Design Verification

SYPHILIS TPHA liquid

CONTENTS

1 Assay principle

The SYPHILIS TPHA liquid test kit is used for the diagnosis of treponemal infections. The test allows for a qualitative as well as a semi-quantitative detection of antibodies to *Treponema pallidum* in human serum. The diagnostic test utilizes preserved avian erythrocytes coated with antigens of pathogenic *T. pallidum* (Nichols strain). These bind with specific antibody present in a patient's sample. The cells are suspended in a medium designed to eliminate non-specific reactions. Positive reactions are shown by agglutination of cells, negative reactions settle into a button or small ring. The product is supplied as a set of reagents, including control reagents, positive and negative controls and a diluent.

2 Analytical sensitivity

2.1 Limit of detection

Method

Analytical sensitivity of the SYPHILIS TPHA liquid test kit was determined against the WHO 1st International Standard for human syphilitic plasma IgG and IgM, NIBSC code 05/132. The standard was diluted in sample diluent in the range of 0.0125 – 0.5 IU/ml and assayed semi-quantitatively as specified in the instructions for use.

Materials

Material	Description
International Standard	WHO 1st IS for human syphilitic plasma IgG and IgM, NIBSC code 05/132
HUMAN SYPHILIS TPHA liquid reagents	Test cells, assorted lots Diluent, assorted lots

Results

Lot Test Cells	Lot Diluent	Result						
12052104	12051302	IU/ml	0.2	0.1	0.05	0.025		
		Agglut.	+	+	+	-		
12062901	12062907	IU/ml	0.5	0.25	0.125	0.065	0.0325	0.016
		Agglut.	+	+	+	+	+	-
12071001	12070605	IU/ml	0.1	0.05	0.025	0.0125	0.006	
		Agglut.	+	+	+	-	-	
12081301	12070605	IU/ml	0.5	0.25	0.125	0.065	0.0325	0.016
		Agglut.	+	+	+	+	+	-

Conclusion

HUMAN SYPHILIS TPHA liquid has an expected sensitivity of 0.05 IU/ml against the WHO 1st IS for human syphilitic plasma IgG and IgM, NIBSC code 05/132.

2.2 High-dose hook (prozone) effect

Method

Propensity of the SYPHILIS TPHA liquid test to fail to agglutinate distinctly at a high concentration of analyte – the high-dose hook (prozone) effect – was assessed with high-titre specimens. Three high-titre samples were diluted serially with a negative matrix and assayed with SYPHILIS TPHA liquid according to the instructions for use in the semi-quantitative format. The hook effect was evaluated by assessing the extent of agglutination in wells with high concentrations of sample relative to wells with intermediate and low concentrations of sample.

Materials

Material	Description
High-titre positive samples	PSG 09842, expected titre >40960

	PSG 10188, expected titre 20480 PSG 12948, expected titre >40960
Negative matrix	PSG LS2364156
HUMAN SYPHILIS TPHA liquid	Lot 4J0010TS, Exp. 2016/02

Results

Sample ID	9842		10188		12948		Kit Positive Control		Kit Negative Control	
Titre	Agglut.	Interp.	Agglut.	Interp.	Agglut.	Interp.	Agglut.	Interp.	Agglut.	Interp.
1:80	4+ folding	Positive	4+	Positive	4+	Positive	4+	Positive	- (all wells)	Negative
1:160	4+	Positive	4+	Positive	4+	Positive	3+	Positive		
1:320	4+	Positive	4+	Positive	4+	Positive				
1:640	4+	Positive	4+	Positive	4+	Positive				
1:1280	4+	Positive	4+	Positive	4+	Positive				
1:2560	4+	Positive	4+	Positive	4+	Positive				
1:5120	4+	Positive	3+	Positive	4+	Positive				
1:10240	4+	Positive	2+	Positive	3+	Positive				
1:20480	3+	Positive	1+	Positive	2+	Positive				
1:40960	2+	Positive	+/-	Positive	1+	Positive				

Conclusion

SYPHILIS TPHA liquid does not suffer from a high-dose hook (prozone) effect with specimens with concentrations of treponemal antibodies in a clinically relevant range.

