JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2000, p. 215–219 Vol. 38, No. 1
0095-1137/00/$04.00+0

Validation of the INNO-LIA Syphilis Kit as a Confirmatory Assay for *Treponema pallidum* Antibodies

ANNE EBEL,[1] LIES VANNESTE,[2] MARTINE CARDINAELS,[2] ERWIN SABLON,[2] ISABELLE SAMSON,[2] KATRIEN DE BOSSCHERE,[2] FRANK HULSTAERT,[2] AND MAAN ZREIN[2]*

Institute Alfred Fournier, 75014 Paris, France,[1] and Innogenetics N.V., B-9052, Ghent, Belgium[2]

Received 14 July 1999/Returned for modification 26 August 1999/Accepted 6 October 1999

The commercially available diagnostic tests for syphilis are mostly based on the use of extracted antigens of *Treponema pallidum*. Pronounced cross-reactivities with other spirochete antigens are often reported. The aim of this study was to validate a novel multiparametric assay (the assay performed with the kit) INNO-LIA Syphilis for the confirmation of syphilis antibodies in a set of 840 documented human serum samples. All serum samples were previously tested at the French World Health Organization reference center for venereal diseases (Institute Alfred Fournier, Paris, France), with a consensus result provided for each sample. The study was conducted in two phases, with each phase involving a validation set (500 well-documented serum samples) and an exploratory set (340 serum samples) of serum samples, respectively. By measuring the sensitivity and specificity, we compared the result of the new assay with the consensus result on the basis of the results of a variable number of classical serological methods and clinical information when available. A sensitivity of 99.6% (95% confidence internal [CI], 98.5 to 99.9%) and a specificity of 99.5% (95% CI, 98.1 to 99.9%) were found for the new line immunoassay. Six of seven samples with indeterminate results by classical serology tested positive with the INNO-LIA Syphilis kit. This single multiparametric assay provides reliable confirmatory diagnostic information that must currently be obtained by the performance and interpretation of results of a combination of serological assays.

Syphilis is an infection most often transmitted by sexual contact. The causative agent is the human pathogen *Treponema pallidum* subsp. *pallidum* (9, 13). Serodiagnosis can be performed by two types of methods: (i) nontreponemal assays, such as the Venereal Disease Research Laboratory (VDRL) and rapid plasma reagin assays, and (ii) treponemal assays, such as fluorescent treponemal antibody-absorption (FTA-ABS), the microhemagglutination assay for antibody to *T. pallidum* (MHA-TP) or the *T. pallidum* hemagglutination assay (TPHA), and various enzyme-linked immunosorbent assays (ELISAs), which are typically more sensitive and yield more objective results than agglutination and fluorescence assays (7). All serological techniques have been shown to be cross-reactive with antibodies directed to other treponema species. False-positive reactivities can arise when conditions such as Lyme borreliosis, autoimmune disease, and human immunodeficiency virus (HIV) infection are present (2, 5, 10, 11, 15). Therefore, the use of only one type of test is insufficient for an accurate diagnosis (16). Moreover, the development of new sensitive assays such as ELISAs points to the questionable empirical threshold values that have been used in agglutination assays (i.e., for TPHA, 1:80) (8, 12, 17).

Although insensitive and of poor specificity, the nontreponemal antibody titers have been correlated with disease activity (1, 11). By contrast, treponema-specific antibody titers correlate poorly with disease activity and cannot be used to assess disease stage. Syphilis disease stage was previously evaluated by the *T. pallidum* immobilization assay (TPI), but it is rarely used today because it is difficult to perform, time-consuming, and, therefore, very expensive. Anti-*T. pallidum* titration by FTA-ABS has replaced TPI in many laboratories. However, the interpretation remains subjective and FTA-ABS is not suitable for large-scale testing situations (5, 14). Although Western blotting methods have been suggested as confirmatory assays, they include nonrelevant proteins, thereby inducing indeterminate and false-positive reactivity patterns (6). Furthermore, the reported sensitivity of about 94% (4) has resulted in their limited use.

