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GUIDELINES FOR ORGANIZING NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEMES FOR HIV SEROLOGICAL TESTING

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I. Introduction

These guidelines are destined for policy makers and programme planners wishing to introduce national external quality assessment schemes (NEQAS) for serological testing for human immunodeficiency viruses (HIV). They describe some important basic principles and the main practical aspects of NEQAS. The objectives of external quality assessment schemes are briefly discussed below and elsewhere (References 1 and 2 in bibliography, Annex 2). It is now widely accepted that quality assurance, quality control and quality assessment constitute an essential part of HIV testing and of diagnostic testing in general. Quality assessment is one component of a total quality assurance programme.

The availability of excellent HIV tests does not automatically guarantee reliable laboratory results. Many steps are involved between the moment when a specimen enters the laboratory and the moment when the result of the test is reported to the physician, and at each step something can go wrong. Therefore each government should ensure that sufficient support is made available for a National Reference Laboratory to provide a suitable programme to monitor and if necessary improve the quality of HIV testing in the country. A well-functioning national programme is an important step towards achieving high-quality laboratory performance nationwide.

1. Terminology

In order to avoid confusion distinctions need to be drawn between three commonly used terms.

Quality assurance (QA) is the total process that guarantees that the final results reported by a laboratory are as accurate as possible. This involves inspecting specimens, reviewing transcriptional measures, using the most reliable assays and verifying final reports.

Quality control (QC) comprises those measures that must be included during each test run to verify that the test is working properly. This includes ensuring correct temperature conditions, kit controls, etc. Thus QC indicates whether the test run was valid and has produced acceptable results. QC does not, however, indicate that the results are accurate, nor that they have been reported properly.

Quality assessment is a means of determining the quality of results. It is usually an external evaluation of a laboratory's performance using proficiency panels. Quality assessment is undertaken to evaluate the effectiveness of the quality assurance programme. **A good QA/QC programme may make** quality assessment in some situations **less important**; however, quality assessment is **never** a substitute for good QA/QC. Failure with quality assessment specimens usually indicates that there is a problem with QA/QC procedures. Furthermore, quality assessment schemes are much more efficient at detecting differences in performance between participating laboratories than differences between test methods and techniques.

II. Establishing a national external quality assessment scheme (NEQAS)

1. General outline

A typical national external quality assessment scheme (NEQAS) is described below (see also organigram in Annex 3).

In collaboration with the Ministry of Health, the National AIDS Control Programme should select one qualified laboratory as the organizing laboratory for the NEQAS programme. In large countries more than one such laboratory might need to be identified. However, close collaboration between those laboratories is vital, as data will need to be compiled and merged to provide annual national overviews. In practice the National Reference Laboratory (or laboratories) for HIV which is the best qualified for this task will be selected. All laboratories carrying out HIV tests should participate in the NEQAS, including regional and district hospitals, blood transfusion centres and small dispensaries in the public and private sectors. It is advisable that the Government issue regulations requiring the participation of all laboratories in the NEQAS.

When establishing an external quality assessment scheme for HIV testing the organizer should explore whether linking with an existing laboratory assessment scheme in microbiology is feasible in order to combine and/or facilitate logistical efforts and to decrease costs. Unfortunately, in many developing countries such programmes do not exist. To ensure the success of the scheme in this setting, it is extremely important to motivate chiefs and staff of participating laboratories and to discuss in detail its objectives and benefits in order to gain their support.

The purposes and benefits of introducing a NEQAS are multiple and of mutual interest to both organizers and participants. The programme will generate valuable information on the types and brands of assays used in the country. It will provide officials with a better overview of laboratories carrying out HIV tests and of their other activities. The NEQAS will make it possible to monitor the performance of each laboratory over time and to identify those laboratories which require training to improve their performance. Participating laboratories will learn from the results; either they will be reassured that their performance is up to standard or they will be made aware of a problem that would otherwise have gone unnoticed. The laboratories will be offered the opportunity to identify and to remedy their weaknesses with regard to HIV testing. Consumers (patients and physicians) also benefit from NEQAS, because they are reassured that they can rely on the laboratory results. The programme should also provide updated information to participants regarding HIV testing and related matters.

2. Objectives

The primary objectives of a national external quality assessment programme are:

1. to assess the quality of laboratory performance on a nationwide basis;

2. to provide assurance to consumers (physician and patients), that laboratory results are reliable.

Secondary objectives are:

1. to identify common errors and recommend corrective measures;

2. to encourage good laboratory practice, using standardized procedures, appropriate definitions and high-quality reagents;

3. to encourage the implementation of quality assurance and control measures in the participating laboratories;

4. to stimulate information exchange and networking among laboratories at the national and/or international level;

5. to provide updated information on new developments in HIV diagnostics and related matters.

The characteristics and the tasks of the organizing and participating laboratories are outlined in sections 3 and 4 below.

3. The steps to be taken

Considerable human and financial resources are required in order to set up a successful quality assessment scheme. Laboratories organizing quality assessment must be adequately funded on a continuing basis. This can be achieved by demanding a participation fee and/or by government funding.

In the absence of regulations it is difficult to motivate all laboratories (public and private) to participate in the scheme. Nevertheless, every effort should be undertaken to secure their participation. A flowchart showing the steps to be taken to establish a quality assessment scheme is given in Annex 4. These steps involve:

- advertising the scheme widely;
- trying to enlist the support of professional societies;
- explaining the purpose of the scheme;
- stressing the educational benefits;
- emphasizing that the purpose of the scheme is to give participants a tool to help them improve their own results;
- offering advice and follow-up visits in case of persistent problems;
- introducing a module on quality assurance in training courses for laboratory technicians and students.

III. The organizing laboratory

In order to be capable of managing a NEQAS, the organizing laboratory needs to have technical competence, the necessary facilities (staff, space, equipment, computer) and access to suitable biological material.

1. Technical competence

The organizing laboratory should have the necessary expertise in the field, as it will act as a reference and will provide training and updated information on new developments in the area of HIV diagnosis. It must be acquainted with the full range of diagnostic assays used in the country and have access to a supply of such diagnostic assays. The specimens of the panel should be fully characterized by different assays in order to establish whether they are HIV-1 and/or HIV-2 positive, negative or indeterminate.

It is essential that participating laboratories have confidence in the quality of the NEQAS and in the quality of the panel, and the organizing laboratory must devote considerable time and effort to ensuring this quality. Therefore, it is strongly recommended that the organizing laboratory should participate in an international scheme for quality assessment offered by institutions such as the Centers for Disease Control, Atlanta, USA, or the Central Public Health Laboratory, Colindale, United Kingdom, to validate their competence (Annex 1).

Each participating laboratory should be identified by a unique confidential numerical code. Results of the performances of individual laboratories should be kept confidential. Mechanisms used to help poorly performing laboratories should be described to participants in advance. The success of the NEQAS depends on mutual trust and mutual interest.

2. Staff

Starting up an external quality assessment scheme requires considerable time and managerial effort. Once the initial phase with its inevitable problems and misconceptions has been completed, the time spent on operating the scheme should be kept within acceptable limits. To ensure the success of the scheme, it is important that the staff considers quality assessment activities as an integral component of HIV testing and, therefore, a normal part of their duties.

Staff will be required:

- to prepare the panel, dispense and characterize the panel specimens;
- to pack specimens for dispatch;
- to prepare paper-work (report forms, summaries, etc.), record and collate returned reports, and maintain an address register of participating laboratories;
- to analyse data, prepare summaries of results;
- to provide feedback in case of poor performance;
- to oversee the whole operation.

Tasks should be as far as possible assigned to already existing staff members. Depending on the personnel situation of a laboratory, recruitment of additional staff might become necessary.

It is crucial that scientific staff will be available to undertake follow-up visits to participating laboratories, especially in case of persistent poor performance. Visits with training of laboratory staff on the spot have been shown to be very effective. However, such visits are time-consuming and difficult to incorporate into the routine work schedule of laboratory staff. Alternatively workshops at the national reference laboratory may be organized. Therefore, special arrangements might need to be considered, such as collaboration with the National AIDS Control Programme.

