Snibe Diagnostic

CE REF 130269003M:100 tests/kit
130669003M: 50 tests/kit
130769003M: 30 tests/kit

MAGLUMI® Syphilis (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the qualitative determination of total antibodies to *Treponema pallidum (T. pallidum)* in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used for an aid in the diagnosis of Syphilis infection.

SUMMARY

Syphilis, a genital ulcerative disease caused by the bacterium *Treponema pallidum*, is associated with significant complications if left untreated and can facilitate the transmission and acquisition of HIV infection[1]. *T. pallidum*'s only known natural host is the human. *T. pallidum* subsp. *pallidum* belongs to a family of spiral-shaped bacteria, the *Spirochaetaceae* (spirochetes), and is related to other pathogenic treponemes that cause nonvenereal diseases[2].

Syphilis is a systemic, sexually transmitted disease. If it is not treated in the primary, acute stage, it becomes a chronic disease. Syphilis has three stages: a) The primary stage usually starts 21 days (range: 10–90 days) following infection; the infected person develops a painless genital ulcer, which lasts 2–6 weeks; b) The secondary stage is characterized by a skin rash over the whole body, often with fever and muscle pain. This stage also lasts 2–6 weeks, and is followed by a latent phase of many years, during which there are no signs or symptoms. However, even during the latent phase, spirochaetes may occasionally circulate in the blood, though this happens less frequently as time goes on; as a result, virtually all the organs of the body may become infected; c) The tertiary stage occurs several years to several decades after infection, and can take the form of neurosyphilis (in which the brain or spinal cord is affected), cardiovascular syphilis (involving the aorta and heart), or late benign syphilis (involving primarily the skin)[3].

Syphilis is transmitted from person to person by direct contact with a syphilitic sore, known as a chancre. Chancres can occur on or around the external genitals, in the vagina, around the anus, or in the rectum, or in or around the mouth. In addition, pregnant women with syphilis can transmit the infection to their unborn child, causing congenital syphilis[4]. Congenital syphilis is a leading cause of stillbirth and perinatal mortality, adverse pregnancy outcomes occur in up to 80% of women with acute syphilis, including stillbirth (40%), perinatal death (20%) and serious neonatal infection (20%)[5,6].

Syphilis can affect the conjunctiva, sclera, cornea, lens, uveal tract, retina, the retinal vasculature, the optic nerve, pupillomotor pathways, and cranial nerves involved in extraocular movements[7]. Multiple studies have shown that syphilis infection is associated with an increased risk of acquiring HIV[8].

TEST PRINCIPLE

Sandwich chemiluminescence immunoassay.

The sample, ABEI labeled with *T. pallidum* specific recombinant antigen, buffer and magnetic microbeads coated with *T. pallidum* specific recombinant antigen are mixed thoroughly and incubated, performing a wash cycle after a precipitation in a magnetic field. ABEI labeled with *T. pallidum* specific recombinant antigen are then added and incubated, reacting to form sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of *T. pallidum* antibodies present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with *T. pallidum* specific recombinant antigen (~12.0 μg/mL) in PBS buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	1.0 mL
Calibrator Low	A low concentration of *T. pallidum* antibody in PBS buffer, NaN_3 (<0.1%).	3.0 mL	2.0 mL	1.0 mL
Calibrator High	A high concentration of *T. pallidum* antibody in PBS buffer, NaN_3 (<0.1%).	3.0 mL	2.0 mL	1.0 mL
Buffer	Tris-HCl buffer, NaN_3 (<0.1%).	7.5 mL	5.0 mL	3.3 mL
ABEI Label	ABEI labeled with *T. pallidum* specific recombinant antigen (~41.7 ng/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	22.5 mL	12.0 mL	7.8 mL
Negative Control	PBS buffer, NaN_3 (<0.1%).	2.0 mL	2.0 mL	2.0 mL
Positive Control	A high concentration of *T. pallidum* antibody (10.0 mIU/mL) in PBS buffer, NaN_3 (<0.1%).	2.0 mL	2.0 mL	2.0 mL
All reagents are provided ready-to-use.				

