

example, lack of manpower in agrarian societies may lead to reductions in agricultural production. The health systems will surely face major increases in the demand for services and may face this demand with reduced manpower and impaired infrastructures. Any of these outcomes will have major implications for food, nutrition, and health planning, and will increase the need for capital

funding while reducing the ability to repay debts. The Subcommittee therefore recommended that governments and the United Nations agencies monitor not only the development of the AIDS epidemic but also the evolution of its structural effects, so that national and international actions can be set in motion to compensate for the wide-ranging impacts on health and development.



Measurement of Antibodies to Human Immunodeficiency Virus: An International Collaborative Study to Evaluate WHO Reference Sera

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Human immunodeficiency virus (HIV), the causative agent of the acquired immunodeficiency syndrome (AIDS) (1, 2, 3), is transmitted primarily through sexual contact or the injection of contaminated blood or blood products such as anti-hemophilic factors (4). Since 1985, the screening of blood donations for anti-

HIV has been instituted in many countries in order to minimize the risk of transmission of AIDS via blood transfusions or treatment with blood products. The detection of antibodies to HIV is also of major importance as a relatively simple and rapid determination of the extent and spread of HIV infections (5), and many commercial and "in-house" immunochemical tests are now in use throughout the world. At present, the most commonly used assays are based on enzyme-linked or radioimmunosorbence, immunofluorescence, immunoblotting, or immunoprecipitation, and variations in the specificities and sensitivities of the techniques reflect inherent differences between the principles of the assays as well as batch-to-batch variations in the preparation of reagents and kits (6,

Source: *Bulletin of the World Health Organization* 66(2):197-202, 1988. © World Health Organization.

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7). Thus, there is an urgent need for well-characterized reference materials for use in defining the reliability and sensitivity of the tests, for quality control of batches of kits or reagents, and as common references between laboratories.

This report presents an assessment of two proposed reference preparations of sera, one reactive and the other unreactive to HIV, in a collaborative study involving 21 laboratories in 11 countries.

MATERIALS AND METHODS

Proposed Reference Materials

The preparations of antibody-positive and antibody-negative human sera, freeze-dried in sealed glass ampules, were supplied by Professor K. O. Habermehl (Institute for Clinical and Experimental Virology, Berlin (West)). Each preparation was derived from a single donor; one an asymptomatic carrier of HIV and the other a donor with no known risk factors. Each serum was unreactive when tested for hepatitis B surface antigen (HBsAg) by a standard immunoassay and was heated at 56 °C for one hour before freeze-drying. When reconstituted as recommended (in 0.2 ml water) these preparations had concentrations one-twentieth of the original sera; this dilution factor is not included in the calculations presented in this report.

Coded Preparations

Seven freeze-dried serum preparations, coded A to G, were supplied to each participant. Preparations A and C were duplicate samples of the reactive proposed reference material, preparation E was the nonreactive proposed reference material. Samples D and F were prepared by the Central Public Health Service Laboratory, Colindale, London.

Sample D, derived from a single donor, showed weak reactivity in immunosorbent assays. Sample F, derived from sera pooled from several donors, was highly reactive. Samples B and G, which were National Institute for Biological Standards and Control (England) reference preparations freeze-dried from pooled sera in 1973 and 1967, respectively, were both unreactive.

Design of the Study

The study was designed to identify the coded preparations that reacted with HIV antibodies and to ascertain the minimum amount of the reactive samples that could be detected in the methods routinely used by the participants.

The 21 participating laboratories (see Annex) were supplied with duplicate sets of the coded preparations and requested to assay them by the procedures usually employed in their laboratories.

Assay Methods

All but three participants assayed the preparations by indirect or competitive ELISA. Nine different commercial kits for ELISA were used: Abbott, Dupont, ENI, Genetic, Organon, Ortho, Pasteur ("ordinary" and "Rapide"), Travenol, and Wellcome. Two laboratories carried out ELISAs using their own "in-house" methods and one included in its series of assays the Abbott "confirmatory" ELISA, a competitive ELISA based on envelope and core antigens derived from recombinant DNA.

Immunoblots were carried out by 15 laboratories. All except two, whose techniques involved the use of a mouse monoclonal antibody specific for human IgG and labelled with ^{125}I , or protein A labeled with ^{125}I , used peroxidase-linked anti-human IgG for the identification of antigen-antibody complexes. Eight used

biotin-avidin amplification of the enzyme system.

