests is a WHO international reference reagent. The serum is a lyophilised pooled preparation; it is used to calibrate in house standards used in routine serological tests for treponemal infections.

CALIBRATING IN HOUSE STANDARDS FOR THE TPHA AND VDRL TESTS

The WHO reference serum is not a consumable product to be used in every batch of tests. It is therefore necessary to establish in house standards whose titre relative to that of the WHO reference is measured using precisely the conditions and reagents used for routine tests. The remainder of the reference material is stored as a master serum and used periodically to check the in house standard for evidence of drift, to establish a new batch of local standard material, or for recalibration when modifications or new reagents have been introduced into the existing method. Preparations are made in advance of the proposed date of calibration to ensure that sufficient volumes of serum are available. This entails using a serum bank ($-70°C$) with the relevant records of the source, volume, and serological profile of serum specimens. All banked serum samples are HBsAg negative.

In house standards for the TPHA and VDRL tests are calibrated against the WHO reference serum. An in house standard titre does not have to equal the reference titre. Whenever further comparisons are made between the two serum samples, however, the in house standard is expected to maintain its titre in relation to the WHO reference serum. Serum samples

TABLE II *Interpretation and designation of categories of test results*

	Appearances and titres of test reactions designated:					
			Positive or reactive			
	Negative or non-reactive	*Borderline*	*Minimal*	*Moderate*	*High*	*Very high*
VDRL flocculation	No clumps		Small clumps	Small clumps	Medium or large clumps	
VDRL titre	1/0		1/1-1/2	1/4-1/8	1/16-1/64	⩾1/128
TPHA titre	1/0-1/40		1/80-1/160	1/320-1/640	1/1280-1/5120	>1/5120
FTA-ABS fluorescence (shades of apple green)	0	± (faintly visible)	+	+ +	+ + + (very bright)	+ + + + (brilliant)

VDRL = Venereal Disease Research Laboratory test. TPHA = *Treponema pallidum* haemagglutination assay. FTA-ABS = fluorescent treponemal antibody absorbed test.

with an antibody titre designated "moderate" in TPHA and VDRL tests are chosen for in house standards (see table II).

Titrations of the reference serum and the proposed in house standard are performed in parallel at least 30 times, from which the geometric mean titre and geometric standard deviation are calculated. Each in house standard is further tested to establish its suitability under routine bench conditions. A blind series of tests is carried out to eliminate bias. Satisfactory standards are sterilised by membrane filtrations. Aliquots of 25 μl serum are distributed into vials and stored at −70°C. The unused WHO reference serum is also distributed in similar volumes and stored in the serum bank. In house standards are included in routine test batches at random so they are not identifiable by the laboratory worker performing the tests. The reactivity of each batch of freshly prepared VDRL antigen is checked using an aliquot of in house standard before being used in routine tests.

THE FLUORESCENT TREPONEMAL ANTIBODY ABSORBED TEST

The FTA-ABS test is a confirmatory test for detecting antitreponemal IgG. It is extremely sensitive, and unofficial alterations of laboratory protocols will lead to unreliable results. Evaluation of all reagents is necessary. Laboratory bench records for FTA-ABS tests must include a record of the working titre and batch number of the reagents used on each occasion and the reading score for each test serum.

The FTA-ABS antigen

T pallidum (Nichols strain) maintained by passage in rabbits is the source of FTA-ABS antigen. Antigen suspensions develop hyperreactivity after storage for six weeks at 4°C. Slide preparations of antigen have a limited shelf life; reduced reactivity occurs after a period of time. Antigen is assessed by comparing it with known material using in house standards. At least 30 treponemal organisms per microscope field (× 40 objective) are used for each FTA test. Inadequate numbers of treponemes make it difficult for the microscopist to distinguish shades of apple green. The preparation of antigen smears is a tedious but important job, as they need to be spread evenly before being fixed with 10% methanol. Each smear is checked under the dark field microscope to ensure that sufficient treponemes are present. Fixed preparations are stored in airtight plastic bags at −20°C.