3 Accuracy

3.1 Precision (between-run)

Method

Precision of a quantitative assay is expressed as the coefficient of variation (%CV = standard deviation / mean, expressed per cent). In order to evaluate the between-run (between-assay) precision of the SYPHILIS TPHA liquid test, a QC 1280 positive sample (target titre of 1280 +/- one dilution) was assayed quantitatively in 10 replicates with the same test kit according to the instructions for use.

Acceptance Criteria

%CV ≤ 10%

Results

Titre of 10 replicate assays of the QC 1280 positive sample using reagents of the same SYPHILIS TPHA liquid test kit and statistical analysis relevant to the assessment of between-run precision and bias of the test.

Replicate	Titre
1	1280
2	1280
3	1280
4	1280
5	1280
6	960
7	1280
8	1280
9	1280
10	1280
Mean	1248

Standard deviation	101.1
%CV	8.1%
Bias	-2.5%

Conclusion

10 replicate semi-quantitative assays of a QC 1280 positive control sample with the same SYPHILIS TPHA liquid test kit yielded a within-run coefficient of variation of 8.1%, which is well within the acceptance criteria.

3.2 Bias (between-run)

Method

Bias (trueness) is the agreement between the best estimate of a quantity and its true value. Data presented under 6.1 Precision (between-run) above were used to calculate the between-run bias of the assay. Bias was calculated as per cent of the difference between the measured and the target titre values relative to the target value (|mean – 1280|/1280).

Acceptance Criteria

Bias < 5%

Results

see the results under 6.1 Precision (between-run)

Conclusion

10 replicate assays of a QC 1280 positive control sample with the same SYPHILIS TPHA liquid test kit yielded a between-run bias of 2.5%, which is well within the acceptance criteria.

4 Diagnostic sensitivity and specificity

4.1 Diagnostic sensitivity

Method

Clinical diagnostic sensitivity of the SYPHILIS TPHA liquid test kit was determined using two independent panels of samples collected from syphilis-positive patients. Specimens were assayed qualitatively as specified in the instructions for use.

Materials

Material	Description
Positive syphilis panels	Promedic Syphilis 1 - 80
	St. Georges Syphilis 1 - 20
HUMAN Syphilis TPHA reagents	Test cells, lot 12052104
	Diluent, lot 12051302

Results

Diagnostic sensitivity of the Syphilis TPHA liquid test kit was determined using two panels of samples from patients with diagnosed syphilis.

		SYPHILIS TPHA liquid	
		+	-
Reference diagnostic outcome	+	100	0
	-	0	0

Conclusion

SYPHILIS TPHA liquid showed a diagnostic sensitivity of 100% (95% CI* 96.6 – 100%) on a panel of 100 samples from patients with diagnosed syphilis.
*Clopper-Pearson interval

4.2 Diagnostic specificity

Method

Clinical diagnostic specificity of the SYPHILIS TPHA liquid test kit was determined on a panel of specimens from healthy transfusion blood donors. Specimens were assayed qualitatively as specified in the instructions for use.

Materials

Material	Description
Transfusion blood donor specimens (National Blood Authority, UK)	300 serum samples 300 EDTA plasma samples
HUMAN Syphilis TPHA reagents	Test cells, lot 12041701 Diluent, lot 12051302
Microtiter plates	Greiner U-well

Results

Diagnostic specificity of the Syphilis TPHA liquid test kit was determined using a panel of samples from healthy transfusion blood donors.

		SYPHILIS TPHA liquid			
		Sera		EDTA Plasma	
		+	-	+	-
Reference diagnostic outcome	+	0	0	0	0
	-	0	300	0	300

Conclusion

SYPHILIS TPHA liquid showed a diagnostic specificity of 100% (95% CI* 98.8 – 100%) on a panel of 300 serum samples and 300 EDTA plasma samples from healthy transfusion blood donors.
*Clopper-Pearson interval

5 Cross-reactivity

Method

Following the requirements for WHO prequalification of Syphilis diagnostic tests, potential of SYPHILIS TPHA liquid for false-positive results arising from cross-reactivity was determined for clinically categorized samples of a number of relevant infections or conditions.