One participant used the Karpas method (8) and one used an assay based on particle agglutination (PA).

Method of Analysis

For each test the reactivities of the coded samples A to G and the end-point titers for samples A, C, and F were taken to be those recorded by the participants. End-points were defined as the reciprocals of the highest dilutions of the reconstituted original materials in normal serum (not the final dilutions in the assay wells) that gave positive responses in the assays.

Potency ratios of C and F were expressed as ratios of their titers to that of A in the same assays.

RESULTS

Classification of Sera by Reactivity in ELISA and Immunoassays

Samples A, C, and F were found reactive in all tests and samples B, E, and G

were reported as negative in all but one test. The exception was a test based on particle agglutination (PA) in which sample E was judged to be weakly reactive. Sample D was found to be reactive or weakly reactive in 40 of the 48 ELISAs performed, in both the PA and Karpas test, and in two of the four fluorescence microscopy (FM) tests (Table 1). In the eight ELISAs in which sample D was identified as unreactive, it had a higher optical density (OD) than the negative control (although not, of course, as high as the OD of the cut-off limit), and in all but one the ODs were at least twice that of the negative control.

Titration of Samples A, C, and F

Participants carried out single or duplicate assays for individual manufacturers' ELISAs or by their local methods. Some laboratories used several manufacturers' ELISAs; in particular, laboratory 1 used seven different kits. For each kit, the geometric means of the titers for A, C, and F and of the potency ratios for C and F obtained by individual laboratories were calculated. The frequency distributions of

Table 1. Assessment of reactivity of sample D by immunoassays.

Assay method	No. of laboratories	No. of assays	No. with stated reactivity		
			+	±	—
ELISA kits:					
Abbott	9	12	9	2	1
Dupont	2	3	3	—	—
ENI	1	2	—	—	2
Genetic	1	2	—	2	—
Organon	3	4	1	1	2
Ortho	2	3	3	—	—
Pasteur ^a	5	8	6	1	1
Travenol	1	1	—	—	1
Wellcome	6	10	8	2	1
“In-house” ELISA	2	3	1	1	1
All ELISAs	18	48	31	9	8
Fluorescence microscopy	4	4	1	1	2
Karpas method	1	1	1	—	—
Particle agglutination	1	2	2	—	—

^aIncludes assays using the "Rapide" version.

these values are shown in Figures 1 and 2. One of the participants using the Abbott kit obtained much higher titers for A and F than did the other participants using both this and other kits. Further, its

titers for C were ten-fold and 100-fold lower than those for A. The results from this participant were, therefore, considered atypical and excluded from the subsequent analyses.

