

# Evaluation of microhaemagglutination assay to determine treponemal antibodies in CSF

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**SUMMARY** The microhaemagglutination assay for *Treponema pallidum* antibodies (MHA:TP-CSF), the Venereal Disease Research Laboratory (VDRL-CSF), and the fluorescent treponemal antibody (FTA-CSF) tests were used to detect treponemal antibodies in 3157 samples of cerebrospinal fluid from patients with syphilis, with neurological, metabolic, or immunological disorders, and with undetermined diagnoses. An agreement of 99.5% between the results of the MHA:TP-CSF and FTA-CSF tests was obtained for all the CSF samples whereas that of only 95.5% was obtained for samples from the known cases of syphilis. The MHA:TP-CSF test is simple, economical, rapid, and specific but not as sensitive as the FTA-CSF test.

## Introduction

The *Treponema pallidum* microhaemagglutination assay (TPHA), a relatively simple, highly specific, and sensitive test, was developed to determine the presence of syphilitic antibodies in serum (Rathlev, 1967). Later, many laboratories throughout the world started evaluating this test using a commercial antigen (manufactured by Fujizoki Pharmaceutical Company, Tokyo, Japan). Various authors compared the results of this test with those of the fluorescent treponemal antibody absorption (FTA-ABS) test, which until now was considered to be the confirmatory test, and found an agreement from 85% to 100% for antibodies present in the serum of syphilitic and nonsyphilitic patients (Cox *et al.*, 1971; Garner *et al.*, 1972; Kopecky *et al.*, 1977; Luger and Spendingwimmer, 1973; O'Neill *et al.*, 1973; Sequeira and Eldridge, 1973). Studies indicate that the TPHA test is less sensitive than the FTA-ABS test in detecting cases of primary syphilis (Tomizawa, 1969; Sequeira and Eldridge, 1973; Lesinski *et al.*, 1974). False positivity to the TPHA test in sera of healthy individuals is very rare (Coffey *et al.*, 1972; Garner *et al.*, 1973; O'Neill *et al.*, 1973; Young *et al.*, 1974), but it may frequently be observed in patients with autoimmune diseases (Lesinski *et al.*, 1974).

While much information is available on the evaluation of the TPHA test in serum, little is

described about its application in cerebrospinal fluids (CSF). Jaffe *et al.* (1978) found no reactivity for the TPHA test in CSF from 15 patients with latent syphilis, whereas Monsiorska-Borowska (1977) found it to be the most sensitive of the Venereal Disease Research Laboratory (VDRL), *Treponema pallidum* immobilisation (TPI), and FTA-ABS tests carried out on 110 samples of spinal fluid and 49 sera from patients with suspected or diagnosed late syphilis.

The present study was undertaken to evaluate the reactivity of the microhaemagglutination test in cerebrospinal fluid (MHA:TP-CSF) from non-syphilitic and syphilitic patients at different stages and to compare it with that of the VDRL-CSF and FTA-CSF tests.

## Material and methods

During the period of two years, from July 1976 to July 1978, cerebrospinal fluid specimens from 3157 patients were examined in the central laboratory for routine syphilis serology. Of these patients, 356 were known cases of syphilis confirmed by clinical examination, case history, and serology. The remaining 2801 specimens came from patients with undetermined diagnoses and from patients with neurological, metabolic, or immunological disorders. Most of the specimens were collected from the local hospitals and brought to the laboratory within 24 hours of collection. The specimens were kept at  $-20^{\circ}\text{C}$  until tested. No information was sought regarding the reactivity of the VDRL, FTA-ABS, and MHA:TP tests in the sera of these cases.

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Standard techniques were used for the VDRL-CSF (CDC, 1969) and MHA:TP-CSF (CDC, 1973) tests, whereas the FTA-CSF test was carried out according to the methods described by Duncan *et al.* (1972) and Leclerc *et al.* (1978). During this period all the samples were examined simultaneously by the three tests. The MHA:TP-CSF test was carried out on unactivated CSF using antigens and serum controls (supplied by Fujizoki Pharmaceutical Company, Tokyo, Japan).

The test was performed and interpreted according to the directions of the manufacturer as recommended for serum; the initial dilution of 1/20 of the CSF sample was brought to a final dilution of 1/80 after addition of the cells. The FTA-CSF reagents (*T. pallidum* antigen, conjugate, and sorbent) were supplied by Beckman Company, Fullerton, California, USA. The FTA-CSF test was carried out routinely on undiluted, unabsorbed CSF. All the CSF samples giving a reactive result in the routine test were retested after dilution to 1/5 in sorbent (supplied by the manufacturer) before being reported as giving a reactive result. Monospecific IgG conjugate was used in a dilution of 1/2300 with unabsorbed CSF and of 1/600 with absorbed CSF to get the same intensity of fluorescence with strongly reactive, weakly reactive, and non-reactive CSF controls selected in the laboratory. A Leitz Orthoplan microscope equipped with an incident light system was used according to the method described by Kasatiya and Birry (1972).

