INSTITUTE OF TROPICAL MEDICINE ANTWERP

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Evaluation report
STANDARD Q HIV/Syphilis Combo Test
(SD BIOSENSOR)

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Table of Contents

Introduction and description of the assay (from the kit insert)

Introduction

STANDARD Q HIV/Syphilis Combo Test is a rapid chromatographic immunoassay for the qualitative detection of antibodies specific to HIV-1 including subtype O, HIV-2 and Syphilis (*Treponema pallidum*) in human serum, plasma or whole blood.

Description of the assay

STANDARD Q HIV/Syphilis Combo Test has "H1", "H2", "SYP" and "C" line pre-coated with recombinant HIV-1 GP41 protein / recombinant HIV-1 subtype O GP41, recombinant HIV-2 GP36 protein, recombinant p17 Treponema pallidum protein (recombinant TPP17 protein) and monoclonal anti-HIV-1 / monoclonal anti-syphilis respectively. The anti-HIV-1/anti-HIV-1 subtype O in patient sample interacts with the recombinant HIV-1 GP41-gold / recombinant HIV-1 subtype O GP41-gold and the anti-HIV-2 in patient sample interacts with the recombinant HIV-2 GP36-gold in the conjugation pad. The anti-syphilis in patient samples interacts with the recombinant TPP 17-protein gold. The complex moves along the membrane chromatographically with assay diluent and is captured by the recombinant HIV antigens and/or recombinant TPP 17 protein on each test line (H1, H2, SYP). If the antibodies against HIV and/or syphilis are in the patient sample, visible lines are formed in the test region. The control line should always appear if the test procedure is performed properly.

Kit components

A kit of 25 tests contains:

- Test device, individually pouched in an aluminum pouch with a desiccant
- Assay diluent
- Capillary tube (20µl)
- Lancet
- Alcohol swab
- Instructions for use

Remark: The volume of assay diluent and number of capillary tubes, lancets and alcohol swabs is not specified in the instructions for use.

Storage and stability

- Kits must be stored at 2-40°C.
- Kits materials are stable until the expiration date printed on the outer box.
- Do not freeze kits.

Specimen collection and preparation

The Standard Q HIV/Syphilis Combo Test can be performed using whole blood (venous or capillary), serum or plasma.
Venous whole blood:

- Venous whole blood samples must be collected in tubes with heparin, EDTA or sodium citrate as anticoagulant.
- If stored in the refrigerator (2-8°C) it must be used for testing within 1-2 days after collection.
- Do not use hemolyzed blood samples.

Capillary whole blood:

- Capillary whole blood should be collected aseptically by fingertip.
- Capillary whole blood must be tested immediately after collection.

Serum/plasma:

- For plasma, venous whole blood samples must be collected in tubes with heparin, EDTA or sodium citrate as anticoagulant.
- For serum, venous whole blood samples must be collected in plain tubes NOT containing anticoagulants.
- If specimens are not tested immediately, they should be stored at 2-8 C° and tested within 1 week. For prolonged storage, the specimens must be stored at below -40°C.
- Plasma and serum specimens should be brought to room temperature prior to use.

Remark: Room temperature is not specified in the instructions for use.

Assay procedure

For venous whole blood/serum/plasma specimen:

- Add 20µl of venous whole blood or 10µl of serum/plasma to the sample well (using a micropipette).
- Add 3 drops of assay diluent into the sample well.
- Read the results at 15 minutes. Do not read the test results after 20 minutes.

For capillary whole blood specimen:

- Add the collected 20µl of capillary whole blood to the sample well.
- Add 3 drops of assay diluent into the sample well.
- Read the results at 15 minutes. Do not read the test results after 20 minutes.

Limitations and remarks of the assay (from the kit insert)

- The 3 test lines (H1, H2 and C) may develop when tested with samples containing high titers of HIV-1 antibodies. Hence, reactive test bands for both HIV-1 and HIV-2 may not indicate mixed infection but may result from the cross-reactivity of HIV-1 an HIV-2 because of the similarity of their genomic structure.
- A negative test result may occur if the level of extracted antibody in a sample is below the sensitivity of the test or if a poor-quality specimen is obtained.