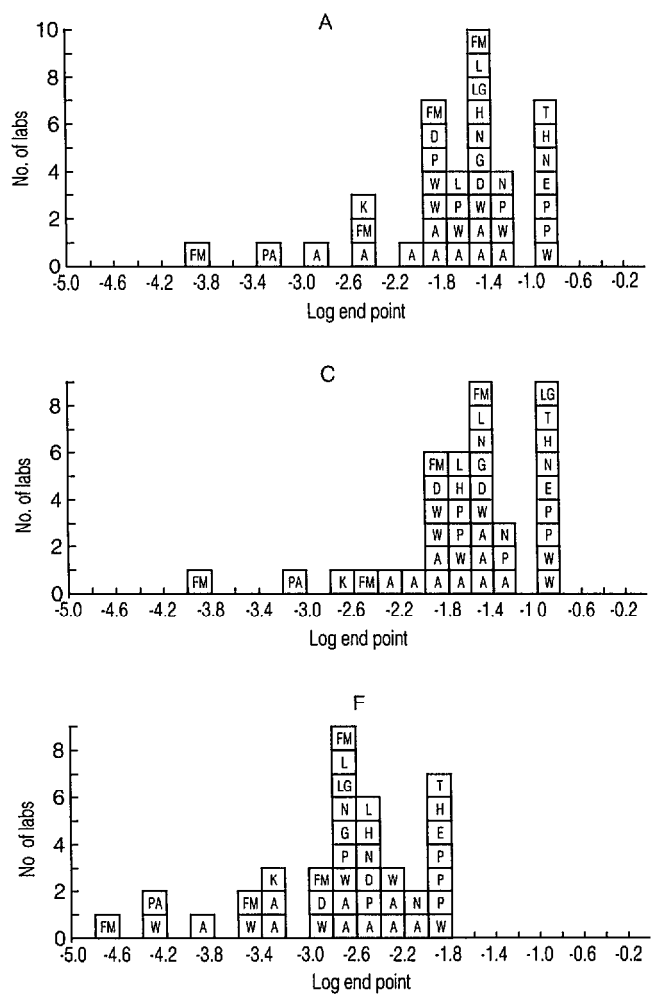


Figure 1. Frequency distributions of the end-point dilutions obtained for samples A, C, and F. Each square denotes an estimate from one test; the letters in the squares refer to the type of assay. A, D, E, G, H, N, P, T, and W denote the following commercial kits of ELISA: A, Abbott; D, Dupont; E, ENI; G, Genetic; H, Ortho; N, Organon; P, Pasteur; T, Travenol; and W, Wellcome. L and LG denote "in-house" versions of ELISA. Letters FM, K, and PA denote the following methods other than ELISA: FM, fluorescence microscopy; K, Karpas; and PA, particle agglutination test.

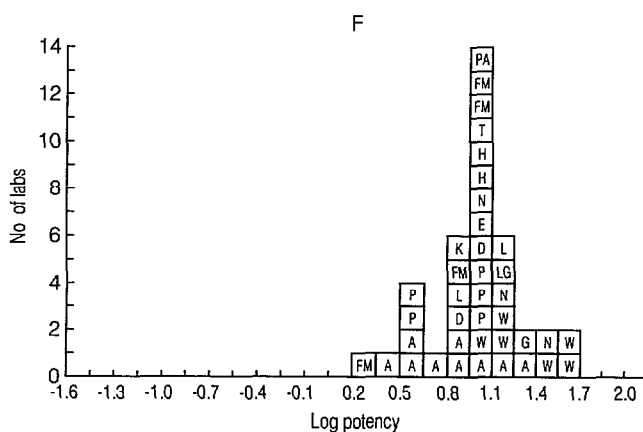
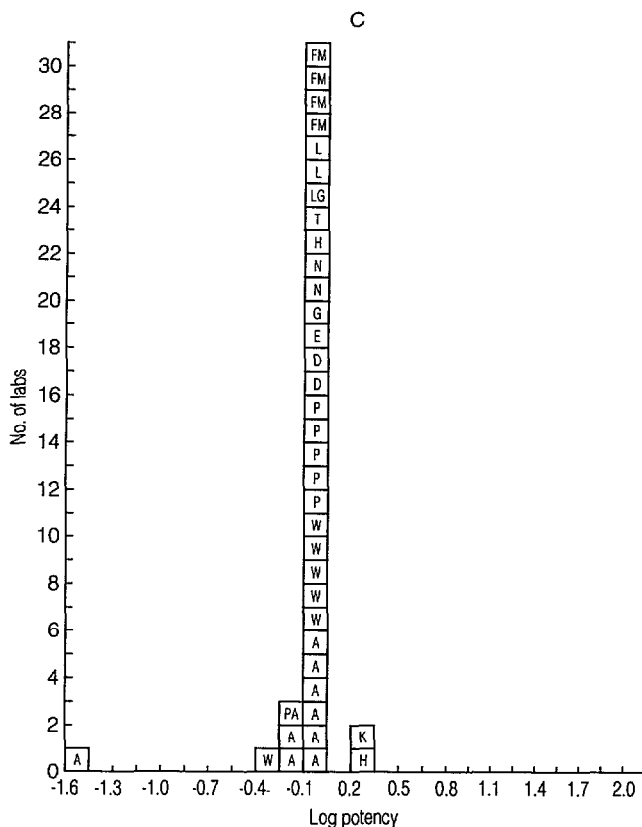


Figure 2. Frequency distributions of the potency ratios of samples C and F in terms of A obtained from individual tests. (See legend in Figure 1 for explanation of the letters in the squares.)

The laboratory mean titers varied considerably over about a 20-fold range. There were, however, no obvious differences between the titers from different kits: for instance, the ranges of titers for Abbott, Pasteur, and Wellcome overlapped with each other.

