

**American Red Cross**

National Headquarters

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December 27, 1999

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

**RE: Suitability Determination for Donors of Human Cellular and Tissue Based Products [Docket No. 97N-484S, 64 FR 52696 (September 30, 1999)]**

Dear Docket Officer:

The American Red Cross (ARC) is pleased to submit comments on the proposed rule Suitability Determination for Donors of Human Cellular and Tissue Based Products. This proposal outlines the Food and Drug Administration's (FDA) plans to require manufacturers of certain human cellular and tissue-based products to screen and test the donors of cells and tissue used in those products for risk factors for clinical evidence of relevant communicable disease agents and diseases.

The American Red Cross, through its National Tissue Services, supplies approximately 20 - 25% of the nation's tissue needs for transplantation. ARC supplies cardiovascular, musculoskeletal, as well as skin, allograft tissue to physicians and dentists for patient treatment. ARC thus has an interest in regulation of human tissues intended for transplantation. ARC is committed to working with FDA in its efforts to develop a regulatory program for human cellular and tissue-based products.

In general, ARC supports the proposed rule. It is clear that FDA has thoroughly considered the appropriate issues. Thus, we wish to provide public comments, in the spirit of adding further information that hopefully will contribute to a carefully planned structure for the oversight of tissue products in line with the nation's public health protection goals.

97N 484S

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### **Timing of Obtaining the Sample**

The proposed rule contains a specified time frame for collecting the donor test sample. This requirement is describe in section 1271.80(b) page 52722, which states:

... the donor specimen shall be collected at the time of recovery of cells or tissue from the donor or within 48 hours of recovery...

The Agency explains that the reason for obtaining the specimen so close to the time of procurement is due to the need to reduce the risk of transmission of communicable diseases. Presumably, potential tissue or cellular donors may receive transfusion of blood-related products during a hospital stay. To be able to test for the possible transmission of communicable diseases from such infusions, FDA proposed to require obtaining the donor specimen as close as possible to the actual time of death.

In many cases, the same donors who are tissue donors are also organ donors, and a sample for organ donation purposes is drawn well before asystole. We believe that the same sample used for testing by the organ procurement organizations would provide acceptable test results for tissue donors under the proposed regulations. Moreover, it is inappropriate to establish a two tiered system -- one for tissue donation, and the other for organ donation, particularly when the donor is the same individual.

First, many, if not most potential donors are receiving considerably aggressive treatment prior to their deaths. Such treatment can include infusions of crystalloids and/or colloids, and create a situation where the patient is hemodiluted to the extent that the sample obtained at the time of recovery or within 48 hours of recovery would not be useful for its intended purposes, and loss of the donor will occur. However, a preinfusion sample, if available, would provide for reliable test results and thereby avoid possible loss of an otherwise acceptable donor.

Additionally, if transfusion of blood products takes place while the patient is hospitalized, it is unlikely that the potential donor receiving the transfusion will remain a patient prior to death long enough to meet the testing "window period". Thus, the desire to select a sample in order to detect the most recent potentially transmitted disease will not be met, even if the sample is obtained very close to the time of death.

There is also a growing recognition within the Blood Community, the Federal Government, and the public at large, that the blood supply is safer than it has ever been. New donor screening and testing regimes, such as the recent implementation

of Nucleic Acid Testing (NAT), are being implemented routinely, leading to an ever increasing assurance of only the safest possible blood and tissue supply. Thus, the concern about the potential transmission of an unsafe blood to a potential tissue donor is infinitesimal, and continues to decrease.

ARC also believes that this requirement is not necessary, may significantly reduce the number of potentially available donors, and will not appreciably reduce the associated risks.

### **Definition of Establishment**

There is a significant gap in this proposal that is, in all likelihood, a result of proposing this regulation prior to finalization of the regulation titled *Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products* [63 FR 26744 (May 14, 1998)].

