
GUIDE TO INSPECTIONS OF INFECTIOUS DISEASE MARKER TESTING FACILITIES

10/96

TABLE OF CONTENTS

[Introduction Pg 1](#)

[Background Pg 1](#)

[List of Memoranda Pg 2](#)

[Operations Pg 2](#)

[Facilities Pg 4](#)

[Equipment Pg 4](#)

[Personnel Pg 5](#)

[Scientific Principles Pg 5](#)

[Viral Marker Test Methods Pg 5](#)

[Infectious Disease Testing Pg 6](#)

[RIA Procedures Pg 8](#)

[Syphilis Testing Pg 9](#)

[Records Pg 9](#)

[Disposition Pg 10](#)

[Biosafety Pg 10](#)

[Disposal of Infectious Waste Pg 10](#)

INTRODUCTION

This guide provides the current interpretation of certain regulations and guidelines. It was prepared by the FDA's Office of Regulatory Affairs (ORA) and Center for Biologics Evaluation and Research (CBER). The guide is an inspectional reference intended to be used in conjunction with the Investigations Operations Manual (IOM); the Code of Federal Regulations, Title 21 (21 CFR), Parts 600 and 211; the Compliance Programs for the Inspection of Blood Banks and Source Plasma Establishments (CP 7342.001 and .002); the Compliance Policy Guides for biologics (CPGs 7134) and inspections (CPGs 7150s); and the Guides to Inspections of Blood Banks and Source Plasma Establishments.

In several instances, the manual refers to memoranda published by CBER and sent to registered establishments. These memoranda are available at the FDA District Offices in hard copy and on CD

ROM, "The Gold Disk." Investigators may also request a copy from ORA, Division of Emergency and Investigational Operations (DEIO), (301) 443-3340.

Questions should be addressed to CBER, OC, the Division of Inspections and Surveillance (HFM-650) at (301) 594-1191.

BACKGROUND

Tests described in the Circular of Information or on labeling of blood components or products are subject to Current Good Manufacturing Practices (cGMPs) regulations. This includes tests that are required by the regulations (i.e., HBsAg, anti-HIV, and syphilis), recommended by FDA (e.g., HIV-Ag, anti-HCV, anti-HTLV-I and anti-HBc), commonly performed by industry (e.g., ALT), or performed by physician request (e.g., anti-CMV).

All tests performed to determine donor or product suitability, including tests for donor reentry, must be performed with an FDA licensed or pre-market cleared (i.e. 510(k)) test kit. There are no licensed confirmatory or supplemental tests for anti-HTLV-I and anti-HBc at this time. Refer to the compliance programs for information on the specific identity of licensed test kits for viral marker testing. The kits for syphilis testing are not licensed by FDA but are cleared by the 510(K) reviewer process; however, product inserts must indicate they are intended for testing blood products.

Licensed establishments manufacturing Whole Blood and components may, with CBER approval, have infectious disease marker testing performed at licensed testing facilities other than their own. Source Plasma establishments may utilize an unlicensed, CLIA approved laboratory with CBER approval. Unlicensed blood establishments may use licensed or unlicensed testing facilities without CBER approval. Any licensed blood establishment using facilities not approved by CBER should be reported to the Division of Inspections and Surveillance at 301-594-1191. This approval from CBER only applies to what is referred to as the test of record which are the screening tests. CBER approval is not needed for laboratories performing confirmatory or supplemental tests in another facility.

Currently, clinical laboratories that are approved for Medicare reimbursement and that perform hepatitis and anti-HIV testing on donor blood for unlicensed, registered facilities are exempt from theregistration requirements of 21 CFR 607.65(g). However, such laboratories should be encouraged to voluntarily register, and should be sent Form FDA-2830, Blood Establishment Registration and Product Listing, in accordance with the procedures described in Field Management Directive 92 (available in each district through ORA/ORM/DMO).

LIST OF MEMORANDA

The following is a list of specific recommendations for testing for infectious disease markers issued as memoranda to registered blood establishments. Copies of these memoranda have been sent to district offices and may be obtained as noted previously:

HEPATITIS

"Recommendations for Labeling and Use of Units of Whole Blood, Blood Components, Source Plasma, Recovered Plasma or Source Leukocytes Obtained from Donors with Elevated Levels of Alanine Aminotransferase (ALT)," August 8, 1995.