3. Equipment and space

Apart from general laboratory equipment and specialized serological equipment, such as ELISA washers and plate readers, the following are also required: filtration equipment, a device for dispensing specimens into vials, and a means of labeling the vials. If a lyophilizer is available, it may be possible to freeze-dry the samples, so they can be mailed without deterioration even in countries with hot climates.

Equipment and space are required as follows:

- freezers (-40°C or -70°C) for storage of bulk sera or plasma;
- refrigerators (4°C) for storage of the panel before dispatch;
- bench space for processing bulk material (recalcification, filtration, dispensing into vials, etc.);
- equipment for performing various diagnostic HIV assays;
- bench space for characterization with diagnostic assays;
- general space for packing of panel for distribution;
- storage space for vials, paper and packing materials.

The appropriate universal safety regulations and guidelines should be respected (e.g. bench space in containment facilities when handling potentially infectious material).

4. Computing resources

A computer and a photocopier are essential for producing and multiplying the necessary documents for distribution of the panel, for analysing of the results and for preparing of the report. Generally the data analysis does not require very sophisticated software; database programmes such as Epi-Info, Dbase or similar are adequate.

The computer should be used for:

- maintaining an address register of participating laboratories;
- recording the characteristics of specimens;
- recording of different distributions;
- analysing data for the global summary of results;
- preparing performance statistics for individual participants;
- word-processing of documents.

5. Access to biological material

The organizing laboratory must have access to biological material in sufficient quantity to prepare the proficiency panels. Details regarding the panels are given in section 5, below.

IV. Participating laboratories

The aim of every national scheme should be to secure the participation of every laboratory in the country that is performing serological testing for HIV. It is probably best for the NEQAS programme to be phased in and for participation at this stage to be restricted to a few volunteers,

while inevitable early problems are dealt with. Once the programme is running smoothly, participants should be encouraged to enroll. A mandatory system may be considered if all laboratories do not join voluntarily.

The laboratory supervisor should discuss with staff members the purposes and benefits of participation in an external quality assessment scheme prior to enrolment and give them the opportunity to ask questions. It is essential that staff members do not view quality assessment as a way of penalizing technicians for bad work. It is important to encourage the staff members to feel responsible for their work and to feel proud if they have done well and to stimulate them to improve if they have done less well. Participating laboratories should have no objection to undergoing training if this proves necessary.

On receipt of the proficiency panel the staff of the participating laboratory should read the accompanying documents and check that the specimens are in good condition and that no leakage has occurred. The panel should be kept at 4^{o}C until use. The panel should be integrated in the daily routine work as soon as possible, preferably the next working day. Quality assessment specimens should provide participants with an insight into their performance with routine specimens. Therefore, participating laboratories should treat the panels exactly the same way as routine specimens. The report form and questionnaire should be returned to the organizing laboratory promptly.

On receiving the intended results, laboratory chiefs should compare these with their own results. If there is 100 % agreement, the chief may congratulate the technicians on a job well done and advise them to continue to work in the same manner. The chief should investigate all discordant results and try to identify the source of the differences. This might be a transcriptional error, it might be related to the assay used, or it might be due to non-optimal test conditions (incubation temperature, washer, reader, pipettes, etc.).

Once the problem has been identified, measures to solve it can be taken. Advice or training might be requested from the organizing laboratory. The organizing laboratory can also act continuously as an advisory body for problems related to HIV diagnostics.

V. Preparing quality assessment panels

1. Biological material

It is easy to specify characteristics of ideal HIV serology specimens for quality assessment, taking into account various factors known to affect the results of some tests. However, in practice it is often difficult or impossible to obtain ideal specimens.

Ideally, the material used should be serum. Although many diagnostic kits allow the use of either plasma or serum, plasma tends to be unstable on storage and in transport and may clot spontaneously. The minimum volume required for each specimen will depend on the number of participating laboratories. For every 100 participants at least 50 ml of each specimen is required in addition to the amount necessary for the full characterization of the specimen. Therefore, in practice, the only available source of supply is likely to be blood transfusion donations. This will be plasma unless special arrangements are made to collect into packs without anticoagulant. If

serum is not available, plasma can be used as second best; however, it is less stable and may give problems related to clotting, especially after freezing and thawing. If possible, plasma should be defibrinized with thrombin and/or recalcified (see below) to overcome these problems.

The material should be fresh and processed as soon as possible. Blood transfusion laboratories often store plasma that is unsuitable for transfusion until their freezers are full before making it available for quality assessment purposes. Blood bags should be collected promptly from the blood transfusion centre, and plasma immediately separated (for recalcification). Whatever the HIV result given at the blood transfusion centre (this might be false positive), the HIV serostatus of the specimen should be confirmed.

Positive material should be representative of the HIV viruses circulating in the country and of the different stages in HIV infection. West African countries should more frequently include HIV-2 positive specimens than Latin American countries in their panel. However, weakly positive material from early seroconversion is rarely available and the collection of large volumes of material from AIDS patients is clinically contraindicated.

It is recommended that panel specimens should not be diluted as there is a risk of diluting out antibodies detected by some test systems but not by others. However, as weakly positive material may be difficult to obtain it may be necessary to dilute a strongly positive sample with negative serum. The diluent should be human serum, preferably from a single donor, tested and found to be negative in all available tests. Specimens should be retested after dilution.

In general, a single blood donation of an adult donor will provide approximately 300 ml of plasma. Only in rare cases where the NEQAS programme would involve more than 350-400 participants, single-donor volumes may be inadequate. Here the best solution is to work with two different proficiency panels for each distribution.

2. Conversion of plasma to serum

If serum is not available, then plasma must be converted to serum. Two methods are available for conversion of plasma to serum, thrombinisation and recalcification (as described below). These are hazardous and technically difficult operations, which should be carried out in a biological safety cabinet. Personnel should practise universal precautions and wear protective clothing and other safety devices during such operations. Working under sterile conditions will also prevent contamination with micro-organisms. Thrombinisation is the preferred method as micro-clots are less likely to form during storage and a “cleaner” product is obtained. Excessive recalcification should be avoided as this adversely affects some assays, such as gelatin particle agglutination.

Thrombinisation

Reconstitute dried human or bovine thrombin in deionised water to a concentration of approximately 500 units per ml (stock solution). Use at the rate of 1 unit of bovine thrombin per 2ml of plasma or 1 unit of human thrombin per 1ml of plasma.

1. Add the appropriate volume of thrombin to the plasma at room temperature and mix gently. Incubate at 37°C for 1-2 hours.

2. After incubation, check that the plasma has clotted, if not, further incubation may be required. Occasionally plasma does not clot, but it can still be used.

3. Remove plasma from the incubator and allow to cool at room temperature for 1 hour before transferring to a freezer at -30°C.

4. Remove from the freezer after 2-3 days.

5. Allow the serum to thaw at room temperature, placing the bottles in a beaker to contain any spillage resulting from cracked bottles.

6. Pour the serum into labelled, empty bottles, holding the clot back with a pipette. Extract any remaining serum from the clot by pressing the clot at intervals over 1 hour. Serum will continue to be expressed as the clot retracts.

7. If serum is to be used soon, store at 4°C, otherwise store at -20°C /-40°C.

Recalcification

The following method has been used with some success:

1. Make a 2 mol/l solution of $CaCl_2.2H_20$ by adding 3 g of $CaCl_2.2H_20$ to 10 ml of distilled water.

2. Add 0.5 ml of the freshly prepared $CaCl_2$ solution to 100 ml of plasma, mix and incubate in a water bath at 37^oC for 1 hour (final concentration 0.01M $CaCl_2$). Larger volumes may require several hours to clot.

3. If the plasma has not clotted, more $CaCl_2$ may be added (not exceeding a total of 1% of the 2 mol/l solution) and the mixture incubated further.

4. When the plasma has clotted, remove from the water bath, cool, place the bottle in a strong plastic bag or beaker (in case it cracks) and place in a freezer preferably at below -20^oC, overnight.