The present study was designed to validate the new INNO-LIA Syphilis kit as a confirmatory assay for *T. pallidum* antibodies. We evaluated the assay in a two-phase study. First, we used well-characterized sera to assess sensitivity and specificity. In a second phase, we evaluated the performance with a somewhat less well documented collection of samples and studied any samples with discrepant results in detail. The specificity of the assay was also challenged by testing serum samples from patients with biological conditions, such as autoimmunity and pregnancy, who often test false-positive by assays for *T. pallidum*. Overall sensitivity and specificity were calculated with reference to consensus diagnostic assay results.

MATERIALS AND METHODS

Study design and sera. This study involved a total of 840 human serum specimens tested by the French National Reference Center for Venereal Diseases (Institute Alfred Fournier, Paris, France). The samples had been collected over the past 5 years either for a first screening or for confirmatory testing following a reactivity in a first screening test. All samples were stored at −20°C prior to testing. In the first phase of the study, a unique collection of samples (all tested by TPI) was included (n = 500); of these, 276 were clearly negative (TPHA negative at 1:80 dilution, VDRL negative, and TPI negative) and 224 were clearly positive (TPHA positive at a dilution greater than or equal to 1:80 and TPI positive). The second phase involved the remaining 340 serum samples. In addition to serum samples, 13 cerebrospinal fluid (CSF) samples obtained from patients with clinically documented syphilis at different stages were also evaluated; this collection was tested by classical methods with mixed results, including equivocal serologic results for syphilis. Furthermore, sera from patients with the most common biological conditions that result in false-positive reactivities by serologic assays for syphilis were also investigated by various assays such as assays for anticardiolipin antibodies (n = 8), antinuclear antibodies (n = 10), and rheumatoid factors (n = 11). In addition, Lyme serology-positive sera (n = 12,

* Corresponding author. Mailing address: Department of Immunochemistry, Innogenetics N.V., Industriepark Zwijnaarde 7, Box 4, B-9052, Ghent, Belgium. Phone: 0032-9241-0711. Fax: 0032-92410907. E-mail: maanzre@innogenetics.be.

Epstein-Barr virus capsid antigen immunoglobulin M (IgM)-positive sera (n = 8), sera from pregnant women (n = 10), and isolated sera with VDRL reactivities (n = 31) were tested. The Lyme serology-positive samples were screened by an in-house immunofluorescence technique with *Borrelia burgdorferi* B31; the results for six samples were further confirmed by *Borrelia* Western Blot IgG (Gull Laboratories, Bois d'Arcy, France).

Screening assays. The VDRL assay used in this study uses a colloidal suspension of cholesterol-lecithin sensitized with beef heart-extracted cardiolipin (Sypal, Diagast, Loos, France) as a nontreponemal antibody detection system. In addition, treponema-specific antibodies were detected in an initial screening with sensitized sheep erythrocytes (RBCs; MHA-TP; Ames-Bayer, Puteaux, France). Repeat testing, in addition to the previous assay, if needed, was performed by using avian RBCs (LD Serokit TPHA, Labor Diagnostika GmbH, Heiden, Germany). Finally, a screening ELISA was used for further investigations; we used a kit with treponema recombinant antigens (ICE Syphilis; Murex Diagnostics B. V., Aalst, Belgium). All test procedures and interpretation of the results were performed according to the manufacturer's instructions.

Fluorescence methods (FTA-ABS). Samples reactive by a screening method are usually tested by a fluorescent *T. pallidum* assay with cultured treponema organisms (TrepoSpot IF; BioMérieux, Marcy l'Etoile, France). Specific antibodies are detected with a fluorescein-labeled anti-human immunoglobulin after the absorption of nonspecific antibodies on *T. phagedenis* extracts (5). If specific fluorescence can be perceived at a sample dilution of 1:100, the sample is considered positive.

IgM detection methods. Two methods have been used to determine IgM in human sera. A commercial assay (FTA-M; Sanofi Diagnostics Pasteur, Marnela-Coquette, France) and an in-house IgM solid-phase hemadsorption assay (IgM-SPHA) with *T. pallidum*-sensitized RBCs were used.

