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FROM: Director, Center for Biologics Evaluation and Research
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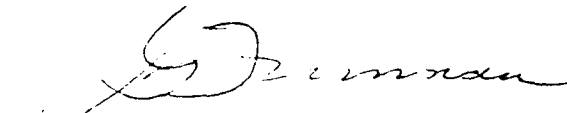
SUBJECT: Draft of "Points to Consider in the Manufacture and
Clinical Evaluation of In Vitro Tests to Detect
Antibodies to the Human Immunodeficiency Virus, Type 1
(1989)"

TO: Manufacturers of In Vitro Tests to Detect Antibodies to
the HIV-1 and Other Interested Parties

This document represents the first draft provided to industry and other interested parties for comment. In preparing this document, special emphasis was placed on addressing the most frequent questions directed to CBER and problems encountered by manufacturers. However, in common with other "Points to Consider" circulated by CBER, the HIV-1 antibody detection test kit "Points" are not intended to be all inclusive for this product type (e.g., requirements for facilities and ELAs are not discussed in detail), and certain items may not be applicable in all situations.

We invite your written review of this draft document. Please address your comments to:

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DRAFT

Points To Consider in the Manufacture and
Clinical Evaluation of In Vitro Tests
to Detect Antibodies to the
Human Immunodeficiency Virus, Type 1 (1989)

Center for Biologics Evaluation and Research
U.S. Food and Drug Administration

I. INTRODUCTION

In vitro test kits for the detection of HIV antibodies are biological products subject to licensure under the provisions of the United States Public Health Services Act (Federal Register Vol. 49, pages 18899-18900, May 3, 1984). Investigational new drug (IND) applications, product license applications (PLAs), and establishment license applications (ELAs) for these products are reviewed by the Center for Biologics Evaluation and Research (CBER).¹ As of June, 1989, there were ten U.S. FDA licensed in vitro test kits (eight ELISAs, one particle agglutination test, and one WB) for the detection of antibodies against HIV-1. The HIV ELISA tests have been used to screen blood and plasma donors, as a diagnostic aid and for epidemiology. The particle agglutination test is a rapid assay that can be used by properly trained personnel as a screening test in hospital laboratories, medical clinics, and in blood banks or other settings where ELISA tests are not practical or available. The WB for the detection of antibodies against HIV-1 is intended for use as an additional, more specific test on samples found to be repeatably reactive using a screening procedure.

These "Points to Consider" are intended to facilitate communication between CBER and individuals interested in making applications to FDA; they are not regulatory requirements. These "Points" discuss aspects of manufacture and testing which CBER has considered important in review of earlier submissions in greater detail than is specified in existing regulations. There are no specific "Additional Standards" specifying requirements for procedures or processes which manufacturers must apply in

¹ Abbreviations used in this text:

- a) Scientific terms - Acquired Immunodeficiency Syndrome (AIDS), AIDS-related complex (ARC), colony forming units (CFUs), enzyme-linked immunosorbent assay (ELISA), Human Immunodeficiency Virus (HIV), immunofluorescence assay (IFA), optical density (OD), radioimmunoprecipitation assay (RIPA), recombinant (r), signal to cut-off (S/CO), restriction enzyme (RE), standard operating procedure (SOP), Western blot (WB).
- b) Administrative terms - Center for Biologics Evaluation and Research (CBER), Code of Federal Regulations (CFR), Division of Biological Investigational New Drugs (DBIND), Division of Product Certification (DPC), Establishment License Application (ELA), Good Manufacturing Practice (GMP), Institutional Review Board (IRB), Investigational New Drug (IND), Product License Application (PLA), U.S. Food and Drug Administration (FDA).

order to be licensed for test kits to detect HIV antibodies. Therefore, approaches to manufacture and testing which are consistent with generally applicable regulations are acceptable. Data used in support of PLAs should be collected in compliance with applicable FDA regulations and policies.

Other documents may also be helpful to individuals who are preparing applications. Copies of the various biological "Points to Consider" and an up-to-date IND application package including Form FDA 1571, Form FDA 1572, and certain relevant sections of the CFR can be obtained from: DBIND, HFB-230, Bldg. 29, 8800 Rockville Pike, Bethesda, MD 20892 (301-443-4864). Manufacturers may also obtain Form FDA 3314 "PLA for the Manufacture of HIV for In Vitro Diagnostic Use" and Form FDA 3210 (ELA) from: DPC, HFB-240, Bldg. 29, 8800 Rockville Pike, Bethesda, MD 20892 (301-443-5433). Sponsors planning to export their product for research or clinical use prior to PLA approval should comply with 21 CFR 312.110 and the "Drug Export Amendments Act of 1986." Export requests are obtained from, and submitted to: International Affairs Staff, OHA, HFY-50, Room 11-47, Parklawn Bldg., 5600 Fishers Lane, Rockville, MD 20857 (301-443-4480). The "Summary Basis for Approval" for licensed biological products can be requested from: Freedom of Information Office, FDA, HFI-35, Room 12A-16, Parklawn Bldg. (301-496-9508).