Evaluation method and panels

The evaluation was performed according to the agreement 'HIV/Syphilis Test Kit Evaluation – ITM/QARAD-SD BIOSENSOR HIV/Syphilis' dated January 2018 and 'Clinical Performance Study Plan - CPSP nr. BSS-ITM 17-033'. All manipulations were carried out in a restricted laboratory level 2 in accordance with the Belgium law and in conformity with the quality standards of ISO 17025.

Study design

The aim of this evaluation is to provide an objective measure of the ability of the test to identify the presence of HIV-1, HIV-2 and/or Syphilis antibodies by the use of seven archived (frozen) testing panels (consisting of well characterized HIV positive/negative and syphilis positive/negative serum/plasma samples) and one fresh collected EDTA whole blood/plasma panel.
Briefly, the results obtained with the STANDARD Q HIV/Syphilis Combo Test will be compared with the outcome of the combined reference tests used for characterization of the different panels.

Evaluation panels

All reference assays were interpreted according to the instructions given by the manufacturer.

HIV-1 positive / Syphilis negative specimens, n=210

Frozen (± -25°C) serum/EDTA plasma specimens (Annex 1).
All specimens are screened with one or two of the following CE marked ELISAs; Vironostika HIV Uni-Form II Ag/Ab (bioMérieux), Vironostika HIV Ag/Ab (bioMérieux), Genscreen ULTRA HIV Ag-Ab (BIO-RAD) or VIDAS HIV DUO Quick (biomérieux) and further characterized by the CE marked INNO-LIA HIV I/II Score (Fujirebio).
All specimens are found negative for Syphilis using VITROS® Syphilis TPA Assay (Ortho Clinical Diagnostics) and a non-treponemal test: BD Macro-Vue™ RPR Card Tests (Becton Dickinson).

HIV/Syphilis positive serum/plasma samples, n=150

Frozen (± -25°C) serum/EDTA plasma specimens (Annex 2).
For HIV, all specimens are screened with one or two of the following CE marked ELISAs; Enzygnost Anti-HIV 1/2 Plus (SIEMENS), Genscreen HIV-1/2 Version 2 (BIO-RAD), Genscreen ULTRA HIV Ag-Ab (BIO-RAD) or VIDAS HIV DUO Quick (biomérieux) and further characterized by the CE marked INNO-LIA HIV I/II Score (Fujirebio).
For Syphilis, all specimens are tested with both CE-marked reference tests: VITROS® Syphilis TPA Assay (Ortho Clinical Diagnostics) and non-treponemal test: BD Macro-Vue™ RPR Card Tests (Becton Dickinson) and found positive with one or both reference tests. In case of discordant results between TPA and RPR, specimens are further characterized with a second treponemal test: Serodia® TP-PA (Fujirebio) .

HIV negative / Syphilis positive serum/plasma samples, n=150

Frozen (± -25°C) serum specimens (Annex 3).
All specimens are found HIV negative based on screening with at least one of the following CE marked ELISAs; Enzygnost Anti-HIV 1/2 Plus (SIEMENS), Genscreen HIV-1/2 Version 2 (BIO-RAD), Genscreen ULTRA HIV Ag-Ab (BIO-RAD) or VIDAS HIV DUO Quick (Biomérieux).
For Syphilis all specimens are tested with both CE-marked reference tests: VITROS® Syphilis TPA Assay (Ortho Clinical Diagnostics) and non-treponemal test: BD Macro-Vue™ RPR Card Tests (Becton Dickinson) and found positive with one or both reference tests. In case of discordant results between TPA and RPR, specimens are further characterized with a second treponemal test: Serodia® TP-PA (Fujirebio) .

HIV-1 positive samples belonging to different subtypes, n=40

Frozen (± -25 °C) EDTA plasma specimens with known subtype (Annex 4). The plasma samples were selected retrospectively on the basis of their HIV-1 subtype from patients attending the clinic of the Institute of Tropical Medicine in Antwerp. The plasma samples were characterized the same way as the other HIV-1 positive specimens.
The isolates from the patients were subtyped by sequencing and bootstrap analysis of the *pol* region.