TPI. TPI was performed at the Institute Alfred Fournier by an in-house procedure, as follows. A 1/10 dilution of human serum was incubated with a suspension of freshly prepared treponema in the presence of complement proteins for 18 h at 37°C. A control procedure with inactivated complement proteins was also performed for each test. TPI determines the ability of antibody to immobilize live *T. pallidum* (Nichols strain), as visualized under a dark-field microscope and expressed as a percentage (number of immobilized spirochetes/total count). A sample is considered positive by TPI if at least 70% of *Treponema* are immobilized compared to the rate of immobilization for the corresponding control. The sample is considered negative if the value by TPI is less than 30% of that for the control, and TPI values between 30 and 70% of that for the control are considered inconclusive. For some TPI-positive samples, titration is obtained at the end dilution that shows immobilization of at least 50% of the *T. pallidum* spirochetes.

Consensus results by classical tests. For the 340 less well documented samples, we established a consensus serological diagnostic for all samples on the basis of the available results of the classical assays (TPHA, FTA-ABS, and TPI). In some cases, if enough serum was available, repeat testing, in addition to a screening enzyme immunoassay (ICE Syphilis), was performed. In such cases, the global information was considered to define the consensus results. Since VDRL is often negative for patients with true late infections (who are positive by other techniques), VDRL data were not used to determine the consensus results. These consensus results were obtained as follows. A sample was considered positive if all available results were positive; a sample was considered negative if all available results were negative. When discrepant results were shown, the most predominant result was considered the consensus result; if discrepant results were present in equal numbers, no consensus could be reached and the result for the sample was therefore considered to be equivocal.

INNO-LIA Syphilis kit. The INNO-LIA Syphilis kit uses recombinant antigens and synthetic peptides derived from *T. pallidum* (Nichols strain) membrane proteins. Briefly, the antigens used consisted of three immunodominant proteins (TpN47, TpN17, and TpN15) expressed as full-size proteins in *Escherichia coli* and one synthetic peptide (TmpA) derived from transmembrane protein A. In brief, the TmpA antigen was synthesized on a solid phase by using Tentagel S resin (Rapp Polymere GmbH, Tübingen, Germany) by standard 9-fluorenylmethoxycarbonyl amino acid chemistry. After completion of peptide synthesis, the peptide was cleaved from the resin and was then purified by reverse-phase high-performance liquid chromatography. The primary amino acid sequence of this antigen was optimized and included an N-terminal biotin residue to allow peptide immobilization on the nylon strips by using streptavidin binding properties.

The full-size *T. pallidum* genes expressing TpN47, TpN17, and TpN15 (accession nos. M88769, M74825, and M30941, respectively) were isolated from *T. pallidum* Nichols by PCR with specific primers based on the data bank sequences. Appropriate restriction enzyme sites were incorporated into these primers. The resulting sequences were cloned in the pBluescript SK(+) vector (Stratagene, La Jolla, Calif.) and were verified by DNA sequence analysis. The coding sequences were subsequently inserted in an *E. coli* expression vector and were transformed into the expression strain *E. coli* MC1061(pAcI). Both TpN47 and TpN15 were expressed as N-terminal fusion proteins with the first 25 amino acids of mouse tumor necrosis factor followed by a purification tag. The TpN17 protein was expressed as an N-terminal fusion with a purification tag only.

All proteins were purified by a similar procedure. The frozen cell pellets (from a 15-liter fermentation) were lysed in 6 M guanidine hydrochloride (GnHCl)–50 mM phosphate (pH 7.2) overnight at 4°C. The extracts were clarified by centrifugation after a freeze-thaw cycle of 2 h at −70°C. The supernatants were loaded in the presence of 20 mM imidazole on a chelating Sepharose FF column previously equilibrated with $NiCl_2$ and loading buffer (6 M GnHCl, 50 mM phosphate, 20 mM imidazole [pH 7.2]). After loading of the proteins, the column was washed with wash buffer containing 50 mM imidazole. The fusion protein was eluted from the column with elution buffer (6 M GnHCl, 50 mM phosphate, 200 mM imidazole [pH 7.2]). After immobilized metal-affinity chromatography, TpN15 and TpN17 were desalted on a Sephadex G25 column with a 2 M urea buffer, while TpN47 was desalted with a 6 M urea buffer. Sodium dodecyl sulfate-polyacrylamide electrophoresis and Western blotting were used to estimate the purities of the recombinant proteins.