II. SEQUENCE, TIMING AND CONTENT OF APPLICATIONS

A. Overview of IND Applications

When the purpose of the studies proposed in an IND application is to develop data to be used in support of licensure, CBER will review the submission and advise the sponsor regarding the potential adequacy of the manufacturing, testing, preclinical evaluation, and clinical trial design to support licensure. It is hoped that this approach will minimize the performance of unnecessary studies. Thus, IND applications should include well-organized information about manufacture, lot release testing, "preclinical" evaluation, the proposed clinical trial and a description of the intended labeling claims as generally outlined in the following items:

1. If the test kit is intended for subsequent licensure, the product used in the IND clinical trial should be manufactured and tested by the same methods intended for use with the licensed product. The product and manufacturing process should be described in sufficient detail to assure that the product used for an IND trial is the same product described in the subsequent PLA, and to identify any concerns about potential safety and efficacy. Any change in the product during the clinical trial could

be problematic because clinical data obtained with variants of the product described in the PLA might not be acceptable to support licensure of that PLA. A fundamental change in the product, e.g., a change to a different recombinant antigen construct or a different solid phase, necessitates a separate IND application.

Flow charts for manufacturing stages and an overview of manufacturing strategy for all kit components, including monoclonal antibodies, should be provided to facilitate review. See sections on MANUFACTURE, LOT RELEASE TESTING, and STABILITY.

2. "Preclinical evaluation" of an HIV antibody test kit can include testing of "unlinked" human sera and/or plasma to determine what the kit's performance is likely to be in a clinical setting. "Preclinical" data should be provided in the IND application. See section on PRECLINICAL STUDIES.
3. The term "clinical trial" used in this context refers to studies performed to evaluate the use of the test in ways that will affect the people tested. For example, tests of blood or plasma donated for transfusion or further manufacture, and the use of the test for diagnosis or establishing prognosis would be evaluated in a clinical trial. Such studies are said to be "linked" when the results of the tests performed can be related to the specific individual who donated the specimen tested, and "unlinked" when the converse is true. IND proposals should include a copy of the informed consent form(s) and evidence of Institutional Review Board approval for "linked" studies involving human subjects. See section on CLINICAL TRIALS.

Please note that the use of the term "random" refers to an unbiased sample selection so that the samples are truly representative of their category, e.g., sensitivity samples should not be selected for use in preclinical studies or the clinical trial based on previous favorable testing results with the investigational test. A precise description of how the samples are selected, especially for repository samples, should be provided in the IND application and the PLA. See section on CLINICAL TRIALS and PRECLINICAL STUDIES.

4. The IND application should include a detailed description of the intended labeling claims. The proposed clinical trial should be designed to provide adequate product performance data to support licensure with consideration of the proposed labeling claims. See section on CLINICAL TRIALS.

5. Actual experimental evidence (i.e., validation data) of the inactivation of the viral lysate and other potentially HIV infectious material (e.g., positive control sera) in the kit should be included in the initial IND application. See sections on MANUFACTURE, LOT RELEASE TESTING and BIOSAFETY.

B. Overview of PLAs and ELAs

1. The manufacturing and facilities sections of PLAs and ELAs are normally more detailed than those sections in IND applications. A completed Form FDA 3314 and Form FDA 3210 should be submitted with a PLA and ELA, respectively. Data are required to demonstrate that the kit or product can be manufactured in a consistent manner. Complete and detailed descriptions of manufacturing, including critical SOPs, quality control procedures and specifications, actual production records for one lot, and lot release testing procedures should be provided. Well planned tables, summaries and flow charts may expedite review. Ongoing stability, sterility and bioburden testing during the IND trial should be submitted in a PLA. See sections on MANUFACTURE, LOT RELEASE TESTING, BIOSAFETY, AND STABILITY.
2. PLAs are submitted after adequate clinical data are obtained and the sponsor's analysis of all of the data (which should be included in the PLA) shows that the data could support licensure. See "Presentation of Clinical and Preclinical Data" in the CLINICAL TRIALS section. Post-licensure modifications that affect test performance should be supported by clinical trials.

III. MANUFACTURE

It is essential that the manufacturing process which is used to make the lots for the licensure-supporting clinical trial be well characterized, well controlled and identical to the manufacturing process that the sponsor intends to use after PLA approval. The demonstration of lot to lot consistency of all kit components is essential for licensure. The source and acceptance criteria for all raw materials and a list of all ingredients and their final concentrations should be stated. The continued availability of components, including ones purchased from outside vendors, and continuity of production should be addressed in detail.

Information such as the source, and if applicable, HIV antibody test results by licensed tests, should be provided for all serum/plasma samples used as a component of the kit and samples used to evaluate the kit. These samples include the kit control reagents, proficiency panel components, and in-house QC panel components.

Throughout the MANUFACTURE section an attempt is made to indicate when one time testing/qualification by the sponsor is likely to be adequate.

A. Antigen Derived from Virus Produced in Cell Culture

The sponsor may find it useful to review the "General Information" section of the "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1987)." However, safety and toxicology testing discussed in the "Quality Control" section such as tumorigenicity testing and testing for oncogene expression is not applicable to in vitro test kits.