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date

Opened at 2-8°C	6 weeks
On-board	4 weeks

Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 10-30°C	6 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Types	Collection Tubes
Serum	Tubes without additive/accessory, or tubes containing clot activator or clot activator with gel.
Plasma	K2-EDTA, Na2-EDTA, Na-heparin or Li-heparin

- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- Do not use heat-inactivated samples or grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some serum specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the serum specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results.
- Samples must be free of fibrin and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips are recommended.

Preparation for Analysis

- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give reliable results and must be centrifuged prior to testing. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- The sample volume required for a single determination of this assay is 40 µL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 16 hours at 10-30°C or 4 days at 2-8°C, or 3 months frozen at -20°C. Frozen specimens subjected to up to 2 freeze/thaw cycles have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

Syphilis (CLIA) assay, control barcode labels.

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3, MAGLUMI X6 or Integrated System Biolumi 8000, Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on ordering quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing.. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

Traceability: This method has been standardized against the WHO 1st International Standard 05/132.

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 14 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other published guidelines[9]

Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the Syphilis assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi systems and only used matching with the same top eight LOT numbers of corresponding reagents. For each target value and range refer to the label.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order Syphilis (CLIA) Controls (REF: 1602011013MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the Syphilis concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in mIU/mL. For further information please refer to the Analyzer Operating Instructions.

Interpretation of Results

The expected results for the Syphilis assay was obtained by testing 325 Syphilis positive patients and 535 Syphilis negative people in China, gave the following expected value by ROC curve:

- Non-reactive: A result less than 1.00 mIU/mL (<1.00 mIU/mL) is considered to be negative.
- Reactive: A result greater than or equal to 1.00 mIU/mL (≥1.00 mIU/mL) is considered to be positive.

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own reference interval.

LIMITATIONS

- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the Syphilis results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- 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[10].
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- No diagnostic test provides absolute assurance that a sample does not contain low levels of antibodies to *Treponema pallidum*, such as those present at a very early stage of infection. Therefore, a negative result at any time does not preclude the possibility of exposure to infection with syphilis. Additional information may be required for diagnosis.
- Use of only one type of serologic test (nontreponemal or treponemal) is insufficient for diagnosis and can result in false-negative results among persons tested during primary syphilis and false-positive results among persons without syphilis or previously treated syphilis[11].
- None of the serological tests for syphilis differentiate between venereal syphilis (caused by *T. pallidum* subspecies *pallidum*) and the other treponematoses yaws (*T. pallidum* subspecies *pertenue*), endemic syphilis (*T. pallidum* subspecies *endemicum*) and pinta (*T. pallidum* subspecies *carateum*)[12].

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n = 180). The following results were obtained:

Sample	Mean (mIU/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (mIU/mL)	%CV	SD (mIU/mL)	%CV	SD (mIU/mL)	%CV
Serum Pool 1	0.505	NA	NA	NA	NA	NA	NA
Serum Pool 2	1.956	0.072	3.68	0.025	1.28	0.096	4.91
Serum Pool 3	9.845	0.288	2.93	0.166	1.69	0.444	4.51
Plasma Pool 1	0.509	NA	NA	NA	NA	NA	NA
Plasma Pool 2	2.002	0.072	3.60	0.032	1.60	0.097	4.85
Plasma Pool 3	10.135	0.268	2.64	0.147	1.45	0.445	4.39
Negative Control	0.307	NA	NA	NA	NA	NA	NA
Positive Control	10.004	0.297	2.97	0.179	1.79	0.429	4.29

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Hemoglobin	1500 mg/dL	Ampicillin Sodium	6.0 mg/dL
Intralipid	3000 mg/dL	Ascorbic Acid	6.5 mg/dL
Bilirubin	66 mg/dL	Cyclosporine	5.0 mg/dL
HAMA	40 ng/mL	Cefoxitin	66.5 mg/dL
ANA	398 AU/mL	Levodopa	2.0 mg/dL
Rheumatoid factor	2000 IU/mL	Methyldopa	2.0 mg/dL
Human albumin	12 g/dL	Metronidazole	12.0 mg/dL
Systemic Lupus Erythematosus Plasma	/	Phenylbutazone	44.0 mg/dL
IgA	4.8 g/dL	Doxycycline	3.5 mg/dL
IgG	8.0 g/dL	Acetylsalicylic acid	65.5 mg/dL
IgM	2.5 g/dL	Rifampicin	6.0 mg/dL
IgD	1.1 g/dL	Acetaminophen	21 mg/dL
K2-EDTA	22.75 µmol/mL	Ibuprofen	50.5 mg/dL
Na2-EDTA	22.75 µmol/mL	Theophylline	4.0 mg/dL
Heparin sodium salt	80 IU/mL	Azithromycin	1.2 mg/dL
Heparin lithium salt	80 IU/mL	Ceftriaxone	97 mg/dL
Biotin	0.5 mg/dL	Minocycline	5.0 mg/dL

