

Guidance for Industry

Recommendations for Screening, Testing, and Management of Blood Donors and Blood and Blood Components Based on Screening Tests for Syphilis

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I. INTRODUCTION

We, FDA, are providing you, blood establishments that collect Whole Blood or blood components, including Source Plasma, with recommendations for screening and testing of donors and management of donations based on screening tests for syphilis. Licensed blood establishments must report the implementation of the recommendations contained in this guidance in accordance with 21 CFR 601.12. For additional recommendations, see “Guidance for Industry: Changes to an Approved Application: Biological Products: Human Blood and Blood Components Intended for Transfusion or for Further Manufacture,” dated July 2001.

This guidance finalizes the draft guidance of the same title, dated March 2013, and supersedes the memorandum of December 12, 1991, entitled “Clarification of FDA Recommendations for Donor Deferral and Product Distribution Based on the Results of Syphilis Testing” (Ref. 1).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

A. Transfusion-Transmission of Syphilis

Syphilis, caused by the spirochete *Treponema pallidum* (*T. pallidum*), is most often acquired after sexual contact with an infected individual. Syphilis can also be transmitted from mother to child or, rarely, transmitted by transfusion of blood or blood components from donors with active syphilis (Ref. 2). The last reported case of transfusion-transmitted syphilis in the United States (U.S.) occurred in 1966 (Ref. 3). Universal

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testing of blood donors may have played a role in the disappearance of transfusion-transmitted syphilis. Other possible explanations for the decline in transfusion-transmitted syphilis include: that direct donor-to-recipient transfusions no longer take place; inactivation of *T. pallidum* (a cold-sensitive microorganism) in refrigerated blood components; the decline in rates of syphilis in the general population, which in turn is reflected in the donor population; self-deferral of blood donors who are ill during spirochetemia (presence of spirochetes—the causative agent of syphilis infection—in the circulating blood); deferral of potential donors who demonstrate high risk behavior for acquiring syphilis infection (e.g., persons who received money, drugs, or other payment for sex) through donor eligibility screening processes; wide use of antibiotics among transfusion recipients; and difficulties in diagnosing transfusion-transmitted syphilis in recipients (Ref. 4). However, none of these explanations has been quantified or adequately validated.

Current testing requirements for *T. pallidum* are included in Title 21 of the Code of Federal Regulations (CFR) Part 610, Subpart E (Testing Requirements for Communicable Disease Agents), specifically under § 610.40(i) (21 CFR 610.40(i)) and in § 640.65 (21 CFR 640.65) for Source Plasma donors. FDA has sought public comments on the continued need for syphilis testing on several occasions, including in the proposed rule, entitled “Requirements for Human Blood and Blood Components Intended for Transfusion or for Further Manufacturing Use,” November 8, 2007, (72 FR 63416) (Ref. 5).

Information from the American Red Cross on serological testing of donors revealed that there were 324 cases of syphilis infections among American Red Cross repeat allogeneic donors in 2007-2008, a figure several times higher than the numbers of repeat allogeneic donors identified with human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and human T-cell lymphotropic virus (HTLV) infections, which were 92, 47, 127 and 9, respectively, during the same time period (Ref. 6). Several published studies (Refs. 7 through 12) that investigated the presence or absence of *T. pallidum* nucleic acid in blood samples from individuals with confirmed or possible syphilis detected spirochete nucleic acid in blood samples from persons with syphilis, some of whom had a latent infection with no symptoms. This suggests that some asymptomatic blood donors might have spirochetemia. Donations from such blood donors may have the potential to transmit syphilis to recipients. Although syphilis testing has a low sensitivity and positive predictive value as a surrogate marker for detecting known transfusion-transmitted viruses, in 2009, the American Red Cross published data (Ref. 13) indicating that there were significantly higher rates of HIV-, HCV-, HBV-, HBsAg (hepatitis B surface antigen)-, and HTLV-positive donations among donors with positive syphilis test results compared to donors with negative syphilis test results.

