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REPORT OF THE WHO MEETING ON CRITERIA FOR THE EVALUATION AND STANDARDIZATION OF DIAGNOSTIC TESTS FOR THE DETECTION OF HIV ANTIBODY

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1. INTRODUCTION

A WHO meeting was held under the auspices of the Global Programme on AIDS (GPA) at the National Bacteriological Laboratory in Stockholm, Sweden, on 7-8 December 1987 to discuss criteria for the comparative evaluation and standardization of diagnostic tests for the detection of HIV antibodies. It was attended by 26 scientists from 14 countries (see list of participants). The meeting was opened by Dr José Esparza, Acting Chief of the Biomedical Research Unit of GPA. Professor Lars Kallings (Sweden) was elected chairman, with Drs Peter Piot (Belgium) and Fred Mhalu (Tanzania) as vice-chairmen. Dr Ian Gust (Australia) was rapporteur.

2. EVALUATION OF ASSAYS

To advise Member States, WHO needs to have objective information on the characteristics of the large number of assays which are available for detecting infection with the Human Immunodeficiency Viruses (HIV-1, HIV-2). Evaluation of the sensitivity, specificity and reproducibility of these assays and definition of their operational characteristics should be undertaken through a project involving WHO Collaborating Centres on AIDS in different parts of the world.

2.1. Structure of the project

2.1.1. Preliminary assessment

An initial assessment of new assays to define their major operational characteristics should be made using the WHO Collaborating Centres on AIDS. This assessment would involve the use of each assay on a standard panel of 500 sera of diverse geographical origin. It should be stressed that information obtained from such a study would not be used to define sensitivity or specificity. Factors that would be evaluated include:

a) the packaging and labelling of the test kit;
b) the clarity of the manufacturer's instructions;
c) presentation and ease of use of the reagents;
d) the time required to perform a single assay and to process batches of sera;
e) whether predilution of sera is required;
f) the number, temperature and times of incubation;
g) how the test results are read and recorded;
h) the quantities of reagents provided;
i) requirement for specialized reagents (such as distilled water) or specialized equipment;
j) the cost of the kits and ancillary equipment;
k) the possibility of recycling materials;
l) storage requirements.

2.1.2 Formal evaluation

If the test appears promising, (laboratory performance is similar to or better than currently available assays) a large-scale evaluation should be undertaken in more than one centre in the area or areas in which it is intended to use the test. Although theoretically all tests should perform equally well anywhere in the world, regional factors such as the prevalence of other endemic diseases or hypergammaglobulinaemic sera, or the co-circulation of other human retroviruses, may interfere with the outcome of the tests. In this evaluation the sensitivity, specificity and reproducibility of the test will be determined by using sera obtained regionally or locally from several hundred infected individuals and several thousand sera from people with no evidence of infection. Wherever possible, sera that have produced false positive results with other assays should be included.

All the sera should be tested under code and the results returned for analysis to the coordinating laboratory that has organized the study. Sufficient quantities of all specimens should be retained, so that in the event of discrepant results aliquots may be referred to international reference laboratories and to the manufacturers for further study.

2.2. Reports

WHO Collaborating Centres which undertake evaluation of assays for detecting infection with HIV should submit regular reports to GPA.

Information on test performance should be presented in a standard format and contain at least the following information:

a) manufacturer and name of the kit
b) type of assay
c) format
d) source of antigen
e) protocol used - including source and characterization of the test panels
f) sensitivity, specificity and reproducibility (expressed with 95% confidence limits where relevant; statistically acceptable methods for comparing performance of different assays should be used).
g) evaluators' comments.

Manufacturers will be given the opportunity to comment on the results of the evaluation of their product before the report is made available to interested parties. Where relevant, manufacturer's comments may be entered in the report.

3. USE OF HUMAN SERUM/PLASMA PANELS

3.1 General

Panels of carefully characterized human sera or plasma have an important place in the evaluation of diagnostic tests for HIV infection, but they are useful in only one aspect of a strategy to determine acceptable performance standards for diagnostic tests. Other means include:

- evaluation of manufacturer's data on performance and quality control of the test
- monitoring lot-by-lot consistency of the product
- maintaining test performance at the point of use.