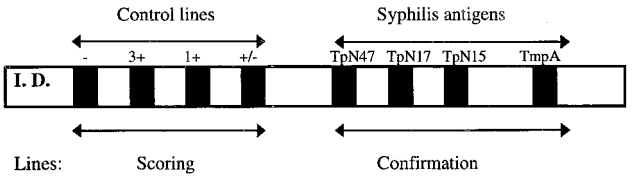

FIG. 1. Layout of INNO-LIA Syphilis kit strip.

In addition to the syphilis antigens, control lines are used for a semiquantitative evaluation of the results as well as for the verification of sample addition and reagents. A schematic layout of the INNO-LIA strip is shown in Fig. 1. The assay procedure can be summarized as follows. Serum or plasma samples were diluted 1:100 and were incubated at room temperature (20°C) overnight, followed by three washing steps with washing buffer before the addition of a goat anti-human IgG (heavy and light chains) conjugated to alkaline phosphatase. Three washing steps were again performed, followed by the addition of a chromogen. Color development was then stopped with an appropriate stop solution. The use of color-coded reagents makes the different steps of the assay clearly distinct. In a visual reading protocol, after color development, each line was compared to the control lines, and the intensities were scored as follows: 0, no line or a line less intense than the +/− cutoff line; 0.5, a line as intense as the +/− cutoff line; 1, a line with an intensity between that of the cutoff line and equal to that of the 1+ control line; 2, a line with an intensity between that of the 1+ control line and that of the 3+ control line; 3, a line with an intensity equal to that of the 3+ control line; 4, a line with an intensity greater than that of the 3+ control line.

The scoring of the results obtained with the INNO-LIA Syphilis kit was done blinded without knowledge of the consensus test results. Samples were scored independently by two people (investigator A.E. and technician L.V.). Weakly reactive samples were scored 100% concordantly.

The interpretation algorithm of the INNO-LIA Syphilis kit was initially optimized for visual reading with an independent set of negative and positive samples (unpublished data). A sample is considered *T. pallidum* antibody negative if no band or an isolated band with a maximum intensity equal to 0.5 is present. If multiple bands with a minimum intensity equal to 0.5 are visible, the sample is considered *T. pallidum* antibody positive. Finally, a sample is considered indeterminate if a single band is visible with a minimum intensity equal to 1. Several representative INNO-LIA Syphilis kit reactivity patterns are shown in Fig. 2.

Sample I.D.	Classical assays Consensus results	INNO-LIA syphilis INNO-LIA Patterns (3+ 1+ ± 47 17 15 TmpA)	Results
547	indeterminate		positive
800	indeterminate		positive
820	indeterminate		positive
601	weak positive		positive
612	weak positive		positive
668	weak positive		positive
207	negative		indeterminate
518	positive		indeterminate
768	Lyme positive		negative
769	Lyme positive		negative
770	Lyme positive		negative

FIG. 2. Representative patterns of reactivity with INNO-LIA Syphilis kit. Control lines are indicated (3+, 1+, and ±); antigen lines are those for the TpN47, TpN17, TpN15, and TmpA antigens, respectively. Sera correspond to the following (from top to bottom): six positive samples with indeterminate (samples 547, 800, and 820) or weakly positive (samples 601, 612, and 668) consensus results, two samples with indeterminate results with one negative (sample 207) and one positive (sample 518) consensus result; and three negative samples (samples 768, 769, and 770) with positive serology for Lyme borreliosis.

TABLE 1. Comparison of INNO-LIA Syphilis kit results and classical testing consensus results

Consensus result	No. of samples with the following INNO-LIA Syphilis kit result:			
	Negative	Indeterminate	Positive	Total
Negative	369	2[a]	0	371
Equivocal	0	0	5[b]	5
Positive	0	2[c]	462	464
Total	369	4	467	840

[a] One sample (sample 666) was positive by FTA-ABS and ICE Syphilis EIA and negative by agglutination assays.
[b] The five samples were positive by at least one of the classical assays.
[c] Doubtful or negative by agglutination assays (samples 518 and 599); consensus positive on the basis of the FTA-ABS and the ICE Syphilis EIA results.

Statistics. StatMate software (version 1.01; GraphPad, San Diego, Calif.) was used for the calculation of 95% confidence intervals (CIs) for proportions.