Fluorescein isothiocyanate (FITC) conjugate

The working dilution of an FITC conjugate must be ascertained to evaluate its suitability. This criterion cannot be met by the manufacturer as the ultimate value depends on the microscope, substrate, and reagents used in any individual laboratory. To measure the working dilution a two dimensional (chessboard) titration is performed using a known middle layer reactive control (see fig 1). A pooled normal serum control is included to detect "serum induced" non-specific staining, and a buffer (phosphate buffered saline (PBS)) control to detect non-specific staining by the conjugate. A satisfactory chessboard titration would require a titre of in house serum (the middle layer) at which minimal fluorescence reactivity (see table II) occurs over a range of at least three conjugate dilutions.[4] This critical serum titre is the "plateau level". The end of the range of conjugate dilutions over which fluorescence occurs at the plateau level is reported as the plateau end poiont. The working titre of the conjugate is calculated as being twice the concentration, or half the titre, of the plateau end point when using doubling dilutions. The length of the plateau level under the conditions described above is important. The higher the concentration of antibody in a conjugate the more it can be diluted but still retain its plateau level (titre). Conjugates with long plateau levels, and hence high working

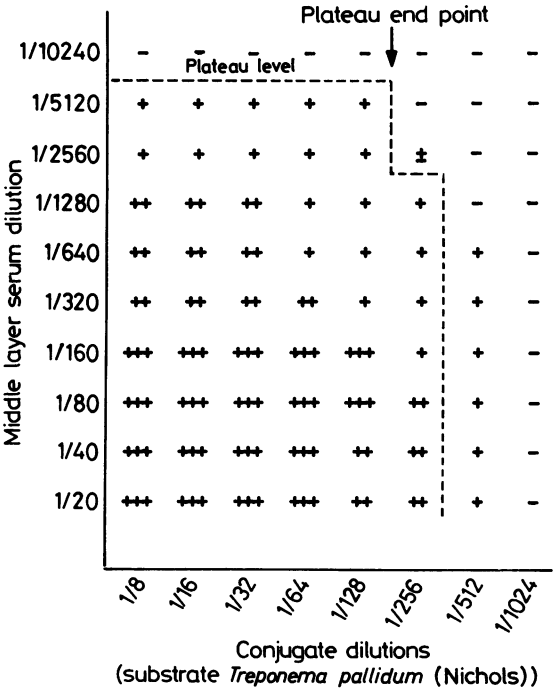

FIG 1 *Chessboard titration indicating plateau level and plateau end point.*

dilutions, are more efficient, and also more economical, because they reduce background staining. Acceptable FITC conjugates are aliquoted into working volumes and stored at −20°C.

Middle layer reactive controls

Middle layer reactive controls are included with each batch of FTA-ABS tests. These reactive controls are used in a different way from the middle layer control referred to in the evaluation of the FITC conjugate. They are calibrated by comparison with the WHO reference serum using the same batch of antigen. Dilutions of the serum are selected that will give the required reactions. Middle layer controls are sterilised by membrane filtration, distributed in working volumes, and stored at −20°C.

The following reactive controls are used in each test batch: standard minimum reactive control (designated +); reactive (+ + + +) control in PBS; reactive (+ + + +) control in sorbent. The minimal reactive control serum is used as an indicator of the minimum fluorescence that is regarded as showing a positive result (the reading standard).

Batch testing of sorbent

New batches of sorbent are quality controlled using a non-syphilitic serum that is reactive when diluted in PBS but non-reactive when diluted in sorbent.

Non-reactive control

A non-reactive control serum is included in each batch of tests. Serum samples are confirmed as being non-reactive by testing dilutions of them in PBS and sorbent.

