Performance evaluation of the Elecsys® Syphilis immunoassay in blood screening

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Background:

- Although syphilis is sometimes considered to be an old and often forgotten infection, epidemiologic data estimate a global incidence of infection per 1,000 of 3.19 in men and 3.02 in women, with 11 million new cases in 2005 and 36 million people infected with syphilis.[1]
- Due to a lengthy latent period, many people with syphilis are asymptomatic.[2] Syphilis testing is, therefore, important to prevent transmission. Screening of groups such as pregnant women (to prevent transmission to the fetus), individuals attending sexually transmitted disease clinics, and blood and organ/tissue donors is therefore warranted, as well as testing for the diagnosis of suspected syphilis and treatment monitoring.[3,4]
- To prevent infection from blood transfusions, World Health Organization and International Union against Sexually Transmitted Infection guidance recommend mandatory screening of all donations for specific treponemal antibodies.[5,6]
- Serologic tests are the method of choice for diagnosing syphilis and such tests exploit the antibodies generated during infection.[2] *Treponema pallidum* testing algorithms for screening vary between countries and there is no 'gold standard' for confirmation. In Europe, an enzyme immunoassay test or agglutination assay is recommended for screening followed by confirmation with a treponemal antigen test of a different type from the primary test.
- The Elecsys® Syphilis assay (Roche Diagnostics, Mannheim, Germany) is a newly developed immunoassay for the in vitro qualitative determination of total antibodies to *T. pallidum* in human serum and plasma.[7]

Aims:

- To evaluate the specificity and sensitivity of the Elecsys® Syphilis immunoassay in blood donations, including Asian Pacific populations, and compare its performance with that of state-of-the-art screening tests.

Materials and methods:

- This study was performed at four independent centers in Italy, Austria, Thailand and Turkey.

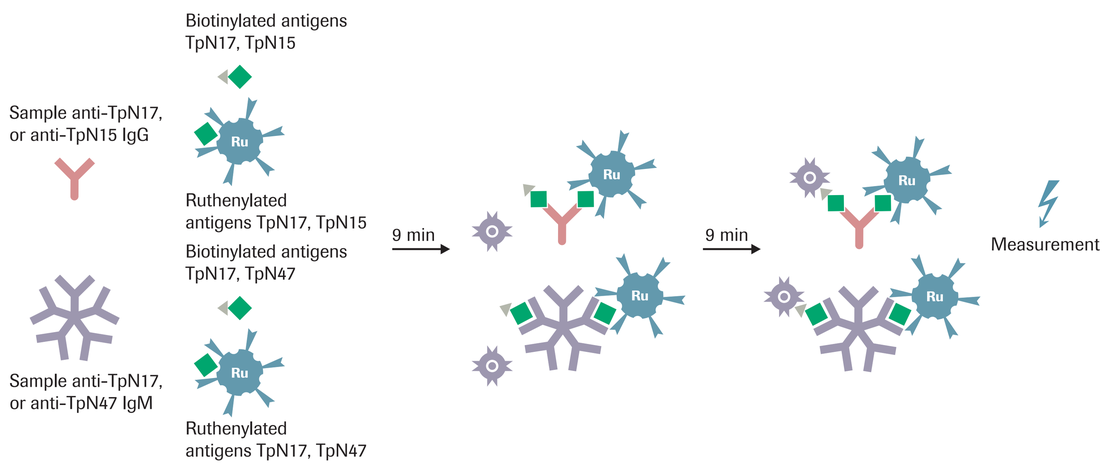
Samples

- A total of 6,257 fresh, seronegative blood donations, as well as 180 archived potentially cross-reacting seronegative samples and 342 archived seropositive samples were tested.

Description of the Elecsys® Syphilis assay

- Elecsys® Syphilis is a double-antigen sandwich (DAGS) assay that simultaneously detects anti-treponemal IgG and IgM antibodies. The sample is incubated with a mixture of biotinylated and/or ruthenylated TpN15, TpN17 and TpN47 antigens to form a DAGS in the presence of the corresponding antibodies.
- Streptavidin-coated microparticles are then added and the immune complexes bind to the solid phase by biotin–streptavidin interaction. The microparticles are magnetically captured on the electrode and a voltage applied to induce chemiluminescence, which is measured by a photomultiplier. The total assay time is 18 minutes (Figure 1).
- The Elecsys® Syphilis assay can be performed on the Elecsys® 2010, **MODULAR** *ANALYTICS*® E170, and **cobas e** 411, **e** 601 and **e** 602 platforms.