B. Testing of Blood and Blood Components for Syphilis

Under § 610.40(i), you must perform a serological screening test for syphilis on each donation of blood.¹ Donors who test reactive² with a screening test for syphilis must be deferred (§ 610.41(a)) and notified of their deferral (21 CFR 630.6). Additional testing to requalify the donor in accordance with § 610.41(b) is described below in sections IV.B and C of this document. In accordance with § 610.40(h)(2)(vi), FDA allows use of blood and blood components, excluding Source Plasma, that test reactive by a screening test for syphilis, if the donation is further tested by an adequate and appropriate test which demonstrates that the reactive screening test is a biological false-positive, and the blood or blood component is labeled with both test results. Under § 610.40(h)(2)(vii), you may use Source Plasma from a donor who tests reactive by a screening test for syphilis, if the donor meets the requirements of § 640.65(b)(2).

FDA requirements regarding syphilis testing specific to Source Plasma are as follows:

1. Current collection, testing and labeling requirements related to results of serologic tests are found in §§ 640.65, 640.67, 640.71 and 606.121 (21 CFR 606.121).
2. A sample of blood must be drawn from Source Plasma donors on the day of the first medical examination or plasmapheresis, whichever comes first, and at least every 4 months thereafter, and these samples must be tested for syphilis (§ 640.65(b)(1)(i)).
3. A donor with a reactive test result for syphilis must not be plasmapheresed again until the donor tests nonreactive, except as stated in points 4 and 5, below (§ 640.65(b)(2)(ii)).
4. A donor with a reactive biologic false-positive syphilis test result may be plasmapheresed, provided that the donor's file: (a) identifies the reactive serologic test and the results used to confirm the biologic false-positive results; and (b) indicates that the physician on the premises has determined the false-positive reaction is not the result of an underlying disorder that would disqualify the donor from participating in the plasmapheresis program (§ 640.65(b)(2)(iii)).
5. A donor with a reactive syphilis test result may be plasmapheresed only to obtain plasma to be used for further manufacturing into control serum for the serologic test for syphilis, provided that: (a) the physician on the premises approves the donation; and (b) the donor's file contains a signed statement

¹ Additional regulatory requirements related to syphilis are found under 21 CFR 606.121, 610.41, 640.5(a), 640.14, 640.23(a), 640.33(a), 640.53(a), 640.65(b)(1) and (b)(2), and 640.67.

² The term "reactive" includes "repeatedly reactive" results. You should follow the instructions in the package insert of the assay you are using.

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from a physician or clinic establishing that treatment for syphilis has commenced and that continuance in the plasmapheresis program will not interfere with or jeopardize the syphilitic donor's treatment (§ 640.65(b)(2)(iv)).

6. Plasma collected from a donor with a reactive test result for syphilis must be appropriately labeled, as stated in § 606.121(e)(5)(iv).

III. CHARACTERISTICS OF SEROLOGIC ASSAYS FOR SYPHILIS

There are two different types of serologic assays for syphilis: (A) nontreponemal assays; and (B) treponemal assays.

(A) ***Nontreponemal assays***, such as the rapid plasma reagin (RPR) test, the venereal disease research laboratory (VDRL) test, and the automated reagin test (ART), are non-specific tests that detect “reagin” antibodies directed against an antigen called cardiolipin that is present in a variety of tissues. Antibodies to cardiolipin appear in the serum of persons with active syphilis and with some other conditions. However, some individuals who were previously infected with syphilis but successfully treated maintain low levels of antibody to cardiolipin for a long time; such persons are classified as being “serofast.”

Sera and plasma from individuals previously infected with syphilis who were successfully treated do not generally remain reactive in nontreponemal tests for more than one to two years after successful treatment. Therefore, persons with active or recently treated syphilis infections generally have reactive results in nontreponemal tests, while uninfected persons or persons successfully treated years earlier usually have nonreactive nontreponemal test results. Nontreponemal assays are useful in identifying recent syphilis infection, and to monitor the progression of syphilis and response to antibiotic therapy.