Subtype *pol*	Number of samples
A	2
A1	2
C	3
CRF01_AE	3
CRF02_AG	3
CRF06_cpx	3
CRF36_cpx	3
D	3
F1	2
F2	1
G	3
H	3
J	3
K	3
Group O	3

http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html

HIV-2 positive specimens, n=60

Frozen (± -25°C) serum/EDTA plasma specimens characterized according to the ARL's testing algorithm. All specimens are screened with one or two of the following CE marked ELISAs; Vironostika HIV Uni-Form II Ag/Ab (bioMérieux), Vironostika HIV Ag/Ab (bioMérieux), Enzygnost Anti-HIV 1/2 Plus (SIEMENS), Genscreen HIV-1/2 Version 2 (BIO-RAD) or Genscreen ULTRA HIV Ag-Ab (BIO-RAD) and further characterized by the INNO-LIA HIV Confirmation or the CE marked INNO-LIA HIV I/II Score (Fujirebio). See Annex 5.

Fresh paired HIV-1/Syphilis positive EDTA whole blood/plasma samples, n=100

To collect the paired EDTA whole blood/plasma samples, the EDTA blood taken for routine follow-up of known HIV-1 infected patients, attending the clinic of the Institute of Tropical Medicine, was used. See Annex 6.
The samples, both EDTA whole blood and plasma, were tested within 24 hours after drawing the blood and the plasma specimens were used to characterize for HIV with Genscreen HIV-1/2 v2 (BIO-RAD). For Syphilis the specimens were characterized using VITROS® Syphilis TPA Assay (Ortho Clinical Diagnostics) and BD Macro-Vue™ RPR Card Tests (Becton Dickinson) and, in case of discordant results between TPA and RPR, with a second treponemal test: Serodia® TP-PA (Fujirebio).

Fresh paired HIV-1 positive/Syphilis negative EDTA whole blood/plasma samples, n=37

To collect the paired EDTA whole blood/plasma samples, the EDTA blood taken for routine follow-up of known HIV-1 infected patients, attending the clinic of the Institute of Tropical Medicine, was used. Inclusion in the panel was based on the result of the BD Macro-Vue™ RPR Card Tests (Becton Dickinson) of the previous follow-up sample of that HIV-1 infected patient.
The samples, both EDTA whole blood and plasma, were tested within 24 hours after drawing the blood and the plasma specimens were used to characterize for HIV with Genscreen HIV-1/2 v2 (BIO-RAD). For Syphilis the specimens were characterized using VITROS® Syphilis TPA Assay (Ortho Clinical Diagnostics) and BD Macro-Vue™ RPR Card Tests (Becton Dickinson) and, in case of discordant results between TPA and RPR, with a second treponemal test: Serodia® TP-PA (Fujirebio). See Annex 6.

Commercial seroconversion panels, n = 20

All panel members of the commercial available seroconversion panels (PRB930, PRB934, PRB947, PRB950, PRB951, PRB952, PRB953, PRB954, PRB955, PRB956, PRB958, PRB959, PRB966, PRB968, PRB969, PRB970, PRB973, PRB977, available from SeraCare Diagnostics, HIV9089 and HIV 9096 available from Zeptometrix) were screened with 2 CE marked EIAs: Vironostika HIV Ag/Ab (bioMérieux), Vironostika HIV Uniform II Ag/Ab (bioMérieux) or Vidas HIV Duo Quick (bioMérieux) as fourth generation assay and Enzygnost Anti-HIV 1/2 Plus (Siemens) or Genscreen HIV ½ v2 (BIO-

RAD) as third generation assay and the INNOTEST HIV Antigen mAb (Fujirebio) and further characterized by the CE marked INNO-LIA HIV I/II Score (Fujirebio). See Annex 7.

Potentially cross-reacting HIV/Syphilis negative samples, n=40

Frozen (± -25°C) serum specimens (Annex 8).
Potentially cross-reacting serum samples were selected retrospectively from patients attending the clinic of the Institute of Tropical Medicine in Antwerp.
All specimens are found HIV negative based on screening with at least one of the following CE marked ELISAs; Vironostika HIV Uni-Form II Ag/Ab (bioMérieux), Vironostika HIV Ag/Ab (bioMérieux), Enzygnost Anti-HIV 1/2 Plus (SIEMENS), Genscreen ULTRA HIV Ag-Ab (BIO-RAD) or VIDAS HIV DUO Quick (Biomérieux) and negative for Syphilis using VITROS® Syphilis TPA Assay (Ortho Clinical Diagnostics) and a non-treponemal test: BD Macro-Vue™ RPR Card Tests (Becton Dickinson).