(*TREPONEMA PALLIDUM* HAEMAGGLUTINATION ASSAY (TPHA)

This passive haemagglutination test is used to detect antitreponemal IgG, and its sensitivity was reviewed by Notowicz and Menke in 1981.[5] Comparative tests are made on all new batches of reagents, as variation occurs between batches. The recognition of the end point is made absolutely clear to staff, as the subtle changes in the haemagglutination pattern can result in variable reading of the end point. TPHA results are influenced by chemical contamination, and all glass and plastics used must therefore be scrupulously cleaned. During the preparation of reagents and in the actual performance of the test close attention must be given to accuracy in measuring fluid volumes, and in the precise way volumes of liquid are transferred. Manufacturers' instructions to prepare TPHA reagents daily are followed to retain the calibrated sensitivity of the test.

VENEREAL DISEASE RESEARCH LABORATORY TEST (VDRL)

The VDRL test was first reported by Harris *et al* in 1946[6] and has since undergone various modifications. It is a flocculation test, which detects mainly IgM class lipoidal antibody.[7] Reactions are classified according to the appearance of flocculation indicating the concentration of lipoidal antibody (table II). Vigilance is required with this simple test to ensure that no unauthorised modifications, however well meant, are introduced. Speeds of rotation, volumes of liquid, storage of reagents, and methods of reading reactions are factors that differ when comparing manufacturers' protocols.

A VDRL carbon added reagent, a modification used in this laboratory, enhances the end point reading of the test. The shelf life of VDRL reagents is important, as false reactions will occur if they are used after the stated expiry date. Serum samples exhibiting prozones are uncommon. To exclude this phenomenon, however, samples from patients with primary and secondary syphilis that give negative results to the VDRL test require retesting in dilution to ascertain whether they have any lipoidal antibody concentrations.

WALL CONTROL CHARTS

Quality control wall charts indicating limits of acceptance are used to plot in house standard values for each batch of tests performed. Recording the distribution of values monitors trends in precision and accuracy. Precision is used to describe the ability to obtain closely related values for duplicated specimens (such as in house standards). Accuracy is an expression of the nearness of one of a series of estimates to what is accepted to be the true value of the substance being measured. A wall chart is prepared by taking the geometric mean and geometric standard deviation values. Fig 2 shows

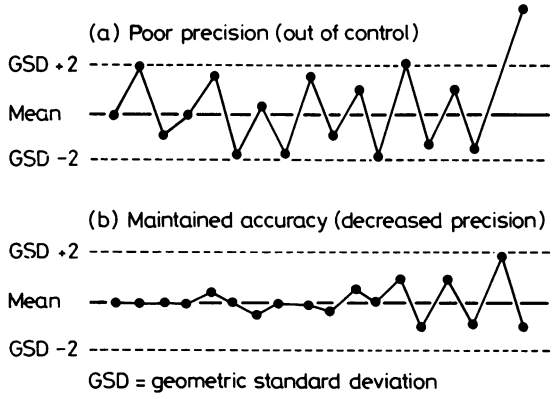

FIG 2 *Example of wall charts used for plotting in house standard values.*

examples of results with differing precision. The distribution should indicate that:

95·5% of reactive control results are within two geometric standard deviations from the mean.

Mean and standard deviation values may not coincide with actual titres, but that in no way invalidates the result.

GENERAL ASPECTS OF INTERNAL QUALITY CONTROL

Scrutiny of incoming blood specimens

Identification of a blood specimen with the corresponding request form appears to be simple enough, but for a variety of reasons that include misreading of names or numbers, inadequate information, and sorting of specimens, mistakes do occur. Ideally, permanent staff should be used to prepare specimens. If temporary help is given because of increased workloads or staffing problems, intensive supervision must be used to control the clerking and preparation of the blood. Laboratory meetings should be held periodically to discuss this area of control with the objective of improving the system.