In that regulation, FDA proposed to require registration and listing of establishments engaged in the procurement or recovery of cells or tissues, but excluded those under contract to other organizations. Specifically, page 26750 of that regulation states:

FDA anticipates that individuals engaged solely in the procurement or recovery of cells or tissues ***and under contract*** to organizations that coordinate procurement or recovery of human cells or tissues will not have to independently register under part 1271. Registration will be the responsibility of the employer or contracting organization, which will also be required under future rulemaking to ensure that its employees, agents, and contractors that engage in the recovery of cells or tissues comply with applicable regulations or procedures regarding the collection, safe handling, and proper shipment of human cells or tissues. (Emphasis added)

ARC believes that the exclusion of individuals engaged solely in the procurement or recovery of cells or tissues under contract is a serious omission from that proposal. It is clear that this omission is being carried forward into the Suitability Determination Regulation. This proposal explicitly states on page 52690:

Establishment means a place of business... that engages in the manufacture of human cellular or tissue-based products... The term also includes any individual, partnership, corporation, association, or other legal entity

engaged in the manufacture of human cellular or tissue-based products, *except that an individual engaged solely in the procurement or recovery of cells or tissues or under contract to a registered establishment is not required to independently register.* (Emphasis added)

ARC is troubled by this statement since it appears that the Agency does not plan to extend the registration and listing regulation to include registration of individuals performing procurements under contract, and we do not understand the justification for allowing some procurers to be subject to FDA rulemaking and inspection, while others are not.

ARC discussed this concern at length in its comments on filed under the proposed registration and listing rule. ARC believes that FDA requirements and inspection oversight should extend equally to all types of procurement organizations, regardless of whether these tasks are performed by tissue banks or organizations or individuals that contract their services to tissue banks.

The potential for disease transmission lies in the performance of the actual activity, without regard to affiliation or contractual arrangement with any organization. We therefore believe that the FDA should revise its definition for screening and/or manufacture to require that organizations that are involved in one or more of these two critical parameters also be required to register, to be subject to the donor screening regulations once finalized, and be subject to FDA inspection under all Tissue related regulations. FDA oversight is especially important for contract organizations since they may frequently change their affiliations, or contracts, from one tissue bank to another. Ensuring appropriate oversight can only be conducted consistently by FDA.

ARC's concerns with this omission are magnified under the proposed Suitability Determination regulation.

First, the procurement usually involves obtaining a sample from the donor for disease testing, a key element of this proposed Suitability Determination regulation. Without a registration and listing requirement, FDA will not have an ability to conduct appropriate agency investigations of the standard operating procedures (SOPs), storage, labeling, shipping and other aspects of the sample collection activity when a non-manufacturing organization performs the procurement. It is not only important to state when a sample should be collected, but to ensure other all other aspects of its quality adequacy are maintained when collected by the procuring individual. ARC is concerned about the fact that FDA will inspect some facilities to assure quality practices, but will not inspect others.

Second, while FDA appears to be relying on the manufacturer to ensure that the procurer maintains regulatory safety requirements, there are no FDA standards or guidances for manufacturers for conducting that oversight.

There are numerous activities that should be a part of a manufacturer's oversight program. Quality Assurance inspections, prior review of certain procurement and medical history SOPs, and review of adverse experience reporting and corrective action, are only a few of the activities that should be conducted under a manufacturer oversight program. Yet, FDA is silent on its expectations for all of them. Without extensively detailed specifications, there can be no assurance that oversight will be conducted with the same level of rigor that FDA would perform, or that an organization subject to full FDA investigation, would perform. Further, there is no possibility that screening and obtaining medical history as part of the donor screening process will be conducted with the same level of uniformity by all manufacturers or at the same level of detail for all procurers. Since uniformity and level of detail are key reasons for issuing these regulations, ARC does not believe these goals will be achieved without uniform application to all procurers.

Third, the Donor Suitability proposal is a positive indication of FDA's further movement on its Tissue Action Plan (TAP). The Donor Suitability regulation, and the others anticipated under the TAP, appropriately expands responsibilities for safety assurances in the field of tissue and cellular procurement. Yet, by excluding those who procure under contract, FDA has inadvertently created a direct and clearly defined loophole for those who wish to avoid the regulations. Those who are excluded will be able to operate with less rigorous safety measures and thereby reduce expenses, with full confidence that FDA's inspection and enforcement "clout" is likewise reduced.

