

Effect of Storage on *Treponema pallidum* Infectivity in Whole Blood and Platelets

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Abstract

Background: Universal testing of blood donations for evidence of syphilis has been in place since 1938. Testing and other factors, including routine refrigeration of blood and components, contributed to the disappearance of transfusion-transmitted syphilis (TTS) in the US. Cold temperatures are thought to inactivate *Treponema pallidum*, the spirochete bacterium causing syphilis, rapidly. Some authorities have concluded that universal syphilis testing no longer adds to blood safety.

Purpose. The goal of the project was to reassess whether routine storage of blood and platelets reliably eliminates the risk of TTS. The results of this study should inform future FDA discussions about whether the requirement for universal testing of donor blood for evidence of syphilis warrants reconsideration.

Methodology. We conducted studies mimicking three FDA-approved storage conditions of whole blood and platelets. We spiked fresh human whole blood with treponemes and stored them at 1-6 °C for 9 days. We also spiked fresh human platelets with treponemes and stored them either at room temperature (20-24 °C) or at 1-6 °C for 7 and 14 days respectively. We sampled aliquots at various times after spiking and injected aliquots into rabbit testes, the gold standard model for detecting syphilis infectivity. Control rabbits were inoculated with either uninfected blood samples or inactivated treponemes. We assayed rabbit sera for seroconversion using a commercial treponemal antibody test (TP-PA, Fujirebio) and a non-treponemal (ASI RPR Card Test, Arlington Scientific) and monitored animals for development of overt orchitis. All studies were terminated after 90 or 120 days.

Results. Rabbits developed orchitis and sera tested positive by serology after injection of treponeme-spiked blood and platelets stored up to 7 days. We observed no differences in survival of *T. pallidum* in blood stored cold or at room temperature. No negative control rabbits developed either orchitis or syphilis antibodies.

Conclusions. Treponemes in banked donor blood can survive for at least 7 days. Our data suggest that some Tp remain viable during storage of blood components and offer experimental evidence to inform future discussions about the survival of treponemes in blood.

Introduction

Syphilis, a primarily sexually transmitted disease caused by the spirochete bacterium

Treponema pallidum subsp. *pallidum* (Tp), can also be transmitted through blood transfusions

(transfusion-transmitted syphilis or TTS). Serological testing of donated blood for evidence

syphilis by US blood establishments dates to 1938. The last reported case of TTS in the US

occurred in 1966. The US CFR still requires testing of all donated blood units (and periodic

testing of Source Plasma donations) for evidence of syphilis. Several factors may have

contributed to disappearance of TTS in the US including routine refrigeration of blood and

components, long thought to inactivate spirochetes rapidly. Hence, some in the blood industry

have argued that mandatory screening of blood donations for syphilis no longer adds value

and advocate ending it. However, a few hundred confirmed syphilis-antibody-positive blood

donations are interdicted in the US each year, mostly from first-time donors. This indicates

that a risk of TTS remains and should be addressed before considering the end of universal

syphilis screening of blood. Past studies showed that Tp spiked into whole blood could still

transmit syphilis infections to rabbits in a dose-dependent manner up to 5 days in cold

storage. In these early studies, a maximum of 1 ml of blood was inoculated into each rabbit

and, usually, only 2 or 3 rabbits were used for each test (i.e., 2-3 ml of sample assayed). Our

goal was to assess whether, by increasing the volumes of samples tested, we could detect

infectivity surviving beyond the 5 days of storage reported in the literature. Also, unlike whole

blood, platelet concentrates have not been evaluated to assess survival of Tp in units stored

conventionally at room temperature. (The shelf-life of platelets is 5 days or 7 days when stored

at room temperature.) FDA recently approved a variance to allow use of apheresis platelets

stored cold up to 14 days to treat serious active bleeding when conventional platelets are

unavailable, or their use is impractical. We investigated infectivity recovered from Tp-spiked

platelets stored under both conditions. The results of these studies should inform decisions

about whether to consider discontinuing the requirement for universal syphilis testing of

blood donations.

Materials and Methods

Nichol's strain of *T. pallidum* (NTP) glycerol stocks (obtained from CDC) were propagated in rabbit testes to prepare archival and working stocks. Darkfield microscopy was used to visualize and quantify glycerol stocks. Tp-infected testicular samples were diluted in normal rabbit serum and counted using Nexcelom disposable slides with grids under DF microscopy set at 50X oil magnification

Inoculations

Fresh whole human blood or platelets were spiked with motile Tp or with heat-inactivated Tp (negative control) and stored either in the cold (whole blood and variance platelets) or at room temperature (conventional platelets) temperature for one to two weeks. Aliquots were injected into testes of adult male New Zealand rabbits (1ml per testis, i.e., 2ml total) at various time intervals.