RESULTS

INNO-LIA Syphilis kit performance. Among the 500 samples (224 positive and 276 negative samples) studied in the first phase, only 1 sample with a discrepant result was found, yielding a sensitivity of 100% (95% CI, 98.4 to 100%) and a specificity of 99.64% (95% CI, 98.0 to 99.9%) for the INNO-LIA Syphilis kit. The sample with a discrepant result had a negative serology but was scored as indeterminate by the INNO-LIA Syphilis kit. Unfortunately, no follow-up or further testing could be performed with this specimen. In the second phase of the study, among the remaining 340 serum samples with somewhat less well documented serologies, 8 samples had discrepant results, and 5 of these had equivocal serologies by classical testing. None of the Lyme borrelia-positive sera reacted in tests with the INNO-LIA Syphilis kit (the results for three such samples are shown in Fig. 2).

Comparison of INNO-LIA Syphilis kit results with the consensus results. For the determination of the overall assay performance, we combined the samples studied in the two phases and calculated sensitivity and specificity. A summary of the results obtained with the INNO-LIA Syphilis kit testing compared to the consensus data obtained from classical tests is shown in Table 1. Sensitivity was evaluated for a total of 464 serum samples that were considered positive by a consensus of multiple tests (TPHA, FTA-ABS, and TPI) and clinical information. The INNO-LIA Syphilis kit confirmed the results for 462 of 464 samples positive for *T. pallidum* antibodies, resulting in a sensitivity of 99.6%. Table 2 presents the detailed results available for each sample with discrepant results. Briefly, two samples (samples 518 and 599) considered consensus positive were indeterminate with the INNO-LIA Syphilis kit. Both samples were doubtful by TPHA, positive by FTA-ABS (low titers) and ICE Syphilis EIA, but negative by VDRL. Only one sample was tested by TPI, and it had a negative result. None of the consensus-positive samples was found to be negative with the INNO-LIA Syphilis kit. For evaluating the specificity, 369 of 371 negative serum samples were correctly identified by INNO-LIA Syphilis kit, thereby yielding a specificity of 99.5%. Due to reactivity of a single band, two samples had indeterminate results with the INNO-LIA Syphilis kit. One sample (sample 207) was negative by four techniques but was not tested by the ICE Syphilis EIA; the other sample (sample 666) was negative by VDRL, TPHA, and TPI but positive by FTA-ABS and the ICE Syphilis EIA. None of the samples from this population were false positive. Both overall sensitivity and specificity fit within 95% CIs.

Analysis of discrepant results. Discrepancies between the consensus result and the INNO-LIA Syphilis kit result were again analyzed in the context of their global information. In addition, due to subjective reading of the results obtained by agglutination techniques, if clear-cut positive or clear-cut negative results were not demonstrated at the tested dilution (1:80), a doubtful TPHA result was indicated as D80 (Table 2).

Serum and CSF samples with clinical information. Thirteen CSF specimens obtained from 10 syphilitic patients were also evaluated in this study. Of these, 11 samples were classified as positive by the consensus result as well as with the INNO-LIA Syphilis kit (100% sensitivity). The two remaining CSF samples were considered equivocal by the consensus result (doubtful by TPHA, negative by FTA-ABS, and positive by the ICE Syphilis EIA); with the INNO-LIA Syphilis kit, one sample (sample 699) was positive and one sample (sample 701) was indeterminate (Table 3).

DISCUSSION

In the course of the 20th century, syphilis serology testing has significantly contributed to limiting the spread of this venereal disease (15). Furthermore, disease activity has been linked to the results of multiple tests: TPHA-positive and VDRL-negative results are thought to be indicative of latent syphilis, while TPHA-positive and VDRL-positive results are usually indicative of infectious syphilis (1). To establish a conclusive diagnosis, TPI was often used to resolve conflicting results. This complex and expensive methodology has been progressively abandoned and substituted by other strategies for confirmation. For instance, several laboratories perform either