Tissue establishments will be hard pressed to remain competitive where the regulatory expenses are encountered so unevenly. ARC believes that some may be forced to either choose to go out of business, or to employ the non-regulated contractors themselves, to reduce their expenses to a competitive level. The net impact of the regulations will be far less safety gain than is FDA's intent and expectation. Thus, if the regulations do not apply to *all* entities, there is no reason to issue them to *any*.

### **Syphilis Testing**

In its proposed rule *Requirements for Testing Human Blood Donors for Evidence of Infection* published on August 19, 1999 [64 FR 45339, Docket No. 98N-0581], FDA indicated an interest in examining the need for testing of blood donors for Syphilis. Specifically, on page 45343, FDA stated that:

The agency is soliciting comments, with supporting data, from the public in regard to the value of donor testing for syphilis as a marker of high risk behavior, as a surrogate test for other infectious diseases, and in preventing the transmission of syphilis through blood transfusion. If the agency receives comments with adequate data supporting the removal of the requirement for a serologic test for syphilis, FDA may proceed with rulemaking to remove the requirements for a serologic test for syphilis, including treponemal and nontreponemal based tests, from part 640.

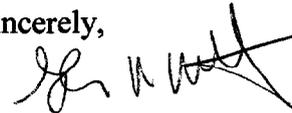
ARC filed public comments on this proposal and included data pertaining to the question of whether syphilis testing could be eliminated as a separate requirement. ARC has attached a copy of the syphilis testing and related data under the Blood Donor Testing proposal to this letter regarding the Suitability Determination for tissue donors.

We request that that as FDA reaches its decisions regarding syphilis testing for blood donors, the Agency reconsider the requirement for syphilis testing for tissue donors. Should FDA eliminate the test for blood donors, we ask FDA to consider eliminating the requirement for tissue donors, as well.

### **Closing**

In closing, Red Cross appreciates the opportunity to comment on the proposed rule. If you have any further questions on this letter, please contact Anita Ducca, Director, Regulatory Relations of Quality Assurance/Regulatory Affairs at (703) 312-5601.

Sincerely,



Glenn M. Mattei, Esq.  
Sr. Director  
Quality Assurance/  
Regulatory Affairs

Attachment

## **Syphilis Testing**

ARC supports FDA's efforts to review relevant data and consider eliminating the requirement for syphilis testing. ARC also acknowledges that sufficient data will be required as described in section A on page 45342 and 45343:

If the agency receives comments with adequate data ... FDA may proceed with rulemaking to remove the requirements for a serologic test for syphilis...

Red Cross has begun research that we believe will support this step. Our initial findings were presented at FDA's meeting on this proposal held at NIH on November 22, 1999, and at a private meeting with FDA/CBER staff on December 15, 1999. We have provided a summary description of our findings below and have attached copies of our presentation materials for additional review by FDA.

We recognize that accurately assessing the value of blood donor serological testing for syphilis in relation to transfusion safety and public health will require extensive quantitative data from multiple sources. Our concerns with blood donor syphilis testing in its present format primarily arise from the very poor predictive value of the test for active syphilis infection. As a result, a very difficult and upsetting result notification message that must be provided to the vast majority of seropositive donors who have never had syphilis infection, or experienced infection many years ago that has long since been treated.

The aspects of this issue that we have explored include: 1) the prevalence of reactive screening tests and positive confirmatory tests among blood donors in our system; 2) the extent to which FTA-ABS confirmed serology among random blood donors does, or does not reflect the presence of circulating T. pallidum DNA; and 3) the relationship of a reactive syphilis serological screening test to unreported behavioral risk in active donors. In the interest of increasing the scientific knowledge base about the potential for transfusion-transmitted syphilis in the US, ARC is willing to consider the funding and implementation of additional studies to expand our current pilot data regarding infectivity of seropositive donor samples, as evidenced by the presence of T. pallidum DNA and RNA. As discussed during the 12/15 meeting with CBER staff, a final sample size for these studies of n = 1000 samples will constitute a sample that is likely to provide infectivity estimates that are reasonably reliable from a statistical standpoint. To examine the possibility of a surrogate relationship between blood donor syphilis seropositivity and infection with other transfusion-transmissible infection, the ARC ARCNET program has also begun an analysis of its systemwide epidemiologic database to determine the extent to which syphilis seropositivity is predictive of prevalent and/or incident HIV, HTLV, HCV, and HBV infection.