Results

Category	No. of samples	Positive	Negative
Potential cross-reactives - Rheumatoid factor positive sera - Epstein Barr virus infections including acute glandular fever - Post Hepatitis B virus vaccination sera - Systemic lupus erythematosus sera - Herpes simples infections sera	50 (10 each)	0	50
Lyme disease	10	0	10
Leptospirosis	10	1	9

Conclusion

SYPHILIS TPHA liquid is highly specific with samples of relevant potentially cross-reacting categories.

6 Interference

SYPHILIS TPHA liquid has been tested for disturbance from common endogenous interfering substances. Furthermore, the probability of flawed interpretation of the results of the assay due to interference of anticoagulant agents has been considered.

Method

Effects of potential interference in haemolysed, lipemic and icteric plasma samples have been assessed in a panel of known syphilis-positive and syphilis-negative plasma samples. Each sample was split into two parts. One part was spiked with pathological concentrations of haemoglobin, bilirubin or triglyceride (see table below). The other part was left without any treatment as a reference. Samples were assayed qualitatively as specified in the instructions for use.

Reference and pathological concentrations of haemoglobin, bilirubin and triglyceride that the samples were spiked to.

Plasma concentration	Haemoglobin, mg/l	Total bilirubin, mg/l	Triglycerid (trioleine), g/l
Normal (adult) range	1.6 – 5.8	2.6 – 14	0.5 – 2.2
Pathological	10000 (Hemolysis)	100 (Icterus)	36 (Lipemia)

Materials

15 syphilis-positive samples
55 syphilis-negative samples
purified haemoglobin, bilirubin, triglyceride
SYPHILIS TPHA liquid kits

Acceptance Criteria

Sample	Assay Result
Syphilis-positive	Positive
Spiked syphilis-positive	Positive
Syphilis-negative	Negative
Spiked syphilis-negative	Negative

Results Summary

All syphilis samples classified as positive tested positive under conditions simulating pathological hemolysis, icterus ad lipemia. All syphilis samples classified as negative tested negative under equivalent conditions.

Conclusion

Haemoglobin, bilirubin and triglyceride at pathological concentrations do not interfere with SYPHILIS TPHA liquid.

7 Stability

7.1 Sample stability

Method

Samples were assayed qualitatively as specified in the instructions for use.

1. 4°C@7 days: A series of positive samples, negative sera and negative plasma were stored at 4°C and tested over 7 days.
2. -20°C@1 month: A series of positive samples, negative serum and negative plasma were stored at -20°C and tested after 1 month.
3. 56°C@3 hours: A series of positive samples, negative serum and negative plasma were split into two parts. One part was incubated at 56°C for 3 hours to simulate a standard virus inactivation procedure in clinical samples by heat treatment. The other part was kept at room temperature for reference.

Results

Effect of extended storage of samples at **4°C** on the quantitative performance of SYPHILIS TPHA liquid.

Sample	Titre				Difference Day 7 vs. Day 0 (4°C)
	Day 0	Day 2	Day 4	Day 7	
QC160	160	160	160	160	none
QC640	640	640	640	640	none
QC1280	1280	1280	1280	1280	none
QC2560	2560	2560	2560	2560	none
QC10240	10240	10240	10240	10240	none
10 negative sera	all negative	all negative	all negative	all negative	none
10 negative plasma	all negative	all negative	all negative	all negative	none

Effect of extended storage of samples at **-20°C** on the quantitative performance of SYPHILIS TPHA liquid.

Sample	Titre		Difference Day 30 vs. Day 0 (-20°C)
	Day 0	Day 30	
QC160	160	160	none
QC640	640	640	none
QC1280	1280	1280	none
QC2560	2560	2560	none
QC10240	10240	10240	none
10 negative sera	all negative	all negative	none
10 negative plasma	all negative	all negative	none

Effect of simulated virus inactivation by incubation at **56°C** for 3 hours on the quantitative performance of SYPHILIS TPHA liquid.

