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Immunoassay

REF **CMJ0201/CMJ0202/CMJ0203/CMJ0204/CMJ0205**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

Anti-TP CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the qualitative determination of Anti-TP (antibody to Treponema pallidum) in human serum and plasma (EDTA, heparin or sodium citrate) as an aid to diagnosis of syphilis.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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CE IVD

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Introduction

The identification of *Treponema pallidum* antibodies aids in the diagnosis of syphilis caused by the microorganisms belonging to the genus Treponema and provides epidemiological information on syphilis[1]. *Treponema pallidum* is a chronic bacterial infection that remains a public health concern worldwide. The WHO estimated that 12 million new cases of venereal syphilis occurred in 1999, more than 90% of them in developing countries, with a rapidly increasing number of cases in Eastern Europe [2, 3].

Syphilis can be transmitted congenitally or by sexual contact. Congenital syphilis is of particular concern in developing nations as it may lead to spontaneous abortion, stillbirth, death of the neonate, or disease in the infant; a recent report from Tanzania estimates that up to 50% of stillbirths are caused by syphilis[4]. Of particular importance to worldwide health is the recognition that syphilis infection greatly increases the transmission and acquisition of human immunodeficiency virus (HIV) [5, 6]. These factors, along with the highly destructive nature of late disease, make syphilis an important public health concern. The disease can evolve into a latent phase in which syphilis is clinically inapparent.

The serological detection of specific antibodies to T. pallidumis of particular importance in the diagnosis of syphilis, as the natural course of the infection is characterized by periods without clinical manifestations[7, 8]. Serological tests are divided into nontreponemal and treponemal tests and neither alone is sufficient for diagnosis, in addition to patients' clinical history, are currently the primary methods for the diagnosis and management of syphilis. Nontreponemal tests can be used to monitor therapy, but owing to their low specificity the positive results obtained by these methods need to be confirmed by treponemal tests. As the positivity at treponemal tests lasts throughout the life, treponemal tests cannot be used in the follow-up of patients. Consequently, the quest for simple, reliable, and money-saving diagnostic methods continues.

This assay intended to be used for the detection of *Treponema pallidum* antibodies in human serum or plasma as an aid in the diagnosis of syphilis.

Measurement Principle

This assay is based upon the principle of the one step sandwich technique for the qualitative detection of the various antibodies associated with *Treponema pallidum* (TP) in human serum or plasma, using chemiluminescent microparticle immunoassay (CLIA Micropaticles) technology. The sample, TP antigen coated paramagnetic microparticles and HRP labeled TP antigen as enzyme conjugate are combined. During the incubation, a complex is generated among the solid phase, the Anti-TP within the sample and enzyme-labeled antigen by immunological reactions. After washing, the substrates are added and the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of Anti-TP in the samples.

Materials provided

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3 mL*2	2.3 mL*5	1.2 mL*2
Enzyme Conjugate	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2

1. Positive Control

1 vial containing 1.0 mL of HEPES buffer, containing heat-inactivated human plasma positive for TP antibody and proteins of bovine origin. Contains a selection of preservatives. Reagent provided ready to use.

2. Negative Control

1 vial containing 1.0 mL of PBS buffer, containing proteins of bovine origin. Contains a selection of preservatives. Reagent provided ready to use.

3. Reagent pack

Reagent pack provided ready to use.

- Microparticles Solution

TP antigen coated microparticles in Tris-EDTA-2Na buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Horseradish-peroxidase labeled TP antigen in PBS buffer containing proteins of ADP and casein. Contains a selection of preservatives.

Note: the indicated reagent volume for the components is only the minimum amount.