Number of samples	Potential cross-reactivity
10	Rheumatic Factor
5	CMV
5	EBV
5	Malaria
5	HSV
5	Borrelia
5	Leptospirose

Results

All samples included in this evaluation were tested with the STANDARD Q HIV/Syphilis Combo Test lot number QHI2017003-1, expiry date 27th of December 2019.
The test was performed by a stagiaire under supervision of an experienced lab technician.
All samples were added following the instructions given by the manufacturer, using a calibrated micropipette.
The results were visually interpreted independently by two operators, within 15-20 minutes after adding the buffer. Results that were discordant between the two readers were interpreted by a third reader. Specimens with a result discrepant from the reference result were repeated in duplicate, the result that occurred the most was reported as final result.

Equipment used

Not applicable.

Validation of the Standard Q HIV/Syphilis Combo Test

The red colored control band was visible on all test devices.
The control samples, run at the beginning of each testing session, were valid.

Interpretation of test results

Negative Result: The presence of only the "C" line indicates a negative result.
HIV-1 Positive Result: The presence of two lines, "C" and "H1", indicates a positive result for HIV-1. In case of the presence of three lines, "C", "H1" and "H2", if the intensity of the "H1" line is stronger than the "H2" line, it should be interpreted as HIV-1 positive.
HIV-2 Positive Result: The presence of two lines, "C" and "H2", indicates a positive result for HIV-2. In case of the presence of three lines, "C", "H2" and "H1", if the intensity of the "H2" line is stronger than the "H1" line, it should be interpreted as HIV-2 positive.
Syphilis Positive Result: The presence of two lines, "C" and "SYP", indicates a positive result for Syphilis.
HIV-1 & Syphilis Positive Result: The presence of three lines, "C", "H1" and "SYP", indicates a positive result for HIV-1 and Syphilis.
HIV-2 & Syphilis Positive Result: The presence of three lines, "C", "H2" and "SYP", indicates a positive result for HIV-2 and Syphilis.
Invalid Result: No presence of "C" line indicates an invalid result.

HIV-1 positive / Syphilis negative samples, n = 210.

STANDARD Q HIV/Syphilis Combo Test							
Initial				Final			
HIV-1 antibodies		Syphilis antibodies		HIV-1 antibodies		Syphilis antibodies	
Reactive	210	Reactive	4	Reactive	210	Reactive	4
Non-reactive	0	Non-reactive	206	Non-reactive	0	Non-reactive	206
Total	210	Total	210	Total	210	Total	210

STANDARD Q HIV/Syphilis Combo Test was reactive for all HIV-1 specimens. Initially 4 specimens were false reactive for Syphilis (HIV_SYPH/392, HIV_SYPH/432, HIV_SYPH/488 and HIV_SYPH/490). These samples were repeated in duplicate and remained false reactive. 29,5% of all specimens also showed reactivity on the test line for HIV-2. In all cases the observed intensity of the HIV-2 line was lower than the HIV-1 line.

HIV-1: Initial and final sensitivity (95% CI): 210/210 X 100 = 100% (98,3% - 100%).
Syphilis: Initial and final specificity (95% CI): 206/210 x 100 = 98,1% (95,2% - 99,5%).

The detailed results obtained with this panel for the STANDARD Q HIV/Syphilis Combo Test are shown in annex 1.

Conclusion: All specimens were correctly classified for HIV-1 antibodies by the STANDARD Q HIV/Syphilis Combo Test. Four samples were classified as false reactive for Syphilis antibodies.

HIV-1/Syphilis positive serum/plasma samples, n=150

STANDARD Q HIV/Syphilis Combo Test							
Initial				Final			
HIV-1 antibodies		Syphilis antibodies		HIV-1 antibodies		Syphilis antibodies	
Reactive	150	Reactive	148	Reactive	150	Reactive	148
Non-reactive	0	Non-reactive	2	Non-reactive	0	Non-reactive	2
Total	150	Total	150	Total	150	Total	150

STANDARD Q HIV/Syphilis Combo Test was reactive for all HIV-1 specimens. Initially 2 specimens were false non-reactive for Syphilis antibodies (HIV_SYPH/080 and HIV_SYPH/147). These samples were repeated in duplicate and remained non-reactive for Syphilis antibodies. 30,7% of all specimens also showed reactivity on the test line for HIV-2. In all cases the observed intensity of the HIV-2 line was lower than the HIV-1 line.