Blood Donor Syphilis Testing in the ARC System

Susan Stramer, Ph.D, ARC National Confirmatory Testing Laboratory

All donated blood is screened for total antibody to T. pallidum by PK-TP (PK7200 Olympus). Repeatedly reactive samples are confirmatory tested by FTA-ABS (Zeus) to an interpretation of Positive (2-4+), Minimally Reactive (1+), or Negative. Non-negative samples are then tested by RPR to assist donor notification of test results. Trends in seroprevalence for each of these assays are provided in the attached data sheets.

Relationship of anti-HBc and Serologic Tests for Syphilis (STS) to Blood Donor Behavioral Risk Factors. A.E. Williams, K. Watanabe, D. Ameti, S. Kleinman, M. P. Busch, S. Orton, G. J. Nemo. NHLBI REDS Study, Rockville, MD

Donor screening tests for anti-HBc and STS have limited value for prevention of post-transfusion hepatitis B and syphilis. It is unknown whether these tests have any value for identification of unreported donor risk behaviors. Anonymous mail surveys to measure donor characteristics and deferrable risk (DR98) were administered to 92,581 recent donors at eight blood centers from 4/98 through 10/98. The survey sample was weighted to over-represent anti-HBc+ and STS+ donors and surveys were pre-coded to reflect these results. Odds ratios comparing DR98 among anti-HBc+ and STS+ donors vs. seronegative donors were tested by Chi-Sq.

DR98 prevalence among respondents (weighted data) was 2.9% among 50,267 seronegative donors, 8.0% among 1726 anti-HBc+ donors (OR=2.9; p<0.001), and 13.7% among 414 STS+ donors (OR=5.5; p<0.001). When the donor screening questions related to history of syphilis or treatment for syphilis were removed from the deferrable risk calculation however, deferrable risk in STS+ donors was no higher than the risk in seronegative controls. Because STS+ and anti-HBc+ seroprevalence in the donor pool is low (0.14% and 0.7% respectively), these tests eliminate only a small proportion of total deferrable risk in the unscreened donor pool (1.0% and 2.2% respectively).

Prevalence of *T. pallidum* DNA in the Blood of Donors Who Are Confirmed Positive by Current Serological Tests for Syphilis

SL Orton, MSPH, PhD candidate, RG Cable, MD, AJ Grindon, MD  
AE Williams, Ph.D. American Red Cross ARCNET Program  
Hsi Liu, Ph.D, Centers for Disease Control and Prevention

Based upon the hypothesis that the blood of STS reactive, FTA-ABS reactive donors does not differ from seronegative controls in terms of syphilis infectivity, our study goal was to determine (on a pilot basis) whether STS reactive, FTA-ABS reactive donors showed any evidence of circulating *T. pallidum* DNA. The sample size tested included 100 STS reactive, FTA-ABS samples; 50 of which were RPR reactive, 50 RPR nonreactive. Aliquots from existing platelet concentrates (PC) from these donors were tested for *T. pallidum* DNA using two PCR test methodologies. The first PCR test is specific for *T. pallidum* and sensitive to 25 organisms per 100 ul of extracted material; the second PCR test is a multiplex test that includes testing for *T. pallidum* DNA and is sensitive to 10 organisms per 100 ul of extracted material. Negative and positive external controls were tested. The positive external control was prepared by spiking a 100 ul sample aliquot from an STS nonreactive platelet concentrate with ~50 organisms. All 100 samples were negative for *T. pallidum* DNA by both PCR tests, and all external control results were appropriate. The study had several limitations which included (1) fresh whole blood is a preferable sample, although PC's were adequate for this study, (2) DNA testing cannot differentiate between live and dead organism (not relevant to these results) and (3) in a study of sample size 100 and all negative test results, there is up to a 3% chance that there is an incorrect interpretation of no infectivity. We concluded that we could not demonstrate circulating *T. pallidum* DNA in STS reactive, FTA-ABS positive blood donors. Further work will include RT-PCR testing for RNA (a more sensitive methodology), and should include further study with a larger sample size.

