Read this insert carefully before performing the assay and keep for future reference.
The reliability of assay procedures other than those described in this package insert cannot be guaranteed.

TS07T ☆ Revised in June, 2015 (Ver. 4)
IVD REF 219126 ☆: Note changes

Reagent for the detection of *Treponema pallidum* antibodies

ESPLINE® TP

■ NAME
ESPLINE TP

■ INTENDED USE
"ESPLINE TP" is based on the principle of immunochromatography for the detection of antibodies to *Treponema pallidum* (TP) in serum or plasma samples and a qualitative assay. The test is intended for professional *in vitro* diagnostic use only and to aid in the diagnosis of syphilis.

Patient population is patients suspected syphilis.

■ PRINCIPLE OF THE PROCEDURE
"ESPLINE TP" employs two recombinant antigens considered to be the main antigens of TP. These recombinant antigens are fixed onto a membrane in the test part of the reaction cassette.

An enzyme-labeled antigen pad, a substrate pad, and a developing solution are also included in the reaction cassette.

If antibodies to *Treponema pallidum* are present in a sample, the antibodies react with the enzyme-labeled antigens.

The mixture continues to migrate on the membrane in the developing solution and combines with the fixed TP antigen.

The enzyme in this complex reacts with the substrate, and forms a blue line visible on an interpretation window.

■ COMPOSITION OF REAGENTS
The reaction cassette contains the following components:

- TP recombinant antigen (TpN47) 0.7μg/cassette
- TP recombinant antigen (Tp15-17) 0.35μg/cassette
- Alkaline phosphatase labeled TP recombinant antigen (TpN47) 17.0ng/cassette
- Alkaline phosphatase labeled TP recombinant antigen (Tp15-17) 14.84ng/cassette
- 5-Bromo-4-chloro-3-indolyl phosphate disodium salt 100μg/cassette

■ PRECAUTIONS
1. Do not press the convex button of the reaction cassette before applying the sample.
2. Do not use a reaction cassette on which the red line is not within the letter "r" or on which the line has disappeared.
3. The developing solution is alkaline (pH 10). Avoid direct contact with the skin, mouth, or eyes. In case of accidental contact with the alkaline developing solution, rinse thoroughly with water and seek medical attention or treatment, if necessary.
4. The TP antigen used in this reaction cassette is a recombinant antigen for which the absence of infectivity has been confirmed. However, the cassette should still be handled with the same care and precautions as samples, for added safety.
5. All samples should be handled as potential carriers of infectious agents such as HBV, HCV, HIV or other hazardous microorganisms. Components and accessories (e.g. tips) should be handled carefully and disposed of properly. Decontamination can be done with sodium hypochlorite (effective chloride concentration 1,000 ppm for more than 60 minutes), glutaraldehyde solution (for more than 60 minutes with 2% solution), autoclaving at 121°C for more than 20 minutes, or by incineration.
6. Do not reuse.
☆7. SAFETY PRECAUTIONS
N-Cyclohexyl-2-aminoethanesulfonic acid: CHES 2.1% (w/v)
EUH210: Safety data sheet available on request.

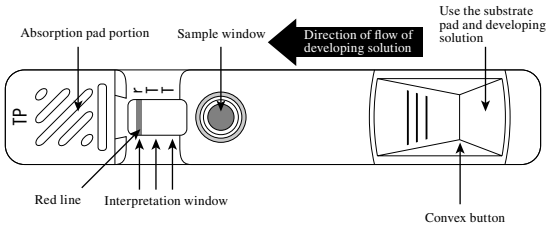
■ STORAGE
2-10°C (Do not freeze). Protect from direct sunlight.

ESPLINE TP is stable through the expiration date printed on the sealed aluminum pouch if it is stored as packaged in the sealed aluminum pouch at 2-10°C. Do not use the reaction cassette beyond the expiration date.

■ MATERIAL REQUIRED BUT NOT PROVIDED
Micropipette

■ SPECIMEN COLLECTION AND PREPARATION
Serum or plasma
1. When storage of a sample is required, store frozen at -20°C or below. Avoid repeated freezing and thawing of sample.
2. Erythrocytes or other visible components in serum or plasma specimen should be removed by centrifugation prior to testing.

■ TEST PROCEDURE
Allow the reaction cassette, serum or plasma specimen to reach room temperature (20-37°C) prior to testing. Do not open the aluminum pouch until ready to perform the assay. Only bring to room temperature the number of reaction cassettes required to assay.
1. Take the reaction cassette out of the aluminum pouch.

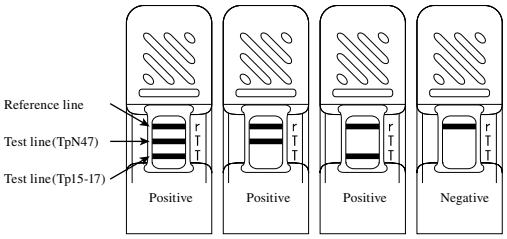


r : reference line
T : anti TpN47 antibodies test line
T : anti Tp15-17 antibodies test line

2. Apply 25 μL of serum or plasma onto the purple sample window of the reaction cassette.
3. Press the convex button of the reaction cassette down immediately after loading the sample to start the reaction.
Make sure that the convex button is completely pressed down.
4. Let the reaction cassette rest at room temperature for 15 minutes and read the result.

■ QUALITY CONTROL
To insure assay validity, a procedure control labeled "r" (=reference) is incorporated in the reaction cassette. If the blue reference line does not appear by assay completion, the test result is invalid and the sample should be retested.

