

Interferon Beta Neutralizing Antibody with Reflex to Titer

Clinical Background

- Interferon beta (IFN β) is used as first-line therapy in relapsing/remitting multiple sclerosis.
- While autoantibodies to IFN β are rarely found in humans, neutralizing antibodies (NABs) are frequently seen in patients receiving prolonged therapy with recombinant human IFN β . NABs neutralize different IFN β biopharmaceuticals as well as natural IFN β . Reduction in therapeutic effects of IFN β and higher relapse rates have been correlated with sustained elevated levels of NABs in multiple sclerosis patients.¹
- In NAB-positive patients, particularly in patients treated with IFN β -1b, NABs may disappear during continued therapy. When NABs disappear spontaneously, patients have been reported to regain the full effect of IFN β therapy with no negative carryover effect from the previous NAB-positive period.²
- This assay is intended for the semiquantitative determination of NABs to IFN β . The test directly measures the presence or absence of NABs against IFN β (i.e., Avonex[®], Rebif[®], Betaseron[®], and Betaferon[®]) without the need for the traditional binding assay. If the specimen is positive for NABs, a semiquantitative estimate of the amount of NABs is determined by titering the serum to a dilution that provides a tenfold reduction in IFN activity.

Indications for Ordering

- This test is indicated for the following individuals:
- Individuals receiving interferon at 12 months and 24 months after initiating therapy.
- Individuals who have never been tested but have been receiving interferon for more than 24 months.
- Individuals experiencing relapse.
- Individual under consideration for a change in therapy (test prior to changing therapy).

Interpretation

- A positive screen indicates the presence of neutralizing antibodies to IFN β . The normal reference range for the screen is > 1 LU (laboratory units)/mL of IFN β activity. Serum specimens with values of ≤ 1 LU/mL will be reported as positive for neutralizing antibodies and reflexed to a titer assay.
- A titration assay will be performed for specimens with a positive screen. The neutralizing potency of a specimen is derived as the dilution of serum that permits interferon activity of 1 LU/mL. The titer is calculated as $Dx (C-1)/9$, where D is the reciprocal of the serum dilution that achieves 1 LU/mL interferon activity, and C is the interferon concentration used in the titer test.³

- In patients with moderate levels of neutralizing antibodies (titers of 20 to <100), therapeutic efficacy of IFN β may still be observed, but continued patient monitoring is warranted.
- Titers of ≥ 100 should be considered high levels of neutralizing antibodies. Change in therapy should be considered in patients who have sustained high titers of neutralizing antibodies at repeated measurements.⁴
- Results of this test should be interpreted within the context of clinical data.

Limitations

- This assay detects neutralizing antibodies to current commercial forms of IFN β . However, because IFN β -1a is used as the reference standard, the quantification of neutralization activity is more precise for IFN β -1a drugs (e.g., Avonex, Rebif). Quantification for neutralization activity against IFN β -1b (e.g., Betaseron, Betaferon) may be less accurate.
- Interference is shown with specimens with high lipid, bilirubin, or hemoglobin content, and with high endogenous interferon alpha, beta, or gamma.
- Specimens need to be collected either before treatment with interferon or more than 48 hours following the most recent dose. Patient should not be receiving steroid therapy (over 10 mg prednisolone or equivalent daily) for at least four weeks prior to testing.

Methodology

- Patient serum is incubated with interferon-sensitive cells and exogenous IFN β . IFN β bioactivity is measured using luciferase-generated bioluminescence. The presence of NABs in serum reduces the bioactivity of exogenous IFN β .⁵
- A screening test is first performed to detect the presence of NABs as indicated by the reduction in IFN bioactivity. Specimens reducing the IFN activity to ≤ 1 LU/mL are reported positive for NABs and will be reflexed to a titer assay to provide semiquantitative data calculated as the serum dilution that permits IFN activity of 1 LU/mL, a tenfold reduction of activity of exogenous IFN provided at about 10 LU/mL (Kawade method).³
- Good correlation has been reported between this method and the cytopathic effect assay (CPE assay), which measures viral neutralization of IFN β and its inhibition in serum of patients.⁵
- Assay performance:
 - Sensitivity: 100 percent (83.4–100 percent)
 - Specificity: 100 percent (92.3–100 percent)
- No cross-reactivity has been reported with antibodies in patients treated with IFN alpha.
- The assay is not FDA-approved but has been validated by ARUP Laboratories.

Related Tests

TEST NAME AND NUMBER	RECOMMENDED USE
Oligoclonal Band Profile with MBP (0080341)	Assist in the clinical assessment of suspected multiple sclerosis (MS)
Immunoglobulin G, CSF Index (0050676)	Assist in the clinical assessment of suspected multiple sclerosis (MS)
Cell Count, CSF (0095018)	Rule out presence of infectious agent (meningitis)

References

1. Goodin DS et al. Neutralizing antibodies to interferon beta: assessment of their clinical and radiographic impact: an evidence report: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology* 2007;68(13):977–84.
2. Sorensen PS, et al. Is the treatment effect of IFN- β restored after the disappearance of neutralizing antibodies? *Mult Scler* 2008;14(6):837–42.
3. Kawade Y. An analysis of neutralization reaction of interferon by antibody: a proposal on the expression of neutralization titer. *J Interferon Res* 1980;1(1):61–70.
4. Sorensen PS et al. Guidelines on use of anti-IFN-beta antibody measurements in multiple sclerosis: report of an EFNS Task Force on IFN-beta antibodies in multiple sclerosis. *Eur J Neurol* 2005;12(11):817–27.
5. Lallemand C, et al. Quantification of neutralizing antibodies to human type I interferons using division-arrested frozen cells carrying an interferon-regulated reporter-gene. *J Interferon Cytokine Res* 2008;28(6):393–404.
6. Polman CH, et al. Recommendations for clinical use of data on neutralising antibodies to interferon-beta therapy in multiple sclerosis. *Lancet Neurol* 2010;9(7):740–50.

Test Information

2003390 Interferon Beta Neutralizing Antibody with Reflex to Titer

For specific collection, transport, and testing information, refer to the ARUP website at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.