1. Virus

The virus should be managed using a virus seed lot system, and information should be provided regarding the virus strain, master seed lot and working seed lot, as outlined in the following:

- a. Virus Strain. Source, isolate name, passage history and known characteristics.
- b. Master Seed Lot - qualify on a one time basis.
 - i. Identity - e.g., SDS-PAGE, WB, RE mapping.
 - ii. Potency - e.g., titer.
 - iii. Storage conditions and locations.
- c. Working Seed Lot - qualify each time a new working seed lot is created from the master seed lot. (It is anticipated that this qualification will be performed infrequently, since a working seed "lot" can consist of multiple vials).
 - i. See Master Seed Lot, b.i., b.ii., and b.iii., above.

2. Cell substrate

- a. Cell line. Source and passage history.
- b. Master Cell Bank - qualify on a one time basis.
 - i. Source and passage history.
 - ii. Identity - RE mapping of integrated proviral DNA. Cell surface markers, if applicable.

- iii. Purity - Demonstration of freedom from adventitious agents (e.g., mycoplasma), other viruses and other cell lines.
 - iv. Storage conditions and location. Storage in sufficient quantity and at two or more locations to ensure consistency in the manufacturing process for an indefinite period of production is suggested.
 - v. Description of testing schedule, methodology, results, and acceptance criteria.
- c. Working cell bank - qualify each time a new working cell bank is created from the master cell bank. (It is anticipated that this qualification will be infrequent because a working cell "bank" can consist of multiple vials).
- i. See Master Cell bank b.ii. - b.v., above.
 - ii. The working cell bank should be organized and maintained to minimize the total number of cell culture passages involved in virus production.
- d. Production cell culture:
- i. Steps, rationale, and scale.
 - ii. Culture maintenance (e.g., media formulation).
 - iii. Quantitative antigen yield for a production run.
 - iv. Acceptance criteria with supporting data for passage number and/or cell doubling and/or time of production culture.
 - v. Testing schedule, methodology, results and acceptance criteria.
3. Purification and characterization:
- a. Steps, rationale and scale.
 - b. Qualitative/quantitative analysis, results. Original photographs of SDS-PAGE gels of the HIV antigens at various stages of purification should be provided. WB following purification.
 - c. Acceptance criteria.
4. Inactivation profiles (by validated methods).
- Experimental data with positive and negative controls should be provided to demonstrate that the antigen is not infectious after processing.

B. Antigen Produced by Recombinant DNA techniques

The sponsor may find it helpful to review the "Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology," with regard to expression vectors, the master cell bank, production and purification. The following comments provide an outline of the type of information necessary to evaluate the manufacture of rDNA antigens:

1. rDNA product cloning strategy including a summary and flow diagram of the following steps, if applicable:
 - a. Synthesis of DNA fragment encoding product.
 - b. Nucleotide sequence and RE mapping.
 - c. Construction of expression vectors (e.g., diagrams of precursor and final plasmids).
 - d. Description of the host cell including the source, history and genotype.
 - e. Transformation of host cell by recombinant constructs.
 - f. Gene copy number of the recombinant construct per cell.
 - g. Selection of cells expressing product (i.e., antibiotics or other chemicals used to exert selective pressure on an organism to prevent reversion to a different form).
2. rDNA product master cell bank - qualify on a one time basis:
 - a. History of subculture.
 - b. Storage conditions and locations.
 - c. Identity. Sequence analysis and RE mapping of recombinant construct, stability of genotype and phenotype, auxotrophic markers.
 - d. Purity. Sterility, lack of adventitious agents.
3. rDNA product working cell bank - qualify each time a new working cell bank is created from the master cell bank:
 - a. History of subculture, the exact number of passages beyond the master cell bank.
 - b. See rDNA product master cell bank 2.b. - 2.d., above.
4. rDNA product production culture (after an experience basis is developed, e.g., after 3 lots, it may be appropriate to streamline or eliminate some of the qualification testing, such as item 4.c., below):
 - a. Steps and rationale. Details of fermentation, if applicable, as follows: Operating parameters for the bioreactor including equipment type and manufacturer, production scale, pH, temperature, effectiveness of

- mixing, media component concentrations, viscosity, O₂ and CO₂ concentrations and partial pressures, cycle time.
 - b. Quantitation of antigen yield for each production run.
 - c. Genetic stability in production culture, including the rate of plasmid loss from the host cell (i.e., reversion rate), at the beginning of production and after a defined time at least equal to maximum production culture time.
 - d. Identity at beginning and end of production run.
 - e. In process quality control.
 - f. Acceptance criteria.
5. rDNA product purification and characterization - please also refer to second paragraph under LOT RELEASE TESTING:
- a. Steps, rationale and scale.
 - b. Identity, potency and qualitative/quantitative analysis of structure of product before and after processing. One approach is to perform the following testing: SDS-PAGE electrophoresis; reverse phase HPLC; amino acid analysis (correspondence of peptide to coding DNA sequence); peptide mapping; N-terminal sequence; C-terminal sequence; glycosylation; WB to evaluate recombinant polypeptide (e.g., reactivity of purified recombinant proteins compared with viral lysate using different human sera).
 - c. Purity at beginning and end of production, e.g., WB to detect contaminating cell substrates (residual host proteins should be reduced to the greatest extent possible).
 - d. Antigen yield following purification.