**The following attachment has been submitted to Docket No.  
98N-0581**

**It is also being submitted to Docket No. 97N-484S.**

## Relationship of anti-HBc and Serologic Tests for Syphilis (STS) to Blood Donor Behavioral Risk Factors

AE Williams, K Watanabe, DI Ameti,  
S Kleinman, MP Busch, S Orton, GJ Nemo

Retrovirus Epidemiology Donor Study (REDS)

## Background - anti-HBc

- ◆ Assays for anti-HBc have low specificity and high donor loss (0.7 - 1.8%) when used for screening of donated blood
- ◆ Value of anti-HBc for detection of HBV infection is limited
- ◆ Surrogate value of anti-HBc for behavioral risk detection is speculative

## Background - STS

- Current STS assays detect long term *T. pallidum* antibodies in 0.1 - 0.2% of healthy blood donors.
- No well-documented cases of transfusion-transmitted syphilis have occurred in the US in over 30 years
- Surrogate value of STS for behavioral risk detection is speculative

## Background - STS (cont.)

- 1995 NIH Consensus Conference debated the value of continued blood donor STS screening
- August 1999: FDA seeks data regarding the value of donor STS (Proposed Rules: Requirements for testing....)
  - as a marker of high risk behavior
  - as a surrogate test for other infectious diseases
  - in preventing the transmission of syphilis through transfusion

## Study Objective

- ◆ Assess the value of anti-HBc and STS as surrogate indicators of blood donor risk behaviors

## REDS 1998 Donor Survey

- ◆ ARC, Greater Chesapeake and Potomac Region
- ◆ ARC, Southeastern Michigan Region
- ◆ ARC, Southern California Region
- ◆ Blood Centers of the Pacific - Irwin/UCSF
- ◆ Oklahoma Blood Institute
- ◆ New York Blood Center
- ◆ Blood Bank of San Bernardino
- ◆ Lifeblood (Memphis)
  
- ◆ Medical Coordinating Center - Westat, Inc.

### REDS 1998 Donor Survey (cont.)

- ◆ Anonymous mail survey
- ◆ Allogeneic donors; ≥18 years.
- ◆ Monthly probability sample of donors April through October 1998.
- ◆ 92,581 sampled donors at eight sites
- ◆ 57% survey response rate

### REDS 1998 Donor Survey (cont.)

- ◆ Survey sample included four laboratory test strata:
  - anti-HBc+
  - STS+
  - other lab reactivity
  - seronegative
- ◆ all anti-HBc+ and STS + donors surveyed

### REDS 1998 Donor Survey - Content

- Demographics
- Donation history/experiences
- Deferrable Risk Assessment (DR)
- Multiple Investigations
  - » Surrogate value of STS and anti-HBc
  - » Incentives
  - » Hemochromatosis
  - » HIV test-seeking

### Deferrable Risk

- ◆ A risk that should have resulted in deferral according to blood donor screening criteria at the time of the survey

### Results: Deferrable Risk (DR)

	<u>DR Prev</u>	<u>OR</u>	<u>Adj. OR*</u>
◆ Neg	2.9%	1.0	1.0
◆ anti-HBc	8.0%	2.9†	2.7†
◆ STS+	13.7%	5.4†	5.5†
◆ Other†	11.5%	4.4†	3.3

\* Odds ratios adjusted for gender, age, race/ethnicity, education, center, FT donors (all p < .001)  
 † p < 0.001

### Proportion of Overall DR Associated with anti-HBc and STS (%)

	<u>DR Prev</u>	<u>% of Overall DR</u>
◆ Neg	2.9	94.4
◆ anti-HBc	8.0	2.4
◆ STS+	13.7	1.0
◆ Other†	11.5	2.2

**Proportion of Overall MSM and IDU Risks Associated with anti-HBc and STS (%)**

	MSM	s/MSM	IDU	s/IDU
◆ Neg	94.1	96.5	87.0	93.7
◆ anti-HBc	3.0	2.1	2.5	1.9
◆ STS+	0.3	0.5	0.2	0.5
◆ Other+	2.6	1.0	10.3	3.9

**STS-Related Risks Included in Deferrable Risk Calculation**

Q48. In the past 12 months, have you had a positive test for syphilis?