"Revised Recommendations for Testing Whole Blood, Blood Components, Source Plasma and Source Leukocytes for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV)," August 5, 1993.

"Clarification of the Use of Unlicensed Anti-HCV Supplemental Test Results in Regard to Donor

Notification," August 19, 1995.

"Revised Recommendations for Testing Whole Blood, Blood Components, Source Plasma and Source Leukocytes for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV)," April 23, 1992.

"Disposition of Blood Products Intended for Autologous Use That Test Repeatedly Reactive for Anti-HCV," September 11, 1991.

"FDA Recommendations Concerning Testing for Antibody to Hepatitis B Core Antigen (anti-HBc)," September 10, 1991.

Recommendations for the Management of Donors and Units that are Initially Reactive for Hepatitis B Surface Antigen (HBsAg)," December 2, 1987.

HIV

"Additional Recommendations for Donor Screening With a Licensed Test for HIV-Antigen," March 14, 1996.

"Recommendations for Donor Screening with a Licensed Test for HIV-1 Antigen," August 8, 1995.

ANTI-HIV

"Revised Recommendations for the Prevention of Human Immunodeficiency Virus (HIV) Transmission by Blood and Blood Products," April 23, 1992.

"Use of Fluorognost HIV-1 Immunofluorescent Assay (IFA)," April 23, 1992.

ANTI-HTLV-I

"HTLV-I Antibody Testing," November 29, 1988.

SYPHILIS

"Clarification of FDA Recommendations for Donor Deferral and Product Distribution Based on the Results of Syphilis Testing," December 12, 1991.

OPERATIONS

The investigator should determine which tests the establishment performs. Determine how samples are received (i.e., in-house vs. shipped from contracting blood establishment) and tracked through the testing process, and how the test results are transmitted to the blood product manufacturer. Some laboratories may also perform infectious disease marker testing on specimens that are not from blood donors (e.g., patient or insurance specimens). Evaluate how the blood establishment ensures that testing of blood donor specimens meets donor screening testing specifications (e.g., use of licensed test kits).

Blood establishments and contract testing laboratories should have a written agreement or SOP with specifications including, but not limited to: -the use of licensed or unlicensed test kits;

-sample handling;

- transmission of test results and/or,
- interpretation of test results in accordance with FDA requirements and recommendations;
- repeat testing; and,
- the use of symbols, phrases, or abbreviations used in reporting results.

The agreement should also include a statement which allows for inspection by FDA.

Standard Operating Procedures

All laboratories must have standard operating procedures (SOP) on the premises readily available to personnel in each critical area. The SOP manual, including copies maintained in work areas, should be consistent, current, reflect actual practices and test methods, and should include procedures for all functions the laboratory performs. SOPs should be in compliance with cGMPs.

SOPs should be reviewed to assure that test procedures are performed according to the test kit manufacturers current instructions (product inserts), and instrument performance conditions are monitored. Observe testing procedures, and review records to determine that samples and controls are diluted properly. Time and temperature of incubation must be within the range specified by the manufacturer's instructions. The time a test is incubated is important, but slight variances have been taken into account by the manufacturers. Thus, only variation beyond the intraassay variation would be considered potentially critical. Establishments should monitor the testing procedure to determine that the manufacturer's instructions are being followed. This shifts the focus of error detection from post analytical record review to a real time evaluation of employee competency and the effectiveness of the establishment's training program.

Verify that instrument and equipment settings (e.g., the mode of the spectrophotometer, amount delivered by the automatic dilutors, etc.) are correct; calculations are accurate (e.g., cutoff); initially reactive results are repeated in duplicate; and results are interpreted correctly.

All laboratory personnel should be familiar with current laboratory procedures and manufacturers' instructions for their assigned areas. There should be established procedures describing how new test kit inserts are distributed in a timely manner to all affected laboratory personnel. Manufacturers' instructions and revised SOPs that reflect changes should be easily accessible and available to all personnel. New test kit inserts should be compared to current records, SOPs, and equipment to determine if any changes are necessary.

Furthermore, there should be a procedure describing how personnel may provide information regarding problems with laboratory systems to management and to the Quality Assurance unit of the blood establishment.

Proficiency Testing

Most laboratories will be participating in a proficiency testing program. Programs may be developed in-house or purchased from organizations such as the College of American Pathologists (CAP), American Association of Blood Banks (AABB), or the Centers for Disease Control and Prevention (CDCP). Proficiency testing records should be reviewed to determine that the facility performs appropriate corrective actions if test results are unacceptable.

