

Department of Bacteriology

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Date

Detection of specific antibodies against *Treponema pallidum*.

EVALUATION OF FUJIREBIO, SERODIA®-TP•PA

The evaluation was performed in accordance with an agreement between Euro-Diagnostica AB, S-205 12 Malmö, Sverige, authorised representative of diagnostic kits from Fujirebio Inc., and the Department of Bacteriology, National Institute of Public Health, Oslo, Norway.

Test principle

TPPA is a passive particle agglutination assay test basically similar to the TPHA (*Treponema pallidum* haemagglutination assay). However, the blood cells have been substituted by red-coloured gelatin particles. These particles have been coated with purified specific antigen from *Treponema pallidum* (Nichols strain). Specific antibodies in the test sample react with antigen on the surface of different gelatin particles thereby creating agglutination of the particles visualised by a typical sedimentation pattern in the bottom of the wells where the reaction takes place.

The test may be run qualitatively at single serum dilution of 1:80, or quantitatively where the amount of antibodies present in the sample is defined as the inverse value of the highest two-fold dilution giving a clear positive reaction in the test. As in the TPHA test a control well is included in the TPPA for each sample. In this well particles without specific antigen coating is added to a final serum dilution of 1:40 so that non-specific agglutination of the particles may be detected.

The test can be used both on serum specimens and on plasma with different anticoagulants present. The kit insert does not state if and how spinal fluids may be tested.

A procedure for absorption of sera agglutinating non-coated particles is given.

Purpose of the evaluation

The purpose of the evaluation was to compare the performance of the TPPA and the TPHA from the same manufacturer. Until recently we have been routinely using the TPHA test at our laboratory. We wanted by own experience to know if the TPPA test had any advantages regarding sensitivity and practical test procedure. The evaluation comprised a limited number of test kits. Therefore evaluation of specificity, that would need testing of a large number of defined seronegative samples, could not be done. In the evaluation, the results of the samples included, were not correlated to the clinical diagnosis of the patients such as primary, secondary or tertiary syphilis, latent infection or a post treatment situation. However, most of the samples were from asymptomatic patients which means either latent infection or previously treated syphilis.

Material

Sera. A total of 92 sera were analysed. All sera were selected from the ordinary diagnostic routine in our laboratory in the period 1994 to 1996. 80 sera were selected on the basis of the primary TPHA result so that every two-fold titre step from 80 to >10240 was covered. In addition 12 TPHA negative sera were included

All sera were stored at -20°C until tested and had at most been thawed only once prior to the evaluation.

Analyses. All 92 sera were examined quantitatively both in TPPA (lot VN 50803) and in TPHA (lot FZ 50403). The manufacturers recommendations regarding test procedure and reading were followed meticulously. Sera with different amount of specific antibodies were tested randomly but in both tests at the same time. One skilled technician performed all analyses.

Sera found positive in one test and negative in the other were retested and also tested in an *Treponema* specific IgG-EIA (Captia Syphilis-G, Centocor). This additional test can *not* be taken as a "gold standard".

Results

Performance. The kit insert is written in English. The information is in great detail and is easily understood. Both tests use in principle the same procedure. Preliminary tests showed that shaking of the microplates were important for clear reading and reproducible results. This is also pointed out in the kit insert where it is stated that a vibrator and not an agitator must be used for approximately 30 seconds after all reactants have been added to the wells. We found that 60 seconds of shaking gave more easy and stable reading than 30 seconds. A coloured sheet showing different agglutination patterns to illustrate reading of end-point titre was very useful. Such a sheet did not follow the kits but was delivered by the kit representative.

It was definitely more easy to read the endpoint titre in the TPPA test than in the TPHA test.

Test results. A comparison between the results obtained by testing the 92 sera in the TPPA and TPHA tests, are shown in figure 1 and 2. Seventyseven sera gave positive results in both tests. The 12 selected negative sera were negative in both tests. Three sera showed positive-negative discrepancy. Two sera were TPPA positive (titre 80 and 320) but TPHA negative, and one serum was TPPA negative but TPHA positive (titre 160). All three sera were Captia Syphilis-G EIA positive (2) or equivocal (1).

Giving sera with negative results (titre < 80) the titre value of 40, the geometric mean titre of all sera in the TPPA was 1,9 times higher than the GMT of the TPHA results. If sera negative in both tests were excluded the GMT of the TPPA results were 2,1 times higher than the GMT of the TPHA results.

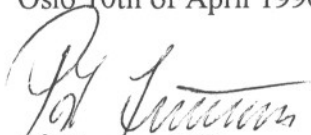
None of the sera positive in both tests (77 sera) had a lower titre in the TPPA test than in the TPHA test. For 16 sera (21%) the titre was equal, for 41 sera (53%) the TPPA titre was one titre-step higher, for 17 sera (22%) two titre-steps higher and for 3 sera (4%) three titre-steps higher than in the TPHA test.