3.2 Types of serum collection

Collections of sera for purposes of evaluation of assays are of two types:

a) Validation panels, and
b) WHO International Reference Sera.

3.2.1 Validation panels

Panels of sera or plasma should be established on a regional or national basis with the assistance of the WHO Collaborating Centres on AIDS.

The purposes of such panels are:

- to allow rapid assessment of new assays and identify those with major problems of sensitivity and specificity

- to assist local laboratories in familiarizing themselves with a new assay, by providing specimens with known activity.

These panels are limited in their uses. They can not be used for formal evaluation of the assay. While data obtained with the panels could exclude an assay from consideration, such data should never be used to select a test for use in the field.

Validation panels should contain at least 50 unequivocally anti-HIV-positive sera and 50 unequivocally negative sera, from individuals whose clinical condition and risk of infection have been well documented. Some of the positive samples should have low or indeterminate reactivity. Local factors (such as malarial infections, visceral leishmaniasis or other conditions which cause unusual immunoglobulin profiles) that may result in false positive reactions may be taken into account by the inclusion of some specimens known to produce equivocal reactions. All specimens in the panel should be thoroughly characterized by well-defined assays such as ELISA, immunofluorescence and immunoblot.

Each serum or plasma sample should be available in sufficient volume to meet local demand for at least one year.

The validation panels should be regularly modified or replaced, to ensure that they contain sera which are likely to be representative of the current stage of the epidemic.

Smaller panels, including dilution series of well characterized antibody-positive sera, should be prepared by the WHO Collaborating Centres on AIDS and issued to regional or national laboratories. These panels should be used to monitor lot-by-lot variation of kits and assess the proficiency of testing procedures.

Careful consideration should be given to procedures for the preparation, storage and distribution of the panels. Caution should be exercised in heating sera or exposing them to multiple freeze-thaw cycles, since this changes the reactivity of some samples. Storage of non-lyophilized samples should be at $-20^{\circ}C$ or lower. All samples should be maintained in stoppered vials rather than glass ampoules.

3.2.2 WHO international reference sera

International reference sera for comparing assays performed in different laboratories need to be kept continuously up-to-date so that they remain representative. This should be done with the assistance of the WHO Collaborating Centres on AIDS, as an activity of the AIDS Reagent Project. The collection of international reference sera should contain representative samples from each WHO region. Pooling of sera from different donors is not recommended. Sera should be prepared in at least 1000 replicate stoppered vials containing 0.25 ml and lyophilized. Use of heating or another method to inactivate HIV is advisable.

Before its establishment each WHO international reference serum should be subjected to an international collaborative study involving testing in several laboratories. These studies should be coordinated by the WHO Collaborating Centres on AIDS.

Consideration needs to be given to the development of reference HIV antigen preparations once the technology is available and affordable.

4. CONFIRMATORY (SUPPLEMENTARY) TESTS

4.1. Uses and types of confirmatory (supplementary) tests

Serological tests are now widely used to screen blood donors, to confirm clinical suspicion of HIV infection, and for surveillance purposes. Because of the importance of a

positive result and the lack of specificity of the earliest assays, a two-tier system of testing has been established, in which sera found to be reactive on initial testing are re-assayed with a second (confirmatory) test.

Differences in the epidemiology of HIV infection, in available resources and in technical skills affect the choice of confirmatory test. In general, confirmatory tests which employ a different physical principle from that of the screening test are preferred. Of these the most common are:

1. Western blot (Immunoblot)
2. Indirect immunofluorescence (IF)
3. Radioimmunoprecipitation (RIPA).

The confirmatory test must be at least as sensitive as the screening test. This can be ensured by checking the performance of both tests on a standard panel of sera. Such a standard panel should include several well-characterized positive samples and a dilution series of known positive sera.

4.2. Standardization of confirmatory tests

There is currently no standard confirmatory test, no standard method of performing any of the confirmatory tests, and no standard interpretation of the results. WHO should strive to ensure that, wherever possible, techniques and interpretations are standardized within countries and regions.

4.3. Standardization and interpretation of confirmatory tests

4.3.1. Western blot

There are several aspects of standardization of Western blot

- standardization of the production of test strips;
- performance of the test in a standard manner;
- standardization of the readings and interpretation of the results.