Cross-Reactivity

The assay is highly specific for *T. pallidum* antibodies, with no observed cross-reactivity to Toxo IgM, Toxo IgG, CMV IgM, CMV IgG, HSV -1 IgM, HSV -1 IgG, HSV -2 IgM, HSV -2 IgG, Rubella IgM, Rubella IgG, Anti-HAV IgM, Anti-HAV IgG, Anti-HBs, HBeAb IgG, HBcAb IgM, HBcAb IgG, Anti-HCV, Anti-HEV, Anti-HIV, EBV VCA IgM, EBV VCA IgG, EBV EA IgG, EBV NA IgG, *M.Pneumoniae* IgM, *M.Pneumoniae* IgG, *C.Pneumoniae* IgM, *C.Pneumoniae* IgG, VZV IgM, VZV IgG, Influenza A virus IgM and Influenza A virus IgG.

High-Dose Hook

No high-dose hook effect was seen for Syphilis concentrations up to 3000 mIU/mL.

Clinical Sensitivity

The clinical sensitivity of the Syphilis assay was determined in China by testing 323 samples collected from expected positive population with commercial assay confirmation of Syphilis positive result.

N of samples	Reactive	Sensitivity	95% CI
323	322	99.69%	99.08%-100.00%

Clinical Specificity

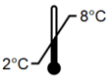
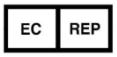
The clinical specificity of the Syphilis assay was determined in China by testing 511 samples collected from expected negative population with commercial assay confirmation of Syphilis negative result.

N of samples	Non-reactive	Specificity	95% CI
511	510	99.80%	99.42%-100.00%

REFERENCES

1. Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance 2018[R]. Atlanta: U.S. Department of Health and Human Services, 2010.
2. LaFond R E, Lukehart S A. Biological Basis for Syphilis[J]. Clinical Microbiology Reviews, American Society for Microbiology, 2006, 19(1): 29–49.
3. World Health Organization. The global elimination of congenital syphilis: rationale and strategy for action[R]. 2007.
4. Centers for Disease Control and Prevention. STD Facts - Syphilis (Detailed) [R]. 2021.
5. Stamm L V. Global Challenge of Antibiotic-Resistant Treponema pallidum[J]. Antimicrobial Agents and Chemotherapy, American Society for Microbiology, 2010, 54(2): 583–589.
6. Schmid G. Economic and programmatic aspects of congenital syphilis prevention[J]. Bulletin of the World Health Organization, 2004, 82(6): 402–409.
7. Kiss S, Damico F M, Young L H. Ocular Manifestations and Treatment of Syphilis[J]. Seminars in Ophthalmology, Taylor & Francis, 2005, 20(3): 161–167.
8. Tobian A A R, Quinn T C. Herpes simplex virus type 2 and syphilis infections with HIV: an evolving synergy in transmission and prevention[J]. Current opinion in HIV and AIDS, 2009, 4(4): 294–299.
9. CLSI. Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions. 4th ed. CLSI guideline C24. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
10. Boscato L M, Stuart M C. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988;34(1):27-33.
11. Centers for Disease Control and Prevention. Syphilis - STI Treatment Guidelines[R]. 2021.
12. French P, Gomberg M, Janier M, et al. IUSTI: 2008 European Guidelines on the Management of Syphilis[J]. International Journal of STD & AIDS, SAGE Publications, 2009, 20(5): 300–309.

SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
	Temperature limit (Store at 2-8°C)		Use-by date
	Contains sufficient for<n> tests		Keep away from sunlight
	This way up		Authorized representative in the European Community
	*In vitro* diagnostic medical device		Kit component
	Catalogue number		Batch code
	CE marking		

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