C. Antigen Consisting of Synthetic Peptides

A detailed scheme for the peptide synthesis including all chemical parameters should be presented. It is recommended that the synthetic product be purified from contaminating peptides. The level of contaminants should be minimized, including chemicals used during the synthetic process as well as those chemicals used during the deprotection and work-up of the final peptide. The content of modified peptides due to inefficient coupling or derivatization of amino acid side chains should be minimized. There should be determinations of purity, identity, and potency (e.g., test results or immunological activity) for each lot of synthetic antigen.

The purity of the final product should be verified using at least 2 analytical methods, such as ion exchange chromatography and capillary zone electrophoresis. The identity of the peptide may be verified by using analytical methods for the determination of molecular weight and

structure, such as mass spectral analysis, amino acid sequence and amino acid analysis. In addition, immunoassays should be performed to demonstrate equivalent immunoreactivity of each production lot of antigen with that of a reference lot.

D. Other Test Kit Components

The quantitative compositions of all test kit components should be provided.

1. Solid phase components

A description of the manufacture of any antigen anchoring component (e.g., plates, beads), the concentration of antigen(s) on that component, and the source of the conjugating reagent, if applicable, should be provided. If more than one antigen is used, specifications for the coating ratio should be provided. If the sponsor purchases the solid phase component (e.g., a microtiter plate), the source, a description of the quality assurance evaluation (e.g., use of magnifying equipment to detect defects), and the acceptance criteria should be provided.

2. Control sera

The source of the control sera and the plan for continued renewal should be included. The investigational kit should include routine use of a weak positive control (i.e., reactivity just above cut-off). Validation data should demonstrate the inactivation of all potential infectious HIV in sera. In addition, both the positive and negative control reagents should be nonreactive for hepatitis B surface antigen.

E. Sterility/Bioburden

CBER recommends manufacturing as many components as possible under sterile conditions. At a minimum, all manufacturing processes should be performed in a controlled environment and should be designed to minimize initial product microbial loads. Nonsterile liquid components should contain an appropriate preservative to minimize proliferation of microorganisms. These preservatives should be shown to be effective in accordance with the USP XXI Antimicrobial Preservatives-Effectiveness test. The sponsor should also evaluate the effectiveness of preservatives for opened components and kits.

Data accumulated by various in vitro diagnostic manufacturers have shown that elevated levels of bioburden (e.g., 10^5 CFUs/ml) of test kit components will compromise kit

performance. Elevated bioburden levels of this magnitude should not occur in a controlled manufacturing environment. If the manufacturer can assure a controlled environment (via viable/nonviable monitoring of the manufacturing environment by sampling walls, floors, air, etc.), spiking experiments may not be necessary.

The test used for sterility or bioburden for each kit component should be described in detail inclusive of acceptance criteria limits, as follows:

1. The naturally occurring bioburden should be determined for each of the non-sterile kit components. The profile generated should identify and quantify (i.e., CFUs/ml) each organism.
2. An environmental monitoring program should be established to evaluate the bioburden of all manufacturing areas. Again, the profile generated should identify and quantify each organism.
3. Experimental results from 1. and 2. above should provide sufficient information to generate appropriate alert/action limits for bioburden. These limits plus corrective action/retest procedures should be outlined in an SOP. Bioburden results should be submitted, in lieu of sterility results for nonsterile components, for HIV test kits submitted to CBER for review and release. Sterility results for those components purported to be sterile should also be submitted.

F. Facilities

The following information should be presented:

1. Detailed diagrams of each facility showing the locations of rooms and room airflow patterns (e.g., negative or positive).
2. All narrative describing activities should be referenced to the rooms in the diagram.
3. Flow of material and personnel within the facility (e.g., which personnel can enter specified rooms).
4. Location and description of containment facilities.
5. Description of segregation of production activities in a given area and facility, if applicable.

6. List of all products and other infectious agents processed at each facility and procedures to prevent cross-contamination.
7. In the event that manufacturing is performed at more than one facility, the flow diagram of manufacturing should clearly indicate where each step is performed.

Questions concerning facilities should be directed to DPC. These would include questions concerning potential problems associated with using more than one manufacturing facility, and which parties involved in a "divided" manufacturing arrangement would eventually obtain establishment licenses (e.g., one company purchases antigen from a vendor and performs the remainder of the manufacturing).

IV. LOT RELEASE TESTING

Lot release testing for each lot should include tests for identity, specific activity, purity, potency, and sterility or bioburden. For applicable components, a procedure which has been established to be effective based on actual data generated by the applicant should be used for routine lot to lot monitoring of virus inactivation (referencing the scientific literature without actual data is inadequate). In addition to designating a kit lot number and an expiration date for each complete kit, there should be a lot number and an expiration date assigned to each kit component. This allows clear cross-referencing because the same lot of a kit component could be a part of multiple complete kit lots (e.g., the same lot of microtiter plates could provide components for multiple kit lots).

Generally, the routine lot release testing is not as extensive as the initial component qualification. For example, for routine lot release testing of antigens produced by rDNA techniques, it might be adequate to select a subset of the testing suggested in III.B.5. such as SDS-PAGE, WB, reverse phase HPLC and peptide mapping.