HIV-1: Initial and final sensitivity (95% CI): 150/150 X 100 = 100% (97,6% - 100%).
Syphilis: Initial and final sensitivity (95% CI): 148/150 x 100 = 98,7% (95,3% - 99,8%).

The detailed results obtained with this panel for the STANDARD Q HIV/Syphilis Combo Test are shown in annex 2.

Conclusion: All specimens were correctly classified for HIV-1 antibodies by the STANDARD Q HIV/Syphilis Combo Test. Two were classified as false non-reactive for Syphilis antibodies.

HIV-1 negative / Syphilis positive serum/plasma samples, n=150

STANDARD Q HIV/Syphilis Combo Test							
Initial				Final			
HIV-1 antibodies		Syphilis antibodies		HIV-1 antibodies		Syphilis antibodies	
Reactive	2	Reactive	149	Reactive	1	Reactive	149
Non-reactive	148	Non-reactive	1	Non-reactive	149	Non-reactive	1
Total	150	Total	150	Total	150	Total	150

STANDARD Q HIV/Syphilis Combo Test was initially false reactive for HIV-1 antibodies for 2 specimens (HIV_SYPH/004 and HIV_SYPH/022) and false non-reactive for Syphilis antibodies for 1 specimen (HIV_SYPH/029). These samples were repeated in duplicate. Specimen HIV_SYPH/022 became concordant with the reference result, the others remained discordant.

HIV-1: Initial specificity (95% CI): 148/150 X 100 = 98,7% (95,3% - 99,8%).
Final specificity (95% CI): 149/150 X 100 = 99.3% (96.3% - 100.0%).
Syphilis: Initial and final sensitivity (95% CI): 149/150 x 100 = 99,3% (96,3% - 100%).

The detailed results obtained with this panel for the STANDARD Q HIV/Syphilis Combo Test are shown in annex 3.

Conclusion: STANDARD Q HIV/Syphilis Combo Test misclassified one HIV-1 negative specimen as false reactive for HIV antibodies and 1 Syphilis positive specimen as false non-reactive for Syphilis antibodies.

HIV-1 positive samples belonging to different subtypes, n = 40.

STANDARD Q HIV/Syphilis Combo Test			
HIV-1 antibodies		Syphilis antibodies	
Reactive	40	Reactive	3
Non-reactive	0	Non-reactive	37
Total	40	Total	40

STANDARD Q HIV/Syphilis Combo Test was reactive for HIV-1 antibodies for all specimens. 25% of all specimens also showed reactivity on the test line for HIV-2. In all cases the observed intensity of

the HIV-2 line was lower than the HIV-1 line. All 3 HIV-1 non-B subtype specimens with reactivity on the Syphilis test line were positive in the Syphilis reference test TPA.

HIV-1: Sensitivity (95% CI): 40/40 X 100 = 100% (91.2% - 100%).

The detailed results obtained with this panel for the Standard Q HIV 1/2 Ab 3-Line Test are shown in annex 4.

Conclusion: All HIV-1 non-B subtype specimens were correctly classified as HIV-1 by the STANDARD Q HIV/Syphilis Combo Test.

HIV-2 positive samples n = 60.

STANDARD Q HIV/Syphilis Combo Test							
Initial				Final			
HIV-2 antibodies		Syphilis antibodies		HIV-2 antibodies		Syphilis antibodies	
Reactive	60	Reactive	13	Reactive	60	Reactive	12
Non-reactive	0	Non-reactive	47	Non-reactive	0	Non-reactive	48
Total	60	Total	60	Total	60	Total	60