The final rule proposed by HCFA, which regulates laboratories receiving Medicare and Medicaid reimbursement, was published in the February 28, 1992, Federal Register. This final rule requires laboratories to have policies and procedures for an ongoing program to assure that employees are

competent and maintain competency to perform their assigned duties.

Proficiency samples should be tested by all personnel who routinely perform the tests using the laboratory's usual methods and equipment, and under routine conditions and workload. If testing is not performed the regional HCFA contact should be notified.

Errors and Accidents

Licensed establishments are required to submit reports to the Director, Office of Compliance, CBER, of all errors and accidents in manufacturing which may affect the safety, purity, or potency of any product. Unlicensed, registered blood establishments are requested to voluntarily report errors and accidents in the March 20, 1991, memorandum, "Responsibilities of Blood Establishments Related to Errors and Accidents in the Manufacture of Blood and Blood Components." Reports should be submitted promptly after errors and accidents are discovered. "Promptly" will be defined in the regulations based on a recommendation in an Inspector General's report. Contact Division of Inspections and Surveillance to discuss appropriate time frames for reporting.

Errors or accidents that may affect the safety, purity, or potency of a product include, but are not limited to:

- use of an incorrect test (e.g., use of an unlicensed Western blot test for donor reentry);
- incorrect interpretation of test results;
- testing errors related to improper use of equipment;
- failure to follow the test kit manufacturer's instructions;
- use of outdated test kits, improper sample or reagent handling; and,
- miscalculation of cutoff values, etc.

Blood establishments that perform their own testing should report errors and accidents to CBER. Contract testing laboratories should notify client blood establishments of errors in testing promptly, so that the blood establishments may take appropriate action on distributed products promptly. The client blood establishment will need to determine if a recall and/or error/accident report to CBER is necessary.

Refer to the March 20, 1991, memorandum, "Responsibilities of Blood Establishments Related to Errors and Accidents in the Manufacture of Blood and Blood Components," for additional guidance.

Impact of Testing Errors

When screening donated blood for blood-borne pathogens, three categories of errors can occur; pre-analytical, analytical and post-analytical. Typical examples of pre-analytical error are events such as: incorrect sample identification, incorrect sample type (serum vs plasma), and computer errors. Some typical examples of analytical errors are: incorrect sample volumes, incorrect incubation timing, or incorrect incubation temperatures. Finally, post-analytical errors may consist of: data mixup, failure to invalidate a run with controls not meeting acceptance criteria, retesting of donors beyond package insert recommendations, or failure to defer repeatedly reactive donors.

Errors must be evaluated on a case by case basis because the blood-borne pathogen diseases:

- 1) differ substantially in their nature and

2) test kit procedures differ accordingly.

In addition, test kits for the same markers, but from different manufacturers may have very different procedures.

Evaluation of the impact of an error typically includes:

- 1) frequency of the error,
- 2) frequency of the disease in the donor population, and
- 3) the quantitative degree of the analytical error.

For example, in the case of an anti-HIV testing mix-up involving 20,000 released units, it would be possible that 1 of the 20,000 units might be truly positive for anti-HIV. This error would be viewed as significant relative to transfusion. However, if there was a timing error, for example, -7% (i.e. 26 minutes vs. a package insert minimum of 28 minutes) for an incubation procedure, the likelihood of missing a true positive would be very low as this degree of error would be within the normal intraassay variation expected when using such tests.

FACILITIES

All rooms and work areas where manufacturing processes, controls, and storage are performed must meet cGMP requirements, including orderliness, cleanliness, good lighting and ventilation. Hand-washing facilities should be conveniently located, with soap, towels, and hot water available.

Ideally, laboratory procedures should be conducted in a room separated from other manufacturing procedures; however, it is acceptable if a specific area is designated for infectious agent testing and is clearly labeled as such. Areas used for radioimmunoassay (RIA) procedures should be physically separated from other areas such as component preparation.

Work areas, such as counter tops, should be constructed of nonporous materials and designed to permit thorough cleaning and disinfection.

Testing laboratories should observe recommendations as published in the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) publication entitled Biosafety in Microbiological and Biomedical Laboratories. The booklet is available through the Department of Health and Human Services (DHHS), publication no. (CDC) 88-8395, 94-95, 2nd Edition, Washington, D.C.: U.S. Government Printing Office, 1988.