From a manufacturing viewpoint the Western blot is one of the most difficult assays to standardize, since different preparations of virus contain differing quantities of each of the major gene products. Some licensing authorities have promulgated strict guidelines for the evaluation of test strips; they include checking at least two strips obtained from every transfer with a known negative and a known positive serum. To be of acceptable quality, each batch of strips must not only correctly identify these two sera, but also provide a consistent band pattern.

Ideally, the Western blot procedure would be standardized with respect to a reference panel of sera containing defined titres of antibodies to a variety of antigens and known reactivity against standard antigen preparations. This is not currently possible.

In the absence of any agreed interpretation of Western blots, the following criteria are widely used:

"positive" —
- env + gag + pol
- env + gag
- env + pol
- env alone (with minimum of 2 env antigens recognized)

"indeterminant" —
- gag + pol
- either gag or pol alone

These reactions may be seen in early infections with HIV-1, may represent cross reactions with HIV-2, or may be nonspecific. Additional testing by RIPA (see below) will usually give conclusive results. If RIPA is not available, testing of repeat specimens after some time may also give valuable additional information.

"negative" absence of all three classes of antigen

These criteria are likely to be revised in the light of increasing experience.

4.3.2. Immunofluorescence

This assay is relatively simple and inexpensive to perform but requires a special microscope and a high degree of training and expertise to interpret. The use of uninfected cell antigen (on the same slide as the infected cell antigen) is recommended as an important negative control. Immunofluorescence is not recommended as a universal confirmatory test since in some geographical areas and clinical settings it has been reported to be less sensitive than the screening test and hence to produce false-negative results.

Standardization of immunofluorescence may be based on a reference panel of sera which have defined characteristics when tested by other confirmatory tests.

4.3.3. Radioimmunoprecipitation (RIPA)

RIPA, although a highly sensitive and specific confirmatory assay and an elegant research tool, is not generally available even in developed countries. The technique is expensive and requires equipment and training suitable for only sophisticated laboratories. To perform this assay, virus-infected cells must be grown on-site. Standardization of RIPA may be provisionally achieved through use of a reference serum panel and a standardizing quantity of trichloro acetic acid precipitable protein and a quantity of radioactivity in the antigen preparation. When using radio-labelled whole-cell lysates, nonspecific reactions can be recognized by the use of control uninfected cell lysate.

4.3.4. Other

In some countries, confirmation of the detection of anti-HIV is carried out by the use of a second ELISA, based on a different principle. The validity of this approach is under study. In addition, assays based on antigens produced by recombinant DNA technology or synthetic polypeptides have been developed. These assays are currently being evaluated as alternative confirmatory tests in a number of centres.

5. RECOMMENDATIONS

WHO should use its network of Collaborating Centres on AIDS to assist in the evaluation of assays for infection with HIV-1, HIV-2 and any other human retroviruses which are shown to be of public health importance.

Specifically:

a) - a small number of laboratories with special expertise in evaluating diagnostic tests should be identified in each WHO region; ideally these centres should be located in areas with differing patterns of HIV-1, HIV-2 and HTLV-1 infection.

b) - the laboratories should themselves be capable of evaluating assays and monitoring their performance in blood transfusion centres and other laboratories in their own country and in other parts of the region.

c) - the laboratories should report data to WHO/GPA on the sensitivity, specificity, reproducibility and operational characteristics of the tests.

d) - GPA should regularly review data (and evaluate data from other sources) and provide summaries for circulation. These summaries should be made available to the WHO Collaborating Centres on AIDS, national reference laboratories and relevant non-government and donor organizations. Where appropriate, extracts should be published in the Weekly Epidemiological Record.

e) - the Collaborating Centres should assist in monitoring the performance of assays in the field, detecting problems where they arise and rapidly communicating this information to manufacturers. They should be prepared to assist manufacturers in field evaluation of new tests.

f) - the Collaborating Centres should assist in ensuring that tests performed in their region are of high quality by

i) assembling and distributing control sera and proficiency test panels

ii) training staff responsible for performing tests, preferably by means of regional or national workshops in which the training would take place under the same conditions as those in which the staff normally work

iii) providing prolonged periods of training for selected individuals so that national laboratories can undertake responsibility for approving products for use, monitoring their use, evaluating laboratory proficency and undertaking confirmatory testing.

ANNEX

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