Sample	Positive Samples Titre		Sera Negative		Plasma Negative		Difference RT vs. 56°C
	RT	56°C	RT	56°C	RT	56°C	
P1	80	80	neg	neg	neg	neg	none
P2	80	80	neg	neg	neg	neg	none
P3	160	160	neg	neg	neg	neg	none
P4	160	160	neg	neg	neg	neg	none
P5	160	160	neg	neg	neg	neg	none
P6	2560	2560	neg	neg	neg	neg	none
P7	5120	5120	neg	neg	neg	neg	none
P8	10240	10240	neg	neg	neg	neg	none
P9	20480	20480	neg	neg	neg	neg	none
P10	81920	81920	neg	neg	neg	neg	none

Conclusion

1. Samples are stable for at least 7 days at 4°C.
2. Samples may be stored for at least 30 days at -20°C.
3. Samples may be heat-treated at 56°C for up to 3 hours.

7.2 Shelf life

7.2.1 *Accelerated stability testing*

Short-term data on the stability of a diagnostic device stored at an elevated temperature may be extrapolated to estimate its stability at a lower storage temperature in the long term.

Method

Reagent components of 3 lots of SYPHILIS TPHA liquid were stored at 30°C and tested at 10 occasions over a period of 17 weeks according to the instructions for use.

Acceptance Criteria

Material	Expected outcome	Acceptance
QC 1280	Titre 1280	± 1 doubling dilution (titer level)
WHO 1st IS	Titre 10240	± 1 doubling dilution (titer level)
Kit Positive	Titre 1280	± 1 doubling dilution (titer level)
Kit Negative	negative (neg)	negative
Negative Plasma	negative (neg)	negative

Results

Lot 1

Sample	Weeks									
	0	1	2	4	6	8	10	12	14	17
QC 1280	1280	1280	2560	1280	1280	1280	1280	1280	1280	1280
WHO 1st IS	10240	10240	20480	10240	10240	10240	10240	10240	10240	10240
Kit Positive	1280	1280	640	1280	1280	1280	1280	640	640	640
Kit Negative	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Negative	20x	20x	20x	20x	20x	20x	20x	20x	20x	20x
Plasma	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

Lot 2

Sample	Weeks									
	0	1	2	4	6	8	10	12	14	17
QC 1280	1280	1280	2560	1280	1280	1280	1280	1280	1280	1280
WHO 1st IS	10240	10240	20480	10240	10240	10240	10240	10240	10240	10240
Kit Positive	1280	1280	640	1280	1280	1280	1280	640	640	640
Kit Negative	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Negative	20x	20x	20x	20x	20x	20x	20x	20x	20x	20x
Plasma	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

Lot 3

Sample	Weeks									
	0	1	2	4	6	8	10	12	14	17
QC 1280	2560	2560	2560	1280	1280	1280	1280	640	640	640
WHO 1st IS	20480	20480	20480	10240	10240	10240	10240	10240	10240	10240
Kit Positive	640	640	640	1280	1280	1280	1280	640	640	640
Kit Negative	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
Negative	20x	20x	20x	20x	20x	20x	20x	20x	20x	20x
Plasma	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

Conclusion

For all 3 lots tested, the sensitivity of SYPHILIS TPHA liquid remained within the acceptance criteria. The product is thus stable when stored at 30°C for 17 weeks. 17 weeks at 30°C may be considered equivalent to 24 months at 2...8°C.

7.2.2 *Real-time stability testing*

A shelf life of 24 months derived from the accelerated stability study above has been confirmed by real-time stability testing on SYPHILIS TPHA liquid kits or reagents stored at 2...8°C. The data has been collected in two real-time stability studies:

Study 1 – a real-time shelf life study for the Test and Control Cells reagents

Study 2 – a real-time shelf life study for the Diluent, Positive and Negative Control reagents

Study 1

Performance of the SYPHILIS TPHA liquid Test and Control Cells was tested on a least three lots of each reagent after 25-27 months of storage at 2...8°C.