STANDARD Q HIV/Syphilis Combo Test was reactive for HIV-2 antibodies for all specimens. 50% of all specimens also showed a reactivity on the test line for HIV-1. For specimen HIV_SYPH/HIV-2/48 the observed intensity of the HIV-1 line was higher than HIV-2 and is therefore classified as HIV-1. For specimen HIV_Syph/HIV-2/57 the observed intensity of the HIV-2 line was equal to HIV-1 and is therefore classified as HIV. Both were repeated in duplicate and the final result for HIV remained the same.
All 13 HIV-2 specimens with reactivity on the Syphilis test line were positive in the Syphilis reference test TPA. For specimen HIV_Syph/HIV-2/57, initial testing was observed reactive for Syphilis but final testing showed no reactivity for Syphilis. This sample was weak in the Syphilis reference test TPA (S/CO 6.0) and might explain the discordance between initial and final testing using the STANDARD Q HIV/Syphilis Combo Test.

HIV-2: Sensitivity (95% CI): 60/60 X 100 = 100% (94.0% - 100%).

The detailed results obtained with this panel for the STANDARD Q HIV/Syphilis Combo Test are shown in annex 5.

Conclusion: STANDARD Q HIV/Syphilis Combo Test detected HIV antibodies in all HIV -2 specimens. For two specimens the intensity of the HIV-2 test line was equal or weaker than the HIV-1 test line.

Paired HIV-1/Syphilis positive EDTA whole blood/plasma couples, n=100.

STANDARD Q HIV/Syphilis Combo Test					
	EDTA Whole blood	EDTA Plasma		EDTA Whole blood	EDTA plasma
Reactive for HIV-1 antibodies	100	100	Reactive for syphilis antibodies	98	98
Non-reactive	0	0	Non-reactive	2	2
Total	100	100	Total	100	100

STANDARD Q HIV/Syphilis Combo Test was reactive for HIV-1 antibodies for all EDTA WB/plasma specimens. 10 % of all specimens also showed reactivity on the test line for HIV-2. In all cases the observed intensity of the HIV-2 line was lower than HIV-1.
The test was reactive for Syphilis antibodies for 98 out of 100 EDTA WB/plasma specimens. Specimens 2018_03_14 and 2018_03_88 were false non-reactive for Syphilis antibodies. The EDTA plasma of specimen 2018_03_14 was repeated in duplicate and remained false non-reactive for Syphilis antibodies. The whole blood specimen could not be repeated because it was too old at the time the combined reference data were available, to be retested. Specimen 2018_03_88 could not be retested because it was too old at the time the combined reference data were available. For both specimens the reference TPA (VITROS® Syphilis TPA Assay) and TPPA (Serodia TPPA) were reactive but RPR (BD Macro-Vue™ RPR Card Tests) was non-reactive.

HIV-1: Sensitivity on EDTA whole blood (95% CI): 100/100 X 100 = 100.0% (96.4% - 100%).
HIV-1: Sensitivity on EDTA plasma (95% CI): 100/100 X 100 = 100.0% (96.4% - 100%).

Syphilis: Sensitivity on EDTA whole blood (95% CI): 98/100 X 100 = 98.0% (93.0% - 99.8%).
Syphilis: Sensitivity on EDTA plasma (95% CI): 98/100 X 100 = 98.0% (93.0% - 99.8%).

The detailed results obtained with this panel for the STANDARD Q HIV/Syphilis Combo Test are shown in annex 6.

Conclusion: The STANDARD Q HIV/Syphilis Combo Test detected HIV-1 antibodies correctly for all specimens but was false non-reactive for Syphilis antibodies for 2 specimens.

Paired HIV-1 positive / Syphilis negative EDTA whole blood/plasma couples, n=37.

STANDARD Q HIV/Syphilis Combo Test					
	EDTA Whole blood	EDTA Plasma		EDTA Whole blood	EDTA plasma
Reactive for HIV-1 antibodies	37	37	Reactive for syphilis antibodies	2	1
Non-reactive	0	0	Non-reactive	35	36
Total	37	37	Total	37	37

Deviation from the protocol: for this panel 37 specimens were collected instead of 25.
STANDARD Q HIV/Syphilis Combo Test was reactive for HIV-1 antibodies for all EDTA WB/plasma specimens. 5% of all specimens also showed reactivity on the test line for HIV-2. In all cases the observed intensity of the HIV-2 line was lower than HIV-1.
STANDARD Q HIV/Syphilis Combo Test was false reactive for Syphilis antibodies for two EDTA WB/plasma specimens (2018_03_42 and 2018_03_56). Specimen 2018_03_42 was false reactive for Syphilis antibodies only for the whole blood specimen and remained false reactive after repeat testing in duplicate. Specimen 2018_03_56 was false reactive for Syphilis antibodies for both the whole blood and plasma specimen. Once the combined reference results were available, the specimen was too old to be retested. For both specimens the reference TPA (VITROS® Syphilis TPA Assay) was also reactive, while RPR (BD Macro-Vue™ RPR Card Tests) and TPPA (Serodia TPPA) were non-reactive.

