CHEM.CENTAUR.ASSAY.22.0 SYPHILIS BY ADVIA CENTAUR

PRINCIPLE

The ADVIA Centaur Syphilis (SYPH) assay is intended to be a fully automated, antigen sandwich assay, using direct chemiluminometric technology. The ancillary pack reagent containing acridinium-ester-labeled *T. pallidum* recombinant antigens is added to the sample. These *T. pallidum* recombinant antigens complex with the antibodies in the sample. The solid phase reagent, containing biotinylated *T. pallidum* recombinant antigens preformed to streptavidin-coated magnetic latex particles, is then added to the sample. Antibody-antigen complexes will form if syphilis antibodies are present in the sample. The particles capture the *T. pallidum* recombinant antigen-antibody complexes.

A direct relationship exists between the level of antibodies to *T. pallidum* present in the patient sample and the amount of relative light units (RLUs) detected by the system. A result of reactive, nonreactive, or equivocal is determined according to the Index Value established with the calibrators.

The ADVIA Centaur and ADVIA Centaur XP systems automatically perform the following steps:

1. Dispenses 100 µL of sample into a cuvette.
1. Dispenses 40 µL of Ancillary Pack Reagent and incubates for 5 minutes at 37°C.
2. Dispenses 100 µL of Solid Phase and incubates the mixture for 18 minutes at 37°C.
3. Separates the Solid Phase from the mixture and aspirates the unbound reagent.
4. Washes the cuvette with Wash 1.
5. Dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction.

The ADVIA Centaur systems report results according to the selected option, as described in the system operating instructions or in the online help system. The system reports SYPH results in Index Values and as reactive, equivocal, or nonreactive.

OWNERS

Manager, Regional Chemistry

RELATED DOCUMENTS

SPECIMEN COLLETION AND HANDLING

A. Specimen requirements
   1. Serum
   2. Plasma (EDTA, lithium or sodium heparin, and citrate)
B. Stability
   Refrigerated (2-8°C): 7days
   Freeze (-20°C): >7days

C. Special instructions

1. Before placing samples on the system, ensure that:
   a. Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation.
   b. Samples are free of bubbles or foam.

REAGENTS

- Store the reagents upright at 2–8°C.
- Protect reagent packs from all heat and light sources. Reagent packs loaded on the system are protected from light. Store unused reagent packs at 2° to 8°C away from heat and light sources.
- Discard reagent packs at the end of the 60-day onboard stability interval.

- Solid Phase and Ancillary Reagent are stable at 2–8°C until the expiration date on the pack label or for 60 days onboard the system.
- Calibrators are stable at 2–8°C until the expiration date on the vial or for 8 hours onboard the system.
- Ancillary Probe Wash 1 is stable at 2–8°C until the expiration date on the pack label or for 14 days onboard the system.
- Wash 1 is stable at 2–25°C until the expiration date on the pack label or for 1 month onboard the system.

Reagent	***Volume***	***Ingredients***
Solid Phase	20.0 mL/reagent pack	Streptavidin-coated paramagnetic microparticles preformed with biotinylated recombinant Tp15 antigen (~1.35 µg/mL) and biotinylated recombinant Tp17 antigen (~1.65 µg/mL) in buffer with surfactant, bovine gamma globulin, goat serum, and preservative
Ancillary Reagent	10.0 mL/reagent pack	Recombinant Tp15 antigen (~0.1 µg/mL) and recombinant Tp17 antigen (~0.15 µg/mL) labeled with acridinium esters in buffer with surfactant, goat serum, and preservative
Calibrators	2.0 mL/vial	Human plasma positive for *Treponema pallidum* antibodies in phosphate buffer with sodium azide (< 0.1%)
Ancillary Probe Wash 1	25.0 mL/reagent pack	0.4 N sodium hydroxide
Wash 1	1500 mL/pack or 2500 mL/pack	phosphate buffered saline with sodium azide (< 0.1%), surfactant

CALIBRATION

For calibration of the ADVIA Centaur SYPH assay, use ADVIA Centaur SYPH Calibrators provided with each kit. The calibrators provided in this kit are matched to the ReadyPack primary and ancillary reagent packs. Do not mix calibrator lots with different lots of reagent packs

The ADVIA Centaur SYPH assay requires a Master Curve calibration when using a new lot number of Solid Phase and Ancillary Pack Reagents

Ensure that the lot number on the *Master Curve* card matches the lot number of the ReadyPack.