5. Remove the bottle from the freezer, allow to thaw at room temperature, and separate the serum from the fibrin clot. For small volumes, if collection bags are used separation can be accomplished by centrifugation in a standard blood bank centrifuge.

3. Heat inactivation

For safety reasons, HIV positive panel specimens should be heat-inactivated (60 minutes at 56^oC) before the filtration step. HIV negative specimens should **not** be heat-inactivated as this may cause false positive reactions.

Safety precautions recommended for the handling and distribution of large volumes of HIV-positive material should be adhered to. The recommended literature is given in Annex 2.

4. Filtration

Filtration of large volumes of serum is technically difficult. In many cases it can be avoided by carrying out thrombinisation under aseptic conditions. If the resulting serum appears clear and is bacteriologically sterile, filtration is not necessary. In some cases filtration may be necessary to clarify the serum and to remove any micro-organisms. After centrifugation, a connecting line with an in-line filter can be inserted in the sterile ports of both the centrifuged bag and a new collection bag. The donor material can then be transferred sterile to a new bag and any fibrin particles are trapped by the in-line filter. For larger scale operations, the donor material should be centrifuged (using closed containers for biological safety) to remove most of the clotted fibrin. Donor material can then be filtered through a clarifying filter (3.0 micron or 1.0 micron) that is piggy-backed onto a pharmaceutical grade sterilizing filter (0.22 micron). For large scale operations, or when the donor material is not handled in a closed environment, heat treatment should be undertaken before filtration.

5. Use of biocides

Although filtration should remove bacteria and moulds, serum is easily contaminated in subsequent manipulations. Bronidox L (0.05%) (5-bromo-5-nitro-1,3-dioxane in propylene glycol, Henkel Chemicals) may be added to prevent growth of contaminants. Thiomersal (0.01%) may be used but is effective only for a few weeks because it loses activity, especially if exposed to light. Sodium azide should not be used as it will inactivate peroxidase conjugate and cause problems in some ELISA formats.

6. Selection of panel specimens

The distribution panel should contain 5 to 10 well-selected and fully characterized specimens. Each distribution panel should include HIV-1 strong and weak reactive samples, and HIV negative samples. Occasionally, the panels should include one or two duplicate samples, to monitor the reproducibility of the results. These duplicate samples should have different identification numbers. Depending on the geographical region HIV-2, HIV-1 specific variants might be regularly or occasionally included.

Over time also duplicate distributions might be organized, i.e. exactly the same samples of the previous panel distribution are sent, but with, the specimen identification numbers altered. The composition in terms of absolute numbers of HIV positives and negatives should not be predictable. Nonetheless, the panel should not include too challenging samples; the HIV serostatus of each specimen should be clearly defined and not disputable.

7. Characterization of panel specimens

The stock sera should be characterized with a comprehensive range of diagnostic assays available in the country. It is important that the selection and range of tests used is adequate to establish the sera as HIV-1 and/or HIV-2 positive, negative, or indeterminate according to a consensus definition. The minimal requirement is characterization with two ELISA tests (at least one should be a combined HIV-1/HIV-2 assay) and two simple and/or rapid HIV tests. The serostatus of the specimens should be confirmed with HIV-1 and HIV-2 Western blots or with HIV-1/HIV-2 Line Immuno Assays.

A selection of specimens can then be made to compose a particular panel for a certain distribution. An example of the pre-distribution testing results is given in Annex 10.

In addition to HIV testing the sera should be tested for the presence of hepatitis B antigen (HBsAg) in order to minimize the risk for laboratory staff. HBsAg positive sera should not be used for a proficiency panel, unless it is a combined HIV, HB proficiency panel. The panel can also be sent to other reference laboratories (if available) to check the results.

8. Storage

Good record keeping of available stock (quantity and specifications of specimens) is important. Stock material should be stored at -20 °C or at -40°C in volumes sufficient for one distribution (20 to 50 ml vials). Repeat freezing and thawing should be avoided.

At the time of distribution to the participating laboratories the stock specimens should be thawed and dispensed into leak-proof, screw-capped plastic vials which have been properly labeled with a consecutive number for each specimen and the distribution number. The volume required will depend on the number of assays to be performed by participants; 0.5 ml (maximum 1 ml) is adequate for most purposes.

Before distribution, each panel specimen should be retested with the full set of diagnostic assays as storage might have slightly altered the results, especially in the case of weak positive samples. The results obtained should be summarized on the intended result form.

Vials should be stored at +4°C prior to dispatch. The panels should be dispatched as quickly as possible. A flow-chart for the preparation of panels is given in Annex 6.

If plenty of -20°C/-40°C space is available, vials (0.5 – 1 ml) already filled for testing panels and labeled for distribution can be stored. Just prior to shipping to the laboratory, vials can be removed from the freezer and placed in appropriate panels for distribution. Consequently, specimens will gradually thaw during the shipping process.

VI. Operating an external quality assessment scheme

1. Stability during transportation

Before introducing a quality assessment scheme, the organizers should establish the stability of specimens during transportation to participating laboratories. If specimens are found to deteriorate during transportation then alternative distribution channels or lyophilization might be considered. However, the organizer would need to demonstrate both the stability of the lyophilized specimens and its unaltered behaviour in any of the test systems in local use.

A small number of participating laboratories (1-3), preferably those to whom delivery is expected to take longest, should receive two panels and be requested to return one panel unopened to the organizing laboratory. The organizing laboratory will test this panel again to check that the specimens have remained stable during the stringent conditions of twice the normal period of transport.

2. Dispatch of panels

In most cases the postal service is the only realistic means of transmitting quality assessment specimens, although a courier service or other existing communication network may be used where appropriate. In some settings, delivery by the organizing laboratory may be the only solution. The specimens must be packed to avoid leakage, and local postal regulations on packing and labeling must be observed. Vials may be sealed in a polythene tube, using a heat sealer to seal the tube between each vial. The polythene-sealed vials are then wrapped in cellulose wadding, which is sealed in a plastic bag and placed in a cardboard box, which is in turn sealed with paper tape.

3. Frequency of distribution of specimens

Frequency of distribution of specimens to participating laboratories must be dictated by local circumstances and the resources available. Ideally, to maintain interest, a minimum of one to two distributions a year should be made, each distribution containing between 5 and 10 specimens. Occasional quality assessment distributions are better than none, and small frequent distributions are better than occasional distributions of a large panel size. In addition to the NEQAS, proficiency panels provided by HIV diagnostic companies might be included in the overall quality assessment measures taken by laboratories.

Together with the quality assessment panel, an instruction sheet and a report form should be sent to participating laboratories. The intended result form should be sent to participants after receipt of their completed report form or after the closing date and the report of the distribution should be sent to participants as soon as possible.

4. Instruction sheet

The instruction sheet should give general information on how to process the specimen and how to record the results. The participating laboratories must be reminded that they will benefit from a quality assessment scheme only if they treat the specimens in a routine manner. In addition there should be a statement about potential risk of infection despite heat inactivation of the specimen. Finally, a deadline for receiving results should be included. When the time allowed for examination has expired, a brief note of the intended results should be sent to participating laboratories. Reports received after this time cannot be accepted. An example of an instruction sheet is given in Annex 7.

5. Report form

Participants should record their results on a specially designed report form. The distribution number as well as the identification number for each participating laboratory should be clearly indicated on this document. Laboratory chiefs should check the code and correct it if necessary. In

order to assess the possible influence of a prolonged transportation period the date of arrival of specimens should be recorded on the document.

The forms should include two sections: the first, in which the tests used are identified and the results recorded; and the second, for recording the final result on each specimen as HIV-1/HIV-2 positive, negative or indeterminate (equivocal), and for indicating whether the specimen would normally be referred and if a repeat would normally be requested. The information given by participants in the second section of the form provides a useful basis for assessment of the results.

In addition to the results, space should be foreseen for eventual questions or remarks from the participants. An example of a report form is provided in Annex 8.

Organizers may find that report forms are incorrectly completed during the early stages of a quality assessment scheme. The design of the forms should be reviewed to ensure clarity and to minimize these problems.