Method

Testing of TPHA Test and Control Cells stability in real time was carried out according to the instructions for use on multiple lots of standard production material. All reagents were stored at 2...8°C in primary packaging. Moreover, all containers were opened prior to storage. All batches were tested at time of manufacture and again up to 27 months later. Each lot of TPHA Test Cells and Control Cells has been run against the TPHA QC panel (QC Low, Medium and High) for sensitivity, against the kit positive and negative controls to validate the assay and against 20x negative donor plasma for specificity. The QC materials and kit controls were not older than 3 months and validated against a fresh lot of TPHA Test Cells. In addition, each TPHA Test Cells lot has been run against the WHO 1st IS for syphilis (NIBSC 05/132) to determine the analytical sensitivity. All lots under evaluation have additionally been plated on agar to confirm acceptable bioburden level.

Materials

Five lots of TPHA Test and Control Cells tested at 25, 26 or 27 months post manufacture:

Reagent	Lot	Expiry	Post manufacture
TPHA Test Cells	15120801	2017-06	25 months
TPHA Test Cells	15110301	2017-05	26 months
TPHA Test Cells	15112601	2017-05	26 months
TPHA Test Cells	15102101	2017-04	27 months
TPHA Test Cells	15102102	2017-04	27 months
TPHA Control Cells	15120301	2017-05	26 months
TPHA Control Cells	15120302	2017-05	26 months
TPHA Control Cells	15111301	2017-05	26 months
TPHA Control Cells	15110401	2017-04	27 months
TPHA Control Cells	15102002	2017-04	27 months

Approved TPHA Test Cells, Control Cells and kit controls used where required to validate the assay:

Reagent	Lot	Expiry
Approved TPHA Test Cells	18010901	2019107/09
Approved TPHA Control Cells	17121101	2019/06/11
Approved TPHA Positive Control	17121501	2019/12/15
Approved TPHA Negative Control	17121502	2019/12/15

Panel of internal TPHA quality control (QC) materials to evaluate the sensitivity of the assay:

Reagent	Lot	Expiry
QC Low	170821-L	2021-08
QC Medium	170821-M	2021-08
QC High	170821-H	2021-08

Further materials:

Material	Description / Lot No.
International Standard	WHO 1st IS for human syphilitic plasma IgG and IgM, NIBSC code 05/132
Negative donor plasma	Lot 30112/17#3 G1-10 + H1-10; Anticoagulant: EDTA; number of samples: 20

Results

Performance of five lots of **TPHA Control Cells** tested qualitatively at 25-27 months post manufacture against a panel of positive QC samples, kit controls and healthy donor plasma.

Sample	15120301	15120302	15111301	15110401	15102002	Acceptance
QC Low	negative	negative	negative	negative	negative	negative
QC Medium	negative	negative	negative	negative	negative	negative
QC High	negative	negative	negative	negative	negative	negative

Kit positive control	negative	negative	negative	negative	negative	negative
Kit negative control	negative	negative	negative	negative	negative	negative
20x Donor plasma	all negative	all negative	all negative	all negative	all negative	all negative

Performance of five lots of **TPHA Test Cells** tested semi-quantitatively at 26-27 months post manufacture against a panel of positive QC samples, kit controls and healthy donor plasma.

Sample	15120801	15110301	15112601	15102101	15102102	Acceptance
QC Low	80	80	80	80	160	80 – 320
QC Medium	640	640	640	640	1280	640 – 2560
QC High	5120	5120	5120	5120	10240	5120 – 20480
Kit positive control	640	640	320	640	1280	640 – 2560
Kit negative control	negative	negative	negative	negative	negative	negative
20x Donor plasma	all negative	all negative	all negative	all negative	all negative	all negative

Analytical sensitivity of five lots of **TPHA Test Cells** tested semi-quantitatively at 26-27 months post manufacture against the **WHO 1st IS for syphilitic antibodies**, NIBSC 05/132.

Lot	Titre	IU/ml	Acceptance Titre ≥ 80 / IU/ml ≤ 0.1
15120801	80	0.1	80 – 320
15110301	160	0.05	640 – 2560
15112601	80	0.1	5120 – 20480
15102101	80	0.1	640 – 2560
15102102	80	0.1	negative

Colony-forming units per unit volume of reagent lot counted on agar plates prepared and incubated in line with standard procedures for quantification of bioburden in liquid materials.

