Design Verification

SYPHILIS RPR TEST

CONTENTS

1 Function

SYPHILIS RPR TEST has been designed as a Rapid Plasma Reagin test, a screening test for the detection of reagin antibodies, which are indicative for syphilis infection. The test is based on the reaction of anti-reagin antibodies which bind to charcoal-cardiolipin particles leading to visible agglutinates. The test may be performed either as qualitative or semi-quantitative. In the latter case, positive samples are diluted serially with a physiological saline buffer. The titer is expressed as the final dilution producing a positive result .
The test comprises a RPR antigen suspension consisting of cardiolipin and charcoal particles, positive and negative controls.
The test is performed on a 10-area slides. Slides, RPR antigen, dispenser needle and dispenser bottle are provided with the test.

2 Sensitivity and Specificity

• Description of Control Materials
The standard material Anti-syphilis plasma IgG and IgM (human) WHO Standards NIBSC 05/132 has been employed for sensitivity tests (3 IU per ampoule relative to HS, the 1st International Standard for human syphilitic antibodies, the WHO Reference Serum for Serodiagnostic tests for Treponemal Infections- Ref 3-1980).
The standard material has been prepared according to the instructions of the manufacturer and has been serially diluted up to a 1 to 64 ratio by volume.

• Test Performance
1 drop (50 µl) of the standard dilutions is pipetted onto one area of the slide. 1 drop (approx. 16 µl) of RPR antigen suspension is directly applied to the sample drop using the antigen dispensing needle. The slide is slowly tilted back and forth or placed on an automated rotator at low speed (100 rpm) for 8 minutes. Results are interpreted after 8 minutes incubation under direct light.

• Results
Agglutinations are rated according to the following: SR (strong reactive), R (reactive), WR (weakly reactive), TR (trace reactive), NR (non reactive). The results are shown in the table below:

Titre/ Syphilis RPR Lot	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Positive Control	Negative Control
IU/ml	1.5	0.75	0.375	0.1875	0.0937	0.0468	0.0234	0.0117	N/A	N/A
7037502	SR	SR	SR	SR	R	WR	TR	NR	SR	NR
7037701	SR	SR	SR	SR	R	WR	TR	NR	SR	NR
7037325	SR	SR	SR	SR	R	WR	TR	NR	SR	NR

Syphilis International Standard 05/132 testing

• Positive and Negative Kit Controls
The kit controls are employed for function test. The positive control is diluted up to 1 to 8. As controls, the RPR Positive control (Lot 7036410) and the RPR Negative control (Lot 7036992) have been used.

• Results
The results are summarised in the following table:

Titre/ Carbon Antigen Lot	Neat	1/2	1/4	1/8	Negative Control
7037502	SR	SR	WR	NR	NR
7037701	SR	SR	WR	NR	NR
7037325	SR	SR	WR	NR	NR

• Conclusion

The results show all Syphilis RPR lots meeting the expected titre with the positive and negative controls, and obtaining a positive result up to a dilution of 1 to 128 with Syphilis International Standard 05/132. Syphilis RPR detects up to 0.234IU/ml as defined by Syphilis International Standard 05/132 = 1/128 dilution of 3IU/ml.
RPR Positive Control lot 7036410 can be detected up to a dilution of 1/4 which would indicate that it possessed a concentration of 0.936 IU/ml (0.234 X 4).

• Patient Panel Positive and Negative

In-house panels have been established consisting of 8 positive and negative samples each. The positive and negative samples have been prepared from patient sera which have been tested for reagin antibodies. Respective serums have been pooled and aliquoted and are kept deep-frozen (< -20°C). Positive specimens have been pooled in such way to produce weak positive to strong positive results. The panels are employed for testing of each production lot.

3 Correlation

Comparison of SYPHILIS RPR TEST (HUMAN) with a Competitive RPR Test (Becton Dickinson)

25 patient samples from individuals suspected for syphilis infection have been tested with both tests. The relative titres have been compared. Aim of this study was to demonstrate, that SYPHILIS RPR TEST is fully comparable with other RPR tests based on the recommendations of VDRL.