Assay Analyzers on which the kit can be used

- AutoLumo A2000
- AutoLumo A2000 Plus
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tube(s) or cup(s) for sample containing
4. Chemiluminescent Substrate
5. System Buffer for washing the pipetting needle
6. Wash Buffer used in washing procedure
7. Distilled or deionized water.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the positive control contains human sourced components, which have been tested and found non-reactive for HIV-1/HIV-2, HCV antibodies and HBsAg, but reactive for TP antibodies by CE-marked IVD reagents. It is recommended that all materials of human origin be considered potentially infectious. This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Conduct the assay away from bad ambient conditions. e. g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
9. Do not use reagents beyond the labeled expiry date.
10. Do not mix or use components from kits with different batch codes.
11. When storing the controls, be certain the vials are securely sealed.
12. Ensure the microparticles are resuspended before loading it on the

analyzer.

13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining positive or negative control to 2-8 ℃, under that conditions the stability will be retained for 1 month, for longer use, store opened controls in aliquots and freeze at -20 ℃. Avoid multiple freeze-thaw cycles.

Sample

1. Collect samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy etc. in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (un-punctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample tubes on the sample rack, 50 μL of sample for each test. But consider the sample container and system dead volumes; more sample volume may be needed.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticle Solution and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine Anti-TP in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibration

- Analyzer can read the bar code on the reagent pack automatically to obtain the essential information for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the Anti-TP CLIA Microparticles positive and negative controls into the sample tubes and place the sample tubes on the sample rack. They are automatically tested in triplicate or duplicate (triplicate for positive controls and duplicate for negative controls) at the beginning of each batch. The Assay Analyzer system will not generate results when controls values do not meet specifications. This may indicate either deterioration or contamination of reagents, or analyzer failure.
- Load the sample rack and input negative & positive control information on the system software interface.
- Select "run" to start the test, calibration is required every 28 days.
- Once the control results is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of calibration
  - Important parts of the analyzer are replaced or repaired.
- Refer to the Assay Analyzer's operation manual.

Assay Parameter Specifications

The assay parameter specifications have been factory set. These parameters cannot be printed, displayed, or edited.

Measurement Results

- Calculation of Cut-off and S/CO Values

The Assay Analyzer system calculates the Anti-TP CLIA Microparticles assay Cut-off value using the following formula:

Cut-off Values = PCx * 0.2

PCx: the mean of the positive control RLU replicates

Example: Mean PC RLU = 6585896

Cut-off Value = 6585896 x 0.2 =1317179.2

The Assay Analyzer system calculates the Anti-TP CLIA Microparticles assay S/CO for each sample using the following formula:

S/CO = Sample RLU/Cut-off Value

Example: Sample RLU =2210145

S/CO Value =2210145/1317179.2 = 1.68

- Interpretation of Results

• In the Anti-TP CLIA Microparticles assay, samples with S/CO values of less than 1.00 are considered nonreactive and need not be tested further. Nonreactive samples are considered negative for Anti-TP by the criteria of Anti-TP CLIA Microparticles.

• Samples with an S/CO value of greater than or equal to 1.00 are considered initially reactive by the criteria of the Anti-TP CLIA Microparticles assay. All samples that are reactive on initial testing must be centrifuged prior to retesting. Initially reactive samples must be retested in duplicate using the Anti-TP CLIA Microparticles Assay Kit.

• If the sample RLU for both retests is less than the Cut-off value, the sample is nonreactive. Nonreactive samples are considered negative for Anti-TP by the criteria of the Anti-TP CLIA Microparticles.

• If the sample RLU for either duplicate retest is greater than or equal to the Cut-off value, the sample is considered repeatedly reactive. Repeatedly reactive results indicate the presence of Anti-TP by the criteria of the Anti-TP CLIA Microparticles.

• Although the association of infectivity of donated blood or plasma and the presence of Anti-TP is strong, it is recognized that presently available methods for Anti-TP detection are not sensitive enough to detect all potentially infectious units of blood, plasma, or possible cases of TP infection. A nonreactive test result does not exclude infection.