HIV-1: Sensitivity on EDTA whole blood (95% CI): 37/37 X 100 = 100.0% (90.5% - 100%).
HIV-1: Sensitivity on EDTA plasma (95% CI): 37/37 X 100 = 100.0% (90.5% - 100%).

Syphilis: Specificity on EDTA whole blood (95% CI): 35/37 X 100 = 94.6% (81.8% - 99.3%).
Syphilis: Specificity on EDTA plasma (95% CI): 36/37 X 100 = 97.3% (85.8% - 99.9%).

The detailed results obtained with this panel for the STANDARD Q HIV/Syphilis Combo Test are shown in annex 6.

Conclusion: The STANDARD Q HIV/Syphilis Combo Test detected HIV-1 antibodies correctly for all specimens but was false reactive for Syphilis antibodies for 2 specimens.

Commercial seroconversion panels, n = 20 panels.

Panel ID	Enzygnost Anti-HIV 1/2 Plus or Genscreen HIV-1/2 v2* (3rd gen reference test)	Vironostika HIV Uniform II Ag/Ab or Vironostika HIV Ag/Ab** or Vidas HIV Duo Quick*** (4th gen reference test)	Standard Q HIV 1/2 Ab 3-Line Test (serum/plasma/whole blood)
	Number of reactive panel members/total number tested		
PRB930	2/4	2/4**	2/4
PRB934	2/3	3/3	2/3
PRB947	3/4	3/4	3/4
PRB950	1/4	1/4	1/4
PRB951	1/6	3/6	1/6
PRB952	2/6	2/6	3/6
PRB953	1/4	1/4	1/4
PRB954	0/7	1/7	1/7
PRB955	2/5	3/5	2/5
PRB956	0/5	0/5	1/5
PRB958	2/6	2/6	2/6
PRB959	4/7	6/7	5/7
PRB966	2/10	2/10**	3/10
PRB968	2/10	4/10**	4/10
PRB969	3/10*	3/10**	3/10
PRB970	2/4*	3/4**	2/4
PRB973	1/4	1/4**	1/4
PRB977	2/4	2/4**	2/4
HIV9089	2/6*	3/6***	2/6
HIV9096	3/6*	5/6***	2/6
Total score	**37/115**	**50/115**	**43/115**

The STANDARD Q HIV/Syphilis Combo Test detected HIV antibodies in 43 out of the 115 panel members, whereas the 3rd gen reference test detected 37 out of 115 panel members.
The detailed results obtained with this panel for the STANDARD Q HIV/Syphilis Combo Test are shown in annex 7.

Conclusion: For 6 seroconversion panels (PRB952, PRB954, PRB956, PRB959, PRB966 and PRB968) the STANDARD Q HIV/Syphilis Combo Test detected at least one panel member more than the 3rd generation reference test. For 1 seroconversion panel (HIV9096) the STANDARD Q HIV/Syphilis Combo Test detected one panel member less than the 3rd generation reference test.

Potentially cross-reacting HIV-1/Syphilis negative samples, n=40

STANDARD Q HIV/Syphilis Combo Test			
	Reactive for HIV-1 antibodies	Reactive for syphilis antibodies	non-reactive
Rheumatic Factor	0	0	10
CMV	1*	3	2
EBV	0	0	5
Malaria	0	0	5
Borrelia	0	0	5
Leptospira	1	0	4
HSV	0	0	5
Total	2*	3	36

*one specimen is also false reactive for Syphilis antibodies

At first testing, STANDARD Q HIV/Syphilis Combo Test was false reactive for four specimens (HIV_SYPH/CR03, 04, 05 and 38).