No.	Syphilis RPR (Becton Dickinson)	SYPHILIS RPR (HUMAN)	Comparability
1	16	16	0
2	8	8	0
3	32	32	0
4	32	64	1
5	4	8	1
6	4	2	-1
7	8	8	0
8	64	64	0
9	8	4	-1
10	32	32	0
11	32	64	1
12	2	2	0
13	8	8	0
14	16	32	1
15	64	64	0
16	8	8	0
17	64	64	0
18	32	64	1
19	4	4	0
20	0	0	0
21	32	64	1
22	0	0	0
23	0	0	0
24	32	64	1
25	32	64	1

Overall comparability: +6

Conclusion: Results from 25 patient samples correlated exactly in 15 cases. 8 samples gave increased titres with SYPHILIS RPR TEST from HUMAN, while 2 samples gave decreased titres. The overall comparability (see table 1) has been calculated to +6 which confirms the overall agreement but signifies also a superiority of HUMAN´s SYPHILIS RPR regarding the signal strength.

Comparison against a Competitive Cardiolipin Test

In another study 675 serums have been tested and compared against a competitive cardiolipin test. The 675 samples consist of 645 unselected antenatal serums, 20 cardiolipin reactive serums and 10 cardiolipin non-reactive serums. Complete agreement was found with SYPHILIS RPR test and the competitor cardiolipin test, resulting in 100% diagnostic sensitivity and specificity.

Comparison Study with a Reference Panel

A reference panel from Boston Biomedica Inc. (PSS 202), consisting of 20 members, has been employed to compare the sensitivity of SYPHILIS RPR TEST against a competitive products. The results are summarised in the table according below to the following classification: 4+ grade (very strong agglutination), 3+ (strong agglutination), 2+ (distinct agglutination), 1+ (visible agglutination), (+)(weak agglutination, doubtful), 0 (no agglutination)

PSS202	SYPHILIS RPR	Competitor 1	Competitor 2
#1	4+	4+	4+
#2	4+	4+	4+
#3	3+	3+	3+
#4	0	±	0
#5	(+)	1+	(+)
#6	2+	3+	4+
#7	3+	3+	3+
#8	2+	2+	2+
#9	4+	4+	4+
#10	4+	4+	4+
#11	2+	2+	3+
#12	4+	4+	4+
#13	2+	2+	2+
#14	2+	2+	2+
#15	4+	4+	4+
#16	0	0	0
#17	2+	2+	2+
#18	1+	1+	1+
#19	2+	2+	2+
#20	0	0	0

Conclusion: The results obtained with the reference panel demonstrate a very good agreement with the competitive products and with the panel characterisation supplied by BBI.

Serum / Plasma equivalence

The serum / plasma equivalence for this test method has previously been demonstrated and is published in the literature. Respective articles are cited below.
US Department of Health and Human Services
Centres for Disease Control and Prevention
Program Operations Guidelines for STD Prevention
Medical and Laboratory Services
http://www.cdc.gov/std/Program/medlab/ApE-PGmedlab.htm

"The rapid plasma reagin (RPR) 18-mm circle card test is a macroscopic, nontreponemal flocculation card test used to screen for syphilis (Creighton, 1990). The antigen is prepared from a modified Venereal Disease Research Laboratory (VDRL) antigen suspension containing choline chloride to eliminate the need for heat inactivation of serum, ethylenediaminetetraacetic acid (EDTA) to enhance the stability of the suspension, and finely divided charcoal particles as a visualising agent. For the test, the RPR antigen is mixed with unheated or heated serum or with unheated plasma on a plastic-coated card."

The sensitivity and specificity of the test is determined by the VDRL antigen itself.
The buffer (Choline Chloride, pH Buffer and EDTA) are chemical methods of permitting the use of "unheated or heated serum or with unheated plasma."

A second literature can be found in:

Public Health Report
Vol. 72, No. 9, September 1957, page 761 - 766
Rapid Plasma Reagin Test for Syphilis
JOSEPH PORTNOY, Ph.D., WARFIELD GARSON, M.D., M.P.H., and C. A. SMITH, M.D.
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2031388

Additional 10 RPR positive and 10 RPR negative plasma each were tested to confirm plasma use on SYPHILIS RPR TEST. The testing was performed with three different lots and in parallel with BD RPR test.

EDTA-plasma 3061

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Pos control	Neg control
RPR lot 409	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

EDTA-plasma 3062

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Pos control	Neg control
RPR lot 409	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

EDTA-plasma 3063

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Pos control	Neg control
RPR lot 409	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

EDTA-plasma 3064

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Pos control	Neg control
RPR lot 409	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

EDTA-plasma 3065

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Pos control	Neg control
RPR lot 409	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

EDTA-plasma 3066

	Undiluted	1/2	1/4	1/8	1/16	1/32	Pos control	Neg control
RPR lot 409	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	2+	1+	±	Neg	3+	Neg

EDTA-plasma 3067

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	Pos control	Neg control
RPR lot 409	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	1+	±	Neg	3+	Neg

EDTA-plasma 3068

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Pos control	Neg control
RPR lot 409	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