## Results

### REACTIVITY IN CSF SAMPLES

#### *Known and unknown diseases*

Of the 3157 specimens of CSF from patients with (a) neurological, metabolic, or immunological disorders; (b) different stages of syphilis; and (c) undetermined

diagnoses, 3.1%, 6.4%, and 6.9% gave reactive results to the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests respectively (Table 1). All of the 217 CSF samples giving a reactive result to the FTA-CSF test came from known cases of syphilis. An agreement of 96.3% was obtained between the VDRL-CSF and MHA:TP-CSF or FTA-CSF tests whereas that of 99.5% was found between the MHA:TP-CSF and FTA-CSF tests. False-positive results were not obtained in the VDRL-CSF, MHA:TP-CSF, or FTA-CSF tests. All of the 16 CSF samples giving a reactive result only in the FTA-CSF test came from known cases of syphilis.

#### *Syphilis*

The reactivity of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests in CSF samples from 356 known cases of syphilis are given in Table 2. The VDRL test gave a reactive result in only 27.5% of cases whereas in the MHA:TP-CSF and FTA-CSF tests 56.5% and 60.9% of samples respectively gave reactive results. CSF samples from 16 patients gave reactive results in only the FTA-CSF test. A poor agreement of 66.6% was obtained in this group between the VDRL-CSF and MHA:TP-CSF or FTA-CSF tests. CSF samples from 139 (39%) patients with syphilis did not show any reactivity in any of the three tests.

#### *Distribution according to sex*

Percentage reactivity for the three tests in CSF samples from the 275 men and 81 women with syphilis was almost identical. Of the total 356 CSF samples, 29.4% and 21% in the VDRL-CSF test, 57.4% and 53% in the MHA:TP-CSF test, and 60.7% and 61.7% in the FTA-CSF tests gave reactive results respectively in men and women.

#### *Primary syphilis*

All the CSF samples from 32 known cases of primary

Table 1 Results of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests performed on CSF samples from 3157 patients

Test results	Total no. of samples		Agreement between tests							
			VDRL: MHA		VDRL: FTA		MHA: FTA			
			No.	%	No.	%	No.	%		
+	+	+	98	3.1	3054	96.7				
+	+	—	0	0						
+	—	—	0	0						
—	+	+*	103	3.3					3141	99.5
—	—	+	16	0.5						
—	+	—	0	0						
+	—	+	0	0			3038	96.3		
—	—	—	2940	93.1						
Total no. reactive (%)	98 (3.1)	201 (6.4)	217 (6.9)							

\* Includes two patients with borderline results

+ Positive — negative

Table 2 Results of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests performed on 356 CSF samples from known cases of syphilis

Test results			Total no. of samples		Agreement between tests					
					VDRL: MHA		VDRL: FTA		MHA: FTA	
VDRL	MHA	FTA	No.	%	No.	%	No.	%	No.	%
+	+	+	98	27.5	253	71.0			340	95.5
+	+	—	0	0						
+	—	—	0	0						
—	+	+*	103	28.9						
—	—	+	16	4.5						
—	+	—	0	0						
+	—	+	0	0			237	66.6		
—	—	—	139	39.0						
Total no. reactive (%)			98(27.5)	201(56.5)	217(60.9)					

\* Includes two patients with borderline results

+ Positive —negative

syphilis gave negative results to the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests.

#### Secondary syphilis

As many as 50.6% and 64.5% of the CSF samples gave reactive results in the MHA:TP-CSF and FTA-CSF tests respectively, even in cases of secondary syphilis. The VDRL-CSF test gave reactive results in only 20.2% of cases. An agreement between the VDRL-CSF and MHA:TP-CSF or FTA-CSF tests was obtained in 55.7% of cases. A good agreement was found between the MHA:TP-CSF and FTA-CSF tests in this early stage of syphilis. CSF samples from 11 cases of secondary syphilis gave reactive results only in the FTA-CSF test (Table 3).

#### Tertiary syphilis

All cases of asymptomatic and symptomatic neurosyphilis and cardiovascular syphilis were grouped in this category. It is in this category that most of the CSF samples gave reactive results. Of the

121 CSF samples 91.7% gave reactive results in the FTA-CSF test while 88.4% and 57.9% gave reactive results in the MHA:TP-CSF and VDRL-CSF tests respectively (Table 4). Slightly better agreement was obtained between the VDRL-CSF and MHA:TP-CSF or FTA-CSF tests and between the MHA:TP-CSF and FTA-CSF tests than in cases of secondary syphilis. Of the four patients with reactive results in only the FTA-CSF test, one patient had meningovascular neurosyphilis and three asymptomatic neurosyphilis. The VDRL-CSF test was 2½ times more reactive in tertiary than in secondary syphilis.