Organizers of new quality assessment schemes are often tempted to collect large amounts of methodological information from participants. This is time-consuming for participants to provide and for organizers to analyse. The likely usefulness of such information and the manner in which it will be analysed must be considered before it is collected. Collection of data on optical densities obtained and cut-off values is useful, particularly if duplicate samples are included in a distribution. This provides information on the reproducibility of results and serves to stress the importance of adequate quality assurance within the laboratory. Participants may use different criteria for interpreting Western blot results, and it may be useful to ascertain the criteria used by each participant. The World Health Organization criteria for interpretation of HIV-1 and HIV-2 Western blot results are published elsewhere (see bibliography, Annex 2).

6. Intended results

When the time allowed for examination has expired, a brief note of the intended results should be sent to participating laboratories. Reports received after this time cannot be accepted. It is important that participants receive results soon after the distribution while they are still interested and are able to investigate the reasons for any incorrect results. Participants should be encouraged to retest specimens for which they obtained incorrect results. If necessary, repeat specimens should be made available for investigating the reasons for any incorrect results. An example of an intended result sheet is provided in Annex 9.

7. Analysis

Before a report can be produced, results of participants have to be analysed. The analysis of results should have as its basic aims:

1. to produce for each participating laboratory an analysis of its individual performance;

2. to produce an overall summary of results presenting the total number of correct and incorrect results (this will give information on the degree of standardization and the overall quality of testing in a country);

3. to provide useful information on the HIV antibody kits used in the country.

Data analysis should be computerized; this is essential for retrospective analysis of the results of individual laboratories. Only if the number of participants is small (<20) and no computer available, can analysis be done by hand without the aid of a computer.

VII. Report

The results which have been sent by the participating laboratories should then be analysed and documented in a report. Here too, it is very important to preserve the confidentiality of the individual results. Only the supervisor of the scheme should have the key to the code. Examples of ways of presenting the data are provided in Annex 10.

The organizing laboratory should issue a report to participating laboratories, containing the following information:

1. the intended results of that particular distribution(s) and details of the results obtained by the organizing laboratory and how they were obtained;

2. general information including the types of participating laboratories;

3. a list of the various HIV antibody assays used and the number of laboratories using them;

4. a summary of the results for each specimen, with the number of reports of positive, negative, indeterminate (equivocal), results received;

5. a summary of the performance according to participating laboratories (if a scoring system is used, details of how scores are awarded for each category of report);

6. comments on the results.

Care is required in the presentation of results obtained with individual kits or methods. Participating laboratories, and some organizers, tend to treat these results as kit evaluations and to use them as the basis for decisions on the suitability of individual assays. This should be discouraged as kit evaluation requires prospective testing, on large numbers (>200) of samples. On the contrary, the panels used in quality assessment schemes consist of a small number of specimens, preselected on the basis of their behaviour in various anti-HIV assays by the organizing laboratory.

Any advice to participants on methodology or choice of reagents should be made on the basis of scientific evidence from more than one source and should represent a consensus view. The scheme will lose credibility if advice is based only on the organizers' individual preferences.

1. Details of the pre-distribution test results

Specimens of each distribution are characterized with a comprehensive range of diagnotic assays as described earlier (p. 12). Details of the pre-distribution test-results should be included in the report (Annex 10, Tables 1 and 2).

2. HIV assays used by participants

General information such as the distribution of different types of laboratories participating might be useful (Annex 10, Figure 1). A list showing all HIV assays used in the quality assessment should be generated from the information provided by participants. In many countries this may be the only comprehensive overview of HIV tests currently in use. An example is shown in Annex 10, Table 4. A table listing the number of different HIV assays used per laboratory can also be generated (Annex 10, Table 3).

3. Recorded results per specimen

A summary of the results recorded per specimen will identify whether there were specimens which posed particular problems, e.g. specimen No. 22 was misdiagnosed by 10 laboratories (Annex 10, Table 5).

4. Individual performance of participants

Results should be analysed for each laboratory separately in order to assess individual performance and to have comparative data over time. A simple but effective way is to calculate the accuracy of each laboratory according to this formula:

$$\text{Accuracy} = \frac{\text{number of specimens correctly identified by the participant}}{\text{total number of panel specimens}} \times 100$$

Accuracy is the total number of correct results (true positives + true negatives) found by the participant, divided by the total number of specimens in the panel, multiplied by 100. Due to the small sample size of the panel, assessment of performance can only be graded in large intervals. If a panel contains 6 samples, one wrong result represents a decline in accuracy of 17%. It is important to relate the performance of each participant to the global performance, as some panels might turn out to be more difficult than others.

It is also useful to calculate a performance index to allow participating laboratories to evaluate their performance with a single value. One such index is the individual laboratory's cumulative score expressed in terms of the number of standard errors above or below the mean score. Thus a positive value would indicate a better than average performance and a negative value a worse than average performance. Confidence limits can be used to define "poor performance", e.g., at 95% confidence limits a performance index of < -1.96 could be considered as a "poor performance".

Further data analysis can be undertaken when the quality assessment scheme has been operating for some years and enough data have accumulated to show performance over time. This enables organizers to identify poor performers in need of further support and follow-up. Furthermore, these data can be very useful for programme managers in the National AIDS Control Programme in evaluating overall performance in the laboratory sector.

An example of the performance of an individual laboratory over a 2-year period is provided in Annex 10, Figure 2.

5. Scoring of results

Organizers of quality assessment schemes must take a decision together with participants on whether or not a scoring scheme should be used. There are two opinions on this matter. The first emphasizes the judgmental aspects of a scoring system, which might discourage participants. The second recognizes that scoring of results may be unpopular with participants, but argues that without scoring, poor results may be ignored by participants, and no effort made to improve performance.

Scoring certainly encourages a response from participants. Scoring schemes should be designed to suit local circumstances. A simple "right" or "wrong" assessment (1 or 0) may be adequate. Increasing the number of scoring categories, e.g., -1, 0, 1, 2, permits a graded assessment and consideration of the participant's confirmatory or referral practices in scoring. Thus, for example, the scoring scheme can be used to encourage participants to refer difficult or equivocal specimens to confirmatory laboratories (see sample scoring scheme in Annex 10).

VIII. Feedback

1. Poor performance

Quality assessment schemes are educational and aim at self-evaluation and self-help. There will be cases, however, where some laboratories show poor performance over long periods with no evidence of improvement. In such cases it is useful to have a mechanism to provide them with help and advice.

The possibility of additional training and follow-up in case of persistent poor performance should be part of the scheme. This should be mentioned during the initial invitation to join the scheme and should be repeated in the report. Every effort must be made to encourage laboratories to seek help in the event of poor performance. If a particular participant is not taking the first step, it is the responsibility of the organizer to offer them advice discreetly.

Initially, an attempt should be made to identify the cause of the problem. This can be done by discussing common errors which might explain poor performance. A section on common errors in laboratory techniques can be found in the publication by Family Health International *HIV-testing and quality control* (see bibliography, Annex 2).

If it is not possible to identify the problems or if they persist despite the advice given, a visit to the participating laboratory and on-the-job training provided by an experienced technician, may be the only solution. This training should cover every step in the procedure, from the moment the specimen arrives until the result is transmitted by the laboratory.

Individual performances of laboratories should be discussed only with the laboratory concerned. It is essential that participants trust the confidentiality of the scheme.

2. Information

Regular updates on HIV diagnostics or related matters can be provided to participants on the last pages of the report or issued separately. Exchange of information is important and adds to the educational component of the programme. Most participants, especially those from remote areas, will greatly appreciate it.

