

# COMPARATIVE STUDY FOR TPHA IN COMMERCIAL SYSTEMS

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## INTRODUCTION:

Comparative evaluation was done of 3 serological tests for Treponema antibodies by TPHA tests. TPHA are most often used to screen sera for syphilis because of its high degree of sensitivity in all stages of syphilis, its specificity and the possibility of being automated. It remains positive for life unless treatment is given early in disease. A quantitative assay can be performed, but no standardisation on test performance nor quantification exists. Other negative drawbacks are that none of the current tests for syphilis can discriminate between syphilis and the other Treponematoses. Furthermore 4% of the test results are false positive reactions, especially in pregnant woman (who also have in 0,1-0,6% positive non-Treponemal tests!) Depending on the test one requires extensive equipment and additive solutions, trained personel, time to perform and especially to read and standardize the results locally. Other, more simple, testing methods are therefore needed using other carriers to get more rapid and readable testing results, to stabilize the result.

Different antigen preparations as carriers cause discrepancies in test results: Rapidity and readability of tests involve various antigens, coupled to different carrying materials : erythrocytes or gelatin particles. Carbohydrate antigens readily adhere to RBC's but protein antigens require pretreatment with tannic acid or chromium chloride. Tanning facilitates a high density-coating which increases the sensitivity of the test system. Subsequent formalin or glutaraldehyd treated "tanned" erythrocytes coated with proteins or carbohydrates allows longterm storage. This whole process though also allows for faults in reading, as well as influences sensitivity and specificity.

With the new pretreated carriers manufacturers report high sensitivity and specificity. However these assays need further evaluation and comparison particularly with sera which showed positive in other tests.

### Aim of the study:

This study was designed to test 3 selected groups of sera for comparing a.o. sensitivity, specificity, accuracy, ease of use and costs !! in three different TPHA tests which had different carriers for the Treponema pallidum antigen and different readability.

## METHODS AND MATERIALS:

### 1. Tests:

3 tests were compared :

- Medac: sheep erythrocytes are used, sensitized with extract from *Treponema pallidum* through tannine treatment. An absorbent takes away the unspecific reactions.
- Syphagen: stabilized chicken erythrocytes sensitized with *Treponema pallidum* extract. In the diluent is a Reiter's *Treponemal* extract (Nichols strain) that absorbs non-*Treponemal* antibodies.  
Chicken erythrocytes ensure a quick sedimentation with a final reading after one hour incubation.
- Fujirebio: gelatin particle carriers are sensitized with purified pathogenic *Treponema pallidum* (Nichols strain)

### 2. Specimens:

3 groups of test sera were used:

- a 53 sera from *Borrelia* serological positive patients and 43 sera from Rheuma (Rapa/Latex/anti-DNase ) positive patients
- b 100 sera from patients from the CBL who had in the past positive TPHA (using the MEDAC TPHA) and FTA or VDRL/Kolmer;  
all 100 were TPHA +; 81 were also FTA +, 21 were also VDRL +, 6 were also Wasserman +
- c 299 sera from women as sent in routinely for screening during pregnancy.

### 3. Laboratory procedure:

All tests were carried out by a laboratory-technician for acquaintance/familiarisation with titration ( 3 x 30 tests) using plasma and serum.

After that all sera were initially screened at the same time, by the same analyst. Scoring of results was controlled by a second technician and by a microbiologist.

An ease of use survey was conducted by the technologist who performed the tests in the following areas: technical difficulties, requirement of equipment, storage half-life, interpretation of results, simplicity (test presentation and packaging), applicability of use and automatization. For the definitive evaluation of the tests all discordant results (samples that did not give the same result with all three tests) were retested. Samples which gave discordant results after retesting were considered discordant samples and were titrated and tested for FTA, VDRL and Wasserman. These discordant sera were sent to a reference laboratory, RIVM, for TPHA repeat and Western blot. These results were considered as "standard".