EQUIPMENT

The firm should have written procedures regarding equipment calibration, repair, maintenance, monitoring and control.

Equipment qualification is a critical element in establishing the validity of tests performed in a testing laboratory. Equipment should be qualified upon installation to certify that it can perform according to the established specifications. Qualification includes equipment examination, calibration, adjustment, and repair. Certain instruments may not be interchangeable with other manufacturers' equipment or reagents, e.g. Ortho reagents generally cannot be used with Abbott test equipment. Manufacturers' instructions should be reviewed to determine that incompatible equipment, supplies, or reagents are not being used.

Each piece of equipment should be qualified for each of the different functions it performs. It is not

acceptable to test representative pieces of equipment and apply the results to other, similar pieces of equipment.

Firms should requalify equipment after repairing critical equipment features, or changing testing processes, to ensure that changes will not produce inaccurate test results.

Monitoring performance of each instrument should include a trend analysis when appropriate, e.g., spectrophotometric control readings running consistently "low" or "high", but still within acceptable performance limits. A laboratory should not rely solely on the manufacturer's statements regarding the performance and characteristics of a piece of equipment.

Spectrophotometers used during viral marker testing should be checked periodically for linearity, drift, and repeatability according to manufacturers' instructions.

Refer to the December 20, 1994, memorandum, "Recommendations to Users of Medical Devices That Test for Infectious Disease Markers by Enzyme Immunoassay (EIA) Test Systems." This memorandum provides recommendations to assist users of EIA test systems in complying with FDA regulations and recommendations. It addresses installation instructions, validation procedures, calibration, verification and recalibration, precision and accuracy determinations, performance data, problem detection and correction, troubleshooting, and preventive maintenance.

During the course of an inspection the investigator may observe or review instances where equipment or supplies are either being misused or not functioning as designed. Misuse of key equipment or supplies, or lack of adherence to SOPs and manufacturers' instructions, may compromise data validity or operator safety. It is important, therefore, for the firm's overall use of equipment and supplies to be in accordance with the manufacturers' instructions as well as properly maintained and operated.

Testing equipment, supplies, and reagents should be maintained in a clean and orderly fashion. This will help to prevent cross-contamination of specimens and reagents or supply mix-up.

If automated testing equipment interfaces with a computerized system, determine that the equipment is designed and intended for such use e.g. the interfacing software between the equipment and the computer has been validated. Documentation should be available that indicates the equipment was qualified prior to interfacing and that the test system was validated. If data are transferred via interfaces or modems, review records to determine that errors in the data have not occurred as a result of the transfer.

If the computer software is vendor-supplied and errors in testing result from a design defect, the investigator should report the error to the home district office where the software vendor is located for device inspection follow-up.

Calibration, repair, cleaning, and preventive maintenance records should be reviewed to determine if the firm is following the manufacturers' instructions. Refer to the compliance programs for specific instructions regarding documentation of deficiencies relating to the misuse of equipment.

PERSONNEL

Personnel should receive training from qualified individuals in the following areas, as applicable:

-cGMPs;

-SOPs;

- performance of the procedure;
- QA/QC; and,
- computerized systems and equipment.

Training should be documented, identifying the type of training received, results of the training, and personnel receiving and conducting the training.

Personnel performance should be monitored to determine if further training or retraining in a particular area is necessary.

SCIENTIFIC PRINCIPLES

The performance of immunoassays is generally defined in terms of sensitivity, specificity and reproducibility. The analytic sensitivity of a test can be defined as its ability to detect small changes in concentration of analyte or the ability to detect a minimal amount of a given analyte. In the case of antibodies, sensitivity may be the epidemiologic sensitivity (proportion of positive test results obtained when a population of true positives is tested).

The specificity of a test can be defined as the frequency of negative results obtained when the test is used on a population in which the factor is truly absent. The most common source of false positives is nonspecific binding due to contaminants and/or cross-reactions. This is particularly frequent in indirect sandwich immunoassays for antibody detection where certain nonspecific IgG molecules either react with antigen preparation contaminants on the solid phase or cross-react with the specific antigens. The final test signal will be positive because tagged anti-IgG recognizes all kinds of IgG molecules.