Written criteria for passing or failing lots of all components subjected to each proposed test should be promulgated and used for actual lot control. To evaluate performance, the development of an in-house QC serum/plasma panel is essential. An appropriate comparator, such as a reference kit which is constantly rotated with fresh components within specifications should be created. A lot archive (i.e., retention samples) should be maintained through the expiration date.

V. PRECLINICAL STUDIES

The following comments are intended to clarify the minimum amount of "preclinical" testing that should be performed prior to the initiation of the full scale clinical trial. These "preclinical" studies, which can be performed either in-house by the sponsor or at field sites by independent investigators, include preliminary evaluation of specificity and sensitivity with preliminary evaluation of the cut-off value, studies to determine the relative analytical sensitivities of the clinical trial tests, and testing of the CBER lot release panel.

A. Testing of specificity and sensitivity samples for preliminary assessment of the investigational test-performance.

The original IND application should include the results from testing a minimum of 200 specificity specimens (e.g., random normal blood or plasma donor specimens), and a minimum of 50 seropositive sensitivity specimens with the investigational test and licensed comparator tests. These specificity and sensitivity data may include data collected outside the U.S. and the results of testing unlinked samples, and are useful as a preliminary guide to the choice of cut-off value, if applicable, in the clinical trial.

B. Analytical Sensitivity.

The original IND application should include the results of analytical sensitivity studies for all clinical trial tests (e.g., the investigational and licensed screening tests, the licensed WB and other additional, more specific tests). The analytical sensitivity should be evaluated by performing dilution series testing with known reactive seropositive samples (e.g., 15 samples). The highest dilution which can be detected with consistency for each assay system (dilutional endpoint) should be identified. In addition, the sensitivity of the various tests can be evaluated using seroconversion panels, if available. The investigational test should be at least as sensitive (as assayed by dilutional endpoint and/or seroconversion panels) as the comparable licensed test, and additional, more specific tests should be as sensitive as a licensed test.

C. CBER Lot Release Panel.

The CBER HIV lot release panel should be tested using the investigational test kit and the results submitted in an IND supplement prior to licensure. This panel can be obtained from the Division of Blood and Blood Products [(301) 496-2691] only after the IND application has been submitted.

VI. CLINICAL TRIALS

The definitive clinical studies used to support licensure, i.e., specificity, sensitivity, and precision as described in the CLINICAL TRIALS section, should be performed by **independent** investigators at clinical trial sites. However, the sponsor has the option of performing in-house nonspecificity testing (see section VI.C.). As discussed in section II.A.3., the sample selection should be unbiased.

The clinical trial should compare the investigational test kit to at least one licensed test kit with the resolution of discrepant results. An example of discrepant results is when a sample has a nonreactive investigational screening test result and a reactive licensed screening test result. In the event that an appropriate test (e.g., licensed WB) is not by itself sufficient to resolve discrepant results, data from well validated tests that are not licensed should be obtained. In addition to definitive serologic data (e.g., a positive WB result), definitive virologic data (e.g., culture results) or definitive clinical data can be used to resolve discrepancies.

A serum or plasma sample evaluated in the clinical trial can be either "linked" or "unlinked." A sample is "linked" if the test result can be traced to a specific person. A study with linked samples requires IRB approval and the informed consent of the donor. The sponsor should submit a copy of the IRB approval (for each site) and the informed consent form to the IND file prior to initiating a linked study. Discrepant test results should be thoroughly investigated and are more easily resolved when the discrepant sample is linked. The evaluation of discrepant results from an unlinked sample is more problematic. However, it is not a requirement that all components of a clinical trial be linked.

It is critical that the clinical trial be designed to support the intended licensure claim. For example, if a test is intended for the screening of donated blood, a specificity study should be performed in blood banks. In addition, the trial design should take into consideration all features of the test. For example, any test with a result that requires a visual interpretation such as IFA should be evaluated independently by a number of "readers" (e.g., 2 readers for each test) and results should be semiquantitative (e.g., 0 - 3+ reactive). For tests that are based on such non-numeric criteria records should be adequate to allow a statistical comparison of results from each technician.

Documentation of WB and RIPA results by high-quality photography is strongly recommended, especially when the WB and/or RIPA are used to resolve a discrepant case and when the investigational

test is intended to be an additional, more specific test. Such photographs may be requested by CBER (xerographic reproductions are not acceptable).

A minimum of 3 production lots (usually consecutive), manufactured in the same facility and by the same procedures proposed for licensure, should be used in the clinical trial. All such production lots should be used at each major trial site so that statistical center by lot interaction can be determined at the conclusion of the trial. These 3 (or more) lots should be manufactured with at least 3 different lots of critical components such as antigen and conjugates. These lots should meet release specifications.

In addition to the suggested sample numbers below, CBER may request that more samples are evaluated to resolve problems and questions that arise during review of the IND application or clinical data.