Monitoring rabbits

Tp induces antibodies directed both against Tp antigens (basis of treponemal tests for true syphilis infection) and reagin antibodies directed against the non-specific marker cardiolipin (basis of non-treponemal tests detecting both active syphilis and other inflammatory conditions). Thus, rabbits were assayed weekly for seroconversion using two commercial syphilis tests, Fujirebio Serodia TP-PA Test (treponemal) and ASI RPR Card Test (nontreponemal). Serologically positive rabbits were also checked for development of orchitis, and their testes scored as 0, 1, 2 or 3+ based on increase in size, firmness, palpable nodules, and estimated percentage of tissue involved. We considered results of all tests as well as patterns of serological reactivity over time to assign the final status of each rabbit.

Results and Discussion

First cold storage of Tp spiked whole blood study

Whole human blood was spiked with 1.7 x 10⁶ treponemes/ml of blood and stored at 1-6 °C. Starting from day 0, we collected blood every day and inoculated aliquots into 2 rabbits up to day 9. Rabbits were monitored for seroconversion and orchitis. Table 1 summarizes results.

Table 1. Summary of 1st whole blood study.

Storage (day)	Serodia TP-PA seroconversion (week)	ASI RPR seroconversion (week)	Rabbit euthanized (week)	Final orchitis (score)	Final status	Blind passage
0	+ (1)	+ (3)	3	2-3	Infected	ND
0	+ (1)	+ (3)	3	2-3	Infected	ND
1	+ (3)	+ (3)	4	3	Infected	ND
1	+ (3)	+ (3)	4	3	Infected	ND
2	+ (2)	+ (2)	4	3	Infected	ND
2	+ (2)	+ (1)	3	3	Infected	ND
3	+ (2)	+ (3)	4	1-2	Infected	ND
3	+ (6)	+ (3)	8	1-2	Infected	ND
4	+ (2)	-	3	1-2	Infected	ND
4	+ (8)	-	8	0-2	Infected	ND
5	-	-	12	NA	Uninfected	-
5	-	-	12	NA	Uninfected	-
6	-	-	12	NA	Uninfected	-
6	+ (8)	+ (6)	12	0	Infected	+++
7	-	-	12	NA	Uninfected	-
7	-	-	12	NA	Uninfected	-
8	-	-	12	NA	Uninfected	-
8	-	-	12	0	Uninfected	-
9	-	-	12	0	Uninfected	-
9	-	-	12	NA	Uninfected	-

+ reactive, - nonreactive, NA not applicable, ND not done, +++ positive by Serodia, ASI RPR and orchitis

Tp infectivity was detected in blood stored cold up to 6 days. Results show the week when rabbits seroconverted. Sera of all rabbits inoculated with blood sampled on days 0 to 4 in storage were reactive with both serological tests, and those rabbits developed orchitis. Rabbits inoculated with blood stored for 5 days did not seroconvert with either test. However, 1 of 2 rabbits inoculated with blood stored for 6 days seroconverted. Popliteal lymph node extracts of rabbits from days 5-9 were blind passaged into naive rabbits to increase sensitivity of detection. Only the day-6 blind lymph node passage rabbit became positive by all 3 methods. No rabbits in the negative control group developed either orchitis or syphilis

Additional study of Tp spiked into whole blood and stored cold

To confirm our observations from the 1st experiment, we again spiked whole human blood with 1.4 x 10⁶ treponemes/ml and stored at 1-6 °C. On days 4, 5, and 6, we inoculated 2 rabbits each day; on day 7 we inoculated 8 rabbits to enhance sensitivity of detection near the anticipated end point. Rabbits were monitored for seroconversion and development orchitis. Table 2 summarizes results.

Table 2. Summary of 2nd whole blood study

Storage (day)	Serodia TP-PA seroconversion (week)	ASI RPR seroconversion (week)	Rabbit euthanized (week)	Final orchitis (score)	Final status
4	+ (1)	+ (4)	4	2	Infected
4	+ (1)	-	4	1-3	Infected
5	+ (1)	-	5	0-2	Infected
5	+ (1)	-	5	0	Infected
6	+ (1)	+ (6)	12	0-1	Infected
6	+ (6)	+ (6)	16	0-1	Infected
7	+ (8)	-	16	0	Infected
7	-	-	16	NA	Uninfected
7	-	-	16	NA	Uninfected
7	-	-	16	NA	Uninfected
7	+ (1)	+ (4)	16	0-1	Infected
7	-	-	16	NA	Uninfected
7	-	-	16	NA	Uninfected
7	-	-	16	NA	Uninfected

+ reactive, - nonreactive, NA not applicable

Tp in can survive cold storage up to 7 days. All rabbits inoculated with blood stored for 4 to 6 days seroconverted by Serodia TP-PA treponemal test. Only 2 of 8 rabbits inoculated with blood stored for 7 days were reactive with the Serodia TP-PA test (and only 1 of those reactive by RPR test). Not all rabbits were reactive with all the three tests.