VIRAL MARKER TEST METHODS

-E.G. HIV, HBsAg, HCV, HBc, HTLV-1

Radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA or EIA) are immunologic diagnostic techniques based on indirectly detecting antigen or antibody by use of a radio-isotope or enzyme. Most of the techniques employ reagents that use either antigen or antibody, which is provided in either a solid or liquid phase, and employs a variety of reaction systems ranging from plastic tubes or plates to microscopic particles. They also use a separation system to measure the amount of antigen-antibody interaction.

The results are compared to known positive and negative controls which are used to calculate a cut-off value. Samples with a value at or below the cut-off are non-reactive whereas samples above the cut-off are reactive (exception: anti-HBc; in this case below the cut-off is reactive).

One of the difficulties inherent in screening blood donors for blood-borne pathogens is their low prevalence of infection in this population. This low prevalence, when combined with a very sensitive and specific test, results in many "false-positive" tests and decreases the positive predictive value of the screening assay. In blood donors only 10-25% of repeatedly reactive screening assays for antibodies to anti-HIV are found to be truly positive. This means that 75-90% of the donors reactive by screening tests have a false-positive test.

Tests for HIV-1 p24 antigen ELISA test system use a murine monoclonal antibody to HIV-1 p24 antigen coated onto microwells or beads. A specimen of serum or plasma and lyse buffer are added to the coated microwell or beads and incubated. Lyse buffer disrupts virus particles in the specimen and HIV-1 p24 antigen, if present, binds to the monoclonal antibody on the microwell or beads.

Depending on the particular assay, washes and additional reagents are performed/added until an enzyme reagent is bound, signalling the presence of HIV-1 p24 in the plasma or serum.

Supplemental tests, such as the Western Blot, strip immunoassay (SIA) or immunofluorescence (IFA) are used to clarify ELISA or EIA screening test repeatedly reactive results. Western Blots and SIA tests employ cellulose strips impregnated or electrophoresed with viral antigens so that the individual antibodies to specific viral antigens may be visualized. Supplemental tests are an integral part of the re-entry protocol and must be negative for the donor to be an eligible donor for transfusion purposes. Western blot and SIA results fade with time and old strips may not be representative of the actual test. Not all facilities will keep the strips as it is not required since strips are the test medium and results may be transferred to a written record. It is important to determine if the manufacturer's instructions for interpretation of the tests has been followed. Observe reading and recording of results, and review SOP and test kit insert for interpretation of results.

The preparation of antigenic material for use in the Western Blot assay normally involves the use of heat, reducing agents, and detergents, such as sodium dodecyl sulfonate (SDS) to break down the bonds between the proteins. This allows separation of molecular subunits by weight which are related to different viral gene products. A tracking dye is added to permit visual monitoring of the electrophoretic process. If antibody directed against any of the viral proteins is bound to the antigens on a nitrocellulose strip, colored bands will develop.

INFECTIOUS DISEASE TESTING

Test kits may contain microwell plates, beads, controls, reagents, and wash solutions. Kits are released by the manufacturer as a unit referred to as a master lot, and switching reagents between master lots may cause results to be invalid, unless the manufacturer's instructions state that specific components are interchangeable. Kits should be used within the expiration date. Lot numbers and expiration dates may be recorded on the worksheet. If not, a record must be kept to relate the testing to the lot numbers of test kits used. There should be a system in place for tracing individual reagents back to the master lot if the components are removed from the kit. Use of reagents from different master lots may yield invalid test results.

The tests must be performed according to the manufacturer's instructions. Test conditions such as temperature should be evaluated and determined to be acceptable in accordance with the manufacturer's instructions.

Review of control results should precede acceptance of any results of donor samples. Test data must be reviewed by personnel (ideally someone other than the person performing the testing but this is not mandatory) to assure test runs are valid, results are calculated accurately, and any run failures are resolved. Invalidation of results or test runs must be documented, including the reason for invalidation. See CBER memorandum, "Recommendations for the Invalidation of Test Results When Using Licensed Viral Marker Assays to Screen Donors," dated January 3, 1994. This memorandum includes recommendations regarding the use of external controls in addition to internal controls supplied by the test kit manufacturer.

Repeated testing beyond that required by manufacturers' instructions to obtain negative test interpretations i.e. "testing into compliance", is not an acceptable practice. Interpretation of test results should be in accordance with FDA requirements, the manufacturers insert and recommendations as stated in CBER memoranda to blood establishments.