EDTA-plasma 3069

	Undiluted	1/2	1/4	1/8	Pos control	Neg control
RPR lot 409	1+	±	Neg		3+	Neg
RPR lot 729	1+	1+/±	±	Neg	3+	Neg
RPR lot 556	1+	±	Neg		3+	Neg
BD lot 185	1+/±	±	Neg		3+	Neg

EDTA-plasma 3070

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Pos control	Neg control
RPR lot 409	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

EDTA-plasma negative

Plasma lot	3051	3052	3053	3054	3055	3056	3057	3058	3059	3060	Pos cont	Neg cont
RPR lot 409	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3+	Neg
RPR lot 729	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3+	Neg
RPR lot 556	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3+	Neg
BD lot 185	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3+	Neg

Sodium Citrate-plasma 2991

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Pos control	Neg control
RPR lot 334	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 520	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Sodium Citrate-plasma 2992

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Pos control	Neg control
RPR lot 334	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 520	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Sodium Citrate-plasma 2993

	Undiluted	1/2	1/4	1/8	Pos control	Neg control
RPR lot 334	2+	1+	±	Neg	3+	Neg
RPR lot 520	2+	1+	±	Neg	3+	Neg
RPR lot 556	2+	1+	±	Neg	3+	Neg
BD lot 185	2+	1+	±	Neg	3+	Neg

Sodium Citrate-plasma 2994

	Undiluted	1/2	1/4	Pos control	Neg control
RPR lot 334	1+	±	Neg	3+	Neg
RPR lot 520	1+	±	Neg	3+	Neg
RPR lot 556	1+	±	Neg	3+	Neg
BD lot 185	1+	±	Neg	3+	Neg

Sodium Citrate-plasma 2995

	Undiluted	1/2	1/4	1/8	1/16	Pos control	Neg control
RPR lot 334	3+	2+	1+	±	Neg	3+	Neg
RPR lot 520	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	2+	1+	±	Neg	3+	Neg

Sodium Citrate-plasma 2996

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Pos control	Neg control
RPR lot 334	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 520	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Sodium Citrate-plasma 2997

	Undiluted	1/2	1/4	Pos control	Neg control
RPR lot 334	1+	±	Neg	3+	Neg
RPR lot 520	1+	±	Neg	3+	Neg
RPR lot 556	1+	±	Neg	3+	Neg
BD lot 185	1+	±	Neg	3+	Neg

Sodium Citrate-plasma 2998

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	Pos control	Neg control
RPR lot 334	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 520	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Sodium Citrate-plasma 2999

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Pos control	Neg control
RPR lot 334	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 520	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Sodium Citrate-plasma 2998

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	Pos control	Neg control
RPR lot 334	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 520	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Sodium Citrate-plasma negative

Plasma lot	3001	3002	3003	3004	3005	3006	3007	3008	3009	3010	Pos control	Neg control
RPR lot 334	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3+	Neg
RPR lot 520	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3+	Neg
RPR lot 556	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3+	Neg
BD lot 185	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3+	Neg

Lithium Heparin plasma 3031

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Pos control	Neg control
RPR lot 670	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Lithium Heparin plasma 3032

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Pos control	Neg control
RPR lot 670	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Lithium Heparin plasma 3033

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Pos control	Neg control
RPR lot 670	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Lithium Heparin plasma 3034

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Pos control	Neg control
RPR lot 670	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Lithium Heparin plasma 3035

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Pos control	Neg control
RPR lot 670	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	2+	1+	±		Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Lithium Heparin plasma 3036

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	Pos control	Neg control
RPR lot 670	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Lithium Heparin plasma 3037

	Undiluted	1/2	1/4	Pos control	Neg control
RPR lot 670	1+	±	Neg	3+	Neg
RPR lot 729	1+	±	Neg	3+	Neg
RPR lot 556	±	Neg		3+	Neg
BD lot 185	1+	±	Neg	3+	Neg

Lithium Heparin plasma 3038

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Pos control	Neg control
RPR lot 670	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Lithium Heparin plasma 3039

	Undiluted	1/2	1/4	1/8	1/16	1/32	1/64	Pos control	Neg control
RPR lot 670	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	3+	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	3+	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	3+	3+	2+	1+	±	Neg	3+	Neg

Lithium Heparin plasma 3040

	Undiluted	1/2	1/4	1/8	1/16	Pos control	Neg control
RPR lot 670	3+	2+	1+	±	Neg	3+	Neg
RPR lot 729	3+	2+	1+	±	Neg	3+	Neg
RPR lot 556	3+	2+	1+	±	Neg	3+	Neg
BD lot 185	3+	2+	1+	±	Neg	3+	Neg