Figure 1: Principle of the Elecsys® Syphilis assay



Comparator assays, methods and analyses

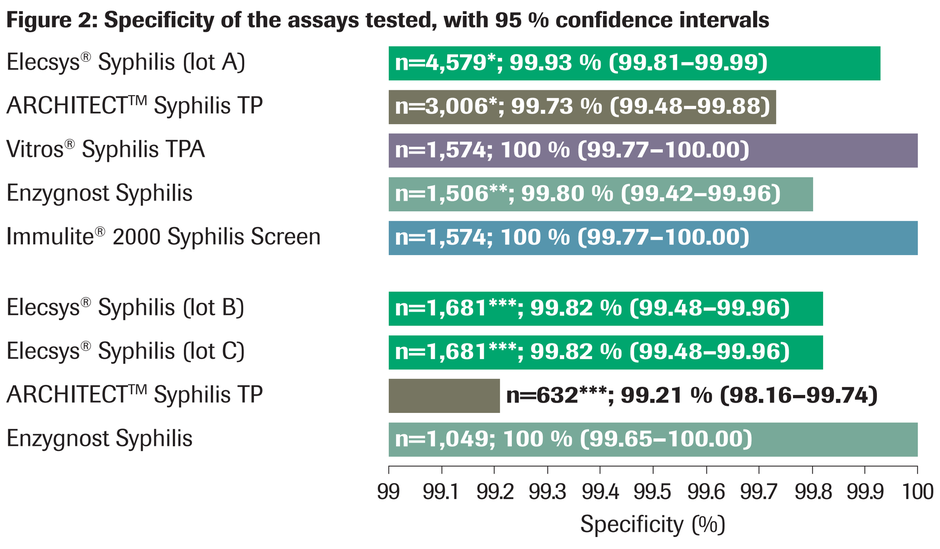
- Each center used the Elecsys® Syphilis assay and at least one of the following comparator assays: ARCHITECT™ Syphilis TP (Abbott Laboratories, Wiesbaden, Germany);[8] LIAISON® Treponema Screen (Diasorin, Saluggia, Italy);[9] Vitros® Syphilis TPA (Ortho Clinical Diagnostics, High Wycombe, UK);[10] Enzygnost Syphilis (Siemens Healthcare Diagnostics, Marburg, Germany);[11] Immulite® 2000 Syphilis Screen (Siemens Healthcare Diagnostics).[12] All assays were performed according to the manufacturers' instructions.[7–12]
- Three different reagent lots of the Elecsys® Syphilis assay were tested (lots A, B and C).
- Elecsys® Syphilis results were considered negative if the signal:cut-off ratio (s/co) was < 1.00 and positive if the s/co was ≥ 1.0.[7] Samples with an initial reactive result were retested in duplicate using the Elecsys® Syphilis assay and considered to be repeatedly reactive, and subjected to confirmatory testing, if either of the results was ≥ 1.0.
- Samples initially reactive or borderline with comparator assays[9–12] were retested (in duplicate or singly), even if the information for users did not specify a requirement for retesting initially reactive samples.[10,12] Samples with a positive result using the ARCHITECT™ Syphilis TP assay were also retested although this is not required by the instructions to users.[8] Samples with at least one repeat positive result or with repeat borderline results were considered to be repeatedly reactive within this study and subjected to confirmatory testing.
- A number of state-of-the-art methods (fluorescent treponemal antibody-absorbance, agglutination tests, immunoblots, non-treponemal tests) were used to confirm all initially or repeatedly borderline and reactive samples from the fresh screening cohorts and to resolve any discrepancies in the other cohorts. The final assessment of a sample's status was based on the MIQ16 algorithm.[13]

Results:

Specificity

- The observed specificity of the Elecsys® Syphilis assay was 99.93 % (4,574/4,577), 99.82 % (1,677/1,680) and 99.82 % (1,677/1,680), respectively, for the three validation lots tested as shown in Figure 2.
- The specificity of the Elecsys® Syphilis assay ranged from 99.52 % to 100.00 % as determined by the individual centers (Table 1). The specificity of the comparator assays was also similar among the centers and ranged from 99.21 % to 100.00 % as shown in Table 1.

Figure 2: Specificity of the assays tested, with 95 % confidence intervals



**Two samples excluded from the specificity calculation due to indeterminate confirmation results; **One sample excluded from the specificity calculation due to an indeterminate confirmation result; ***One confirmed positive sample excluded from the specificity calculation.*

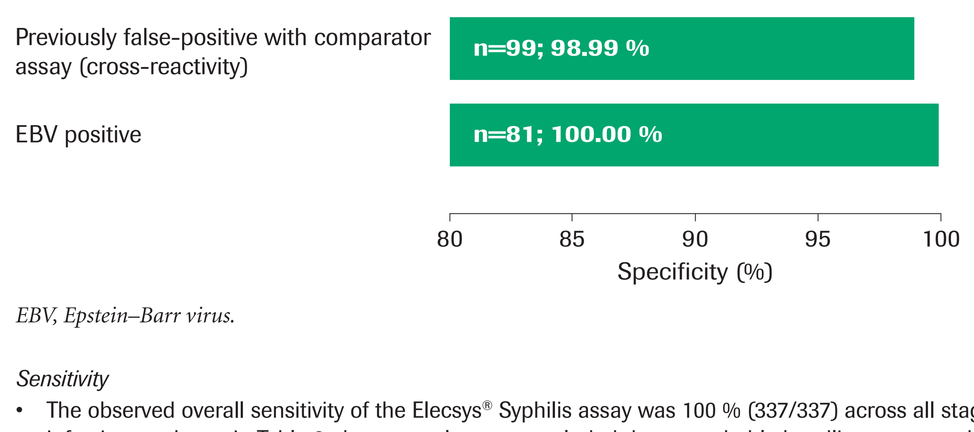
Table 1: Specificity of the assays as determined by the individual centers