A. Specificity Studies for test kits with a proposed labeling claim for routine screening of blood and plasma donors.

Specificity testing should include analyses of a large number of fresh serum and plasma samples from random, normal U.S. donors at multiple distinct centers. Testing should be performed at the clinical trial sites. This testing should include samples from at least 5,000 individuals. It is desirable for the trial centers to include both a high prevalence area and a low prevalence area. It is recommended that the study at the high prevalence site be linked.

In addition, one or more licensed screening tests should be used for comparison to the investigational tests at all sites in the specificity component of the clinical trial. Specimens with putative false results by licensed tests provide the sponsor with the opportunity to validate claims of increased specificity.

Samples found to be repeatably reactive by either the licensed test kit or the investigational test kit should be tested by a licensed WB or functionally equivalent test. In the event of any unresolved discrepancy (e.g., a sample with a reactive investigational test result, a nonreactive licensed screening test result and an indeterminate WB), other tests such as RIPA, IFA, culture and/or nucleic acid hybridization are strongly recommended. Clinical follow-up and further testing of follow-up samples may be the only way to resolve some discrepancies.

To validate the cut-off for an investigational screening test with a numerical result, WB testing should be performed on a

subset of specimens from the specificity studies that are negative but within 15% of the cut-off (15% below the cut-off). In test designs without a cut-off value, testing of random negative specimens by a licensed WB or equivalent test should be performed.

B. Sensitivity Studies for test kits with a proposed labeling claim for routine screening of blood and plasma donors.

Approximately 250 samples representative of the various stages of HIV infection are recommended for the sensitivity component of the clinical trial. All sensitivity samples should be tested with a licensed WB or functionally equivalent test and a licensed screening test. In addition, it is desirable for the sponsor to have, as a cross reference, information such as the clinical history and the results of any virologic testing for each sample tested. This information may be requested during the review of the PLA. While the majority of the sensitivity samples may be repository specimens, it is recommended that some of the sensitivity samples be fresh. The sensitivity trial should include the following (suggested numbers in parenthesis):

1. An unbiased selection of serum or plasma from AIDS and ARC patients (150) preferably from various geographic areas in the US.
2. It is suggested that the trial include fresh samples. One example of a study that would contain unbiased fresh samples is as follows: A prospective study could evaluate a high risk group (e.g., 100 patients at a sexually transmitted disease clinic, Methadone treatment clinic, AIDS clinic, etc.). Ideally, this study should be linked to permit follow-up investigations.
3. Seroconversion panels (consisting of serial samples from an individual prior to and during the seroconversion period). An important component of each seroconversion panel is the first sample that is weakly reactive for HIV antibody. The sensitivity of the investigational screening test to detect HIV antibody in this weakly reactive sample at a minimum should be equal to that of the licensed comparator test. Seroconversion panels provide a different type of test sensitivity assessment than the dilution series studies.

C. Nonspecificity Studies

Testing of approximately 200 samples should be done to determine whether certain specimen types or other factors will produce nonspecific results, reasonably distributed among the following:

1. Samples from individuals with non-HIV viral infections (who could have high levels of immunoglobulin that might interfere with the test), such as CMV, EBV, hepatitis B, NANB hepatitis, and rubella.
2. Samples containing antibodies to other retroviruses, including HTLV-1 and HIV-2.
3. Samples from individuals with autoimmune diseases including active systemic lupus erythematosus (high titer antinuclear antibody) and rheumatoid arthritis (high titer rheumatoid factor).
4. Samples from individuals with polyclonal and monoclonal gammopathies.
5. Samples from multiparous women and recipients of multiple blood transfusions.
6. Seropositive and seronegative samples for each category: hemolyzed, icteric, lipemic, and bacterially contaminated (e.g., skin flora and laboratory contaminants).
7. Seropositive and seronegative samples collected using various anticoagulants (EDTA, heparin, citrate, etc.).
8. Seropositive and seronegative samples frozen and thawed multiple times compared to the "fresh" baseline for each sample.
9. Seropositive and seronegative heated samples (56 degrees Centigrade, 30 minutes).

D. Precision Studies

Representative production lots used at multiple trial sites should be evaluated by each participating technician with the following samples:

1. Dilution series panels. Precision testing may include multiple "blinded" evaluations of out of sequence dilution series of seropositive samples. The dilution series of each seropositive sample should include at least 2 consecutive higher and lower dilutions than the dilutional endpoint and should be done in replicate.
2. Serum panels. These serum panels should include seropositive, seronegative and borderline samples and replicates.

Precision testing should be performed on multiple days. The sponsor should report the mean OD, mean S/CO, SD, and coefficient of variation for the test results for each sample, and review the variation within lots, between lots and between centers.

E. Additional Considerations

1. Tests using Recombinant DNA or Synthetic Peptide Antigens.

Tests based on recombinant DNA antigens or synthetic peptide antigens should be evaluated to assure that the absence of some viral antigens in the kit does not result in reduced sensitivity. One approach to address this potential problem is to have independent investigators test approximately 1000 random samples which are positive by currently licensed tests. Because "viral drift" could potentially result in decreased sensitivity, it would be useful if these sensitivity samples included some geographically diverse samples, especially samples from HIV seropositive individuals who reside in the Caribbean, Africa and South America, or other foreign endemic areas that may be identified.