## Results

Table 1 shows the number of samples for each group of sera that gave discrepant results after the first analysis and after retesting. From the 21 discrepancies in the Lues group 7 samples gave identical results after retesting, one sample could not be retested because of insufficient material, leaving 14 discrepancies in the Lues group. In the Rheuma/Borelia group one sample remained discordant after retesting. In the group with routine samples retesting diminished the number of discrepancies to 8, and in case of one sample there was insufficient material for retesting.

**Table 1: All sera**

Number of sera	Discrepancies	Repeat discrepancies
100 lues	21	13 + 1 x i.s.*
43 rheuma	1	0
53 borrelia	1	1
299 routine	15	8 + 1 x i.s.*
		24

\* i.s. = insufficient sample

Table 2: 24 Discrepancies

number	MEDAC		Syphagen		FUJIREBIO		Remark	VDRL	FTA	WA	RIVM TPHA	RIVM BLOT
	U	S	U	S	U	S						
433146	-	-	-	320	-	320	o.l.	-	1+	-	+	+
508351	+	320	-	-	+	-	o.l.	-	-	-	-	-
508562	-	160	-	-	-	-	o.l.	-	-	-	-	-
508729	-	320	-	-	-	-	o.l.	-	+-	-	-	-
508915	+	80	-	-	+	320	o.l.	+/-	-	1/1	asp	asp
508967	-	-	-	320	-	160	o.l.	is	is	is		
509037	+	640	-	-	+	-	o.l.	-	-	-	-	-
509069	+	320	-	-	+	-	o.l.	-	-	-	-	-
509074	+	320	-	-	-	-	o.l.	is	is	is		
509300	-	160	-	-	-	-	o.l.	-	-	-	-	-
509393	+	80	-	-	-	-	o.l.	1/4	+-	1/1		
509398	+	320	-	640	-	320	o.l.	-	-	ac		
509484	-	320	-	-	-	-	o.l.	-	-	-	-	-
169	+	-	-	80	-	-	Borrelia	>32	-	-	-	-
509237	is	is	is	is	is	is	rout	is	is	is		
517315	-	+	-	-	-	-	rout	-	-	ac	-	-
518010	-	-	-	+-	-	-	rout	-	-	-	-	-
517975	-	+-	-	-	-	-	rout	-	-	-	-	-
517977	-	+	-	-	-	-	rout	-	-	-	-	-
518009	-	+	-	-	-	-	rout	-	-	-	-	-
518087	+	-	-	-	-	-	rout	-	-	-	-	-
518096	+-	-	-	-	-	-	rout	-	-	-	-	-
518156	+-	+-	-	-	-	-	rout	-	-	-	-	-
517356	is	is	is	is	is	is	rout	is	is	is	-	-
TOTAAL	24		24		24		24					

u = unsensitized  
i.s. = insufficient sample  
rout. = routine  
asp. = aspecific

s = sensitized erythrocytes  
o.l. = old lues  
+- = dubious positive  
ac = anticomplementair

Table 2 shows the remaining discordant results. These sera were sent to the RIVM and 1 out of 18 sera was confirmed positive. Unfortunately 6 of these discrepancies had not enough material left to perform a confirmation test. The low number of confirmed positives might be explained by the fact that the RIVM uses a cut-off which is higher than the CBL uses in the Medac TPHA. As a result the CBL will report more false positive results. Compared to Syphagen and Fuji there were very few discrepancies with RIVM results in TPHA and Blot.

Strangely enough the only sample (#433146) which is reported positive by the RIVM is reported negative by Medac. Another sample (#508967) was reported clearly positive by Fuji and Syphagen but negative by Medac, unfortunately there was not enough material for the RIVM to perform a confirmation. Medac shows reactivity with unsensitized erythrocytes in 11 samples and reported 8 samples false positive.

Compared to the RIVM, Syphagen has 2 possible false positives. One (#169) was weak positive in a patient with Borrelia (and a VDRL > 32), and one (#518010) was dubious positive in the routine screening and would have been repeated in normal circumstances! No reactivity in unsensitized erythrocytes was seen with Syphagen.