TABLE 2. Detailed results for sera from Table 1 with discrepant results

Sample no.	Results by classical techniques[a]					Consensus result	INNO-LIA Syphilis kit intensity				INNO-LIA Syphilis kit result
	TPHA	VDRL	FTA-ABS	TPI (%)	ICE (S/CO)		TpN47	TpN17	TpN15	TmpA	
523	D80	Neg	Neg	NT	2.4	Equivocal	1	2	1	0	Positive
547	D80	Neg	Neg	NT	4.1	Equivocal	0	1	2	0	Positive
800	Neg	2	100	NT	1.8	Equivocal	0.5	3	0.5	1	Positive
811	D80	2	Neg	NT	NT	Equivocal	0.5	4	1	0	Positive
820	Neg	16	100	NT	2.2	Equivocal	0.5	3	1	0	Positive
666	Neg	Neg	100	10	4.4	Negative	0	4	0	0	Indeterminate
207	Neg	Neg	Neg	Neg	NT	Negative	0	1	0	0	Indeterminate
518	D80	Neg	100	NT	3.2	Positive	0	0	1	0	Indeterminate
599	D80	1	200	30	2.6	Positive	0	3	0	0	Indeterminate

[a] TPHA, VDRL, and FTA-ABS results are expressed in titers. Neg, negative result, D80, a doubtful reading at a dilution of 1:80; NT, not tested; S/CO, value for the sample/cutoff value.

TABLE 3. INNO-LIA Syphilis kit results for sera with clinical condition documentation

Sample no.	Date, sample	Clinical condition[a]	TPHA titer	VDRL titer	IgM result[b]	FTA-ABS titer	TPI titer	INNO-LIA Syphilis band intensity				INNO-LIA Syphilis kit result
								TpN47	TpN17	TpN15	TmpA	
692	04 Feb 1997, blood	Primary syphilis	10,240	32	Pos[c]	1,600		4	4	4	4	Positive
	19 Feb 1997, blood	First injection of penicillin	5,120	16	Pos	1,600		3	4	4	2	Positive
	26 Feb 1997, blood	Second injection of penicillin	5,120	16	Pos	800		3	4	4	4	Positive
	26 Mar 1997, blood	Third injection of penicillin	5,120	16	Pos	800		3	4	4	3	Positive
	11 June 1997, blood		5,120	16	Neg[d]	800		4	4	4	2	Positive
693	07 Jul 1997, blood	Neurosyphilis	20,480	64	Pos	NT[e]	1,600	3	4	4	4	Positive
	11 Aug 1997, blood	Treatment	20,480	64	Pos	6,400	1,600	4	4	4	4	Positive
	11 Aug 1997, CSF		NT	16	Neg	800	800	3	4	4	4	Positive
	05 Mar 1998, blood	Treatment	20,480	16		NT	400	2	4	4	4	Positive
	05 Mar 1998, CSF		2,560	Neg		NT	25	3	4	4	4	Positive
694	22 Jan 1997, blood	Neurosyphilis	5,120	8		1,600		4	4	4	4	Positive
	22 Jan 1997, CSF		80	Neg		100		1	3	0	0	Positive
	23 Apr 1997, blood	Treatment	5,120	8		1,600		3	4	4	3	Positive
	23 Apr 1997, CSF		80	Neg		NT		1	4	0	0	Positive
695	14 Dec 1996, blood	Neurosyphilis	NT	64		6,400		3	4	4	4	Positive
	14 Dec 1996, CSF		NT	8		1,600		3	3	3	3	Positive
	18 Dec 1996, blood	Treatment	20,480	8		1,600	100	3	4	4	4	Positive
	18 Dec 1996, CSF		5,120	Neg		400	50	3	4	4	4	Positive
696	16 Oct 1996, blood	Neurosyphilis	NT	512		6,400	400	3	4	4	4	Positive
	16 Oct 1996, CSF		NT	NT		1,600	100	3	4	4	4	Positive
697	12 Feb 1997, blood	Tertiary syphilis treatment	10,240	64	Pos	1,600		3	4	4	3	Positive
	07 Jul 1997, blood	Treatment	10,240	16	Pos	800		3	4	4	3	Positive
	03 Sep 1998, blood		5,120	16	Pos	800		3	4	4	3	Positive
699	24 Mar 1998: blood	Primary syphilis	640	64	Pos	800		3	4	4	2	Positive
	05 May 1998, blood	Evolving primary syphilis	5,120	128		NT		3	4	4	3	Positive
	05 May 1998, CSF		D80	Neg		Neg		1	3	0.5	0	Positive
	04 Jun 1998, blood	Treated syphilis	1280	32	Neg	NT		3	4	4	3	Positive
701	28 Aug 1998, blood	Congenital syphilis	5,120	NT	Pos	NT		1	4	3	3	Positive
	28 Aug 1998, CSF		D80	Neg	Neg	Neg		0	3	0	0	Indeterminate
703	23 Jun 1998, blood	Neurosyphilis with HIV positivity	20,480	2,048		NT		3	4	4	4	Positive
	23 Jun 1998, CSF		1,280	1		NT		3	4	3	3	Positive
736	Unknown, blood	Neurosyphilis with HIV positivity	40,560	512	Pos	NT		3	4	4	3	Positive
	Unknown, CSF		5,120	4		NT		3	4	4	3	Positive