Annex 1
International external quality assessment schemes

1. Public Health Laboratory Service, United Kingdom NEQAS for microbiology
Quality Assurance Laboratory, 61 Colindale Avenue,
London NW9 5EQ, United Kingdom
Tel: +44 181 2004400 Fax: +44 181 2051488

2. South African Institute for Medical Research,
Department of Microbiology,
PO Box 1038, Johannesburg 2000, South Africa
Tel: +27 11 725 0511 ext 2192 Fax: +27 11 725 2319

3. Model Performance Evaluation Program,
Centers for Disease Control and Prevention, 4770 Buford Highway,
Building 102, Room 2218 MS G-23, Chamblee, Georgia 30341-3714, USA
Tel: +1 404 639 17 03 Fax: +1 404 488 76 93

4. College of American Pathologists,
Quality Assurance and International Development,
Northfield, Illinois 60093-2750, USA
Tel: +1 800 323 40 40 Fax: +1-708 446 82 66

5. National HIV Reference Laboratory at Fairfield Hospital,
Yarra Bend Road,
Fairfield 3078, Australia
Tel: +61-39 280 2400 Fax +61-39 482 4352

Company Services

6. Abbott
Diagnostics Division,
Max-Planck-ring 2,
D-6200 Wiesbaden-Delkenheim, Germany
Tel: +49 61 22 581 623 Fax: + 49 61 22 581 217

7. Organon Teknika
Veedijk 58,
2300 Turnhout, Belgium
Tel: +32 14 40 40 40 Fax: +32 14 42 16 00

Annex 2
Bibliography

1. Constantine N.T., Callahan J.D., Watts D.J. *HIV-testing and quality control: A guide for laboratory personnel*, available through Family Health International, P.O.Box 13950, Research Triangle Park, NC 27709, USA, fax 919-544-7261.

2. El-Nageh et al. *Basics of quality assurance for intermediate and peripheral laboratories*. Alexandra, World Health Organization, 1992 (WHO Regional Publications, Eastern Mediterranean Series, No. 2).

3. European Committee on Clinical Laboratory Standards. *External quality assessment in microbiology*. Berlin, Beuth Verlag Gmbh 1983, (ECCLS Document 3, No. 6).

4. Proposed WHO criteria for interpreting results from Western blot assays for HIV-1, HIV-2 and HTLV-I/HTLV-II. *Weekly epidemiological record*, 1990, 37: 281-283.

5. Recommendations for the interpretation of HIV-2 Western blot results. *Weekly epidemiological record*, 1990, 10: 74-75.

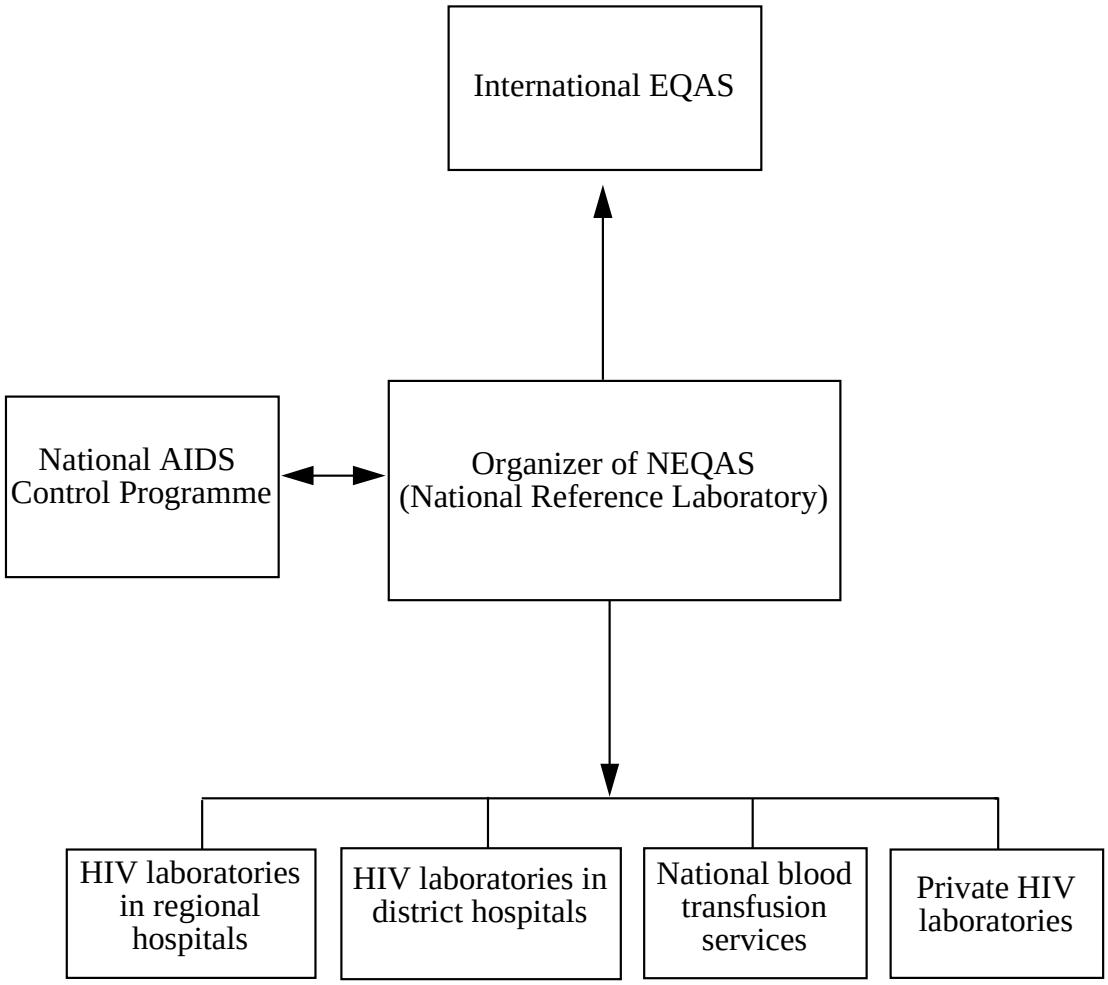
6. World Health Organization, *External quality assessment of health laboratories*, Report on a WHO Working Group. Copenhagen, 1981 (EURO Reports and Studies, No. 36).

7. *Report of the WHO meeting on criteria for the evaluation and standardization of diagnostic tests for the detection of HIV antibody. Stockholm, 7-8 December 1987*. Geneva, World Health Organization, 1988, (unpublished) document WHO/GPA/ BMR/88.1.

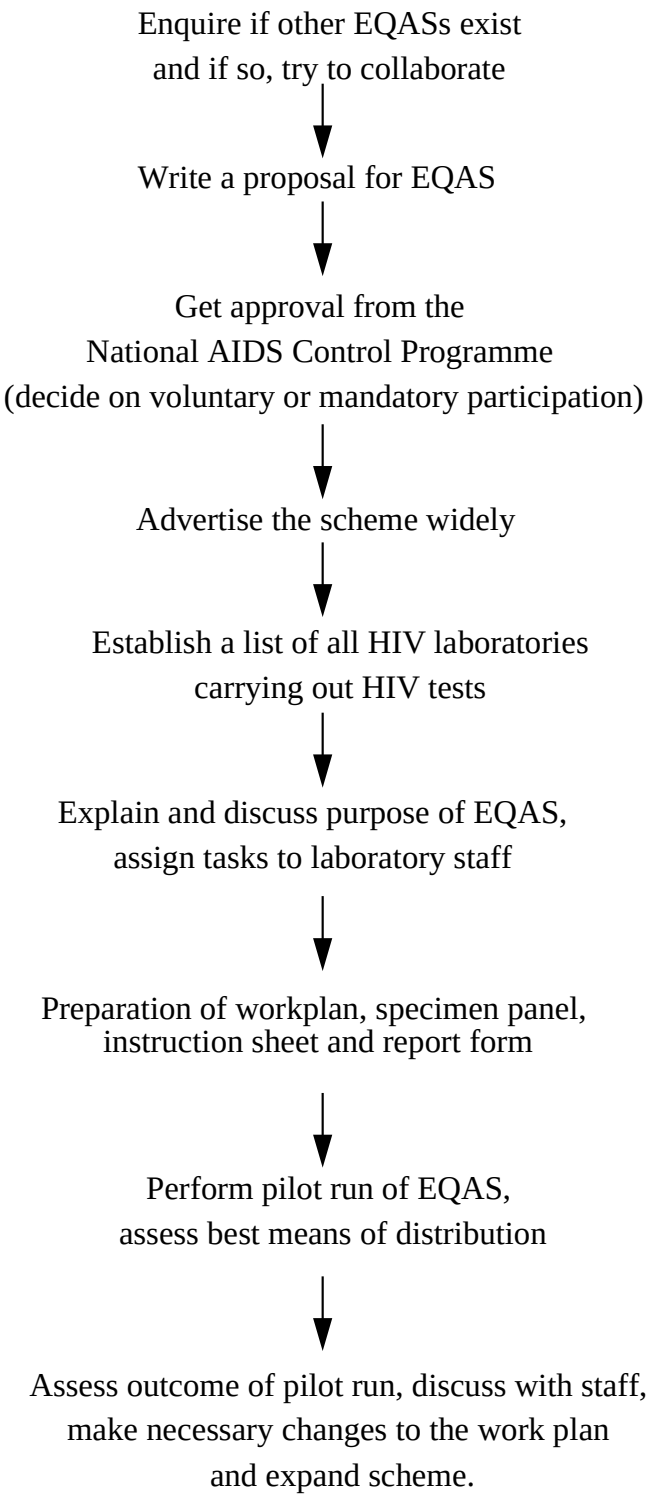
8. World Health Organization. *Biosafety guidelines for diagnostic and research laboratories working with HIV*. Geneva, 1991 (WHO AIDS Series No. 9).

