MAYO CLINIC LABORATORIES

Test Definition: NSAIP

Neurosyphilis IgG Antibody Index with VDRL, Serum and Spinal Fluid

Overview

Useful For

Aid in the diagnosis of neuroinvasive syphilis

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
NSCSF	Neurosyphilis IgG Screen, CSF	No	Yes
NSSER	Neurosyphilis IgG, S	No	No

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
NSAI	Neurosyphilis IgG, Ab Index	No	No
VDSFT	VDRL Titer,CSF	No	No

Testing Algorithm

Testing begins with syphilis IgG screening of the spinal fluid (CSF) specimen. If the screen is negative, no additional testing will be performed.

If the CSF screen is reactive, the paired CSF and serum specimens will be used to establish the antibody index. To do this, the paired serum and CSF samples (collected within 24 hours of each other) are tested on the same run using quantitative assays to determine levels for the following analytes:
1. Anti-*Treponema pallidum* IgG in CSF and serum
2. Total IgG in CSF and serum
3. Albumin in CSF and serum

These additional tests are necessary to normalize the level of anti-*T pallidum* antibodies to total IgG and albumin in the CSF and establish the antibody index ratio of anti-*T pallidum* antibodies in CSF-to-serum. This testing is performed at an additional charge.

Syphilis Antibody Index negative results do not undergo additional testing.

For Syphilis Antibody Index positive or equivocal results, VDRL testing will be performed to establish a semi-quantitative titer.

For more information see Meningitis/Encephalitis Panel Algorithm.

Special Instructions

- Meningitis/Encephalitis Panel Algorithm

Test Definition: NSAIP

Neurosyphilis IgG Antibody Index with VDRL, Serum and Spinal Fluid

Highlights

This test should only be ordered in patients who are seropositive for syphilis in blood.

This test compares the level of IgG antibodies against *Treponema pallidum* in spinal fluid and serum. The level of anti-*T pallidum* IgG is normalized to total IgG and albumin in spinal fluid (CSF) and serum.

This test can help identify whether the presence of IgG to *T pallidum* in the CSF is due to true intrathecal antibody synthesis, suggesting neuroinvasive syphilis, versus antibody presence due to passive diffusion through the blood-brain barrier or possibly, due to blood contamination of the CSF as a result of a traumatic lumbar puncture.

This approach also provides higher specificity over the classic VDRL assay for neurosyphilis.

Method Name

NSCSF, NSAI: Enzyme-Linked Immunosorbent Assay (ELISA)
NSSER: Technical Interpretation
VDSFT: Flocculation/Agglutination

NY State Available

Yes

Specimen

Specimen Type

CSF
Serum

Ordering Guidance

This test should be ordered in patients with suspected neurosyphilis who are confirmed seropositive in blood.

For syphilis testing on serum, order SYPH1 / Syphilis IgG with Reflex, Enzyme Immunoassay, Serum or SYPH2 / Syphilis IgG with Reflex, Enzyme Immunoassay, Serum.

Additional Testing Requirements

Although a small percentage of patients with neurosyphilis may be seronegative, it is recommended that all patients tested by this assay are first confirmed seropositive for syphilis in blood.

Specimen Required

Both spinal fluid (CSF) and serum are required for this test. CSF and serum must be collected within a maximum of 24 hours of each other.

Specimen Type: Spinal fluid
Container/Tube: Sterile vial
Specimen Volume: 2.2 mL

Collection Instructions:
1. The spinal fluid (CSF) specimen **must be** collected within 24 hours of the serum specimen, preferably at the same time.
2. The CSF aliquot should be from the second, third, or fourth CSF vial collected during the lumbar puncture. **Do not submit CSF from the first vial due to the possibility of blood contamination, which will cause specimen rejection**.
3. Label vial as spinal fluid or CSF.
4. Band CSF specimen together with the serum sample.

Specimen Type: Serum
Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 2.2 mL Serum
Collection Instructions:
1. Within 24 hours of collection of the spinal fluid specimen, a serum specimen **must also be** collected, preferably at the same time.
2. Centrifuge and aliquot serum into a plastic vial.
3. Label tube as serum.
4. Band serum specimen together with the CSF sample.