Q49. In the past 12 months, have you had or been treated for syphilis or gonorrhea?

**Results: Deferrable Risk (DR) excluding STS**

	DR Prev	OR	Adj.OR*
◆ Neg	2.7%	1.0	1.0
◆ anti-HBc	7.3%	2.8 <sup>†</sup>	2.5 <sup>†</sup>
◆ STS+	4.7%	1.7 <sup>‡</sup>	1.3
◆ Other+	11.5%	4.6 <sup>†</sup>	3.6 <sup>‡</sup>

\* Odds ratios adjusted for gender, age, race/ethnicity, education, center, FT donors (all p < .001)  
<sup>†</sup> p < 0.001; <sup>‡</sup> p < 0.05

**Summary: Surrogate value of anti-HBc+**

- ◆ When controlled for FT donor status and demographic factors, anti-HBc+ donors have a 2.7-fold higher level of reported deferrable risk than seronegative donors.
- ◆ Qualitatively, anti-HBc-associated risks are similar to those of the overall donor base
- ◆ When anti-HBc prevalence (0.7%) is considered, anti-HBc+ is associated with 2.4% of overall DR

**Conclusion: Surrogate value of anti-HBc+**

- ◆ The value of anti-HBc as a surrogate needs to be considered in the context of other variables that have modestly higher levels of deferrable risk. (males, FT donors, etc.)

**Summary: Surrogate value of STS**

- ◆ When controlled for FT donor status and demographic factors, STS+ donors have a 5.2-fold higher level of reported deferrable risk than seronegative donors.
- ◆ When STS+ prevalence is considered (0.14%), STS is associated with 1.0% of overall DR

#### Summary - Surrogate value of STS (cont.)

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- ◆ However, deferrable risk associated with STS+ is largely due to STS-related risk factors.
- ◆ When STS-related risk factors are not considered, STS has no significant value as a surrogate indicator of behavioral risk

#### Conclusion

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- ◆ **If molecular studies continue to show an absence of *T pallidum* in STS+ blood, the requirement for STS testing of donated blood should be removed.**

# Prevalence of circulating *T. pallidum* DNA in STS+/ FTA•ABS + blood donors:

- American Red Cross ARCNET Program
  - SL Orton, MSPH, PhD candidate
  - RG Cable, MD
  - AJ Grindon, MD
  - AE Williams, Ph.D.
- Centers for Disease Control and Prevention
  - Hsi Liu, Ph.D.

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Factors that influence an infected individual (with spirochetemia) presenting as a blood donor include:

Symptomatology

Incidence

## Background

- **Primary syphilis: chancre/acute local lymphadenopathy present (97%/80%) ~ 3 weeks after exposure with subsequent organism infiltration of the blood stream. Resolution of the chancre occurs at ~ 6 weeks.**
- **Secondary syphilis: infiltration of the blood stream (and peak spirochetemia) causes systemic macropapular rash development in ~ 100% of infected individuals (~ 6 weeks after exposure), with gradual clearing of the spirochete.**

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The phases overlap.

## continued

- It is unlikely that an individual would be asymptomatic during spirochetemia.
- Rabbit infectivity tests indicate that with disappearance of overt symptoms, the blood loses its ability to infect due to migration of the spirochete to the lymphoid tissue.
- STS are positive except very early in the primary phase.