#### Latent syphilis

The reactivity for the VDRL-CSF test dropped markedly to 9.5% in this particular group. The diminution in percentage reactivity was also observed in the MHA:TP-CSF and FTA-CSF tests (Table 5); 53.4% of the CSF samples gave no reactive results in any of these tests. Although agreement between the

Table 3 Results of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests performed on CSF samples from 79 cases of known secondary syphilis

Test results			Total no. of samples		Agreement between tests					
					VDRL: MHA		VDRL: FTA		MHA: FTA	
VDRL	MHA	FTA	No.	%	No.	%	No.	%	No.	%
+	+	+	16	20.2	55	69.6			68	86.0
+	+	—	0	0						
+	—	—	0	0						
—	+	+	24	30.4						
—	—	+	11	13.9						
—	+	—	0	0						
+	—	+	0	0			44	55.7		
—	—	—	28	35.4						
Total no. reactive (%)			16 (20.2)	40 (50.6)	51 (64.5)					

+ Positive —negative

Table 4 Results of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests performed on CSF samples from 121 known cases of tertiary syphilis

Test results			Total no. of samples		Agreement between tests					
					VDRL: MHA		VDRL: FTA		MHA: FTA	
VDRL	MHA	FTA	No.	%	No.	%	No.	%	No.	%
+	+	+	70	57.9						
+	+	—	0	0	80	66.1				
+	—	—	0	0						
—	+	+	37	30.6					117	96.7
—	—*	+	4†	3.3						
—	+	—	0	0						
+	—	+	0	0			80	66.1		
—	—	—	10	8.3						
Total no. reactive (%)			70 (57.9)	107 (88.4)	111 (91.7)					

\* Four cases of +/— reaction reported as a negative result

† One case of meningovascular and three cases of asymptomatic neurosyphilis

+ Positive —negative

Table 5 Results of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests performed on CSF samples from 116 known cases of latent syphilis

Test results			Total no. of samples		Agreement between tests					
					VDRL: MHA		VDRL: FTA		MHA: FTA	
VDRL	MHA	FTA	No.	%	No.	%	No.	%	No.	%
+	+	+	11	9.5						
+	+	—	0	0	74	63.8				
+	—	—	0	0						
—	+	+	42	36.2						
—	—	+	1	0.9					115	99.1
—	+	—	0	0						
+	—	+	0	0			73	62.9		
—	—	—	62	53.4						
Total no. reactive (%)			11 (9.5)	53 (45.7)	54 (46.5)					

+ Positive —negative

VDRL-CSF and MHA:TP-CSF tests remained more or less the same as in the previous cases, the agreement between the MHA:TP-CSF and FTA-CSF tests rose to 99.1%.

#### Congenital syphilis

Of the eight CSF samples from patients with congenital syphilis only one gave a reactive result in all three tests.

#### Diseases other than syphilis

No reactivity with any of the three tests was found in CSF samples from 499 patients with diseases other than syphilis (Table 6).

#### Discussion

Most authors consider that the TPHA technique is as specific and sensitive as the FTA-ABS test in detecting treponemal serum antibodies in syphilis,

except in cases of primary syphilis (Tomizawa *et al.*, 1969; Garner *et al.*, 1973; Kiraly and Prerau, 1974) and that the antibody appears later than the one detected by the FTA-ABS test and probably also by the VDRL test (Rudolph, 1976). On the contrary, Jephcott *et al.* (1977) reported a case in which the TPHA test was the first to give a positive result in serum compared with the VDRL and FTA-ABS tests. Similarly, Monsiorska-Borowska (1977) found the TPHA test to be more sensitive than the FTA, TPI, and VDRL tests.

It is known that the central nervous system is affected early in the course of early syphilis (O'Neill *et al.*, 1973; Lejman *et al.*, 1976; Kopecky, 1977), but efficient serological tests are not available for detecting treponemal antibodies in CSF in the early stages of syphilis. Previous work done in this laboratory has shown that the FTA-CSF test is capable of detecting treponemal antibodies in 65.9% of cases of secondary syphilis (Leclerc *et al.*, 1978) and this is confirmed by the present study.