Specimen Minimum Volume

Spinal fluid/Serum: 1.5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Spinal fluid (CSF) contaminated with blood	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
CSF	Refrigerated (preferred)	10 days	
	Frozen	10 days	
Serum	Refrigerated (preferred)	10 days	
	Frozen	10 days	

Clinical & Interpretive

Clinical Information

Neurosyphilis (NS) caused by the spirochete *Treponema pallidum* can occur at any stage of syphilis. Currently the Centers for Disease Control and Prevention estimate that approximately 2% of patients with syphilis will develop neuroinvasive syphilis if untreated. Early stages of NS may be asymptomatic or symptomatic, with patients typically exhibiting classic meningitis symptoms. Patients with late-stage NS patients may present with dementia paralytica or tabes dorsalis. Other manifestations of neuroinvasive syphilis include ocular or otologic syphilis, which can occur at any stage, however, are more common during early NS.

The diagnosis of NS is challenging due to a number of factors, including the lack of consensus on the relevance of abnormal cerebrospinal fluid (CSF) findings in patients who are seropositive for syphilis but neurologically asymptomatic. With respect to diagnostic testing, numerous treponemal and non-treponemal (lipoidal) assays have been evaluated, alongside CSF protein and pleocytosis findings, however direct comparisons of these assays are limited. The VDRL assay is currently the only assay with US Food and Drug Administration (FDA) clearance as an aid in the diagnosis of NS, however the sensitivity and specificity of this non-treponemal (lipoidal) assay is highly variable, ranging from 66.7% to 85.7% and 78.2% to 86.7%, respectively. Although no treponemal assay has FDA clearance as an aid for diagnosis of NS, studies evaluating the fluorescent treponemal antibody absorption (FTA-ABS) assay performed in CSF from patients with definitive NS was associated with a sensitivity of 90.9% to 100%. Specificity of this approach ranged from 55% to 100% however, primarily due to the issue of passive diffusion of serum antibodies across the blood-brain barrier.

The NS antibody index assay corrects for passive diffusion across an inflamed blood-brain barrier by measuring quantitative levels of anti-*T pallidum* antibodies in serum and CSF and normalizing those to total IgG and albumin in both specimen sources. A positive NS antibody index indicates true intrathecal antibody synthesis of antibodies to *T. pallidum*, which alongside clinical and exposure history can be used to establish a diagnosis of NS. All NS antibody index positive samples are also reflexed for testing by the VDRL assay to acquire a semi-quantitative titer. The NS antibody index should only be ordered in patients who are seropositive for antibodies to *T pallidum* in blood, who also present with neurologic manifestations suspicious for NS or who are at risk for asymptomatic NS.

Reference Values

NEUROSYPHILIS SCREEN, IgG, SPINAL FLUID:
Negative
Reference values apply to all ages.

NEUROSYPHILIS IgG ANTIBODY INDEX:
Antibody Index: 0.6-1.2
Reference values apply to all ages.

VDRL TITER, SPINAL FLUID:
Negative

Reference values apply to all ages.

Interpretation

Negative:
No antibodies to syphilis (*Treponema pallidum*) detected in cerebrospinal fluid (CSF). A negative result in a patient with appropriate exposure history and symptoms consistent with neurosyphilis should not solely be used to exclude infection. If not already performed, testing for antibodies to *T pallidum* in serum should be ordered.

Reactive:
Supplemental testing to determine a syphilis IgG Ab Index in CSF has been ordered. Diagnosis of neurosyphilis should not be established solely based on a reactive screening result.

Cautions

A single negative result should not be used to exclude the diagnosis of neuroinvasive syphilis disease in a patient with appropriate exposure history and symptoms suggestive of infection.

False-negative results may be acquired in patients tested soon after infection, prior to the development of a detectable level of antibodies in the spinal fluid.

False-reactive results may occur in patients with *Borrelia* or *Leptospira* infections. Patient management decisions should not be made on a single reactive result.

Antibody index can remain positive for a prolonged period of time after complete resolution of disease. Therefore, a positive result must be interpreted in light of current, presenting symptoms.