[a] Clinical data were from various hospitals in France or from the sexually transmitted disease clinic of the Institute Alfred Fournier.
[b] IgM determination by FTA-ABS and/or IgM-SPHA.
[c] Pos, positive result.
[d] Neg, negative result.
[e] NT, not tested.

TPHA and VDRL titration by serial dilution or TPHA and FTA-ABS titration for confirmation of syphilis (7).

The INNO-LIA Syphilis kit used in this study is a user-friendly methodology that makes use of color-coded reagents and simple interpretation criteria. It shows a high degree of performance as a multiparametric confirmation method. The assay validated in this study has been optimized to confirm the presence of specific antibodies to *T. pallidum* antigens in human sera and plasma. Nevertheless, testing of a limited series of CSF samples was also possible, and it was suggested that the kit had a higher degree of sensitivity with these samples than agglutination assays do. Since IgM molecules (agglutinins) do not cross the blood-brain barrier, detection of treponemal antibodies was somewhat less sensitive by agglutination techniques (TPHA, VDRL) than by other assays. This was clearly illustrated with the available paired samples from patients (serum and CSF samples) (Table 3). The high degree of sensitivity of the INNO-LIA Syphilis kit allowed the detection of low levels of IgG, like in the CSF of patient 699 (Table 3). Classical testing schemes classified five samples as equivocal due to discrepancies among the different assays (Table 2). Interestingly, these samples were clearly positive with the INNO-LIA Syphilis kit. This can reflect an improved sensitivity on the basis of the use of recombinant antigens versus nontreponemal antigens (in VDRL) (3), extracted treponemal antigens (in TPHA), or whole treponema organisms (in FTA-ABS) (14). The two samples with consensus-negative and INNO-LIA Syphilis kit-indeterminate results may also reflect the high degree of sensitivity of the new assay. On the other hand, since the specificity of the INNO-LIA Syphilis kit has been demonstrated with a large collection of negative samples (data partially shown in the present study) as well as a collection of difficult samples usually prone to false-positive reactivities (Table 4), the two consensus-positive samples that were indeterminate with the INNO-LIA Syphilis kit may illustrate the specificity of the INNO-LIA Syphilis kit. One of these samples (sample 599) had a doubtful result by agglutination techniques, was positive by FTA-ABS and the ICE Syphilis EIA, but was negative by TPI. This can also be due to a differential inter-

TABLE 4. INNO-LIA Syphilis kit results for sera containing antibodies for other infectious or autoimmune diseases

Positive for[a]:	No. of specimens tested	INNO-LIA Syphilis kit results
Cardiolipin antibodies	8	Negative
Pregnancy	10	Negative
Rheumatoid factors	11	Negative
B. burgdorferi	12	Negative
Antinuclear antibodies	10	Negative
Epstein-Barr virus IgM	8	Negative
VDRL (biological false positive)	31	Negative

[a] Tested by immunofluorescence, EIA, or agglutination techniques at the Institute Alfred Fournier.

ference of antibodies to other treponemes that have no clinical significance (5).

In conclusion, the INNO-LIA Syphilis kit can reliably replace various strategies for the confirmation of syphilis infections, which currently requires the performance of several assays. A new prospective study will allow us to determine a possible algorithm for disease staging by this multiparametric assay.

ACKNOWLEDGMENTS

A. Ebel thanks Innogenetics for providing the INNO-LIA Syphilis kits free of charge.

Fred Shapiro is acknowledged for critical review and editorial contributions.

