Date: March 14, 1996

From: Director, Center for Biologics Evaluation and Research

Subject: Additional Recommendations for Donor Screening With a Licensed Test for HIV-1 Antigen

To: All Registered Blood and Plasma Establishments

INTRODUCTION

On August 8, 1995, FDA issued a memorandum to all registered blood and plasma establishments entitled, "Recommendations for Donor Screening with a Licensed Test for HIV-1 Antigen". This memorandum supplements that memorandum and provides additional recommendations regarding storage of samples for HIV-1 antigen testing as well as clarifications of the previous recommendations regarding specific implementation issues.

STORAGE OF SAMPLES FOR HIV-1 ANTIGEN TESTING

Concerns have been raised about the stability of HIV-1 antigen containing samples during shipping and storage. Stability data from studies of both antibody(-) and antibody(+) specimens from seroconversion series which were performed by manufacturers in support of product license applications for HIV-1 p24 antigen test kits suggest that detectable antigen levels in some serum or plasma specimens may be adversely affected by elevated temperatures and long periods of storage. The available data have not demonstrated false-negative results in the **HIV-1 antibody** test if antigen(+)/antibody(+) samples are stored under conditions that would allow significant antigen-antibody complexing or antigen deterioration to occur. The following is a summary of the findings on antigen stability:

All studies confirmed the stability of HIV-1 p24 antigen(s) in antigen(+)/antibody(-) samples. Only one sample showed a loss of reactivity following storage for three days at 4EC and at 26EC. When this sample was stored for longer periods, no

further decrease in reactivity was observed and the sample remained positive for HIV-1 p24 antigen(s).

Storage of some antigen(+)/antibody(+) samples for as little as three days at 4EC and at 26EC resulted in a loss of reactivity on the antigen screening test, including the occurrence of false negatives. When testing was not completed within three days of sample collection with storage at 4EC, an increased likelihood of failure of initially reactive samples to be repeatedly reactive on the antigen screening test, invalid neutralization test results, and false negative neutralization test results occurred.

Instability of the antigen signal in both antigen(+)/antibody(-) and antigen(+)/antibody(+) samples held at 37EC has been clearly demonstrated, suggesting that antigen-antibody complexing or antigen deterioration or both occur to a greater extent at such elevated temperatures.

Overall, the preponderance of the available data suggests that most antigen(+) samples, especially antigen(+)/antibody(-) seroconversion samples which are of critical importance for the HIV-1 p24 antigen test to detect in donor screening, are stable at 4EC for at least seven days and that exposure to room temperatures below 26EC can be tolerated for up to three days. These findings are the basis for the attached recommendations on sample storage prior to antigen testing.

CLARIFICATIONS OF PREVIOUS RECOMMENDATIONS

1. FDA has been asked to clarify its expectations related to the "date of implementation" of the test for HIV-1 antigen(s). In **Recommendation A.1.** of the August 8, 1995, memorandum, the FDA stated that, **"all donations of Whole Blood, blood components, Source Leukocytes and Source Plasma should be screened for HIV-1 antigen(s) by an FDA licensed test labeled specifically for use in donor screening,"** and that, **"...this recommendation should be implemented within three months of the commercial availability of the first such test. Following the date of implementation, only units from donors found negative on HIV-1 antigen screening ... should be released..."**

CLARIFICATION: The "date of implementation" is the date within three months of HIV-1 antigen test licensure that a

manufacturer declares to FDA will be the date following which all units released by the establishment will have been screened for HIV-1 antigen(s).

2. FDA has been asked to clarify how to deal with Source Plasma and Recovered Plasma in inventory for further manufacturing. **Recommendation A.2.** states, **"Within three months of the commercial availability of the first FDA licensed HIV-1 antigen test labeled specifically for use in donor screening, all inventoried units ... available for release should be screened for HIV-1 antigen(s)."**

CLARIFICATION: For Source Plasma and recovered plasma, this recommendation is intended to apply only prospectively, i.e., to units drawn after the implementation date (within three months of licensure of the first donor screening test for HIV-1 antigen). Units collected on or after the implementation date should be tested for HIV-1 antigen and labeled as tested and found negative. Untested units collected prior to the implementation date may be distributed without special labeling. Additional labeling consistent with section B.3. of the August 8, 1995 memorandum may be used at the establishment's discretion.

3. FDA has been asked whether the three month time frame for implementation of the antigen test also applies to inventories held by consignees. **Recommendation A.3.** states that, **"Blood establishments should cooperate with consignees to insure that inventoried within-date units <u>intended for use in transfusion</u> which were distributed prior to test implementation are either replaced with screened units or else tested for HIV-1 antigen(s) as soon as feasible."**

CLARIFICATION: These units are in hospital inventories and otherwise out of the control of the blood establishment. Blood establishments should work with transfusion services and other consignees to complete inventory testing or exchange to the extent possible and as soon as it is feasible. FDA recognizes that this may not occur within the three month period recommended for test implementation. This recommendation does not apply to units already released for transfusion.