Fuji showed reactivity of unsensitized carrier in 4 samples. No false positive samples were reported with Fuji.

Reproducibility was determined by retesting all discrepancies and the positive samples from the routine screening. In total 43 samples were retested. Table 5 shows a summary of the results. The table should be read as follows: Initially 25 samples scored positive for Medac and negative for Fuji and Syphagen. After retesting Medac scored 9+ and 12 - results, three samples reacted positive with unsensitized erythrocytes one sample contained insufficient material for retesting. For this group Fuji and Syphagen first and second test result were identical.

**Table 3: Sensitivity and specificity on routine screening of 299 samples:**

	Medac	Syphagen	Fuji
Sensitivity	100 %	100 %	100 %
Specificity	98,6 %	99,7 %	100 %

**Table 4: Result comparison on all samples**

	Medac	Syphagen	Fuji
Unsensitized erythrocytes positive	11	0	4
False positives	8	2	0

Table 5: Reproducibility

Medac		Syphagen		Fuji	
First	second	First	Second	First	Second
25+	9+ 3 U+ 1 is 12-	25-	24-  1is	25-	24-  1is
1+	1 U+	1-	1-	1 U+	1 U+
2+	1+ 1-	2-	2+	2+	2+
3-	1- 1+ 1 U+	3+	3+	3+	3+
3-	3-	3+	1+ 2-	3-	3-
1-	1 U+	1 U+	1-	1-	1 U+
1-	1+	1-	1+	1+	1-
5+	5+	5+	5+	5+	5+
2-	2-	2-	2-	2-	2-
43	43	43	43	43	43

U+ :positive reaction in unsensitized erythrocytes

is :insufficient sample

Table 6: Kit comparison

	Medac	Syphagen	Fuji
Readability	+	++	+++
Time needed before it can be read	>3 hours	1 hour	2 hours
Stability of reagents	+	++	+
Stability of agglutination	unstable	stable	stable
User guide	clear	dubious	clear
All reagents included	+	+	+
Costs	inexpensive	medium exp.	expensive
Attributes	equal	equal	equal
Technical difficulty	equal	equal	one step extra needed
Test presentation	+	±	++
Shelflife (diluted)	short	long	short
Serum/plasma	yes	yes	yes

## General conclusions:

Three commercial tests for TPHA with different carriers for the Treponema pallidum antigen were compared. These tests were compared for their ease of use, performance and stability. In our hands the performance of Fujirebio and Syphagen was comparable. Both kits had fixed and stable agglutination. Syphagen has the special advantage that results can be interpreted after one hour. Readability was slightly better with Fujirebio, probably because of clear staining, but still very good in Syphagen. All kits are easy to use. However, Fujirebio needs one extra step in preparing sample/reagent. Furthermore, Syphagen reagents are ready to use and stable until expiry date.

A great advantage of Syphagen is the very low reactivity with unsensitized erythrocytes, as a result repeat testing because of aspecific reaction is minimal, which is an important feature when a test has to be automated. Reactivity with unsensitized erythrocytes c.q. carrier is highest in MEDAC and still numerous in Fujirebio.

In the group with Borelia/Rheuma patients no problems were observed with the rheuma samples, Once a weak positive result was obtained with Syphagen for a patient with Borelia (and VDRL>32).

### Resume:

#### Medac

Advantage: low price, clear instructions

Disadvantage: stability of agglutination is short, reagents have a limited stability, reading time > 3 hours. Many aspecific and not interpretable reactions, reproducibility is limited

#### Fuji

Advantage: Clear instructions and good presentation. Test results in two hours.. Readability is very clear, no false negatives. Good sensitivity and specificity

Disadvantage: Limited stability of reagents, one step extra needed in test procedure. A substantial number of not interpretable and aspecific reactions.

#### Syphagen

Advantage: reagents ready to use, longterm shelflife of reagents. Agglutination is very stable and no aspecific nor not-interpretable reactions. No false negatives. Test results in one hour. Good sensitivity and specificity.

Disadvantage: instructions and presentation is poor.