In addition to the suggestions in Section VI.C., nonspecificity testing should also include specimens which contain high titers of antibodies against the host organism (e.g., sera from individuals with E. coli or yeast infections).

2. Additional, more specific tests

Additional, more specific tests are intended to be used to validate the positivity of samples found to be repeatably reactive with licensed screening tests. It is advisable for the sponsor to discuss the clinical investigational plan with CBER prior to submitting an IND application for an additional, more specific test. The following comments are most applicable to an investigational WB with a whole viral lysate antigen.

Concerning specificity testing, testing the following samples from "low risk" individuals may provide adequate information to evaluate test performance:

- a. Samples from a minimum of 200 random normal blood and plasma donors, e.g., prospective studies of fresh samples from 100 consecutive blood donors at one site and 100 consecutive plasmapheresis donors at a second site.
- b. Samples from a minimum of 200 random blood and plasma donors which are repeatably reactive by licensed screening tests, e.g., 100 consecutive repeatably reactive samples from 2 centers. In particular, these samples must be truly representative of repeatably reactive samples, and not be preselected based on testing results with the licensed WB. It is also desirable that screening tests produced by several manufacturers be used in the trial, e.g., a different licensed screening kit at each center.
- c. Samples from a minimum of 100 random blood and plasma donors that are repeatably reactive by licensed screening tests and which have been previously classified by licensed WB testing (but not previously tested with the investigational test). These samples should be roughly equally distributed between licensed WB positive and indeterminate results. These could include repository samples.

Concerning sensitivity testing, a minimum of 500 sensitivity samples may provide adequate information to evaluate test performance. Regarding nonspecificity and precision testing, see sections VI.C. and VI.D.

For additional, more specific investigational tests, a licensed WB is an appropriate comparator for the clinical trial. The investigational plan should include a detailed plan for resolving discrepant results, e.g., when the investigational WB has a different result than the licensed

comparator WB. Because resolution of discrepant results between the licensed WB and the investigational test is essential (e.g., the licensed WB is indeterminate and the investigational test is negative), it is recommended that the trial be predominantly or completely linked. A well validated RIPA may also be useful for resolving discrepant results.

3. Tests with intended labeling claims that differ from currently licensed tests.

If a clinical trial is intended to support licensure with labeling claims that differ from currently licensed products, it might be appropriate to revise the above recommendations for the clinical trial. At the sponsor's request, CBER will comment about the adequacy of a specific proposed clinical trial to support specific labeling claims.

F. Presentation of the Proposed Clinical Trial

1. In addition to a narrative describing the planned study, tabular and graphic summaries are recommended to facilitate IND application review, e.g.:

<u>SITE#</u>	<u>SPECIMENS</u>	<u>LINKED/UNLINKED</u>
#1	Plasma/1000 blood donors	unlinked
#1	Sera/50 AIDS patients (prospective)	linked
#2	Sera/50 AIDS patients (repository)	unlinked
etc.		

2. A precise algorithm for evaluating each type of specimen (normal donor sample, sensitivity sample, etc.) is important to include in the study design. It should be clear exactly what additional tests will be performed and at which step they will be performed. An example is located in Appendix A.

G. Presentation of Preclinical and Clinical Data

"Preclinical" data should be included in the IND application and the PLA. All data accumulated in the clinical trial should be submitted in the PLA. The IND application and PLA data presentations should be well organized and include results obtained for the investigational kit and all comparators. Tables may contain interpretive data (e.g., positive/reactive, negative/nonreactive or indeterminate result); however, raw data such as actual OD values and band by band WB results should also be included in the application. Such line listings should be indexed for easy location. In

addition to tabular presentation of the overall results, the following are examples of data categories that should be presented in separate tables: all specificity data, specificity data by geographic site, all clinical trial sensitivity data, categorical sensitivity data (seroconversion panels, linked prospective data, repository sample data, analytical sensitivity data, etc.), and nonspecificity data. Special attention should be directed to addressing discrepant results.

It should always be clear exactly which data were obtained from "field" investigators and which data were obtained "in-house." In addition, the presentation must clearly separate data obtained with any prototypes from data obtained with the final test configuration intended for licensure.

The summary of the data should include distribution histograms from the preclinical and clinical studies for those tests with a numerical read-out. It is especially useful to analyze the histograms from high risk studies and low risk studies according to the % WB positive in each percentile of OD (or S/CO).

VII. BIOSAFETY

All items in the BIOSAFETY section should be addressed with supporting information in the IND application and the PLA, and the information should be indexed for easy reference. The safety precautions and special facilities used for the protection of manufacturing personnel working with HIV should comply with Biosafety Level 3 in the most recent edition of Biosafety in Microbiological and Biomedical Laboratories, HHS publication # 88-8395. This publication (stock # 17-40-508-3, \$3.75 per copy) may be obtained from: Superintendent of Documents, the US Government Printing Office, Washington, DC, 20402 (202-275-3318). Useful references for biosafety questions include the Division of Safety, Bldg. 31, Room 1C02, National Institutes of Health [(301)-496-1357] and the Office of Biosafety, Center for Disease Control, Atlanta, Georgia [(404)-329-3883]. Plans for serologic surveillance of employees having direct contact with HIV, safety training and emergency decontamination should be described. Demonstration of HIV inactivation in all product components is a safety issue for clinical trial personnel and should be addressed by the sponsor with specific protocols and validation data prior to the initiation of any field studies. Validation of HIV inactivation should include appropriate positive and negative controls. Co-culture experiments for detection of residual infectivity should be maintained for 28 days.