The ADVIA Centaur SYPH assay requires a two-point calibration when:

1. Every 21 days
2. Changing lot numbers of primary reagent packs
3. Replacing system components
4. Quality control results are repeatedly out of range (See CHEM.QC.REGIONAL.1.0)

QUALITY CONTROL

A. Refer to CHEM.QC.REGIONAL.2.0 Quality Control Testing Intervals for QC Testing Intervals for appropriate control materials and testing intervals.
B. Refer to CHEM.QC.REGIONAL.1.0 Regional Chemistry laboratory Quality Control for criteria on accepting/rejecting patients based on control results.
C. Refer to CHEM.QC.REGCHEM.2.2 QC PREP and STABILITY

PROCEDURE

The ADVIA Centaur and ADVIA Centaur XP systems are automated, direct chemiluminescent immunoassay analyzers that offer optimal productivity and efficiency.

Sample racks are loaded in the sample entry queue and the sample start button is pressed to activate the test sequence. The sample entry queue moves the sample racks to the inprocess queue, where the sample is aspirated and dispensed it into a cuvette in the incubation ring.

Reagents are dispensed into the cuvette, the reaction mixture is incubated, and then the cuvette is moved to the wash station where the magnetic particles are washed. Acid Reagent is dispensed into the cuvette and then the cuvette is moved into the luminometer. The addition of Base Reagent causes the chemiluminescent reaction to occur. The PMT measures the chemical light reaction that takes place.

See procedure CHEM.CENTAUR.2.0, Advia Centaur Operating Procedure, for specific details.

Online “HELP” windows are also available for instructions on use.

REPORTING RESULTS

A. Normal Range: Non-Reactive

RESULT ENTRY

A. Sunquest
   1. Function MEM or OEM
   2. Test Code: SYPSC
   3. Worksheet: CI
   4. Method: CI1
B. Toplab (QLS)
   1. Common Pathway: 3,3,1
   2. Test Code: 51374
   3. Worksheet: CIM1

PROCEDURE NOTES

If a system error occurs, and the rocking of the primary reagent compartment is stopped for more than 5 minutes, remove and mix the ADVIA Centaur SYPH ReadyPack primary reagent packs until the particles are in solution before loading onto the system.

LIMITATIONS

A. The ADVIA Centaur SYPH assay is limited to the detection of antibodies to *T. pallidum* in human serum or plasma (EDTA, lithium or sodium heparinized plasma, citrated plasma).

A nonreactive test result does not exclude the possibility of exposure to or infection with syphilis.

T. pallidum antibodies may be undetectable in some stages of the infection and in some clinical conditions.

Assay performance characteristics have not been established when the ADVIA Centaur SYPH assay is used in conjunction with other manufacturers' assays for specific syphilis serological markers.

The performance of the ADVIA Centaur SYPH assay has not been established with neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma, such as saliva, urine, amniotic, or pleural fluids.

Assay interference due to possible circulating antibodies against pinta, yaws, and leptospirosis has not been evaluated.

As with any chemical reaction, you must be alert to the possible effect on results of unknown interferences from medications or endogenous substances. The laboratory and physician must evaluate all patient results in light of the total clinical status of the patient.

B. The ADVIA Centaur SYPH assay was evaluated for interference according to CLSI guideline EP7-A2. The following substances were found not to interfere at the concentrations indicated. A bias less than 10% is not considered a significant interference:

Substance Tested	*Test Concentration*
Hemoglobin	500 mg/dL
Triglycerides (intralipids)	1000 mg/dL
Cholesterol	400 mg/dL
Bilirubin, conjugated	40 mg/dL

For additional information on performance characteristics, see the product information in the ADVIA Centaur SYPH product insert.

For Cross-Reactivity, see the product information or package Insert

C. Alternative Method

Refer to MACL SPP 30.008 and Core Chemistry Procedure E2.12.1 for Computer Downtime.

REFERENCES

Advia Centaur and Advia Centaur XP System Package Insert