9. *Guidelines for quality assurance programmes for blood transfusion services*. Geneva, World Health Organization, 1993.

Annex 3
Organigram of a national external quality assessment scheme (NEQAS)



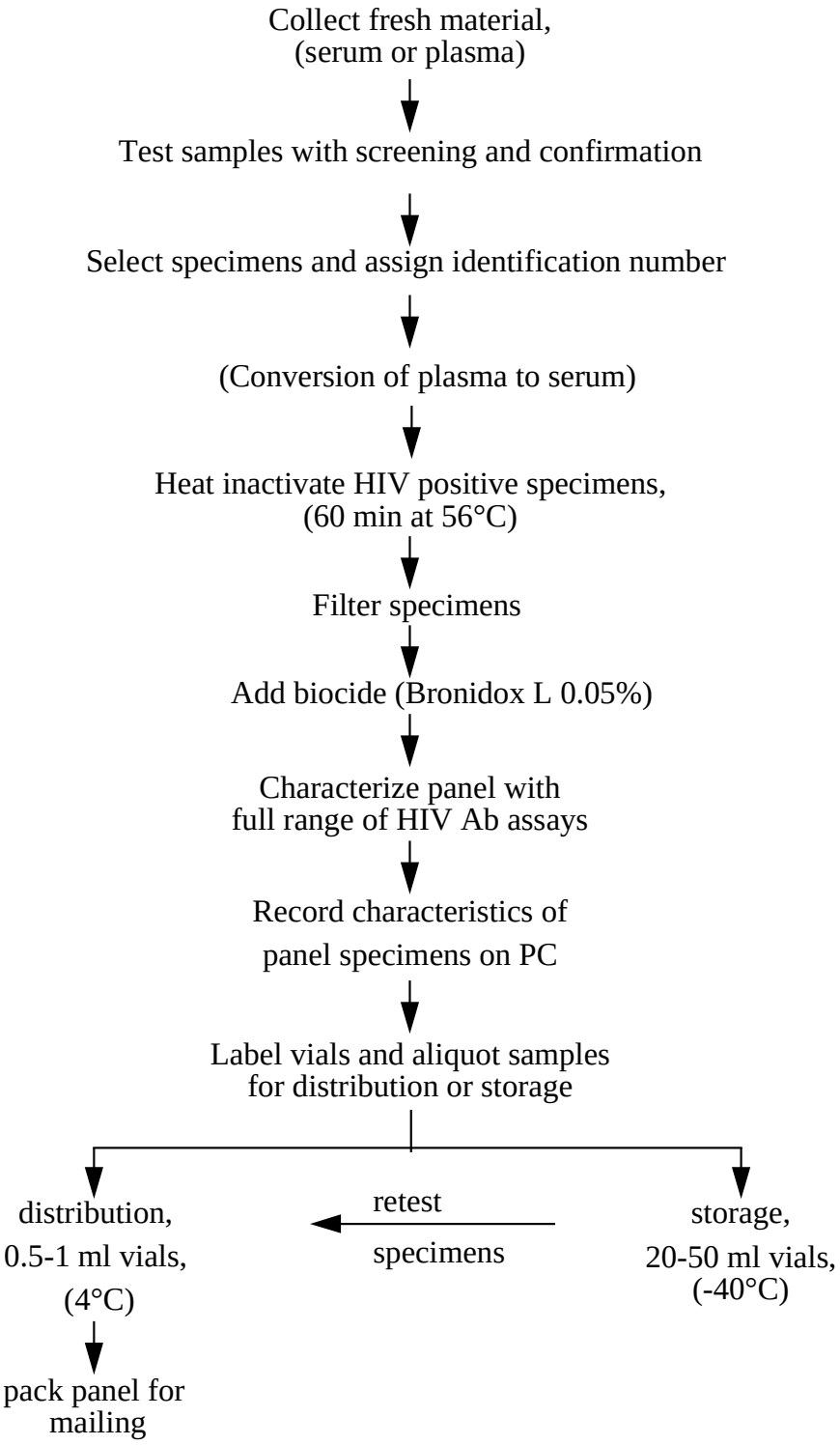
Annex 4
Establishing an external quality assessment scheme



Annex 5
Responsibilities of organizer and participants in external quality assessment schemes

ORGANIZER	PARTICIPANTS
1. Advertise NEQAS programme	
	2. Enrol in the programme
3. Register participants (assign a confidential code)	
4. Prepare distribution (panels, instructions and report forms)	
	5. Test QA panel and return completed report form
6. Send intended results to participants	
	7. Compare results (contact the organizer)
8. Compile data and analyse results	
9. Produce a general report	
10. Intervene where necessary	

Annex 6
Preparation of external quality assessment panels



Annex 7
Sample instruction form

National External Quality Assessment Scheme
(name of country)

INSTRUCTIONS FOR HIV SEROLOGY

1. PLEASE CHECK THAT THE LABORATORY IDENTIFICATION NUMBER IN THE TOP LEFT HAND CORNER OF THE REPORT FORM IS CORRECT.
   Failure to do this may result in your laboratory being credited with results from another laboratory. If the number is not correct please notify the organizer immediately and correct the number on the form.

2. Specimens for HIV antibody testing are enclosed. Any specimens positive for anti-HIV have been heated at 56°C for 30 minutes. **BUT ALL SPECIMENS SHOULD STILL BE HANDLED AS IF CAPABLE OF TRANSMITTING INFECTION.**

3. Please examine the specimens by your **ROUTINE METHODS** for HIV antibody testing, and write the assay outcomes and the final result for each specimen on the report form.

4. Please complete the section of the form relating to methods, including the results relevant to each technique.

5. Laboratories will achieve the maximum educational benefit from these specimens if they are treated as nearly as possible as normal clinical samples without non-routine procedures being used.

6. To ensure that the results are evaluated on laboratories' initial examinations repeat specimens will normally only be supplied after the final date for return of the report form.

7. If you are unable to examine a specimen, please state your reasons on the form and return the form but not the specimens.

8. In the unlikely event of a damaged, leaking or missing specimen, please telephone (name) as soon as possible for a replacement.

9. Please return your results as soon as possible and by **(date) at the latest to:**

   Name of responsible person
   Address of organizer
   Tel.....
   Fax.....

Annex 8
Sample report form

National External Quality Assessment Scheme for HIV serology

REPORT FORM
DISTRIBUTION No...

Laboratory identification No .....
Specimen Nos .....-..... Date of arrival ....

If you do not examine these specimens please specify reason: ....
....
....