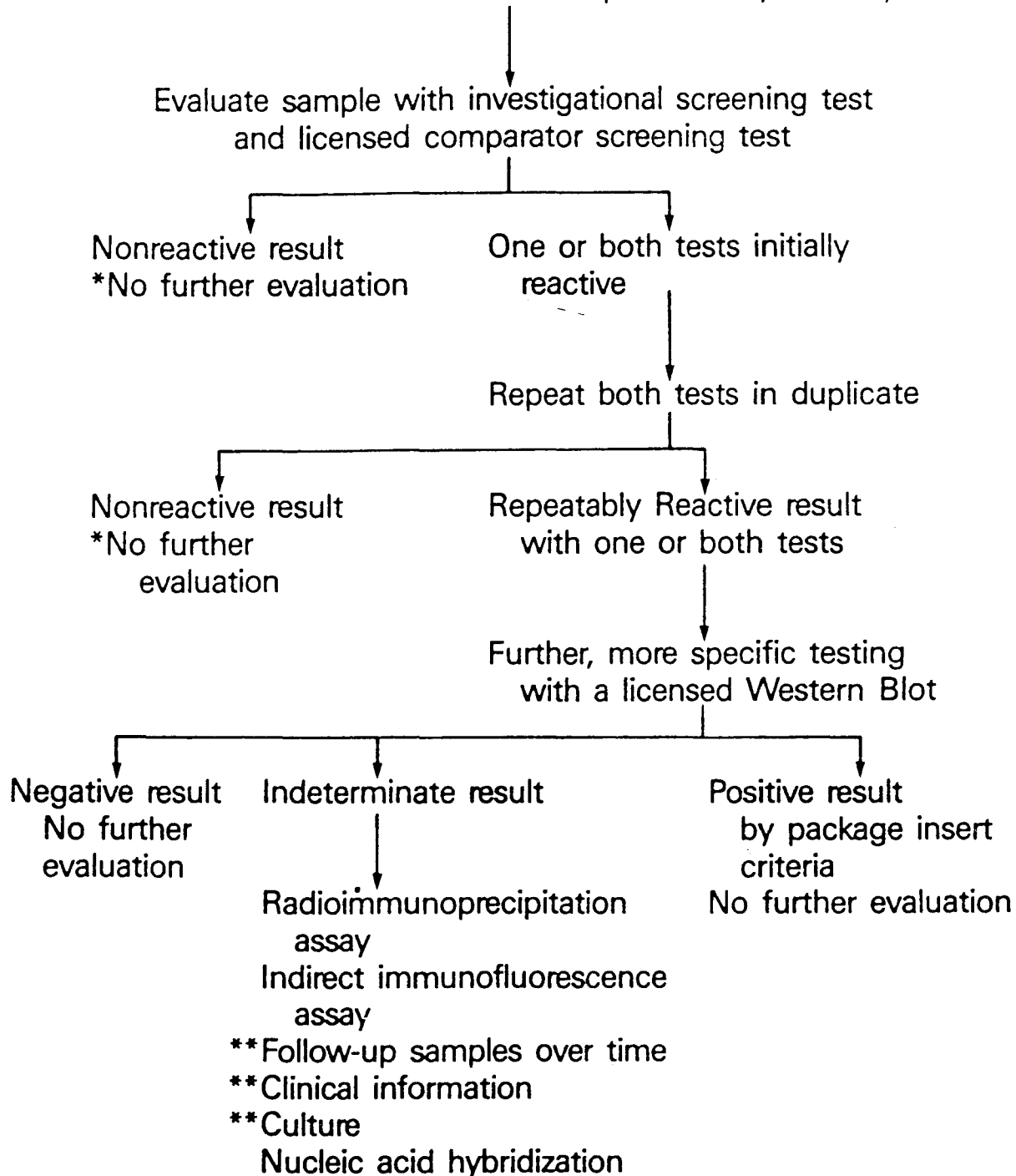
VIII. STABILITY

Stability data should be provided based on storage (including shipping) conditions which will actually be used. Generally, the dating period begins with the date of manufacture of the component. The dating period for the entire kit should be based on the shortest dated kit component. Each component should be studied independently of the entire kit by comparing performance over time with reference samples and a reference kit, i.e., a kit whose components have passed all in-house QC and performance requirements. Data should also be derived for opened components and kits.

Specificity

Example of Specificity Study Algorithm for Screening Tests

Random Blood or Plasma Donor Sample (Serum, Plasma, etc.)



*The choice of "cut-off" value for the investigational screening test assay should be validated during the clinical trial. This validation can include licensed WB evaluation of nonreactive samples having optical density values within 15% of the lowest reactive value (i.e., "grey zone" samples), if applicable, and WB of randomly selected nonreactive samples.

**Linked samples.