**continued**

- **CDC reported that in 1998:**
  - **87% decline in incident syphilis cases between 1990 (20.6/100,000) and 1998 (2.6/100,000)**
  - **14 states reported < 5 cases; 5 states reported 0 cases**
  - **78% (2430/3115) US counties reported 0 cases**
  - **50% of incident cases occurred in 0.9% (31/3115) US counties**

## ARC statistics

- 1,801,505 allogeneic donations tested by PK-TP (after diluent modification) between May 1993 and September 1995; representing 16% of total blood collected
- 2151 (0.12%) STS reactive; 1274 (0.07%) confirmed by FTA-ABS
- 6,000,000 donations annually:
  - ~7,200 lost components
  - ~4,200 temporarily deferred donors

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**How does syphilis testing impact the ARC?**

**This data is extracted from a paper by Aberle-Grasse (ARCNET) in Transfusion, 2/99**

## Goal

Determine if there is any evidence of circulating *T. pallidum* in the blood of donors who are STS reactive, FTA-ABS reactive.

## Methods: ARC laboratory infectivity study

- Target sample size: 100 STS reactive, FTA-ABS reactive donations; 50 RPR reactive, 50 RPR non-reactive (including 16 autologous)
- Collect and freeze daily (within ~24 hours) any existing platelet concentrates from PK-TP (Olympus Corp) reactive blood donations. Ship to HL.
- Upon receipt of confirmatory test results, aliquot platelets and send for DNA testing (maximum of 2 freeze/thaw cycles).

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NE: MA, ME, VT, NH:	5 samples
CT:	7
Southern: GA, South Florida	23, 52
GCP: DC, MD	13

## Testing

- PCR for *T. pallidum* specific DNA
  - pol A gene target: 378 bp band
  - capillary electrophoresis and fluorescent detection
  - read on an ABI 310 Genetic Analyzer
  - sensitivity as low as 25 organisms/100 ul platelet concentrate extracted

continued

- Multiplex PCR kit (Roche) for *T. pallidum*, *H. ducreyi* and Herpes Simplex Virus type 1 and 2.
- 47kd basic membrane protein gene target for *T. pallidum* previously described.
- Both assays included internal and external control samples. Positive external controls were diluted to 50 organisms per 100  $\mu$ L from stock *T. pallidum* (Nichols strain) cultures.

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Same sample volume was used.

## Results

- All 100 samples tested negative for *T. pallidum* DNA by both assays.
- Internal and external control samples results were appropriate.

## Study limitations

- The optimal sample is fresh whole blood.
- One weakness of DNA detection is the inability to differentiate live from dead organisms.
- Because we can never “prove” a negative test result, in a pilot study with a sample size of 100 and all negative test results, there is up to a 3% chance that there is an incorrect interpretation of no infectivity.

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The spirochete can tolerate ~3% oxygen tension and then will die ~12 hours. The oxygen tension of the platelet concentrate bag is ~15%. This is probably not the component we should be concerned about.

For the purposes of our study, however, the slow spin separation of platelet rich plasma followed by the hard spin preparation of the platelet concentrate would yield spirochetes in the platelet concentrate bag. In addition, *T. pallidum* DNA is an extremely stable biopolymer.

## Conclusions

- We did not demonstrate circulating *T. pallidum* DNA in STS reactive, FTA-ABS reactive blood donors in this study.
- Because of the low incidence of syphilis in the population, it is unlikely that an infected individual would present as a blood donor.

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From the literature:

The low incidence of disease in the US population (and the demographics of individuals currently found to be infected with syphilis) make it unlikely that an infected individuals will present as a blood donor.

## Conclusions continued

- It is unlikely that a symptomatic individual would present as a blood donor.
- The data suggests that in the absence of syphilis testing, transfusion transmitted syphilis infection is unlikely to occur.

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**From the literature:**

**Due to the symptomatology of this disease during peak spirochetemia (secondary phase), it is unlikely that a symptomatic individual would donate.**

**AND**

**Current information regarding spirochete survival (or lack thereof) in the various blood components, coupled with a lack of evidence that confirmed positive blood donors actually have spirochetemia, makes the potential risk of transfusion transmitted syphilis small.**

## Work in progress

- RT-PCR testing for RNA: may further verify the absence of viable organisms
  - uses the 16S rRNA as a template for reverse transcription
  - sensitivity to  $10^{-3}$  DNA equivalents
  - 100% sensitivity compared with RIT (20 stock sample strains from clinical isolates previously virulent by RIT).