Specimen No	Assay (company)	OD sample	OD cut off	Results	Sent to ref. lab?
	....	....	....	....	....
	....	....	....	....	....
	....	....	....	....	....
	....	....	....	....	....
	....	....	....	....	....

Return this form at the latest by (date) to
Name of responsible person
Address of organizer
Tel ....
Fax............

Laboratory identification No....

Results of additional tests (Line assays, Western blots)

Specimen No	Assay	Protein bands	Interpretation	Final result
	....	....	....	....
	....	....	....	....
	....	....	....	....
	....	....	....	....
	....	....	....	....

Specify criteria used for interpretation:
....

Comments/questions:
....

Name of responsible person, Address of organizer, Tel: ......, Fax: ....

Annex 9
Example of an intended result form

National External Quality Assessment Scheme
(name of country)

DISTRIBUTION NO.... FOR HIV SEROLOGY

INTENDED RESULTS

Specimen No	**Results**
Specimen No. 18	HIV-1 positive
Specimen No. 19	HIV negative
Specimen No. 20	HIV-2 positive
Specimen No. 21	HIV-1 positive
Specimen No. 22	HIV-1 positive
Specimen No. 23	HIV negative
Specimen No. 24	HIV-1 positive

Annex 10
Example of a distribution report

National External Quality Assessment Scheme
(name of country)

for

HIV Serology

Summary of Results

Distribution Number ...
dispatched (day, month, year)

Name of organizing laboratory, address, telephone and fax number

SPECIMEN DETAILS

Distribution No..
dispatched (date)

Six specimens were dispatched with requests for the determination of HIV antibody status. All specimens were derived from serum or (recalcified) plasma donations. Specimen No 22 consisted of a positive donation diluted 1:50 in negative donations. The positive specimens were heat inactivated at 56^{o}C for 60 minutes. A range of pre-distribution tests was performed on each specimen and the results are tabulated on the following pages. In addition, two sets of specimens returned to the organizing laboratory gave the intended results. Four specimens were positive for HIV-1 antibody, one was positive for HIV-2 antibody, and two were negative. Specimen Nos 18 and 24 were duplicate samples. Details are as follows:

Specimen No. 18/24	HIV-1 positive
Specimen No. 19	HIV negative
Specimen No. 20	HIV-2 positive
Specimen No. 21	HIV-1 positive
Specimen No. 22	HIV-1 positive
Specimen No. 23	HIV negative

Figure 1
Percentage of participants, by laboratory type that reported results for distribution, no., date of shipment

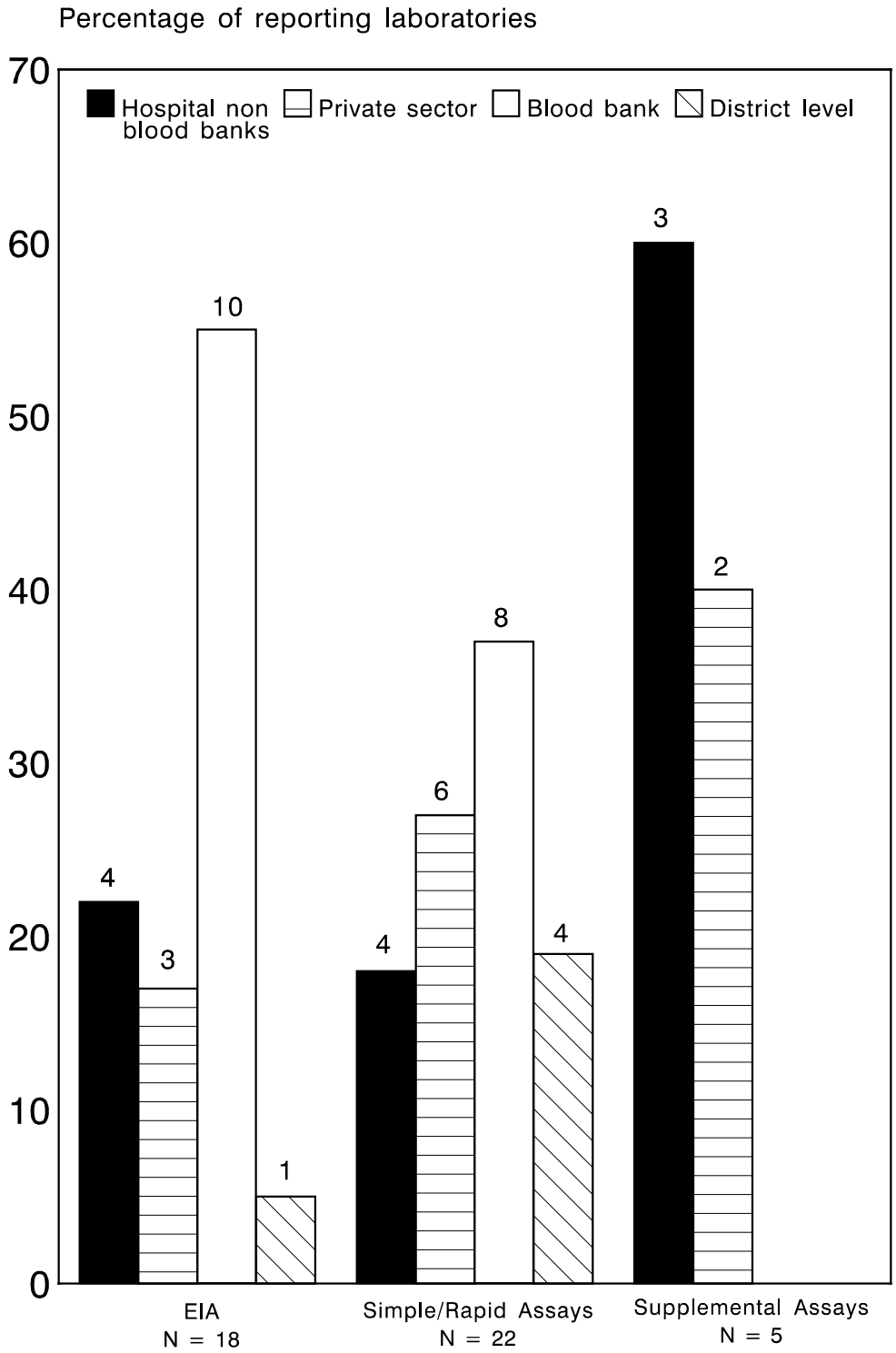


Table 3
Number of kits used by participants (N = 28)

No of kits	No of users
1	14
2	9
3	3
4	2

Table 4
HIV assays used by participants

HIV antibody assays (company)	Number of labs using the test
ELISAs	
Serodia HIV1 particle agglutination (Fujirebio)	13
Wellcozyme HIV1+2 Recombinant EIA (Murex)	5
Wellcozyme HIV Recombinant EIA (Murex)	2
Abbott Recombinant HIV1/HIV2 EIA third generation (Abbott)	2
Vironostika HIV Uni-Form II (Organon Teknika)	1
Enzygnost HIV 1+2 plus (Behring)	1
Genelavia Mixt (Sanofi Pasteur)	6
ETI-AB1+2 (Sorin)	1
Simple and/or Rapid Assays	
Serodia HIV 1+2 particle agglutination (Fujirebio)	3
Capillus HIV 1+2 (Cambridge Biotech)	5
Inno-quick HIV 1+2 (Innogenetics)	1
Immunocomb II bispot (PBS Orgenics)	4
Combaids Visual (Span Diagnostics)	3
Supplemental Assays	
Organon Liatek line assay (Organon Teknika)	2
Western Blot HIV-1 (Genelabs Diagnostics)	2
Lav blot HIV-I1 (Sanofi Diagnostics Pasteur)	1

