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Dockets Management Branch
(HFA-305)
Food and Drug Administration
5630 Fishers Lane
Room 1061
Rockville, MD 20852

Dear Sirs:

We are writing to voice our official comments regarding the "Requirements for Testing Human Blood for Evidence of Infection Due to Communicable Disease Agents", specifically in reference to the serologic test for syphilis.

Our position is opposed to changing the current rule (Secs. 640.5 (a) and 640.65 (b)) requiring that all donated blood be screened with a serologic test for syphilis. Our opposition is based upon the following information.

First, we would like to address the recent and ongoing studies by ARCNET to support discontinuation of the test. We believe that there is insufficient data to warrant such a decision at this time. The data is still lacking related to whether infectious *T. pallidum* organisms are present in the blood of STS positive persons. A recent American Red Cross study reported at the 1999 AABB annual meeting, and at the FDA Conference on November 22nd, attempted to answer this question. Samples from platelet concentrates stored at room temperature for less than 24 hours were obtained from 100 PK-TP, FTA-ABS positive blood donors. Half (n=50) were RPR positive and half were RPR negative. These samples were tested by two different PCR assays that were stated to detect 50 *T. pallidum* organisms per input sample volume and 10 *T. pallidum* DNA copies respectively. All 100 samples tested negative for *T. pallidum* DNA.

There are numerous potential criticisms of this study:

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1. No details of the two PCR assays were described. The sensitivity, specificity, and reproducibility of the assays were not reported. Assay performance data on clinical samples from the various stages of syphilis (incubation, primary, secondary, etc) were not provided.
2. There are no data to correlate the analytic sensitivity limits of the assay (expressed as concentration per ml) with the dose required to cause infectivity. Therefore, it cannot be concluded that units below the limits of detection of the assay are non-infectious. In addition, a platelet recipient would receive approximately 30 to 50 ml of volume, resulting in the actual exposure to T. Pallidum being 30 to 50 times higher than the per ml concentration.
3. It was stated that the optimal sample source for the assays was fresh blood. However, the study used samples obtained from stored platelet concentrates. No data were provided comparing assay performance across these two sample sources.
4. Although the sample size was 100, 50 of these donors were RPR negative and represent cases of treated, resolved syphilis. Thus the effective sample size for detection of infectivity was only 50. Using the statistical rule of 3, this means that the true result ranges from 0 to 3 T. pallidum PCR positives in the 50 RPR positive, PK-TP positive units. In other words, statistics state that this experiment cannot rule out that up to 6% of RPR positive, PK-TP positive units contain T. pallidum at the level of assay detection. In summary, even if the methodological problems of the study could be solved, the sample size is too small to establish that STS testing does not occasionally detect infectious units.

There are a number of additional questions related to the study which we believe must also be addressed.

1. Recovery rate of TP in Platelet fraction: Platelets are usually separated by centrifugation, but platelets are generally not completely separated by centrifugation. If the platelet recovery rate is low, it will effect to sensitivity of PCR (NAT).
2. In case of Antibody test for TP, antibody tests can detect the complex with TP antigen and antibodies in blood, but it is not clear how much TP organism itself is exists in platelet fractions.
3. (PCR) NAT Sensitivity and specificity
Recovery rate of DNA extraction from platelet samples: Usually Thyroid DNA from Sermon (addition of non-Target DNA) is added to the sample to increase the recovery rate of DNA or the addition of polyethylene glycol is necessary to insure a good DNA recovery rate, but there is no information of DNA extraction method. (In the presentation, Herpes I and 2 were also tested, but there is no information of recovery rates of Herpes DNA either).

4. Basic data for selection of primer and probe relating to the amplification site:
There is no information of how the amplification site had been selected, and how primers were selected. It had to be optimized primer concentrations. Is this amplification site widely used to detect TP? How much genetically stable on this amplification site? More basic information is needed to justify this NAT assay itself.
5. Testing population: From statistical point of view, there is certain number required to reject a null hypothesis. We do not believe that predicting a general infected population has been satisfied.
6. What are the qualifications of the assays? Who made it and how was it validated? Are the assays cleared by the FDA?
7. The FDA requires manufactures of cleared assays to test against treated and untreated samples from all stages of syphilis. What, if any, testing was done with this type of samples.
8. What sample types, anticoagulants and sample handling conditions were validated for the assays used in the ARCNET study?
9. The control used in the study was not from actual active syphilis patients, but from a culture. This may not be a valid positive control for the assay. Additionally, the DNA test sensitivity = as low as 25 organism/100µl of platelet concentrate extracted; however, the positive controls were diluted to 50 organism/100µl.
10. According to Sharon Orton during her presentation at the FDA conference, the minimal infective dose is one Treponeme per 100µl of blood, based on Rabbit Infectivity Testing. Since the methods used can not detect this low dose, can any conclusions be drawn from their testing?