Table 6 Results of the VDRL-CSF, MHA:TP-CSF, and FTA-CSF tests performed on CSF samples from 499 patients with diseases other than syphilis

Diseases	Test results			Total no. of samples	Diseases	Test results			Total no. of samples
	VDRL	TPHA	FTA			VDRL	TPHA	FTA	
Anaemia	—	—	—	4	Labyrinthitis	—	—	—	3
Aphasia	—	—	—	4	Lumbar adherence	—	—	—	8
Ataxia	—	—	—	12	Lupus erythymatosus	—	—	—	4
Cardiac insufficiency	—	—	—	7	Medullary compression	—	—	—	4
Cerebral atrophy	—	—	—	6	Meningitis	—	—	—	43
Cerebral metastases	—	—	—	1	Migraine	—	—	—	10
Cerebral syndrome	—	—	—	11	Multiple myeloma	—	—	—	2
Cerebral thrombosis	—	—	—	11	Multiple sclerosis	—	—	—	35
Cerebral tumour	—	—	—	4	Myelitis	—	—	—	13
Dementia	—	—	—	16	Neuritis	—	—	—	12
Diabetes	—	—	—	7	Paralysis	—	—	—	17
Discal hernia	—	—	—	60	Paraplegia	—	—	—	5
Encephalomyeloneuropathy	—	—	—	23	Parkinson's diseases	—	—	—	5
Epilepsy	—	—	—	55	Pick's disease	—	—	—	1
Fever	—	—	—	5	Pneumonia	—	—	—	9
Genital herpes	—	—	—	5	Polyarthrits	—	—	—	3
Hemiplegia	—	—	—	6	Polyneuropathy	—	—	—	5
Hepatitis	—	—	—	1	Portal systemic encephalopathy	—	—	—	8
Hydrocephaly	—	—	—	17	Reticulopathy	—	—	—	1
Hypertension	—	—	—	6	Seizures	—	—	—	19
Hyperthermia	—	—	—	6	Toxoplasmosis	—	—	—	4
Hypophyseal tumour	—	—	—	3	Tuberculosis	—	—	—	6
Hypophyseal disease	—	—	—	5	Vasculitis	—	—	—	3
Infectious diseases	—	—	—	3	Viraemia	—	—	—	1

— Negative

The evaluation of the MHA:TP-CSF test—a simpler and less costly technique than the FTA-CSF test—with 2940 non-syphilitic cases indicates that a false-positive result in this test is very rare and that the test is highly specific for treponemal antibodies. Our data supports the observations of Jaffe *et al.* (1978) regarding the high specificity of the TPHA. It further indicates that this test is sensitive in detecting treponemal antibodies in the early stages of syphilis. It was still not possible to detect antibodies in CSF samples from any of the patients with primary syphilis by the two treponemal tests. At later stages of syphilis the test compares well with the FTA-CSF test, thus supporting the observation of Monsiorska-Borowska (1977). Jaffe *et al.* (1978) did not find any reactivity in patients with latent syphilis, probably because of the small number of cases studied.

The MHA:TP-CSF test results were stable and reproducible even after repetition at long intervals of time, provided that the specimen was stored frozen.

Specificity in the FTA-CSF test increases if the test is performed on CSF samples diluted to 1/5 in sorbent but, in some cases, it may lose sensitivity and give false-negative results. CSF samples from 16 known cases of syphilis, of which 11 were from cases of secondary syphilis, four from cases of tertiary syphilis, and one from a case of late latent syphilis, giving non-reactive results in the VDRL-CSF and MHA:TP-CSF tests and reactive results in the FTA-CSF test, became non-reactive in the latter test when

the CSF was retested after absorption. This finding confirms the results of Duncan *et al.* (1972) who found a reduction in the reactivity of the FTA-CSF test after dilution of the CSF in the sorbent. On the other hand, of the same 16 CSF samples when retested undiluted by the MHA:TP-CSF method, 13 (nine from cases of secondary, three of tertiary, and one of latent syphilis) became reactive, thus indicating that the manufacturer's instructions for serum TPHA, where serum is used at a dilution of 1/20, may not apply to the detection of treponemal antibodies in CSF. Diluting the CSF reduces the concentration of immunoglobulins shown by both the low reactivity in the FTA-CSF test and non-reactivity in the MHA:TP-CSF test. Further studies of the MHA:TP-CSF test using undiluted CSF are in progress.

The reactivity in CSF samples from cases of latent syphilis in all the three tests indicates that those cases were syphilitic and not biological false-positive reactors. In the absence of other features they would have been diagnosed as latent cases without the CSF examination. Some may have been previously treated.

Data indicate that the MHA:TP-CSF test is almost identical in specificity but inferior in sensitivity to the FTA-CSF test. The former test, being simple and more economical to perform, can be easily automated for the routine detection of treponemal antibodies in large numbers of CSF samples.

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