No sera showed unspecific agglutination in any of the two tests.

Conclusions

TPPA seems to represent a clear improvement compared to the TPHA test. The TPPA test is more easy to read and has higher sensitivity than the TPHA test. Sufficient shaking of the testplates before reading is important. Procedure for testing of cerebrospinal fluid is lacking for both tests.

Oslo 10th of April 1996



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FIGURE 1 Comparison of titre in TPPA and TPHA (n=92)

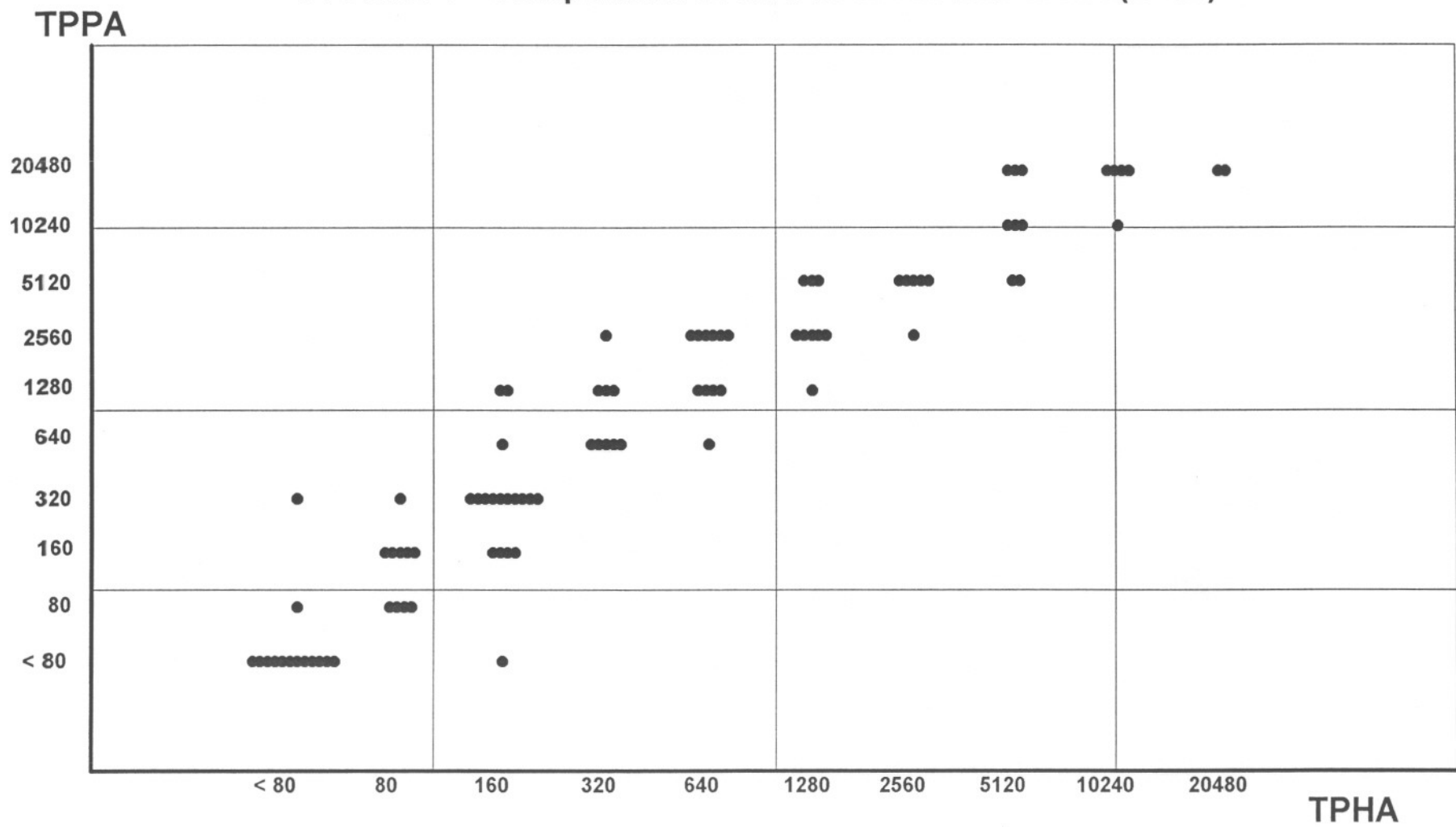


FIGURE 2 Comparison of titre in TPPA and TPHA for 92 selected sera

TPHA titre	TPPA titre										Total
	< 80	80	160	320	640	1280	2560	5120	10240	>=20480	
<80	12	1		1							14
80		4	5	1							10
160	1		4	10	1	2					18
320					5	3	1				9
640					1	4	6				11
1280						1	5	3			9
2560							1	5			6
5120								2	3	3	8
10240									1	4	5
>=20480										2	2
Total	13	5	9	12	7	10	13	10	4	9	92