HIV_SYPH/CR03 (CMV positive) was false reactive for HIV-1 and syphilis, HIV_SYPH/CR04 (CMV positive) and HIV_SYPH/CR05 (CMV positive) were false reactive for syphilis and HIV_SYPH/CR38 (Leptospira) was false reactive for HIV-1. All were repeated in duplicate. The results remained false reactive.

HIV-1: Specificity (95% CI): 38/40 X 100 = 95% (83,1% - 99,4%).
Syphilis: Specificity (95% CI): 37/40 X 100 = 92,5% (79,6% - 98,4%).

The detailed results obtained with this panel for the STANDARD Q HIV/Syphilis Combo Test are shown in annex 8.

Conclusion: STANDARD Q HIV/Syphilis Combo Test classified 4 samples (3 CMV and 1 Leptospira) as false reactive.

Summary

STANDARD Q HIV/Syphilis Combo Test is a rapid immunochromatographic assay for the qualitative detection of antibodies specific to HIV-1 including subtype O, HIV-2 and Syphilis (*Treponema pallidum*) in human serum, plasma or whole blood.

On a panel of 597 HIV positive specimens (210 HIV+/Syph-, 150 HIV+/Syph+, 40 non-B subtypes, 60 HIV-2 and 137 EDTA whole blood/plasma couples), STANDARD Q HIV/Syphilis Combo Test was reactive for HIV antibodies for 597 specimens. This results in an overall HIV sensitivity (95% CI) of 100% (99.5% - 100%).
On a panel of 400 Syphilis positive specimens (150 HIV+/Syph+, 150 HIV-/Syph+ and 100 EDTA whole blood/plasma couples) STANDARD Q HIV/Syphilis Combo Test was reactive for Syphilis antibodies for 395 specimens. This results in an overall Syphilis sensitivity (95% CI) of 98.8% (97.1% - 99.6%).
In one HIV-2 positive sample, tested during the evaluation of HIV sensitivity, an initial Syphilis reactive result was observed with the STANDARD Q HIV/Syphilis Combo Test, repeat testing showed no reactivity for Syphilis. This sample was weak in the Syphilis reference test TPA (S/CO 6.0) and might explain the discordance between initial and final testing using the STANDARD Q HIV/Syphilis Combo Test

The STANDARD Q HIV/Syphilis Combo Test was able to detect 43 out of the 115 seroconversion panel members. The Enzygnost Anti-HIV 1/2 Plus or Genscreen HIV-1/2 v2 (3rd gen reference test), detected 37 out of 115 panel members.

All but four potentially cross reacting HIV/Syphilis negative specimens were correctly classified with the STANDARD Q HIV/Syphilis Combo Test. Four samples (3 CMV positive and 1 Leptospira) were classified as false reactive for HIV and/or Syphilis antibodies. In addition to these false reactive samples, 6 HIV positive and 1 Syphilis positive samples tested during clinical sensitivity obtained a false positive result for respectively Syphilis antibodies and HIV antibodies with the STANDARD Q HIV/Syphilis Combo Test.

Remarks on the instructions for use:

- The volume of the assay diluent and number of alcohol swabs, capillary tubes and lancets is not specified.
- The range room temperature is not specified.
- The interpretation of equal intensity of the "H1" and "H2" line is not specified.

The STANDARD Q HIV/Syphilis Combo Test was evaluated by ITM in the 1st and 2nd quarter of 2018.

This report was approved by

Katrien Fransen
Director HIV/STI Reference Laboratory

Fransen Katrien

Date 25 jun 2018

Legend to annexes

Annex 1: Results of HIV-1 positive/Syphilis negative samples
Annex 2: Results of HIV-1 positive/Syphilis positive samples
Annex 3: Results of HIV-1 negative/Syphilis positive samples
Annex 4: Results of HIV -1 positive samples belonging to different subtypes
Annex 5: Results of HIV-2 positive samples
Annex 6: Results of paired HIV-1 positive/Syphilis positive or negative EDTA whole blood/plasma couples
Annex 7: Results of commercial seroconversion panels
Annex 8: Results of potentially cross-reacting HIV/Syphilis negative samples
Annex 9: Instructions for use Standard Q HIV/Syphilis Combo Test