Results of all testing must be available in written form (i.e. hard copy) or electronically via a computer system before products are released for transfusion or further manufacturing. Facsimile results may also be used.

There should be a system for limiting access to data regarding infectious disease marker testing status to authorized personnel only. A system must be in place to prevent unauthorized changes to test results. In addition, donor confidentiality should be maintained.

Samples

Blood samples for infectious disease testing are collected from the donor at the time of donation. The sample may be drawn directly from the donor, or a segment of tubing from the collection set filled with blood or plasma representative of the contents of the collection may be used. The samples must be identified to accurately relate them to the donor. Accuracy and traceability of sample identification must be maintained throughout the testing process.

Plasma samples may, under certain conditions, become inadvertently diluted with saline in the process of collecting Source Plasma using approved automated plasmapheresis equipment. Plasma sample dilution may be caused by human error in the collection process. There is also the potential for mechanical failures due to changes in tubing specifications or to improper seating of software. The result of diluted samples is the remote possibility of false-negative viral marker test results. Firms should have adequate training programs which:

- 1) highlight the need to follow the manufacturer's instructions when disconnecting the plasmapheresis devices;
- 2) document problems with automated devices; and,
- 3) establish a process to follow-up internally when errors occur.

The testing laboratory should notify the collecting establishment if diluted samples are received.

Some firms have automated sampling equipment and positive identification systems which identify samples by reading a bar code on the test tube. The investigator should determine how the firm identifies bar code reader errors and what procedures are in place to establish correct sample identity and test results when bar code reader errors occur.

Sample requirements vary depending on the type of licensed viral marker test kit. Before testing, samples should be inspected to assess the proper type of sample (serum or plasma), volume, hemolysis, particulate matter, lipemia, or icterus. Hemolysis will cause the serum or plasma to be pink or red rather than straw colored. Lipemic samples will have a milky or cloudy appearance. Icteric samples will be yellow in color. Plasma or serum from more than one donor may not be pooled for viral marker testing or for syphilis testing. SOPs should include directions on how to handle questionable or unsuitable samples.

The investigator should observe if samples are being stored in the refrigerator at the end of a shift or day. It has been observed that some firms keep samples in boxes or cabinet drawers until samples are sent to the laboratory for testing. Some test kit manufacturers require that samples be stored between 2-8°C with specific time/temperature limits. The stability of some viral markers, e.g. HIV-1 p24 antigen, can only be accomplished by strictly following the test kit package insert instruction. Thus storage of samples should follow the manufacturer's insert instructions.

Sample transport for the transit time to the testing laboratory should also follow the test kit instructions. Some plasma centers send samples to central testing laboratories once or twice per week. If the length of time between sample collection and actual testing will be extended, validated shipping and storage SOPs should be in place ensure sample integrity.

There should be established procedures for sample handling. These procedures should describe ways

to detect missing samples and describe methods for investigation if unsuitable products could be released.

Procedures must also define what will be done if samples are not available for repeat testing i.e. not using the unit of for transfusion and placing the donor on a temporary deferral pending further testing.

Other instances of delayed testing may be due hemolyzed samples which would may cause supplemental tests to be invalid.

Reagents

Reagents should be checked against receipt records to ensure that the correct reagents have been received. If reagents are to be stored at specific temperatures, they should be shipped in a manner to ensure that they have not been exposed to temperatures out of the storage range for extended periods.

There should be SOPs in place describing the release of reagents. In large laboratories that receive several kit lots at one time, a system must be in place to prevent the use of reagents from different lot numbers in a single test kit system.

Manufacturers' instructions list critical procedures in handling and using reagents. The investigator should read the manufacturer's insert for instructions regarding acceptance/rejection criteria, e.g., for signs of instability, deterioration, or microbial contamination. All reagents should be within date, properly reconstituted with the proper diluent, e.g., deionized water or purified water, and used within the time specified on the insert. Reagents from one master lot should not be used with other lots of test kits, or test kits with different manufacturers. Do not mix reagents unless it is allowed by the manufacturers instructions unless the package insert specifically states that such interchanges are acceptable. In some instances sulfuric acid and OPD may be used.

Reagents should be stored according to the manufacturer's directions. Most commercially manufactured reagents contain additives that may be affected by freezing. Most manufacturers' instructions specify that the storage requirement for reagents is between 2-8⁰C; however, many licensed viral marker testing kits require that reagents be allowed to reach room temperature before they are used and returned to storage after use. The investigator should observe storage conditions as some reagents cannot be exposed to direct light or stored with a desiccant (e.g., open, unused microplates).