Control Procedure

The recommended control requirement for this assay involves using positive and negative controls to verify assay performance. The result is valid if the control results meet assigned specifications (Mean PC RLU/ Mean NC RLU＞20). When a control fails to meet assigned specifications, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. False-reactive test results can be expected with any test kit. False-reactive test results have been observed due to nonspecific interactions.
4. Some samples that have undergone multiple freeze-thaw cycles or have been stored frozen for prolonged periods may result in erroneous or inconsistent test results.
5. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
6. Performance of this test has not been established with neonatal samples.
7. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
8. This assay was designed and validated for use with human serum and plasma from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
9. Due to limitation of methodology or immunological specificity and other reasons, the test results from different manufacturers' reagents for the same sample may be different, so such results should not be directly compared with each other, so as to avoid the wrong medical explanation. It suggests that the laboratory should indicate the characteristics of the reagents used in the test report issued to the clinician.

Performance Characteristics

1. Measurement Precision

3 samples were assayed in replicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean	Within-run	Total
			%CV	%CV
1	80	3.42	3.96	7.28
2	80	5.69	4.18	5.44
3	80	11.31	3.75	6.93

*Representative data; results in individual laboratories may vary from these data

2. Assay Sensitivity

A total of 1549 serum and plasma samples from individuals known to be positive for TP antibodies were tested with the Anti-TP CLIA Microparticles assay. Of the 1549 samples, all samples were repeatedly reactive, which the overall sensitivity was estimated in these studies to be 100.00%.

3. Clinical Specificity

A total of 3426 fresh serum and plasma samples from volunteer blood donors and medical examination donors were collected and tested at different geographically distinct blood centers and hospitals. Of the 3426 samples, 11 (3415/3426) samples were repeatedly reactive, and based on supplemental test results from a licensed and/ or research immunoblot assay, they were Anti-TP negative. This assay has a specificity of 99.68%.

4. Analysis specificity

Cross reaction: this assay was evaluated for potential cross-reactivity for samples from individuals with medical conditions unrelated to HCV infection. The samples were tested using the Anti-TP CLIA Microparticles assay. The data are summarized in the following table.

Category	No.	Anti-TP CLIA Microparticles assay	
		Reactive	Nonreactive
Anti-EBV positive	3	0	3
Anti-HCV positive	3	0	3
Anti-HBV positive	3	0	3
Anti-HIV positive	3	0	3
Rh.[a] factor positive	3	0	3
Rubella	6	0	6
Anti-CMV positive	6	0	6

a. Rh.[a] factor: Rheumatoid factor

Interference: at the concentrations listed below, bilirubin (conjugated and unconjugated), haemoglobin and triglycerides showed less than 10% interference in the Anti-TP CLIA Microparticles assay for 10 negative samples and 10 positive samples:

- Bilirubin ⩽ 20 mg/ dL
- Haemoglobin ⩽ 500 mg/dL
- Triglycerides ⩽ 3000 mg/dL

5. Tube Type Matrix Study

The following tube types are acceptable for use with this assay:

* Serum and serum separator
* EDTA, heparin or sodium citrate plasma

Different sample types were taken from 15 individuals. The tube types listed in the table below showed no significant difference between serum and plasma.

With the tube types listed in the table below, Anti-TP CLIA Microparticles assay showed the S/CO values for different tube type matrix.

Tube type matrix	Serum	Heparin Plasma	EDTA Plasma	Sodium Citrate Plasma
Mean S/CO	0.02	0.02	0.02	0.02
Maximal S/CO	0.06	0.13	0.06	0.06

6. Performance on BBI Seroconversion Panel

BBI PSS901 Anti-TP Seroconversion Panel

PSS901 Panel member	Days since 1st bleed	BBI Ref. Data		This Assay
		Immunoassay Result, S/CO		
		Assay 1	Assay 2	
PSS901-01	0	NEG	NEG	NEG
PSS901-02	5	NEG	NEG	NEG
PSS901-03	10	NEG	NEG	NEG
PSS901-04	13	NEG	NEG	NEG
PSS901-05	31	NEG	NEG	NEG
PSS901-06	45	POS	POS	POS

The results showed 6 days earlier detection of Anti-TP CLIA Microparticles assay comparing 2 other CE marked commercial kit.

Literature References

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