Clinical Reference

1. Alberto C, Deffert C, Lambeng N, et al. Intrathecal synthesis index of specific anti-*Treponema* IgG: A new tool for the diagnosis of syphilis. Microbiol Spectr. 2022;10(1):e01477-21. doi:10.1128/spectrum.01477-21
2. Papp JR, Park IU, Fakile Y, Pereira L, Pillay A, Bolan GA. CDC Laboratory Recommendations for Syphilis Testing, United States, 2024. MMWR Recomm Rep. 2024;73(1):1-32
3. Klein M, Angstwurm K, Esser S, et al. German guidelines on the diagnosis and treatment of neurosyphilis. Neurol Res Pract. 2020;2:33. Published 2020 Nov 17. doi:10.1186/s42466-020-00081-1
4. We S, Ye F, Wang Y, Li D. Neurosyphilis: insights into its pathogenesis, susceptibility, diagnosis, treatment and prevention. Front Neuro. 2024;14:1340321
5. Reiber H, Lange P. Quantification of virus-specific antibodies in cerebrospinal fluid and serum: sensitive and specific detection of antibody synthesis in brain. Clin Chem. 1991;37(7):1153-1160

Performance

Method Description

Neurosyphilis Screen, IgG, Spinal Fluid
The test kit contains microtiter strips with break-off reagent wells coated with purified recombinant *Treponema pallidum* antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, *T pallidum*-specific IgG antibodies will bind to the antigens. To detect the bound antibodies, a second

incubation is carried out using an enzyme-labelled antihuman IgG (enzyme conjugate), followed by a third incubation using chromogen/substrate, which catalyzes a color reaction that is then measured for optical density (OD) using spectrophotometry.(Unpublished Mayo method)

Neurosyphilis IgG Antibody Index
The test kit contains microtiter strips with break-off reagent wells coated with purified recombinant *T pallidum* antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, *T pallidum*-specific IgG antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled antihuman IgG (enzyme conjugate), followed by a third incubation using chromogen/substrate, which catalyzes a color reaction that is then measured for optical density (OD) using spectrophotometry. The obtained OD values of the paired patient serum and cerebrospinal fluid (CSF) samples are compared against a 4-level calibration curve to quantitatively determine the relative anti-*T pallidum* IgG antibody titers.(Unpublished Mayo method)

The quantitative test results obtained on paired serum and CSF specimens using the *T pallidum* IgG enzyme-linked immunosorbent assay are expressed as relative units (U/mL) and must be used along with the total IgG and albumin levels in the patient's paired serum and CSF samples to calculate the anti-*T pallidum* antibody index (AI), which determines the absence or presence of intrathecal anti-*T pallidum* IgG antibody synthesis. Total IgG and albumin testing on serum and CSF are performed using the Siemens BN II nephelometric testing system.(Instruction manual: Siemens Nephelometer II Operations. Siemens V 2.3, 2008; Addendum to the Instruction Manual 2.3, 08/2017)

To detect an infection of the central nervous system, it is necessary to differentiate between intrathecally produced antibodies and antibodies passed from blood into the CSF. The AI is the value of intrathecal pathogen-specific antibody production. This AI value represents the portion of pathogen-specific antibodies in total IgG of CSF and the portion of pathogen-specific antibodies in total IgG of serum. The patient's AI is calculated using the Reiber and Lange method.(Reiber H, Lange P. Quantification of virus-specific antibodies in cerebrospinal fluid and serum: sensitive and specific detection of antibody synthesis in brain. Clin Chem. 1991;37(7):1153-1160)

VDRL Titer, Spinal Fluid
The VDRL antigen and spinal fluid are mixed on a 180 RPM rotator. The antigen, a cardiolipin-lecithin coated cholesterol particle, flocculates in the presence of reagin.(US Department of Health, Education and Welfare, National Communicable Diseases Center, Venereal Disease Program. Manual of Tests for Syphilis. Centers for Disease Control; 1969; Bennett JE, Dolin R, Blaser MJ, eds. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 9th ed. Elsevier; 2020)

Neurosyphilis IgG Spinal Fluid Screen Technical Interpretation
This interpretation is added by the laboratory information system based on the spinal fluid IgG antibody results for neurosyphilis.

PDF Report
No

Day(s) Performed
Monday, Wednesday, Friday

Test Definition: NSAIP

Neurosyphilis IgG Antibody Index with VDRL, Serum and Spinal Fluid

Report Available
2 to 5 days

Specimen Retention Time
2 weeks

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
86780
82784 x 2-(if appropriate)
82040-(if appropriate)
86780 x 2-(if appropriate)
82042-(if appropriate))
86593-(if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
NSAIP	Neurosyphilis IgG Ab Indx w/VDRL	58031-6

Result ID	Test Result Name	Result LOINC® Value
NSSER	Neurosyphilis IgG, S	69048-7
NSY1	Neurosyphilis IgG, CSF	58031-6
NSY2	Neurosyphilis IgG Interp	69048-7