IBT Laboratories

Specializing in molecular & cellular immunology

Mannan-Binding Lectin Pathway Function Test (C4b Deposition Assay)

Test Name: Mannan-Binding Lectin Pathway Function Test (C4b Deposition Assay)
Test Code: 0034
CPT Code: 86161

Background:
The complement system is a vital component of the body's innate immune system, providing a highly effective means for the destruction of invading microorganisms. The direct binding of complement components (e.g. C1q, MBL) to microbial surfaces initiates the complement cascade and leads to opsonization and pathogen elimination via humoral and cellular mechanisms. In addition, the complement system is not only just essential for the innate immune response, but is closely linked to adaptive immunity, through activation and interaction with its respective receptors on various immune cells.

Three different pathways of complement activation have been described, i.e. the classical pathway, the alternative pathway and the recently elucidated lectin or mannan-binding lectin (MBL) pathway. All three pathways converge at the level of C3, leading to the activation of the terminal complement pathway and formation of the terminal complement complex (C5b-9) which is also known as the cytolytic membrane attack complex (MAC). Impaired function of the complement system is typically due to complement defects that result from genetic abnormalities or acquired deficiencies. Complement defects may lead to a partial or complete blockade of the complement activation cascade depending on the level of the deficiency. Most complement defects are associated with disease, ranging from a relatively mild increase in susceptibility to infections to the occurrence of severe systemic autoimmune disorders.

Complement defects within the classical and alternative pathways are routinely tested for when an immunodeficiency is suspected with clinical diagnostic assays such as CH50 and AH50, respectively. Monitoring of MBL pathway function has not been part of the standard testing panel mainly due to the recent discovery of the pathway and the lack of a reliable testing method. However, recently a method which enables the determination of MBL complement pathway function has been reported in the scientific literature (Peterson et al. 2001). In this assay, patient sera is incubated in mannan-coated microtiter wells. Exogenous C4 is added to the wells and C4b deposition which results from the cleavage of C4 by the MBL-MASP-2 complex is determined with an anti-human C4 monoclonal antibody. The amount of signal generated is proportional to the concentration of the MBL-MASP-2 complex in the patient sera. Thus, this MBL complement pathway function test should elucidate defects which typically result from deficiencies in either MBL or MASP-2.

MBL deficiencies occur in approximately 10-20% of the population and are mainly due to polymorphisms located within the promoter and structural region of the *mbl2* gene. These polymorphisms inhibit MBL oligomeric formation that is required for complement activation via the lectin complement pathway. MASP-2 levels are altered by a single polymorphism within the *CUB1* domain of the MASP-2 gene which causes a substitution of glycine for aspartic acid at position 120 (D120G), preventing MASP-2 from forming a functional complex with MBL. Approximately 10% of the population are heterozygous for the *CUB1* mutation and have 50% less circulating plasma MASP-2, while those rare individuals who are homozygous for this mutation have been reported to have only 10% of normal circulating plasma MASP-2 levels.

Method:
Enzyme Immunoassay (EIA). MBL pathway function is indirectly monitored by determining the specific C4b-depositing capacity of patient sera with an anti-human C4 monoclonal antibody. The specificity and sensitivity of this EIA has been previously reported (Peterson et al. 2001). *The performance characteristics of this test were determined by IBT Laboratories. The test has not been approved or cleared by the FDA.*

Specimen Requirement:
2 mL serum. The specimen can be shipped via overnight courier at ambient temperature. For longer storage, the serum should be kept frozen.

Units Reported:
ng/mL. The assay is calibrated against a purified human MBL protein.

Reference Range:
>200 ng/mL. Results less than 200 ng/mL indicate deficiency. This range is the mean plus 3 standard deviations with sera obtained from a population of deficient individuals.

Interpretation and Clinical Utility:
Deficiency in either MBL or MASP-2 appears to predispose individuals to serious infections (e.g. pneumonitis, sepsis, osteomyelitis, gastroenteritis and meningitis) especially during childhood before the adaptive immunity has fully developed. Furthermore, there is increasing evidence that MBL deficiency plays a role in modulating the severity of an infection. The MBL pathway function test discussed here should be useful for the elucidation of suspected immune defects and as a prognostic indicator alerting to the need for heightened therapeutic or prophylactic measures.

References:

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