(B) ***Treponemal assays*** include enzyme immunoassays (EIA), fluorescent treponemal antibody “absorbed” assays (FTA-ABS), *Treponema pallidum* microhemagglutination assays (MHA-TPA) and *Treponema pallidum* particle agglutination assays (TP-PA). Treponemal assays test for antibodies to antigens that are specific to treponemes. Treponemal assays are most useful in identifying recent and historically remote syphilis infections. They are not generally useful in monitoring the response to antibiotic therapy. With some exceptions, positive results of tests for specific treponemal antibodies remain positive throughout an individual's life regardless of whether the individual is currently infected or has been cured following successful treatment (Ref. 14). Retesting sera that are reactive in nontreponemal assays using a specific treponemal test is valuable in distinguishing true-positive results that indicate active syphilis infection from biological false-positive results due to other conditions.

Since both the nontreponemal and treponemal assays detect antibodies rather than the infectious treponemes themselves, neither assay reliably identifies patients in the “window period” of very

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early syphilis, after infection has been acquired but before antibodies to either treponemal antigens or to cardiolipin have appeared.

Before 1990, blood establishments typically used nontreponemal tests to screen donors for syphilis and then performed a treponemal test on the reactive samples to determine the specificity of nontreponemal test results. In 1990, FDA cleared a 510(k) pre-market notification for an automated modified microhemagglutination test that detects specific antibodies to *T. pallidum*. After this new treponemal assay was implemented for screening donors, some repeat blood donors who had formerly been found suitable based on nonreactive nontreponemal test results were deferred because they were found to have specific antibodies to *T. pallidum*. By largely replacing donor screening with nontreponemal assays, this test eliminated most biological false-positive nontreponemal screening test results, and its use caused no overall increase in the total number of donors deferred (Refs. 15 and 16). Many blood establishments now use automated treponemal assays with high throughput that have been cleared by FDA for purposes of screening donors for syphilis, such as the treponemal assays EIA, MHA-TPA and TP-PA. However, some blood establishments might be using a nontreponemal assay as a screening test for syphilis. Therefore, we are providing recommendations for management of donors and blood and blood components for situations in which a blood establishment would use either a nontreponemal screening test or a treponemal screening test.

To distinguish the results of screening tests for syphilis from the results of additional syphilis tests, we use the terms “reactive²” and “nonreactive” for results of screening tests (both nontreponemal and treponemal) and reserve the terms “positive” and “negative” for results of subsequent treponemal tests (used for donor counseling and requalification) on samples of the same blood specimen previously used for screening. A test result that is read as “indeterminate,” “questionable,” or equivocal” according to the package insert should be considered as equivalent to a reactive test result (for screening tests) or positive test result (for subsequent treponemal tests) for purposes of this guidance document.

Establishments that screen donors using a nontreponemal assay as the test of record may use a treponemal diagnostic test for the purposes of donor counseling, donor reentry, and also for demonstrating that a reactive screening test result is a biological false-positive (§ 610.40(h)(2)(vi)). However, we recommend that in all instances serologic tests used to screen donors for syphilis be cleared by FDA for such intended use (see recommendations in sections IV.B and C of this document.)

IV. RECOMMENDATIONS FOR DONOR TESTING AND MANAGEMENT AND PRODUCT DISPOSITION WHEN USING TESTS FOR SYPHILIS

A. Identification of Donors with a History of Syphilis

1. To assess the eligibility of the donor as required in § 640.3, we recommend that you question prospective donors to determine if they have had syphilis or gonorrhea in the past 12 months or if they were treated for either of these diseases during that time.

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If you are administering an abbreviated donor history questionnaire, we recommend that you ask these donors if they have had any new medical problems or diagnoses and any new medical treatments since their last donation.

2. We recommend that for donors who state that in the past 12 months they have had or have been treated for syphilis or gonorrhea, you defer such donors for 12 months after being told they had syphilis or gonorrhea or after completion of treatment. After this 12-month period, the donor may be eligible to donate again, provided that the donor satisfies all applicable donor eligibility criteria.

B. Donor Testing and Management When Using a Nontreponemal Screening Test as the Test of Record for the Detection of Syphilis (See Figure 1)

You must perform a serological test for syphilis on each donation of blood (§ 610.40(i)).¹ You must test a donor of Source Plasma for syphilis at least every 4 months (§ 640.65(b)(1)(i)). We recommend that all serological tests used to screen donations for syphilis be cleared by FDA for such intended use.