## Proposed study

**Goal:** To determine within specific confidence limits, the extent to which current STS screening protects blood recipients from exposure to transfusion- transmitted syphilis infection.

**Assumption:** Results from the infectivity study indicate that it is unlikely that the blood of STS+/FTA•ABS+ donors is infectious.

## **Proposed study continued**

**Determine prevalence, demographic characteristics and geographic distribution of STS+/FTA•ABS donors within the ARC.**

**Diagnostic work-up will include repeat serology, physical exam and medical history.**

**Research work-up will include additional tests for DNA/RNA.**

## **Proposed study continued**

### **Open questions:**

- **Would demonstrating a lack of infectivity in the donor blood adequately reassure the FDA that the lack of evidence of transfusion transmitted syphilis is because recipients are not contracting syphilis?**
- **Required sample size**
- **Adequacy of proposed laboratory measures**

**Thanks to:**

**ARC ARCNET staff: Jennifer Wolf-Nugent, Margaret Buonanno, Dr. Mark Popovsky, Jonathan Trouern-Trend, Dr. Stan Badon, Kim Munsterman. Nicole Washington, Ingrid Vaquerano, Melinda Tibbals**

**Community Blood Centers of South Florida ARCNET staff: Dr. Bruce Lenes, Angela Buenano, Colleen Reilly, Marilee Olmeda**

**ARC GCP: Monica Reichenbach**

**CDC: Dr. Cheng-Yen Chen**

**Univ of Washington: Dr. Sheila Lukehart**

## Minimal infectious dose

- ~10 spirochetes/ 0.5-1.0 ml is required for a positive RIT.
- DNA PCR would need to detect 1 organism to be comparable (Se ~ 10 organisms)
- Most DNA PCR is sensitive to ~10 organisms
- RT-PCR is sensitive to at least  $10^{-2}$  DNA equivalents
- There is no data on the concentration of organisms in infectious blood

## Syphilis as a surrogate marker

- Herrera, *etal.* Transfusion, 1997.
- Of 4,468,570 donations there might be an estimated 13 HIV window period donations.
- STS testing alone would have removed 0.2 units.
- NAT testing decreases the WP by 27%.
- Using the above donor population and the decrease in the WP, this figure would be even further reduced.

**Syphilis as a surrogate marker:  
ARCNET database**

**Between 1/1/1992 and 3/31/1999:**

- **Of 61,080 PK-TP+, other test negative allogeneic donations, there were 21,485 subsequent donations made (35.2%).**
- **Of these 21,485 subsequent donations, 4 were HIV confirmed positive (.0186%).**
- **With our database capabilities we can have available analyses of other markers and comparisons to the total donor population.**

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Align top of FedEx PowerShip Label or Astra Label here.

RONETTA MITCHELL  
ARC/NHQ  
1616 N FT MYER DR 16TH FL  
ARLINGTON VA 222093100  
(703)312-5827

SHIP DATE: 27DEC99  
ACCOUNT # 173999968  
MAN-WGT: 1 LBS

TO: DOCKET OFFICER  
DOCKETS MANAGEMENT BRANCH (HFA-305)  
FOOD AND DRUG ADMINISTRATION  
5630 FISHERS LANE, RM. 1061  
ROCKVILLE MD 20852

POWERSHIP 3

273 0227 963



273 0227 963

BILL 3rd PARTY

REF: 24700307772

PRIORITY OVERNIGHT

TUE  
AA

CAD # 604845

27DEC99

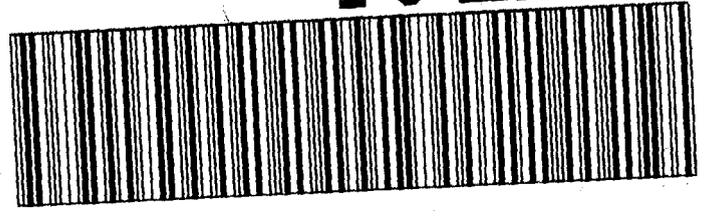
FedEx Pak

Trk#

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