Lithium Heparin plasma negative

Plasma lot	3041	3042	3043	3044	3045	3046	3047	3048	3049	3050	Pos control	Neg control
RPR lot 812	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3+	Neg
RPR lot 729	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3+	Neg
RPR lot 556	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3+	Neg
BD lot 185	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	3+	Neg

4 Interferences

By testing serums containing rheumatoid factors an interference with RF positive specimens could be excluded for concentrations up to 1,074 IU/ml. Potentially interfering substances, such as bilirubin, hemoglobin and triglycerides have been added to pooled negative and positive serum.

Results

Substance	Concentration up to	Result with negative serum	Result with positive serum
Bilirubin	20 mg/dl	negative	positive
Hemoglobin	200 mg/dl	negative	positive
Triglycerides	1,000 mg/dl	negative	positive

Conclusion: SYPHILIS RPR TEST showed no interferences with RF positive specimens, with bilirubin, hemoglobin and triglycerides within the tested concentration range.

5 Stability

Real Time Stability

The stability of SYPHILIS RPR TEST has been confirmed by real time studies on 3 independent production lots. The lots have been tested for correct function with their respective kit controls and a reference positive control. The reagents were stored at 2-8 °C and assessed directly after production (month 0) and at 6, 12, 18 and 24 months. All reagents are stored in primary packaging and were opened prior to storage. The stability is therefore applicable for unopened and opened reagents. The results are summarised in the following tables:

Lot: 120427.1
Prod.: 04.2012

Time point	Pos. ref Undiluted	Pos. ref 1/2 dilution	Pos. ref 1/4 dilution	Pos. ref 1/8 dilution	Pos. ref 1/16 dilution	Pos. ref 1/32 dilution	Pos. ref 1/64 dilution	Pos. ref 1/128 dilution	2x Pos. Control	Neg. Control	10x Neg. Plasma
Month 0 30.04.2012	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	2 x pos.	Neg	10x Neg.
Month 6 30.10.2012	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	2 x pos.	Neg	10x Neg.
Month 12 30.04.2013	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	2 x pos.	Neg	10x Neg.
Month 18 28.10.2013	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	2 x pos.	Neg	10x Neg.
Month 24 30.04.2014	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	2 x pos.	Neg	10x Neg.

Lot: 120420.1b
Prod.: 04.2012

Time point	Pos. ref Undiluted	Pos. ref 1/2 dilution	Pos. ref 1/4 dilution	Pos. ref 1/8 dilution	Pos. ref 1/16 dilution	Pos. ref 1/32 dilution	Pos. ref 1/64 dilution	Pos. ref 1/128 dilution	16x Pos. Control	Neg. Control	10x Neg. Plasma
Month 0 30.04.2012	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	16x pos.	Neg	10x Neg.
Month 6 30.10.2012	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	16x pos.	Neg	10x Neg.
Month 12 30.04.2013	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	16x pos.	Neg	10x Neg.
Month 18 28.10.2013	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	16x pos.	Neg	10x Neg.
Month 24 30.04.2014	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	16x pos.	Neg	10x Neg.

Lot: 120420.2e
Prod.: 04.2012

Time point	Pos. ref Undiluted	Pos. ref 1/2 dilution	Pos. ref 1/4 dilution	Pos. ref 1/8 dilution	Pos. ref 1/16 dilution	Pos. ref 1/32 dilution	Pos. ref 1/64 dilution	Pos. ref 1/128 dilution	2x Pos. Control	Neg. Control	10x Neg. Plasma
Month 0 30.04.2012	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	2x pos.	Neg	10x Neg.
Month 6 30.10.2012	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	2x pos.	Neg	10x Neg.
Month 12 30.04.2013	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	2x pos.	Neg	10x Neg.
Month 18 28.10.2013	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	2x pos.	Neg	10x Neg.
Month 24 30.04.2014	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	2x pos.	Neg	10x Neg.

Conclusion: The results with freshly manufactured reagent kits are identical to the results obtained after 24 months storage. The stability of Syphilis RPR Test kit is up to 24 months when stored at 2-8 °C.

Transport stability

SYPHILIS RPR TEST was tested fresh and after transport simulation at 23°C for 8 days. The tests were done with a Biomerieux standard (lot 201, expected titre 1:64) and 6 positive and 6 negative serum samples. The results are summarised below.