Table 6
Accuracy performance according to participating laboratories

Lab ID No.	No. of specimen	True pos.	True neg.	False pos./ind.	False neg./ind.	Efficiency %
1	7	5	2	0	0	100
2	7	4	2	0	1	86
3	7	3	1	1	2	57
4	7	5	2	0	0	100
5	7	5	1	1	0	86
6	7	5	2	0	0	100
7	7	2	2	0	3	57
8	7	5	2	0	0	100
9	7	5	1	1	0	86
10	7	5	2	0	0	100
11	7	4	2	0	1	86
12	7	5	2	0	0	100
13	7	3	2	0	2	71
14	7	5	2	0	0	100
15	7	3	2	0	2	71
16	7	5	2	0	0	100
17	7	2	2	0	3	57
18	7	5	2	0	0	100
19	6	5	1	0	0	100
20	7	3	1	1	2	57
21	7	5	1	1	0	86
22	7	5	2	0	0	100
23	7	5	2	0	0	100
24	7	5	2	0	0	100
25	7	4	2	0	1	86
26	7	2	2	0	3	57
27	7	5	2	0	0	100
28	7	5	2	0	0	100
Total	**195**	**120**	**50**	**5**	**20**	**87** (Average)

Figure 2
Performance of laboratory No. 5 for years 1994-1995

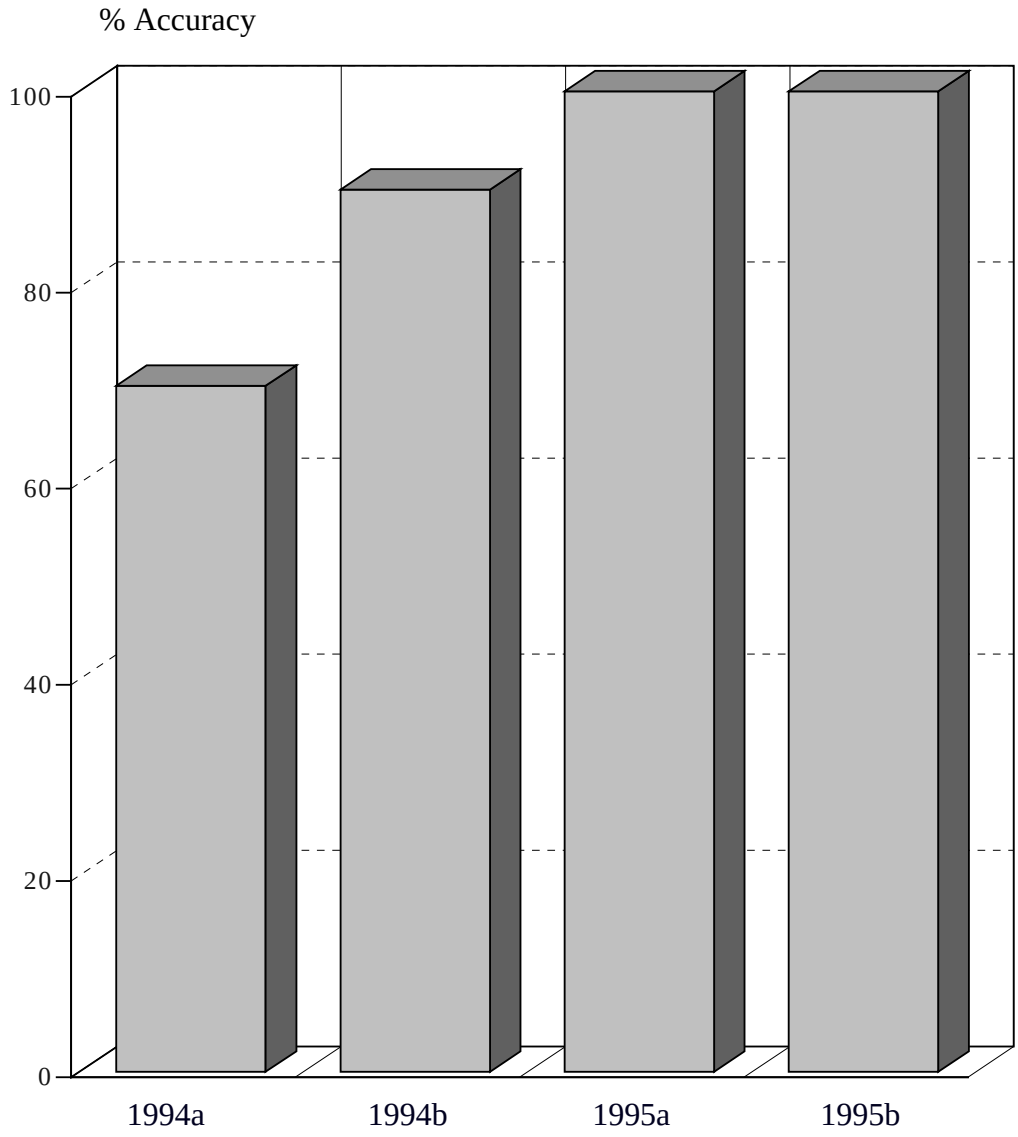


Table 7
Sample scheme for scoring participants' results for anti-HIV serology

Specimen category: anti-HIV positive

Participant's response	Score
Presumptive positive, adequately confirmed or referred	2
Presumptive positive, not adequately confirmed or referred	1
Equivocal/indeterminate, referred	1
Equivocal/indeterminate, not referred	0
Negative	-1

Specimen category: anti-HIV positive (low level)

Participant's response	Score
Presumptive positive, adequately confirmed or referred	2
Presumptive positive, not adequately confirmed or referred	1
Equivocal/indeterminate, referred	2
Equivocal/indeterminate, not referred	1
Negative	0

Specimen category: anti-HIV negative

Participant's response	Score
Presumptive positive, referred	0
Presumptive positive, not referred	-1
Equivocal/indeterminate, referred	1
Equivocal/indeterminate, not referred	0
Negative	2

PRE-DISTRIBUTION TEST RESULTS

Table 1: Screening Assays

Specimen number	Rapid assay 1	Rapid assay 2	Simple assay	ELISA 1		ELISA 2		ELISA 3	
				OD	Ratio	OD	Ratio	OD	Ratio
18/24	reactive	reactive	reactive: HIV-1	2.84	10.8	3.000	16.9	19.76	4.3
19	non reactive	non reactive	non reactive	0.064	0.2	0.016	0.09	0.176	0.4
20	reactive	reactive	reactive: HIV-2	0.734	2.8	1.803	10.2	1.334	2.9
21	reactive	reactive	reactive: HIV-1	1.425	5.4	2.215	12.5	1.048	2.3
22	weak reactive	reative	reactive: HIV-1	0.487	1.8	0.654	3.7	0.553	1.2
23	non reactive	non reactive	non reactive	0.058	0.2	0.011	0.06	0.137	0.3
				cut off: 0.263		cut off: 0.177		cut off: 0.458	

Table 2: Supplemental assays

Specimen number	Western blot HIV-1		Liatek HIV-1/HIV-2		Final result
	proteins bands	result[1]	proteins bands	result	
18/24	p17, p24, p31, gp41, p53/55, p64, gp120, gp160	HIV-1	gp41, p31, p24, p17	HIV-1	HIV-1 positive
19	no bands	negative	no bands	negative	negative
20	p24, p31, p64	indet.	p39, p24, gp36	HIV-2	HIV-2 positive
21	p24, p31, ±gp41, ±p64, ±gp160	HIV-1	gp41, p31, p24	HIV-1	HIV-1 positive
22	p24, gp120, gp160	HIV-1	gp41	HIV-1	HIV-1 positive
23	no bands	negative	no bands	negative	negative

[1] WHO criteria for WB

Table 5
Summary of results recorded per specimen

Category	Specimen number	Number of laboratories reporting as follows			
		Positive (%)	Indeterminate (%)	Negative (%)	Total
HIV positive	18	26 (92.9)	1 (3.6)	1 (3.6)	28
	20	25 (89.3)	0 (0.0)	3 (10.7)	28
	21	27 (96.4)	0 (0.0)	1 (3.6)	28
	22	18 (64.3)	4 (14.3)	6 (21.4)	28
	24	24 (85.7)	2 (7.1)	2 (7.1)	28
HIV negative	19	4 (14.3)	1 (3.6)	23 (82.1)	28
	23	0 (0.0)	0 (0.0)	27 (100)	27[1]

[1] One participant reported a specimen leaking in transit