Room temperature storage of Platelets

Fresh apheresis platelets collected in plasma were spiked with 2 x 10⁶ treponemes/ml and stored at room temperature (20-24°C) with gentle agitation on a rocker plate. Starting from day 0, every day up to day 7 an aliquot was inoculated into 2 rabbits. Rabbits were monitored for seroconversion and development orchitis. Table 3 summarizes results.

Table 3. Summary of platelets stored at room temperature study.

Storage (day)	Serodia TP-PA seroconversion (week)	ASI RPR seroconversion (week)	Rabbit euthanized (week)	Final orchitis (score)	Final status
0	+ (1)	+ (2)	4	1-2	Infected
0	+ (1)	+ (3)	4	3	Infected
1	+ (2)	+ (3)	4	1-2	Infected
1	+ (2)	+ (3)	4	2	Infected
2	+ (3)	+ (4)	4	1-2	Infected
2	+ (1)	+ (3)	4	1-3	Infected
3	+ (1)	+ (6)	6	3	Infected
3	+ (6)	+ (6)	12	0	Infected
4	+ (8)	+ (10)	12	0	Infected
4	+ (4)	+ (4)	12	1-2	Infected
5	+ (8)	+ (8)	12	0	Infected
5	+ (1)	-	12	0	Infected
6	+ (1)	-	12	0	Infected
6	-	-	12	NA	Uninfected
7	-	-	12	NA	Uninfected
7	-	-	12	NA	Uninfected

+ = reactive, - = nonreactive, NA = not applicable

Tp survived up to 6 days in platelets stored conventionally at room temperature. Rabbits inoculated with platelets stored for up to 5 days and 1 of 2 rabbits inoculated with day-6 platelets were reactive with the Serodia TP-PA treponemal test. Not all rabbits were positive with all the three tests.

Cold storage of Platelets

Fresh apheresis platelets collected in plasma were spiked with 7 x 10⁵ treponemes/ml and stored them cold (1-6°C). We removed aliquots of spiked platelets to inoculate 4 rabbits on days 1 and 7 and 2 rabbits on days 3, 4, 5, 6, 8, 9, 12, and 14. We monitored for seroconversion and orchitis. Table 4 summarizes results.

Storage (day)	Serodia TP-PA seroconversion (week)	ASI RPR seroconversion (week)	Rabbit euthanized (week)	Final orchitis (score)	Final status
1	+ (3)	+ (4)	8	1-2	Infected
1	+ (4)	+ (6)	8	3	Infected
1	+ (4)	+ (6)	8	3	Infected
1	+ (4)	+ (6)	8	1-2	Infected
3	-	-	16	NA	Uninfected
3	+ (3)	-	12	0-1	Infected
4	+ (3)	-	12	NA	Infected
4	-	-	16	NA	Uninfected
5	-	-	16	NA	Uninfected
5	-	-	16	NA	Uninfected
6	-	-	16	NA	Uninfected
6	+ (3)	-	16	0-1	Infected
7	-	-	16	NA	Uninfected
7	-	-	16	NA	Uninfected
7	-	-	16	NA	Uninfected
7	-	-	16	NA	Uninfected
7	-	-	16	NA	Uninfected
7	-	-	16	NA	Uninfected
7	-	-	16	NA	Uninfected
8	-	-	16	NA	Uninfected
8	-	-	16	NA	Uninfected
9	-	-	16	NA	Uninfected
9	-	-	16	NA	Uninfected
12	-	-	16	NA	Uninfected
12	-	-	16	NA	Uninfected
14	-	-	16	NA	Uninfected
14	-	-	16	NA	Uninfected

+ = reactive, - = nonreactive, NA = not applicable

Tp infectivity was detected in platelets stored cold for up to 6 days. Rabbits from day 1 were reactive both serologic tests and also developed orchitis. One of 2 rabbits from days 3, 4, and 6 became reactive but only with the Serodia TP-PA test,

Conclusion

- Our experiments increased the sensitivity of detecting infectivity by testing larger volumes of blood than previously used to demonstrate that infectious Tp spiked into human donor blood survived for 7 days—at least 2 or even 3 days longer than in earlier reports.

- We demonstrated for the first time that Tp survived up to 6 days in platelets stored both at room temperature and refrigerated.

- Our data suggest that some Tp remain viable during conventional storage of blood components and offer experimental evidence to inform further discussions about the survival of treponemes in blood.

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