Preparation of serum

Clear serum samples are used from centrifuged clotted blood specimens. House rules require identification of specimens from people known to be in "danger of infection". Blood is separated under a cabinet, and serum is transferred using disposable drinking straws. It is stored at 4°C and is not subjected to heat treatment before being tested. Original clot specimens are kept for seven days.

Scrutiny of laboratory results

Syphilis serology results are scrutinised by an authorised member of staff. A problem area is commenting on an individual result from a large batch of specimens received from a department of genitourinary medicine, antenatal clinic, or blood donor unit. These serum samples will often arrive in batches accompanied by lists of names as opposed to individual request forms with relevant information. Fig 3 shows a record card, which is used to scrutinise results and detect errors in transcription or transfer of liquid. Suspect test results are repeated from the original clot specimen. All serum samples giving positive test results are filed in the serum bank.

Skilled performance

Internal quality control includes monitoring individual skills, the levels of which appreciably influence results. Adequate skilled performance should not be assumed to be maintained all the time. If the performance of a skilled worker is compared with those less skilful, the difference lies not so much in the movements as in the way the senses are channelled and organised and in the precise and timely way decisions are made. Fig 4 shows how a VDRL test can be analysed, with each component part further divided to obtain a critical analysis.

Discussion

Numerous factors can affect syphilis serological tests. Many of these are identified using internal quality control procedures. Other factors that influence results are less easy to define. The style of laboratory management can influence performance. Stress is of particular concern as this is linked with