For transfusion-transmission of syphilis to occur, *T. pallidum* organisms must be present in the unit of blood collected from the donor. From the data concerning spirochetemia, we can infer that infectivity from blood transfusion would be potentially possible from blood collected during each of the following stages: incubation period, primary stage, secondary stage, and latent stage of syphilis. In analogy to transfusion-transmitted viral infections, donors may not be infectious for the entire length of the incubation phase; it may take some time after infection with *T. pallidum* for the organism to reach a sufficient concentration in peripheral blood to represent a human infectious dose.

There are no assays that predict infectivity in humans. The only infectivity assay that has been developed is an experimental animal model using rabbits in which potentially infectious material is injected into the testicle of living rabbits and *T. pallidum* organisms are identified on microscopic examination of this tissue.

It is unknown how many *T. pallidum* organisms are needed for an infectious dose in humans. Since this is not known, it is not possible to determine whether very small numbers of organisms, below the limit of current PCR assay detection, would be sufficient to transmit infection.

In summary, this preliminary study does not adequately address the question of whether STS testing sometimes detects units that are spirochetemic and capable of transmitting infection.

The second area we want to comment on is infectivity and blood storage.

While studies have shown that spirochetes do not show good survival in red blood cells stored at refrigerator temperature (4°C), and would not survive in refrigerator stored citrated whole blood beyond 48 to 72 hours. More recent experiments have suggested that *T. pallidum* may be capable of survival in refrigerated stored blood for up to five days.

There are no experimental data indicating how well *T. pallidum* survives in stored platelet concentrates. The room temperature storage of platelet concentrates favors survival of the organism. Despite the absence of experimental data, most authorities assume that *T. pallidum* survives in platelet concentrates during the five-day storage period. The occurrence of one case of transfusion-transmitted syphilis has been attributed to transfusion of platelet concentrates.

There are a number of potential reasons why transfusion-transmitted syphilis has rarely been reported post 1950, despite its rather common occurrence in the previous decade.

1. The incidence of syphilis in the general population has decreased.
2. Syphilis is rapidly diagnosed and treated so that infected persons do not remain infectious.
3. STS screening of blood donors has been effective in eliminating infectious units from distribution and transfusion
4. Concurrent antibiotic therapy of recipients leading either to suppression of symptoms and/or lack of disease development.
5. Lack of recognition of cases of transfusion-transmitted syphilis
6. Lack of active surveillance for transfusion-transmitted syphilis

Our final area of comment is regarding syphilis as a surrogate marker for other infectious diseases, such as HIV.

Although studies of blood donors, such as the recent REDS study have not shown a significant correlation between a reactive STS and risk of other infectious diseases, such as HIV, studies by public health have shown a significant risk. The differences between this higher prevalence population in public health, and the fact that blood donors are generally low risk, may mask the actual significance of co-infections in blood donors.

The elimination of syphilis serology from blood screening seems to be at odds with the CDC's report to Congress on elimination of syphilis. The CDC calls for continued surveillance even after the disease is eliminated, as it will then be a potential re-emerging infectious disease.

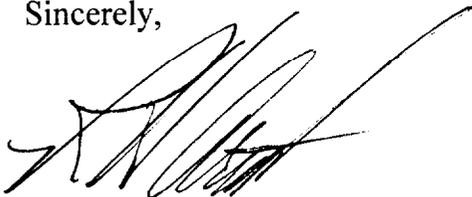
Below are quotes from CDC Report to Congress, by David Satcher, M.D. Ph.D., entitled *Elimination of Syphilis from the United States*.

“Recent studies suggest that heterosexual HIV transmission in the United States still largely follows the geography of the syphilis epidemic of the late 1980's and early 1990's.”

“Recent history suggests that the most likely scenario if elimination is not achieved is an inevitable resurgence of epidemic syphilis, creating new waves of HIV transmission and new outbreaks of congenital syphilis in our most vulnerable populations.”

In closing, we believe that consensus reached in January 1995, at the NIH convened a consensus conference on infectious disease testing for blood transfusions remains valid. The test for syphilis has been used for many years and data are inadequate to ascertain whether it accounts for the rarity of transfusion-transmitted syphilis. Therefore, STS screening of blood donations should continue.

Sincerely,



Richard H. Arnette
Sr. Vice President of Marketing
and Business Development

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