4. FDA has been asked to explain the rationale for imposing controls on the distribution of autologous units for

transfusion in the event of a repeatedly reactive screening test for HIV-1 antigen in the patient/donor.

CLARIFICATION: The procedures described in **section B.2.a.** pertaining to autologous units should be applied to any unit intended for autologous use that tests repeatedly reactive on the HIV-1 antigen screening test. This recommendation has been made because of the possibility of false negative neutralization tests.

5. FDA has been asked to explain its position on the regulatory implication of any follow-up testing performed on a donor prior to eight weeks after a repeatedly reactive screening test for HIV-1 antigen, and the Agency expectation regarding deferral and reentry in the case that a donor has a repeatedly reactive screening test for HIV-1 antigen on more than one independent collection. **Recommendation C.2.** states that, **"Donors whose blood samples are INDETERMINATE based on the neutralization test or**

untested by neutralization should be temporarily deferred from donation for a minimum of eight weeks." Recommendation C.2.a. further states that **"The donor can automatically be reinstated...if all donor screening tests (including HIV antigen) are performed and found NEGATIVE, and the donor meets all other suitability criteria."**

CLARIFICATION: Blood or plasma centers wishing to perform follow-up testing on donors who are temporarily deferred may do so <u>prior</u> to the end of this eight week period for donor notification purposes or for medical reasons. However, if the donor is retested prior to the end of the eight week deferral period and repeatedly reactive EIA results are again obtained, the donor should be permanently deferred. A negative result on the EIA or neutralization test may be used in donor counseling, however, only a negative screening test result obtained at least eight weeks after the latest repeatedly reactive test qualifies as the “test of record” for purposes of donor reentry.

6. FDA has also been asked to clarify **recommendation C.2.b. (the donor is permanently deferred if the screening test for HIV antigen is repeatedly reactive on any subsequent evaluation or donation...)** when a reinstated donor tests repeatedly reactive on a donation subsequent to having been

reinstated.

CLARIFICATION: The donor should be temporarily deferred again and is eligible for reentry eight weeks later as outlined in C.2.a.

The recommendations contained in this memorandum may be implemented without prior approval from FDA. However, licensed establishments implementing these recommendations should submit by official correspondence a statement to their product license file indicating the date that revised standard operating procedures consistent with these recommendations have been established and implemented. If an establishment believes that an alternative approach would provide equivalent protection, the establishment is invited to discuss the approach with FDA for FDA's evaluation.

The recommendations for storage and testing discussed in this memo apply only to HIV-1 antigen testing. In the event that an FDA approved screening test may require different storage conditions, the package insert instructions should be followed.

Although this guidance document does not create or confer any rights, privileges, or benefits for or on any person and does not operate to bind FDA or the public, it does represent the agency’s current thinking with regard to HIV-1 antigen screening.

Questions and comments about the recommendations or clarifications contained in this memorandum may be directed to the Division of Transfusion Transmitted Diseases, FDA/CBER (HFM-310), 1401 Rockville Pike, Rockville, MD 20852; FAX: (301) 480-7928.

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Additional Recommendations for Donor Screening With a Licensed Test for HIV-1 Antigen March 14, 1996

The following recommendations regarding storage of donor samples prior to testing for HIV-1 antigen(s) by licensed tests are intended to supplement those contained in FDA's memorandum to all registered blood and plasma establishments dated August 8, 1995. In order to assure the integrity of donor samples to be tested for HIV-1 antigen(s), the FDA recommends the following:

1. All testing for HIV-1 antigen(s), including the initial screening test, the repeat test, and the neutralization test for repeatedly reactive specimens, should be completed as soon as feasible following sample collection.

2. Serum or plasma samples for testing for HIV-1 antigen(s) should be stored for no longer than seven days at a combination of room temperature (15-26°C) and nominal 4EC (generally accepted as 2-8°C for laboratory samples) following sample collection. This seven day period of storage without freezing should include no more than three days at room temperature.

3. Samples that have to be stored for more than seven days prior to testing should be kept frozen at -20°C or lower. No more than five freeze thaw cycles should be performed on any sample prior to testing for HIV-1 antigen.

4. Samples should not be exposed to temperatures above 26EC during shipping, storage or thawing from the frozen state. FDA encourages blood and plasma establishments to ship samples by methods shown to maintain temperatures at 2-8EC. For shipping at ambient temperatures, shipping conditions should assure that temperatures do not exceed 26EC.