1. If the nontreponemal screening test is nonreactive, the donor is considered to be negative for syphilis infection. You may release the donation, provided it meets all other requirements, and retain the donor.
2. If the nontreponemal screening test is reactive², you must defer the donor indefinitely (§ 610.41(a)) and you must not ship or use the donation, unless an exception for shipment or use is applicable (§ 610.40(h)). However, Source Plasma donors may be allowed to donate plasma for further manufacture into noninjectable products (§ 640.65(b)(2)(ii) through (iv)). Absent an exception under § 610.40(h)(2) being applicable, donations of blood and blood components must not be used (§ 610.40(h)) unless a negative treponemal test result is obtained using the method described below. The donor may be eligible for reentry following the method described below.

Product Management and Reentry under § 610.41(b) of Deferred Donors

3. If the nontreponemal test is reactive², you may perform a treponemal test³ using either a sample from the index donation or a follow-up sample from the donor collected at a later date.
 - a. If the treponemal test result is negative (suggesting that the reactive nontreponemal test result was a biological false-positive result), you may

³ Establishments may use a treponemal diagnostic test for donor counseling, donor reentry or for demonstrating that the reactive screening test is a biological false-positive (§ 610.40(h)(2)(vii)).

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reenter the donor under § 610.41(b). If the sample tested was from the index donation, the donation, excluding Source Plasma, may be released (§ 610.40(h)(2)(vi)), provided the donation meets all other criteria, but must be appropriately labeled under §§ 610.40(h)(2)(vi) and 606.121. Under § 610.40(h)(2)(vii), you may use Source Plasma from a donor who tests reactive by a screening test for syphilis, if the donor meets the requirements of § 640.65(b)(2). You may consider counseling the donor about the potential medical significance of a biological false-positive screening test.

- b. If the treponemal test result is positive, you must continue to defer the donor indefinitely (§ 610.41(a)) and discard the donation unless an exception for shipment or use is applicable (§ 610.40(h)).

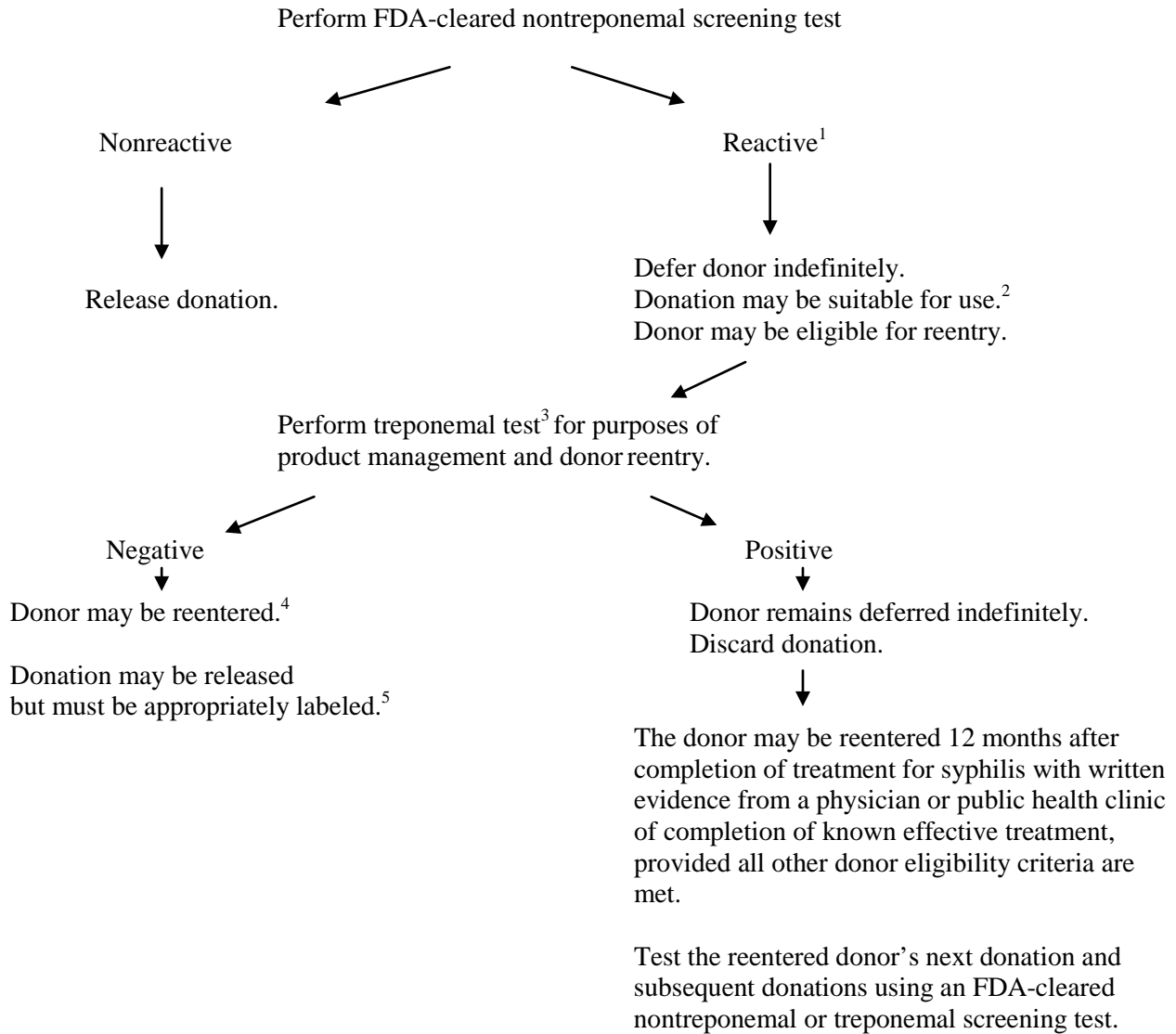
You may reenter the donor under § 610.41(b), if the donor subsequently:

- i. Presents written evidence from a physician or public health clinic of completion of a known, effective treatment for syphilis that was completed at least 12 months before the next donation⁴; and
- ii. Meets all other donor eligibility criteria.

You may use either an FDA-cleared nontreponemal screening test or an FDA-cleared treponemal screening test to test the reentered donor's next donation and subsequent donations.

⁴ If your medical director determines that an individual donor never had a syphilis infection, you may submit a request to FDA to reenter the individual under § 610.41(b).

Figure 1: Donor Testing and Management When Using a Nontreponemal Screening Test as the Test of Record for the Detection of Syphilis



¹The term “reactive” includes “repeatedly reactive” results. You should follow the instructions in the package insert of the assay you are using.

²Source Plasma donations with these test results may be used under some circumstances, but the donor is deferred unless certain conditions apply (§§ 610.40(h)(2)(vii) and 640.65(b)(2)(ii) through (iv)). Absent the applicability of an exception under § 610.40(h)(2), donations of blood and blood components must not be used unless a negative treponemal test result is obtained (§ 610.40(h)).

³For donor counseling, donor reentry or demonstrating that the reactive screening test is a biological false-positive (§ 610.40(h)(2)(vi)), you may use a treponemal diagnostic test for donor counseling, donor reentry or for demonstrating that the reactive screening test is a biological false-positive

⁴Consider counseling the donor about the potential medical significance of a biological false-positive screening test.

⁵Sample tested must be from index donation. Excluding Source Plasma, you must label such donations as reactive by a screening test for syphilis and negative by a treponemal test (§ 610.40(h)(2)(vi)). Under § 610.40(h)(2)(vii), you may use Source Plasma from a donor who tests reactive by a screening test for syphilis, if the donor meets the requirements of § 640.65(b)(2).

C. Donor Testing and Management When Using a Treponemal Screening Test as the Test of Record for the Detection of Syphilis (See Figure 2)

You must perform a serological test for syphilis on each donation of blood (§ 610.40(i)). You must test a donor of Source Plasma for syphilis at least every 4 months (§ 640.65(b)(1)(i)). We recommend that all serological tests used to screen donations for syphilis be cleared by FDA for such intended use.