■ CRITERIA FOR RESULT INTERPRETATION
Interpret the test according to the following criteria:



1. Positive
Blue reference line is observed and one or two blue test line(s) appear in the interpretation window.
2. Negative
Blue reference line is observed but no blue test line appears in the interpretation window.
3. Invalid
If a blue reference line is not observed, regardless of the appearance of the blue test line(s) or the disappearance of the initial red reference line, it can be concluded that the testing procedure is inappropriate or the reaction did not occur in the cassette. Review the test procedure and repeat the test using a new reaction cassette. If the problem persisted, contact your local distributor.

■ LIMITATIONS OF THE PROCEDURE
1. "ESPLINE TP" is designed for the detection of antibodies to *Treponema pallidum* (TP), but not for direct detection of TP.
If a positive result is obtained, another method should be used for confirmation (e.g., a test using syphilis lipid antigen, the FTA-ABS method, etc.), and the data should be considered in light of the patient's history and other clinical and/or laboratory findings.
2. In the early stage of syphilis infection, when TP antibodies are not produced or when its titer is low, a negative may occasionally be obtained. If Syphilis is suspected, the results of other tests and the patient's symptoms should be taken into consideration when making a diagnosis, even if the present test is negative.
3. Samples from patients who have been treated with blood products includ-

ing immunoglobulins may show a positive reaction. Prudence is advised in assessing such cases.
4. Inactivation of samples does not affect the result.
5. EDTA, Heparin and Citric acid can be used as anti-coagulant in the plasma specimen. When EDTA, Heparin and Citric acid were added to a positive sample and tested according to procedure, no effect was found up to 2mg/mL, 300U/mL and 8mg/mL, respectively.
6. When bilirubin F, bilirubin C, hemoglobin and phospholipids were added to a sample and tested according to procedure, no effect was found up to 19.1mg/dL, 19.6mg/dL, 484mg/dL and 2,130 degrees, respectively.
7. In antigen-antibody reactions, the prozone phenomenon may occur. Therefore, close attention to this possibility is warranted. However, the present reagent gave a positive result for a sample with a high titer of 81,920 times by the particle agglutination method, and no prozone phenomenon was detected.

■ PERFORMANCE CHARACTERISTICS

1. Sensitivity and specificity
A total of 935 serum specimens were negative and 145 specimens were positive for Syphilis, as determined by control product (e.g., particle agglutination method).

	Population	Number of specimens	**ESPLINE TP**	SERODIA TPPA
Specificity	Non-syphilis (-)	935	**932** **99.7%** **(932/935[*1,2,3])**	935 100% (935/935)
Sensitivity	Syphilis (+)	145	**145** **100.0%** **(145/145)**	145 100.0% (145/145)

Note) 3 discordant samples were excluded.
*1,2) SERODIA TPPA: weak positive(1:40), FTA-ABS: negative.
*3) SERODIA TPPA: weak positive(1:40), FTA-ABS: indeterminate.

2. Reproducibility
In-house positive control samples[*4)] were tested 3 times and yielded the same results.

*4) Traceability: in-house positive control samples was established with in-house standard, because international reference materials for TP antibody detection kit does not exist.

3. Dilution measurement of TP antibodies (Detection limit)
ESPLINE TP can detect TP antibodies with the same dilution as other commercially available immuno-chromatography rapid tests.

4. Correlation
Using serum samples from 100 patients, the correlation with a control product (e.g., particle agglutination method) was tested and the following results were obtained:

Serum		Reference test			Total
		Positive	Indeterminate	Negative	
ESPLINE TP	Positive	49 (100%)	0	1[*5)] (2%)	50
	Negative	0 (0%)	0	50 (98%)	50
Total		49 (100%)	0	51 (100%)	100

*5) SERODIA TPPA: weak positive(1:40), FTA-ABS: indeterminate.
Using plasma samples from 118 patients, correlation with a control product (e.g., particle agglutination method) was tested and the following results were obtained:

Plasma		Reference test			Total
		Positive	Indeterminate	Negative	
ESPLINE TP	Positive	44 (100%)	1[*6)]	0 (0%)	45
	Negative	0 (0%)	1[*7)]	72 (100%)	73
Total		44 (100%)	2	72 (100%)	118

Note) 2 indeterminate samples were excluded.
*6) ESPLINE TP: Positive, Particle agglutination method: Indeterminate. It was confirmed Indeterminate by FTA-ABS.
*7) ESPLINE TP: Negative, Particle agglutination method: Indeterminate. It was confirmed Negative by FTA-ABS.

■ DISPOSAL PRECAUTION

The reaction cassette should be disposed of as medical waste, industrial waste, etc., in accordance with the regulations pertaining to waste materials.

■ EXPIRY DATE

12 months after manufacturing (refer to the date on the outer box.)

■ PACKING UNIT

2 × 10 tests

■ REFERENCES

1) Norris SJ, et al. Polypeptides of *Treponema pallidum*: Progress toward Understanding Their Structural, Functional, and Immunologic Roles. Microbiol Rev, Vol.57, No.3: 750-779, 1993.
2) Byrne RE, et al. Evaluation of *Treponema pallidum* Western Immunoblot Assay as a Confirmatory Test for Syphilis. J Clin Microbiol, Vol.30, No.1: 115-122, 1992.

■ INQUIRES

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■ GLOSSARY OF SYMBOLS

CE	CE Marking (European directive 98/79/EC on *in vitro* diagnostic medical devices)		
IVD	*In Vitro* Diagnostic Medical Device	LOT	Batch Code
	Consult Instructions for Use	REF	Catalogue Number
EC REP	Authorised Representative in the European Community	SN	Serial Number
	Manufacturer		Use by
	Open here		Temperature Limitation
	Do not reuse		Caution

FUJIREBIO INC.
CE