Reagent	Lot	CFU/ml at 37°C (Acceptance = 5-10 CFU/ml)	CFU/ml at RT (Acceptance = 5-10 CFU/ml)
TPHA Test Cells	15102101, 15102102, 15112601, 15110301, 15120801	0	0
TPHA Control Cells	15120301, 15120302, 15111301, 15110401, 15102002	0	0

Conclusions

TPHA Test Cells

All five TPHA Test Cells lots tested have performed well, achieving post market acceptance criteria against the in-house panel of QC materials and kit controls. 20 samples of healthy donor plasma were non-reactive in all lots as required. All lots tested returned a positive result against the WHO 1st IS for syphilis. Based on the results, the TPHA Test Cells reagent may be assigned a shelf life of 24 months.

TPHA Control Cells

All five lots of TPHA Control Cells included in this real-time stability assessment have fulfilled the quality control acceptance criteria. Testing against the TPHA QC panel (QC Low, Medium and High) and kit positive and negative controls have all returned negative results. The assay was validated using an approved lot of Test Cells. As expected, 20 samples of healthy donor plasma returned a negative result. Based on the results, the TPHA Test Cells reagent may be assigned a shelf life of 24 months.

Study 2

Performance of the SYPHILIS TPHA liquid Diluent, Kit positive control and Kit negative control components was tested on three lots of each reagent after 24 and 25 months of storage at 2…8°C.

Method

A validated lot of TPHA Test Cells has been used to evaluate the materials. Each lot of the diluent has been run against each lot of the positive and negative controls, against a Medium and High internal QC material for sensitivity and against 20x negative donor plasma for specificity. At 25 months, the diluent has also been tested against the WHO 1st IS for syphilis (NIBSC 05/132) to verify the analytical sensitivity. All samples were run in triplicate for each lot of reagents. The endpoint titration for the QC controls and Kit positive control was determined as the lowest reactive concentration for all replicates.

Materials

Lots of SYPHILIS TPHA liquid Kit positive control, Kit negative control and Diluent components tested:

Reagent	Lot	Expiry
TPHA Diluent	17062601	30.06.2019
TPHA Diluent	17080701	31.08.2019
TPHA Diluent	17070301	31.07.2019
TPHA Kit positive control	17072405	31.01.2019
TPHA Kit positive control	17090702	30.09.2019
TPHA Kit positive control	17091502	30.09.2019
TPHA Kit negative control	17072406	30.04.2019
TPHA Kit negative control	17081601	31.08.2019
TPHA Kit negative control	17091503	30.09.2019

Additional validated reagents of the SYPHILIS TPHA liquid assay used to evaluate the performance of the above lots:

Reagent	Lot	Expiry
TPHA Test Cells	19020101	2020-08-01
QC Medium	180906-M	2021-08
QC High	170821-H	2021-08

Further materials:

Material	Description / Lot No.
International Standard	WHO 1st IS for human syphilitic plasma IgG and IgM, NIBSC code 05/132
Negative donor plasma	Lot 30112/17#3 G1-10 + H1-10; Anticoagulant: EDTA; number of samples: 20

Results

Titre or qualitative reactivity of three lots of TPHA Diluent, Kit positive control (PC) and Kit negative control (NC) stored at 2...8°C and tested at **24 months** post manufacture on TPHA Test Cells (lot #19020101) against each other, against a panel of positive QC samples, and against healthy donor plasma:

Sample	Lot	Diluent			Acceptance
		17062601	17080701	17070301	
QC Medium	180906-M	640	640	640	640 – 2560
QC High	170821-H	5120	5120	5120	5120 – 20480
Kit positive control	17072405	1280	1280	1280	640 – 2560
	17090702	640	640	640	640 – 2560
	17091502	640	640	640	640 – 2560
Kit negative control	17072406	negative	negative	negative	negative
	17081601	negative	negative	negative	negative
	17091503	negative	negative	negative	negative
20x Donor plasma	Lot 30112/17#3	all negative	all negative	all negative	all negative