The laboratory mean potency ratios of C, a coded duplicate of A, were mostly unity. All but one laboratory (mentioned above) found the titers of A and C to be within one dilution step (Figure 1), although sometimes these dilution steps were as large as five- and tenfold. The laboratory mean potency ratios for F were more variable than those for C. However, the potency ratios were less variable than the titers (Figures 1 and 2). One laboratory's results gave a potency ratio, based on a single assay, of 256, tenfold higher than the other estimates, and had the highest titer for F (16,384). This titer and potency ratio were, therefore, considered atypical and excluded from the subsequent analysis.

Differences between the laboratories' estimations of titers were found to be significant by analyses of variance, even between those using kits from the same manufacturer. Expressing the reactivities of samples C and F relative to A showed that the differences between laboratories using the same commercial kit were no longer statistically significant. However, there were still significant differences be-

tween the potency ratios from different tests. For example, the overall mean potency ratio, i.e., the geometric mean of the laboratory mean potencies, for Wellcome was 22, about three times higher than those for Abbott and Pasteur kits (6 and 7, respectively). The overall mean potency ratios for the other kits fell within this range.

Immunoblots

Fifteen participants tested samples A to G by immunoblot techniques. The results are given in Table 2. The use of control antigens ("mock" antigens) was reported from only two laboratories. Faint reactive bands in the regions of relative molecular mass (M_r) 24×10^3 and 64×10^3 were detected consistently by one participant using "mock" antigen from H9 cells; more information is required on this important aspect of the assays.

The relative molecular masses recorded in Table 2 are those assigned by the individual participants. For convenience of presentation, bands reported within narrow ranges of M_r are not differentiated and are classified in groups (e.g., $(32-34) \times 10^3$ and $(110-160) \times 10^3$).

Samples A and C were duplicates of the proposed reactive reference serum. As was hoped, they produced identical results for each immunoblot system and are considered together. One participant

Table 2. Detection of anti-HIV by immunoblot.^a

Peptide or glycopeptide (approximate relative molecular mass $\times 10^3$)	Reactive bands/total reports		
	Sample A (C)	Sample D	Sample F
17, 18	11/14	6/14	13/14
24	15/15	13/15	15/15
32, 34	12/14	11/14	11/14
38, 39	9/13	5/14	10/13
41	15/15	6/15	15/15
53, 55	11/14	12/24	14/14
65	13/14	13/14	13/14
110, 120, 160	9/13	7/13	12/13

^aAll participants did not report the presence or absence of each peptide or glycopeptide.

reported on the detection of antibodies to peptides p24 and gp41 only. All participants, except one who reported a weak reaction in the p65 region for sample E and another who observed a reaction to p24 antigen in sample G, detected no antibodies to HIV in samples B, E, and G. Of the positive samples, F reacted most strongly in all immunoblots but, except for less frequent detection of antibodies to the envelope antigens gp110–p160 in A and C than in F, samples A, C, and F were qualitatively identical.

All participants detected antibodies to p24, gp41, and p53/55 in A, C, and F; three reported no antibodies to p17/18; and only one reported no antibodies to p65.

Additional Results

Essex and colleagues included in their study an investigation of the reactivity of samples A to G in immunoblots in which the recently isolated strain HTLV-IV was used as antigen. No reactions with any viral antigens were reported for Western blots and only two reactive regions, gp160 for sample A and p24 for sample F, were detected in radioimmunoprecipitation using ³⁵S-labelled HTLV-IV.

DISCUSSION

ELISA kits from nine manufacturers were used in this study. The sensitivities of the various kits were assessed by comparing end-point titers for the highly reactive samples A and F and by whether or not a weakly reactive serum was found "positive" in the tests. This sensitivity varied between laboratories, even between those using the same commercial kit. Expressing the reactivity of sample F relative to A reduced the variation between tests and resulted in agreement between the laboratories using the same kit. Nevertheless, there were still consist-

ent differences between the kits in the comparison of the reactivity of A and F. This possibly reflected the differences in specificities of the ELISA systems.

Overall, immunoblots revealed reactions of sera A (C) and F to all the expected HIV antigens. The presence of antibodies to p24, gp41, and p55 is considered by most workers an important indication of infection with HIV (9, 10, 11). However, for reproducible results the source of antigens, the standardization of electroblotting procedures, and the provision of "control" antigens require careful attention (9); variations of these factors may well explain the differences shown in Table 2.