Quality Control of each lot of incoming reagents should be performed as described in the firm's SOP which should follow the manufacturer's insert. Each shipment (even if the same lot number has been sent and previously tested) of test kits should be checked with a positive and negative control before use with donor or patient samples to ensure that the reagents perform as expected. The investigator should observe, if possible, if all required run elements, such as the correct number of positive and negative controls, are used for reagent QC. The QC records should be reviewed during an inspection.

Testing

The laboratory must have a system in place which assures that the tests have been performed as specified by the manufacturer. Timers must also be checked for accuracy. The investigator should look for, and investigate, unusual test results (invalidated results, etc.) and determine that the firm is monitoring and evaluating these results. Documentation should be available of the monitoring, with explanation of unusual results and corrective action taken, if needed.

The investigator should:

- 1) make or obtain a list of units with initially reactive test results from raw data;
- 2) verify these are repeated for the correct test and correct number of repeat tests; and,
- 3) verify that units which were confirmed positive for hepatitis, or were positive by a Western Blot or other additional more specific test have been accounted for, and in most cases, destroyed.

Interpretation of Results

Initially reactive: Initial EIA test is reactive

Repeatedly reactive: One or both duplicate EIA retests is/are reactive

Negative: Initial EIA test is non-reactive, or initial EIA test is reactive, and both retests are nonreactive

Positive: Repeatedly reactive EIA test, supplemental test positive, e.g. Western Blot

Supplemental test indeterminate, e.g. Western Blot indeterminate: Western Blot not positive or negative.

Critical Elements of EIA Testing

Critical elements of EIA testing include:

washing

pipetting

incubation

sealing of reaction chamber (e.g., microplate wells)

calibration of equipment and pipettes

reagent reconstitution

correct dilution of samples and reagents

integrity of sample and reagents

reagent temperature

False Positive Reactions

Some causes of false positive reactions include:

high amounts of free hemoglobin

suspended fibrin particles

poor sample quality

cross-contamination

technical error

cross-react with other, non-related IgG antibody

False Negative Reactions

Some causes of false negative reactions include:

no sample/diluted samples

improper dilution

excess washing

incorrect reagent preparation

improper addition of reagents

RADIOIMMUNOASSAY (RIA) PROCEDURES

Special considerations for facilities using RIA procedures include:

proper labeling of storage and testing areas with radiation hazard signs

badges to monitor personnel radiation exposure

records of isotope receipt, use, and disposal according to Nuclear Regulatory Commission (NRC) guidelines

SOPs to include RIA specific procedures regarding training, spill cleanup, decontamination, and radiation exposure monitoring

SYPHILIS TESTING

Post-transfusion syphilis is an extremely rare occurrence as the organism that causes syphilis does not survive longer than 96 hours of storage at 4 degrees C. Blood components stored at room temperature may be capable of transmitting viable T. pallidum even though the blood components have a negative serologic test for syphilis. Potentially infectious blood comes from donors who are in the early stage of syphilis when spirochetes are present and detectable antibody is absent. Serologic tests on these asymptomatic donors with primary syphilis will be negative.

There are two types of nontreponemal antibody tests, Venereal Disease Research Laboratory (VDRL) and the rapid plasma reagin (RPR) test, used in many facilities. The major difference between the VDRL and RPR tests is that unheated serum or plasma may be used in the RPR test which decreases the time needed to perform the test. The RPR kits include reagin suspension; reactive, moderately reactive and nonreactive control sera; plastic-coated test cards that have delineated circles; disposable dispensing and mixing supplies; an antigen-dispensing vial; and a 20 gauge needle.

The procedure requires that 0.05ml of serum or plasma and 1/60ml of antigen suspension are mixed for 8 minutes at 100 rpm at room temperature. A plastic cover with a wet sponge is placed over the

rotating card to provide humidification. Agglutination of the particles in the antigen mixture is clearly visible in reactive samples.

The majority of positive syphilis serology tests on blood donors using nontreponemal based tests are biologic false positives. These positive samples are referred to a laboratory that can perform a more specific test which is usually the fluorescent treponemal antibody absorption (FTA-ABS) procedure. The FTA-ABS is a very specific, sensitive test, and it is widely used as the confirmatory method for the screening tests.