Titre or qualitative reactivity of three lots of TPHA Diluent, Kit positive control (PC) and Kit negative control (NC) stored at 2...8°C and tested at **25 months** post manufacture on TPHA Test Cells (lot #19020101) against each other, against a panel of positive QC samples, and against healthy donor plasma and against the **WHO 1st IS for syphilitic antibodies** NIBSC 05/132:

Sample	Lot	Diluent			Acceptance
		17062601	17080701	17070301	
QC Medium	180906-M	640	640	640	640 – 2560
QC High	170821-H	5120	5120	5120	5120 – 20480
Kit positive control	17072405	1280	1280	1280	640 – 2560
	17090702	640	640	640	640 – 2560
	17091502	640	640	640	640 – 2560
Kit negative control	17072406	negative	negative	negative	negative
	17081601	negative	negative	negative	negative
	17091503	negative	negative	negative	negative
20x Donor plasma	Lot 30112/17#3	all negative	all negative	all negative	all negative
WHO 1st IS	-	0.05 IU/ml	0.05 IU/ml	0.05 IU/ml	≤ 0.1 IU/ml

Conclusion

All lots of TPHA Diluent, Kit positive control and Kit negative control stored at 2...8°C have performed well when tested at 24 and 25 months post manufacture, achieving post market acceptance criteria. Based on the results, a shelf life of 24 months is assigned to these reagents.

7.3 Open vial stability

Open vial stability of HUMAN Syphilis TPHA liquid has been determined by putting the kit in standard use on five occasions at regular intervals spanning a period of 6.5 months.

Method

At each occasion, all kit components were brought to room temperature. A semi-quantitative test has then been performed with 5 QC samples (Accurun 155 Syphilis TPHA standard, 2 positive patient samples, 2 negative patient samples) and both kit controls as per the instructions for use. Accurun 155, positive samples and the PC were titrated serially across the plate. Negative samples and the NC were assessed at a single dilution of 1/80 with the control cells and the test cells. Following the test, all components were packed back in the kit box and stored at 2-8 °C until the next measurement.

Materials

Assay

Assay	HUMAN Syphilis TPHA
REF	50101
Lot	20001
Expiry	30.11.2021

Patient samples

	ID	supplier
negative 1	190701-21	UNI-Klinik MD
negative 2	190701-22	UNI-Klinik MD
positive 1	1855	LG Schenk
positive 2	1859	LG Schenk

Accurun 155 Syphilis TPHA Standard

REF	A155-2010
Lot	10437715
Expiry	2021-08-06
Supplier	SeraCare

Acceptance criteria

Control cells: all samples always negative

Test cells

Kit negative control	always negative
Negative samples	always negative
Kit positive control	titer(0)* as per the instructions for use ± 1 titer level
Positive samples and standards	titer(0)* as determined with fresh kit ± 1 titer levels

*titer(0) is the titer determined for the given sample at time point 0

Results (titer with lowest positive result / cut-off titer)

Time point (months)	**0**	**1.5**	**3**	**4.5**	**6.5**	**Criterion**
Accurun 155	1:2560	1:1280	1:2560	1:1280	1:1280	titer(0) ± 1 titer levels
Kit positive control	1:1280	1:1280	1:1280	1:1280	1:1280	1:1280 ± 1 titer levels
1855	1:2560	1:2560	1:2560	1:2560	1:1280	titer(0) ± 1 titer levels
1859	1:2560	1:1280	1:2560	1:2560	1:2560	titer(0) ± 1 titer levels
Kit negative control	neg	neg	neg	neg	neg	negative
190701-21	neg	neg	neg	neg	neg	negative
190701-22	neg	neg	neg	neg	neg	negative

Evaluation

Control cells always negative?	yes	yes	yes	yes	yes
Test cells within specification?	yes	yes	yes	yes	yes

Conclusion

All acceptance criteria have been met on all occasions. No signs of performance decline have been observed over the period of evaluation. Based on these results, HUMAN Syphilis TPHA liquid is assigned an open vial stability of 6 months.