The results for HTLV-IV confirm earlier findings (11, 12, 13) and emphasize the urgent need for information on the responses of immunoassays to sera from AIDS patients from different geographical areas and for characterization of the genetic and immunological differences between viral isolates. The proposed reference preparation, A, reacted strongly in all ELISAs and related immunoassays and reacted with all the major HIV antigens in immunoblots. Its use will depend on individual requirements, but it may be of value as a qualitative check on the specificity of the assays, to calibrate positive controls included in kits and other assays in arbitrary units, to calibrate detection limits (cut-off) in arbitrary units (as done for HBsAg), and to calibrate immunoblots, particularly for defining the optimal amounts of antigen and for determining the relative mobilities of the major peptides and glycopeptides from HIV. Because the unreactive preparation (E), reconstituted in 0.2 ml water as recommended, represents diluted serum, its use as a reference material will be limited.

The WHO Expert Committee on Biological Standardization reviewed the report of this collaborative study in Decem-

ber 1986 and agreed that preparations A and E would be of value as reference preparations for, respectively, positive and negative anti-HIV sera.

The preparations, under code numbers 86/6302 (reactive) and 86/6238 (unreactive) are available from the Director, National Institute for Biological Standards and Control, South Mimms, Potters Bar, Herts., EN6 3QG, England.

Acknowledgment. We thank Jane Bruce for assisting in the statistical analysis of the data from the study.

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ANNEX

Participating Laboratories

National HIV Reference Laboratory, Fairfield Hospital, Fairfield, Victoria, Australia

Red Cross Blood Transfusion Service, Adelaide, Australia

Laboratory Centre for Disease Control, Ottawa, Ontario, Canada

Laboratoire National de la Santé, Département de Contrôle des Vaccins à Virus et des Produits Dérivés du Sang, Paris, France

Institut Pasteur, Paris, France

Institute for Clinical and Experimental Virology, Free University of Berlin, Berlin (West)

Max von Pettenkofer Institute, Munich, Federal Republic of Germany

Institute for Virus Research, Kyoto University, Kyoto, Japan

Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands

Blood Transfusion Service, Department of Haematology, Singapore General Hospital, Singapore

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England
Department of Haematological Medicine,
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Regional Blood Transfusion Centre,
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School of Public Health, Boston, Mas-
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and Biologics, Food and Drug Admin-
istration, Rockville Pike, Bethesda,
Maryland, USA
AIDS Program, Center for Infectious Dis-
eases, Centers for Disease Control, At-
lanta, Georgia, USA



Animal Models for HIV Infection and AIDS

HIV is a member of the lentivirus sub-family of the retroviruses. Members of the Retroviridae family, or retroviruses, possess enveloped virions containing an RNA genome. The distinctive feature of these viruses, which gave the name to the family, is the presence in the virus particle of a virus-coded RNA-dependent DNA polymerase, or reverse transcriptase; upon infection, this enzyme transcribes the RNA genome into a DNA provirus, which then becomes integrated into the host chromosomal DNA where it may complete the replication cycle by directing the synthesis of infectious virions, or it may express none or only part of its genetic information in a covert infection. Retroviruses are widely distrib-

uted in nature and for many years have been known to infect numerous vertebrate species. Human retroviruses have only been recognized since the late 1970s, and now include the human T-lymphotropic virus types I and II (HTLV-I, HTLV-II) and the human immunodeficiency virus (HIV) (1).

The Retroviridae family is presently subdivided into three subfamilies (Oncovirinae, Spumavirinae, and Lentivirinae), according to their different biological characteristics, which also coincide with different genomic organization. The Oncovirinae subfamily (onco=Greek "tumor"), the largest one, includes viruses most commonly associated with lymphoproliferative disorders in many animal species. The Oncovirinae genome consists of the structural genes, *gag*, *pol*, and *env*. The *gag* gene (for group-specific antigen) codes for the internal proteins that constitute the "core" of the virion; the *pol* gene (for polymerase) codes for the reverse transcriptase; and the *env* gene codes for the glycoproteins found in

Source: World Health Organization. Animal models for HIV infection and AIDS: Memorandum from a WHO Meeting. *Bulletin of the World Health Organization* 66(5), 1988. © World Health Organization. The Memorandum is based on the report of an informal WHO Consultation held in Geneva, Switzerland, on 28-30 March 1988. The names of the participants are given on pages 232-233.