1. If the treponemal screening test is nonreactive, the donor is considered to be negative for syphilis infection. You may release the donation, provided it meets all other requirements and retain the donor.
2. If the treponemal screening test is reactive², you must defer the donor indefinitely and you must not ship or use the donation, unless an exception for shipment or use is applicable (§ 610.40(h)). However, Source Plasma donors may be allowed to donate plasma for manufacture into non-injectable products (§ 640.65(b)(2)(iv)). The donor may be eligible for reentry following the method described below.

Reentry under § 610.41(b) of Deferred Donors

3. Because the possibility exists that a treponemal test might be false-positive for reasons unrelated to the analyte (e.g., failure to remove excess conjugate during the performance of the test), the donor may be eligible for reentry. You may perform another treponemal screening test that is different from the initial treponemal screening test used as the test of record, using either a sample from the index donation or a follow-up sample from the donor collected at a later date.⁵
 - a. If the additional treponemal screening test result is negative, you may reenter the donor.
 - b. If the additional treponemal screening test result is positive, the donor remains deferred indefinitely (§ 610.41(a)). The donor may be eligible for reentry, as described immediately below.

⁵ When treponemal screening test results are reactive, we do not consider negative results on an additional treponemal test to be indicative of biological false-positives on the screening test under § 610.40(h)(2)(vi). Regardless of the result from the additional treponemal screening test, under § 610.40(h) you must not release the index donation unless an exception applies. Source Plasma donations with these test results may be used under some circumstances, provided that the requirements under §§ 610.40(h)(2)(vii) and 640.65(b)(2)(ii) through (iv) are met.

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You may test the sample from the donor which was positive on the additional treponemal screening test using a nontreponemal screening test to assess whether the donor has an active infection.⁶

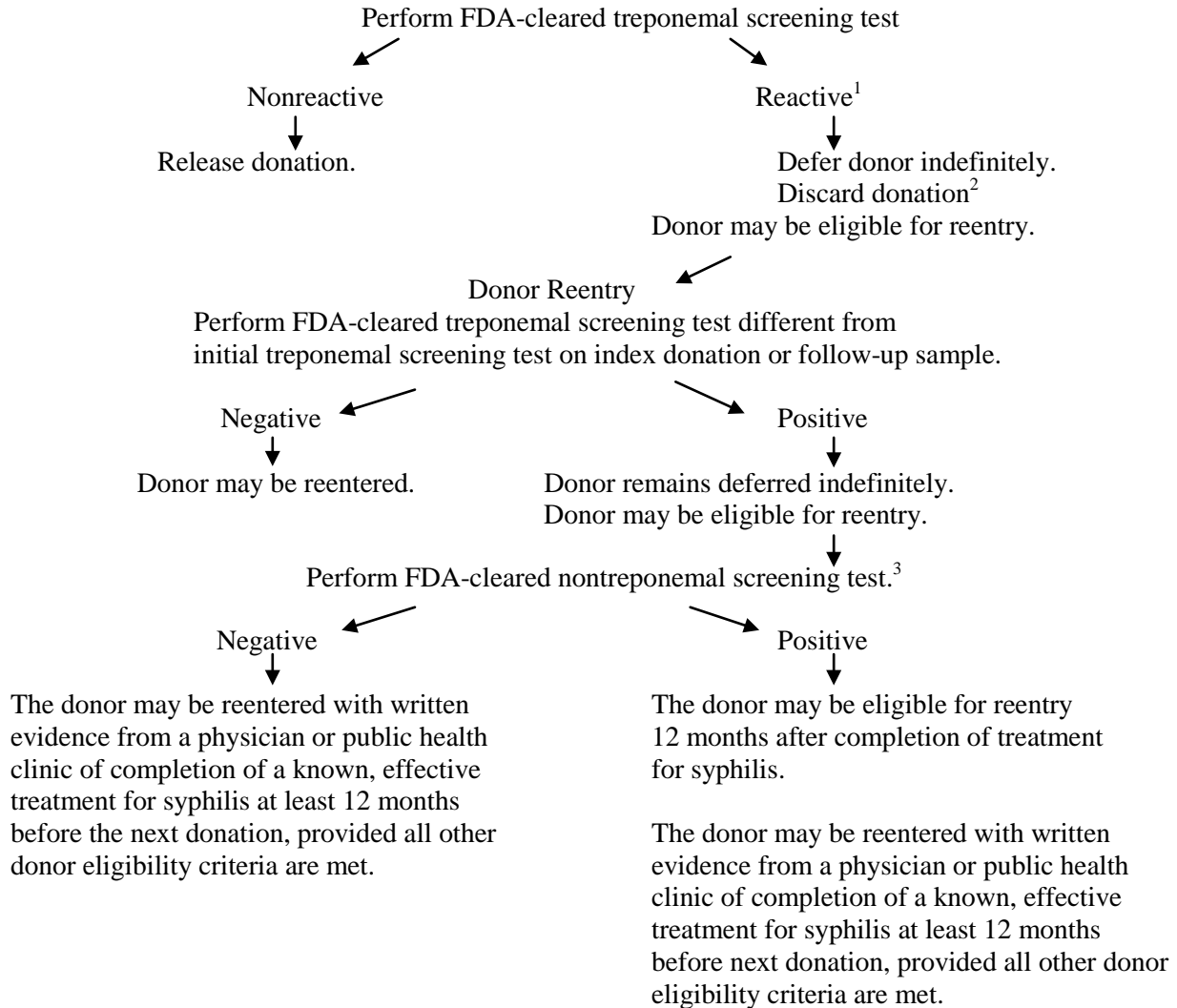
- i) If the nontreponemal screening test result is negative you may reenter the donor under § 610.41(b) if the donor:
 - A) Presents written evidence from a physician or public health clinic of completion of a known effective treatment for syphilis completed at least 12 months before the next donation⁴; and
 - B) Meets all other donor eligibility criteria.