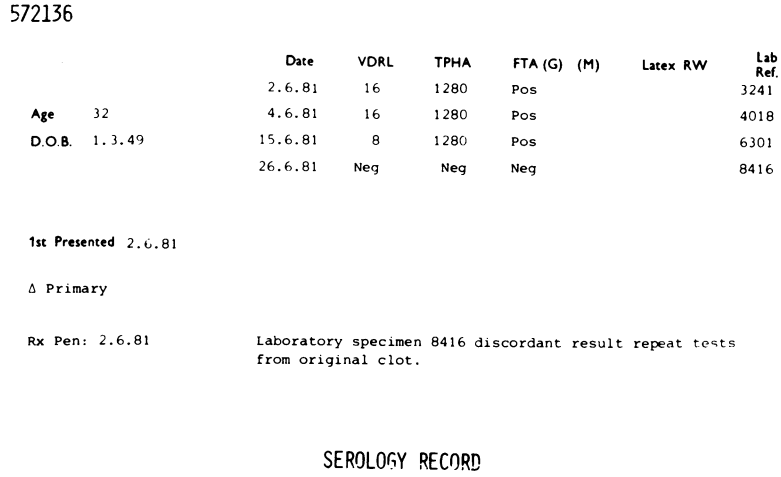

FIG 3 *Sample serology record card.*

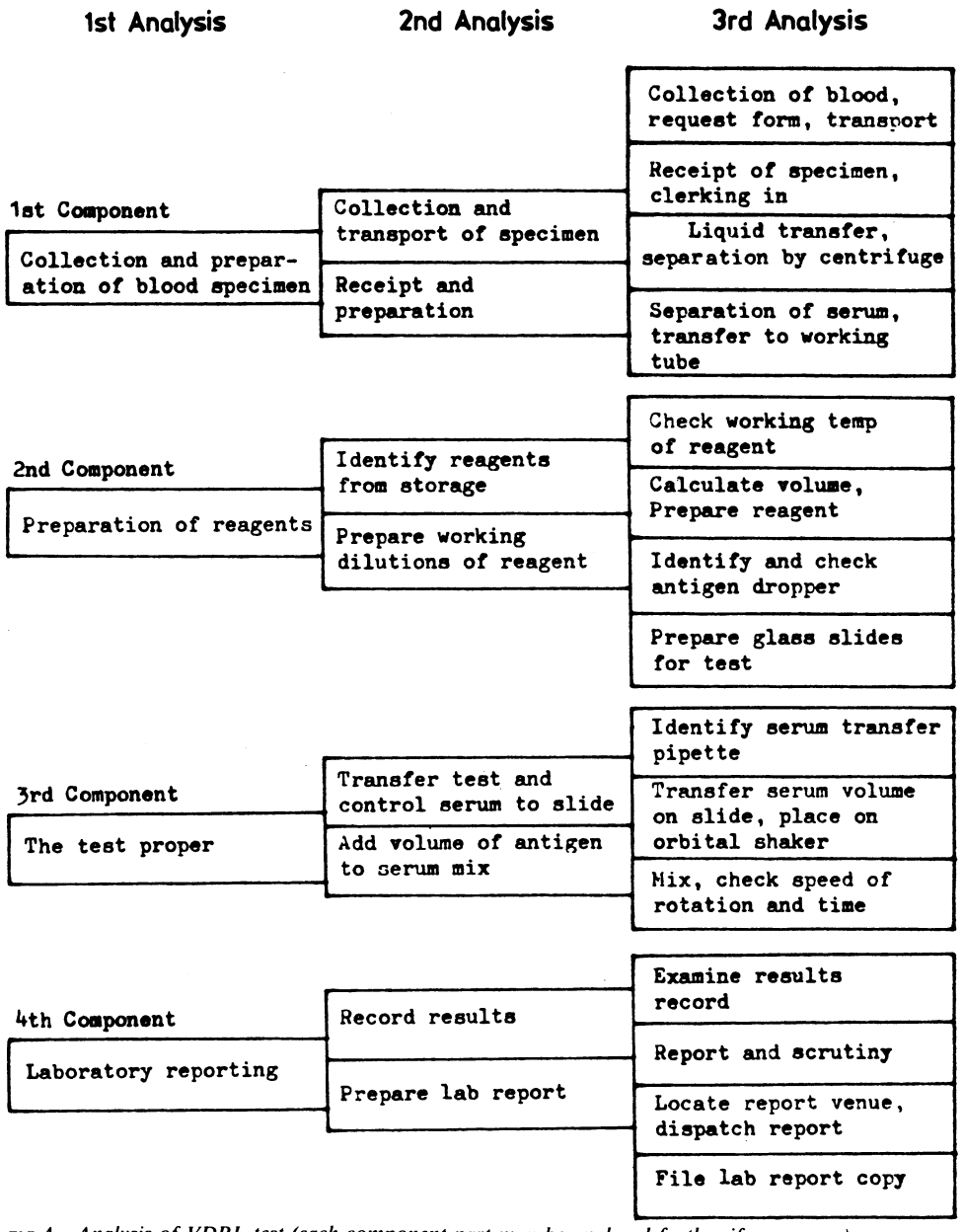

FIG 4 *Analysis of VDRL test (each component part may be analysed further if necessary.)*

the performance of staff.[8] Whether the cost incurred in reducing errors is more than current budgets can afford to pay is a matter of policy. If the current economic climate denies laboratories additional financial resources to implement internal quality control, it may be that this should still be undertaken by reappraising workloads. The availability of the WHO reference serum (ref 3-1980) is an important advance towards achieving standardisation in syphilis serology, and its use as a biological standard should be exploited. Technical excellence is fundamental in achieving consistency; this embraces technique, equipment, tools, reagents, and skills, all of which need periodical review. Serological testing for syphilis, particularly the mass screening of blood, is tedious. This does not alter its importance as a

routine serological test. Confidence in reagents will improve if manufacturers standardise their products based on the WHO reference serum.

I recommend that departments responsible for serological tests for syphilis should implement internal quality control based on the WHO reference serum, and should monitor their daily performance by using wall charts. Internal quality control includes surveillance of all activities that can affect laboratory results. A senior member of the laboratory should be appointed to administer these procedures. The importance of establishing a reliable syphilis serology service in a laboratory must, however, be seen as a basis for obtaining national concordance between laboratories. It is hoped that regional or national reference serum samples will eventually become available to meet this need.

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