Titre	1/2 dilution	1/4 dilution	1/8 dilution	1/16 dilution	1/32 dilution	1/64 dilution	1/128 dilution
Pre-shipment RPR AG	3+	3+	3+	2+	2+	±	neg
Pos sera	703	1588	2489	2487	2491	2492	
	pos	pos	pos	pos	pos	pos	
Neg sera	1057	1471	2876	2877	2035	2039	
	neg	neg	neg	neg	neg	neg	
Post-shipment RPR AG	3+	3+	3+	3+	2+	±	neg
Pos sera	703	1588	2489	2487	2491	2492	
	pos	pos	pos	pos	pos	pos	
Neg sera	1057	1471	2876	2877	2035	2039	
	neg	neg	neg	neg	neg	neg	

Conclusion: The results of the transport simulation show that, under the mentioned conditions, there is no negative impact on the performance of the product.

Serum Stability Study at -20°C

The stability of antibody in serum during storage at -20°C has been confirmed by testing three known positive and three known negative RPR antigen samples fresh and after storage for 2 years at -20°C. The results are summarised in the following.

Lot: X154 Fresh serum samples

Titre	Undiluted	1/2 dilution	1/4 dilution	1/8 dilution	1/16 dilution	1/32 dilution	1/64 dilution	1/128 dilution	1/256	Exp. Titre /Result
Std 1 (bioMérieux)	-	3+	3+	3+	3+	2+	1+	neg	-	1/64
Pos. sample 1	3+	3+	3+	3+	3+	3+	2+	1+	neg	1/128
Pos. sample 2	3+	3+	3+	3+	2+	1+	neg			1/32
Pos. sample 3	3+	3+	3+	2+	1+	neg				1/16
Neg. sample 1	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Neg. sample 2	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Neg. sample 3	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Pos. control	3+	2+	1+	neg						1/4
Neg. control	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

Lot: X232 Serum samples after storage 2 years at -20°C

Titre	Undiluted	1/2 dilution	1/4 dilution	1/8 dilution	1/16 dilution	1/32 dilution	1/64 dilution	1/128 dilution	1/256	Exp. Titre /Result
Std 1 (bioMérieux)	-	3+	3+	3+	3+	3+	2+	1+	neg	1/128
Pos. sample 1	3+	3+	3+	3+	3+	3+	2+	1+	neg	1/128
Pos. sample 2	3+	3+	3+	3+	2+	1+	neg			1/32
Pos. sample 3	3+	3+	3+	2+	1+	neg				1/16
Neg. sample 1	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Neg. sample 2	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Neg. sample 3	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Pos. control	3+	2+	1+	neg						1/4
Neg. control	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

Conclusion: The results with fresh serum samples are identical to the results obtained after 2 years storage at -20°C.

Serum Stability Study at 2...8°C

The stability of antibody in serum during storage at 2...8°C has been confirmed by testing three known positive and three known negative RPR antigen samples fresh and after storage for 7 days at 2...8°C. The results are summarised in the following.

Lot: X232 Fresh serum samples

Titre	Undiluted	1/2 dilution	1/4 dilution	1/8 dilution	1/16 dilution	1/32 dilution	1/64 dilution	1/128 dilution	1/256	Exp. Titre /Result
Std 1 (bioMérieux)	-	3+	3+	3+	3+	3+	2+	1+	neg	1/128
Pos. sample 1	3+	3+	3+	3+	3+	3+	2+	1+	neg	1/128
Pos. sample 2	3+	3+	3+	3+	2+	1+	neg			1/32
Pos. sample 3	3+	3+	3+	2+	1+	neg				1/16
Neg. sample 1	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Neg. sample 2	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Neg. sample 3	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

Pos. control	3+	2+	1+	neg						1/4
Neg. control	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

Lot: X232 Serum samples after storage 7 days at 2...8°C

Titre	**Undiluted**	**1/2 dilution**	**1/4 dilution**	**1/8 dilution**	**1/16 dilution**	**1/32 dilution**	**1/64 dilution**	**1/128 dilution**	**1/256**	**Exp. Titre /Result**
Std 1 (bioMérieux)	-	3+	3+	3+	3+	3+	2+	1+	neg	1/128
Pos. sample 1	3+	3+	3+	3+	3+	3+	2+	1+	neg	1/128
Pos. sample 2	3+	3+	3+	3+	2+	1+	neg			1/32
Pos. sample 3	3+	3+	3+	2+	1+	neg				1/16
Neg. sample 1	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Neg. sample 2	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Neg. sample 3	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Pos. control	3+	2+	1+	neg						1/4
Neg. control	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

Conclusion: The results with fresh serum samples are identical to the results obtained after 7 days storage at 2...8°C.