	Specificity (%) with 95 % confidence intervals (2-sided)				
	Elecsys® Syphilis (lot A)	**ARCHITECT™ Syphilis TP**	**Vitros® Syphilis TPA**	**Enzygnost Syphilis**	**Immulite® 2000 Syphilis Screen**
Central Institute for Blood Transfusion and Immunology, Austria	99.93 % (99.63–100.00)	99.73 % (99.32–99.93)	–	99.80 % (99.42–99.96)	–
n (valid samples)	1,506	1,506	–	1,506	–
RR	1	4	–	3	–
Confirmed positive	0	0	–	0	–
Indeterminate	1*	1*	–	1*	–
National Blood Center, Thailand	99.87 % (99.52–99.98)	99.73 % (99.32–99.93)	–	–	–
n (valid samples)	1,500	1,500	–	–	–
RR	2	5	–	–	–
Confirmed positive	0	0	–	–	–
Indeterminate	1*	1*	–	–	–
Unit of Microbiology – Hub Laboratory – AUSL Romagna, Italy	100.00 % (99.77–100.00)	–	100.00 % (99.77–100.00)	–	100.00 % (99.77–100.00)
n (valid samples)	1,573	–	1,574	–	1,574
RR	0	–	0	–	0
Confirmed positive	0	–	0	–	0
Indeterminate	0	–	0	–	0
	Elecsys® Syphilis (lot B)	**Elecsys® Syphilis (lot C)**	**ARCHITECT™ Syphilis TP**	**Enzygnost Syphilis**	
Central Institute for Blood Transfusion and Immunology, Austria	100.00 % (99.65–100.00)	100.00 % (99.65–100.00)	–	100.00 % (99.65–100.00)	
n (valid samples)	1,049	1,049	–	1,049	
RR	0	0	–	0	
Confirmed positive	0	0	–	0	
Indeterminate	0	0	–	0	
Ege University, Turkey	99.52 % (98.62–99.90)	99.52 % (98.62–99.90)	99.21 % (98.16–99.74)	–	
n (valid samples)	632	632	632	–	
RR	4	4	6	–	
Confirmed positive	1**	1**	1**	–	
Indeterminate	0	0	0	–	

*RR, repeatedly reactive; *Excluded from the specificity calculation due to indeterminate confirmation results; **One confirmed positive sample excluded from the specificity calculation.*

- Furthermore, of the potentially cross-reacting samples, 1/99 samples that had previously given a false-positive result with a comparator assay also gave a false-positive result with the Elecsys® Syphilis assay. All other samples, including 81/81 positive for Epstein–Barr virus, tested negative with the Elecsys® Syphilis assay as shown in Figure 3.

Figure 3: Specificity of the Elecsys® Syphilis assay in potentially cross-reacting samples



EBV, Epstein–Barr virus.

Sensitivity

- The observed overall sensitivity of the Elecsys® Syphilis assay was 100 % (337/337) across all stages of infection as shown in Table 2; three samples were excluded due to probable handling errors and two due to indeterminate confirmation results.

Table 2: Sensitivity of the assays tested as determined by the individual centers

	Sensitivity (%) with 95 % confidence intervals (2-sided)			
	Elecsys® Syphilis (lot A)	**ARCHITECT™ Syphilis TP**	**LIAISON® Treponema Screen**	**Immulite® 2000 Syphilis Screen**
Unit of Microbiology – Hub Laboratory – AUSL Romagna, Italy	100.00 % (98.32–100.00)	– –	100.00 % (98.32–100.00)	100.00 % (98.32–100.00)
n (valid samples)	218	–	218	218
Positive	215*	–	218	218
Non-reactive	0	–	0	0
National Blood Center, Thailand	100.00 % (97.02–100.00)	100.00 % (97.02–100.00)	– –	– –
n (valid samples)	124	124	–	–
Positive	122**	122**	–	–
Non-reactive	0	0	–	–

**Three samples were excluded due to a probable handling error; **Two samples were excluded due to indeterminate confirmation results.*

Conclusions:

- Elecsys® Syphilis is a sensitive and specific assay that allows reliable detection of anti-treponemal antibodies.
- The Elecsys® Syphilis assay is suitable for blood bank use and compares favorably with other well-established screening tests.

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