Some critical factors in the screening test are as follows:

- the use of the correct amount of sample and antigen;
- speed of the rotator;
- correct lighting;
- use of non-hemolyzed or non-contaminated samples; and,
- use of in-date reagents.

Treponemal-based automated procedures qualitatively screen for the detection Treponema pallidum antibodies. Serum is the specimen of choice for the test to lower the false positive rate found when plasma is used for testing.

This test is based on the principle of agglutination pattern recognitions. It differs from other screening procedures in that it detects specific antibodies to T. pallidum. The manufacturer's directions allow for duplicate retesting of initial reactive tests, which also differs from procedures used for the other syphilis screening tests.

RECORDS

Verify that units released for transfusion or further manufacturing are tested as claimed.

When viral marker tests are sent to outside testing laboratories, a printout of raw data may be sent back to the collecting establishment. If this is done, the data should be reviewed for accuracy.

Review the test records for the various tests performed by the facility. To determine the number of records to review see the applicable compliance program. Select a period of time for review when problems are more likely to occur, such as holidays, evening shifts, or when equipment, management, or personnel were new.

It is very important to trace the reactive test results through the system to ensure that the manufacturer's instructions have been followed, and the sample has been retested in duplicate when appropriate. If two of the three tests are reactive the unit may not be used and the donor should be placed on a deferral list.

The next step is the review of the deferral register to see if the donor has been properly deferred, if the unit has been destroyed, and by what method. If the unit has been shipped for further manufacture or for use in research this must be documented.

When computer systems are used for records, changes to records should be traceable as to the date, time, and person making the change so that data integrity is assured. Persons authorized to make changes to data should be specifically identified. Stored data should be audited periodically to assure

that timely retrieval and accurate information reporting are available. Records may be kept entirely electronically if hard copy can be retrieved within a reasonable time frame. This time frame has not been specified in writing previously by CDER or CBER but 24 hours has been discussed and agreed upon as a recommendation.

For additional information, see the FDA "Draft Guideline for the Validation of Blood Establishment Computer Systems," and CBER memoranda: "Recommendations for Implementation of Computerization in Blood Establishments," April 6, 1988, and "Requirements for Computerization of Blood Establishments," September 8, 1989.

Source Plasma and frozen red cells have a ten-year dating period and records of manufacture are required to be retained for 10.5 years. If there is no specified expiration date, records must be maintained indefinitely. Recovered plasma does not have an expiration date.

DISPOSITION

In establishments that both collect blood and perform viral marker tests, determine if components were made from units with repeatedly reactive test results. Verify that all components from units with reactive test results have been appropriately disposed. Recovered plasma with repeatedly reactive test results for anti-HBc may be shipped for further manufacture. Verify that destruction or disposition records for all blood products (i.e. all components made from each unit collected) are maintained.

BIOSAFETY

There should be a written procedure to minimize the spread of infectious agents. This SOP should be consistent with current CDC and OSHA recommendations. OSHA published the final rule for "Occupational Exposure to Bloodborne Pathogens" in the December 6, 1991 Federal Register. The rule includes requirements for facilities to develop procedures to ensure the safety of employees with potential exposure to biohazardous materials, and procedures for medical waste disposal. Routine precautions may include wearing gloves and protective clothing. Masks and face or eye coverings may also be worn as the likelihood of exposure to infectious agents increases. OSHA should be contacted by the district office if biosafety violations are observed.

DISPOSAL OF INFECTIOUS WASTE

FDA advises that state and local laws be followed for disposal of infectious waste. The testing establishment's SOPs should contain specific instructions for bagging or boxing and disposal of contaminated waste. Needles should be disposed of in a container designed to prevent accidental puncturing of personnel. The SOP should contain specific instructions regarding color and size of bags or boxes used for trash, handling of trash bags inside and outside the establishment, and whether autoclaving or incineration is used for contaminated waste. If contaminated waste disposal is contracted out, the testing establishment should have an in-date contractual agreement on file. The contract should specify that biohazardous material is disposed of according to EPA, state, and local regulations. Inappropriate disposal practices should be referred to state authorities for follow-up. This may be listed on FDA Form-483.

Report the firm's provisions, e.g., on-site, off-site, or contract. If the firm contracts out for disposal of infectious waste, report the name and address of the contract firm.