You may use either a nontreponemal screening test or a treponemal screening test that has been cleared by FDA for such intended use to test the reentered donor's next donation and subsequent donations.

- ii) If the nontreponemal screening test result is positive, the donor remains deferred indefinitely (§ 610.41(a)). The donor may be eligible for reentry 12 months after completion of treatment for syphilis. You may reenter the donor (§ 610.41(b)), if the donor:
 - A) Presents written evidence from a physician or public health clinic of completion of a known, effective treatment for syphilis that was completed at least 12 months before the next donation⁴; and
 - B) Meets all other donor eligibility criteria.

⁶ Performing a nontreponemal test after positive treponemal test results are obtained can assist in determining a donor's status in regard to syphilis infection. A positive nontreponemal test result, with positive treponemal test results, indicates an active or a recently treated syphilis infection. A negative nontreponemal test result, with positive treponemal test results, indicates recovery or cure from a previous syphilis infection. Thus, knowledge of the nontreponemal test result, with positive treponemal test results, can assist in counseling the donor and in determining future eligibility of the donor.

Figure 2: Donor Testing and Management When Using a Treponemal Screening Test as the Test of Record for the Detection of Syphilis



Test reentered donor's next donation and subsequent donations using an FDA-cleared nontreponemal or treponemal screening test.

¹The term "reactive" includes "repeatedly reactive" results. You should follow the instructions in the package insert of the assay you are using.

²Source Plasma donations with reactive test results for syphilis may be used under some circumstances, but the donor is deferred, unless certain conditions apply (§§ 610.40(h)(2)(vii) and 640.65(b)(2)(ii) through (iv)). Donations of blood and blood components must be discarded, unless an exception is applicable (§§ 610.40(h)(2)). Note that all applicable labeling requirements must be met, should you be able to use the donations (§§ 606.121 and 610.40(h)(2)).

³Performing a nontreponemal test after positive treponemal test results are obtained can assist in determining a donor's status in regard to syphilis infection. A positive nontreponemal test result, with positive treponemal test results, indicates an active or a recently treated syphilis infection. A negative nontreponemal test result, with positive treponemal test results, indicates recovery or cure from a previous syphilis infection. Thus, knowledge of the nontreponemal test result, with positive treponemal test results, can assist in counseling the donor and in determining future eligibility of the donor.

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