REFERENCES

1. **Aberle Grasse, J., S. L. Orton, E. Notari IV, L. P. Layug, R. G. Cable, S. Badon, M. A. Popovsky, A. J. Grindon, B. A. Lenes, and A. E. Williams.** 1999. Predictive value of past and current screening tests for syphilis in blood donors: changing from a rapid plasma reagin test to an automated specific treponemal test for screening. Transfusion **39:**206–211.
2. **Backhouse, J. L., and B. J. Hudson.** 1995. Evaluation of immunoglobulin G enzyme immunoassay for serodiagnosis of yaws. J. Clin. Microbiol. **33:**1875–1878.
3. **Berkowitz, K., L. Baxi, and H. E. Fox.** 1990. False negative syphilis screening: the prozone phenomenon, non-immune hydrops and diagnosis of syphilis during pregnancy. Am. J. Obstet. Gynecol. **163:**975–977.
4. **Byrne, R. E., S. Laske, M. Bell, D. Larson, J. Phillips, and J. Todd.** 1992. Evaluation of a *Treponema pallidum* Western immunoblot assay as a confirmatory test for syphilis. J. Clin. Microbiol. **30:**115–122.
5. **Carlsson, B., H. S. Hanson, J. Wasserman, and A. Brauner.** 1991. Evaluation of the fluorescent treponemal antibody-absorption (FTA-ABS) test specificity. Acta Dermatol. Venereol. **71:**306–311.
6. **Fujimura, K., N. Ise, E. Ueno, T. Hori, N. Fujii, and M. Okada.** 1997. Reactivity of recombinant Treponema pallidum (r-Tp) antigens with anti-Tp antibodies in human syphilitic sera evaluated by ELISA. J. Clin. Lab. Anal. **11:**315–322.
7. **Larsen, S. A., B. M. Steiner, and A. H. Rudolph.** 1995. Laboratory diagnosis and interpretation of tests for syphilis. Clin. Microbiol. Rev. **8:**1–21.
8. **Lefevre, J. C., M. A. Bertrand, and R. Bauriaud.** 1990. Evaluation of the Captia-Enzyme Immunoassays for detection of immunoglobulins G and M to *Treponema pallidum* in syphilis. J. Clin. Microbiol. **28:**1704–1707.
9. **Lukehart, S. A., and K. K. Holmes.** 1998. Spirochetal diseases, p. 1023–1033. *In* A. S. Fauci, E. Braunwald, K. J. Isselbacher, et al. (ed.), Harrison's principles of internal medicine, 14th ed. McGraw-Hill, New York, N.Y.
10. **Nandwani, R., and D. T. P. Evans.** 1995. Are you sure it's syphilis? A review of false positive serology. Int. J. STD AIDS **6:**241–248.
11. **Rusnak, J. M., C. Butzin, D. MacGlason, and S. P. Blatt.** 1994. False-positive rapid plasma reagin tests in human immunodeficiency virus infection and relationship to anticardiolipin antibody and serum immunoglobulin levels. J. Infect. Dis. **169:**1356–1359.
12. **Silleti, R. P.** 1995. Comparison of CAPTIA Syphilis G enzyme immunoassay with rapid plasma reagin test for detection of syphilis. J. Clin. Microbiol. **33:**1829–1831.
13. **Singh, A. E., and B. Romanowski.** 1999. Syphilis: review with emphasis on clinical, epidemiologic, and some biologic features. Clin. Microbiol. Rev. **12:**187–209.
14. **Sordillo, E. M., B. Hoehl, and J. Belch.** 1998. False-negative fluorescent treponemal tests and confirmation of syphilis infection. J. Infect. Dis. **178:**294–295.
15. **World Health Organization.** 1996. HIV/AIDS and sexually transmitted diseases, WHO policy and strategic orientations. Report WHO/ASD/96.2. World Health Organization, Geneva, Switzerland.
16. **Young, H.** 1998. Syphilis: serology. Dermatol. Clin. **16:**691–698.
17. **Zrein, M., I. Maure, F. Boursier, and L. Soufflet.** 1995. Recombinant antigen-based enzyme immunoassay for screening of *Treponema pallidum* antibodies in blood bank routine. J. Clin. Microbiol. **33:**525–527.