Laboratory Services
Important Test Announcement

ADENOSINE DEAMINASE 2 ENZYME ACTIVITY ASSAY

New Test Code: ADA2 **Live Date:** 12/18/2025

Methodology: Enzyme Activity Assay
Performed: Monday - Friday
Turnaround Time: 36 hours
Specimen Required: Red top, frozen serum

- **Specimen Preparation**: No special collection
- **Stability**: Whole Blood (Red Top): 24 hours at ambient temperature, 48 hours at 4°C. Serum (Frozen): 12 weeks.
- **Storage/Transport/Temperature/Conditions**: Red top tubes can be sent at ambient temperature (24 hours stability) or refrigerated (48 hours stability). Serum should be frozen immediately after isolation. Frozen serum should be sent on dry ice.
- **Unacceptable Conditions**: Wrong collection tubes, sample out of stability, thawed serum samples.

Clinical Utility:

There are two distinct adenosine deaminase (ADA) enzyme in humans, ADA1 and ADA2. While both enzymes catalyze the same reaction (deamination of adenosine to inosine), they differ significantly in their structure, cellular location, tissue distribution and biological functions. ADA1 is primarily present intracellularly (cytosol of nearly cells), while ADA2 is primarily extracellular (plasma, serum and secreted by macrophages/monocytes). The former is a monomer (40kDa), while the latter is a dimer (110kDA). ADA1 has a high affinity for adenosine and deoxyadenosine, while the latter has a lower affinity for adenosine. ADA1 is essential for purine metabolism, and its deficiency causes a form of severe combined immunodeficiency (SCID), while ADA2 is important for immune modulation, macrophage differentiation, inflammation, and its deficiency causes Deficiency of Adenosine Deaminase 2 (DADA2). While ADA1 is ubiquitous in expression, ADA2 is expressed primarily in cells of the myeloid lineage (monocytes, macrophages and dendritic cells, DCs).

This assay is intended for the measurement of ADA2 enzyme activity in serum or plasma.

ADA2 is an enzyme, which catalyzes the deamination of extracellular adenosine (Ado) to inosine (Ino) and a growth factor. It also converts 2'-deoxyadenosine to 2'-deoxyinosine. The primary enzymatic activity of ADA2 involves an irreversible reaction within the purine salvage pathway, mainly in the extracellular space (e.g., plasma, serum). ADA2 helps maintain a proper balance between pro-inflammatory (M1) and anti-inflammatory (M2) macrophages.

DADA2 is an inborn error of immunity or a primary immune disorder, which is autosomal recessive, caused by loss-of-function (LOF) pathogenic variants in the ADA2 gene. This deficiency leads to a multi-faceted phenotype with both immune and other systemic defects, including immunodeficiency, autoinflammation, early and/or recurrent strokes, systemic vasculitis, bone marrow failure with features, such as pure red cell aplasia, among other symptoms. The lack of ADA2 disrupts macrophage balance leading to excess TNFa production and systemic inflammation. Very recently (2025 J. Exp Med, I Meyts team), DADA2 has been reported in individuals with monoallelic (heterozygous) ADA2 variants due to dominant negative effects of the mutant allele on the wild type allele.

Elevated ADA2 activity is observed in a variety of hyperinflammatory conditions including infections, malignancies, and other contexts of immune dysregulation. It can be observed to be increased, along with other

cytokines in macrophage activation syndrome (MAS) and GVHD (graft vs host disease) including other systemic inflammatory contexts.

Measuring ADA2 enzyme activity is crucial for both diagnosing DADA2 and monitoring hyperinflammatory conditions.

The diagnosis of DADA2 is confirmed by very low (less than 5% of normal) or undetectable plasma/serum ADA2 catalytic activity. This functional test is essential because genetic testing alone might miss certain pathogenic variants (e.g., large gene deletions) or yield variants of uncertain significance. The level of residual enzyme activity can also correlate with disease severity and, potentially, response to treatment (e.g. anti-TNF therapy). In general, carriers of DADA2 have higher levels than DADA2 patients but can overlap with DADA2 patients and healthy controls. Genetic testing provides a confirmatory diagnosis in addition to measurement of ADA2 levels.

As mentioned previously, ADA2 enzyme activity levels can also serve as a biomarker for hyperinflammation. In MAS, ADA2 enzyme activity levels appear to correlate well with other MAS biomarkers, like ferritin and IL-18, and can be used to monitor disease activity and therapeutic response. ADA2 has also been reported to be elevated in contexts of chronic GVHD as well as other disorders manifesting with an increased inflammatory response. Pentostatin is a potent, irreversible transition state inhibitor of ADA. It primarily and effectively inhibits both ADA1 and ADA2 isoforms, although its main clinical application leverages its profound effect on ADA1-rich lymphoid cells. However, its significant systemic toxicity and non-specific inhibition along with disruption of normal immune homeostasis makes it undesirable for treating systemic inflammation related to increased ADA2 levels.

ADA2 levels are typically higher in healthy children compared to healthy adults, and therefore, age-specific reference intervals have been established in this laboratory with this assay.

Cladribine treatment can result in a false-positive result for DADA2 as it is a purine analog that is resistant to degradation by ADA1 and can accumulate in lymphocytes. Since ADA2 measures the functional enzymatic conversion of an adenosine substrate to inosine in a patient's plasma or serum sample, cladribine, being an adenosine analog, could potentially act as a competitive substrate or an inhibitor of the ADA2 enzyme and interfere with the assay methodology, potentially leading to inaccurate or suppressed ADA2 activity results. For diagnostic purposes, establishing a patient's natural baseline ADA2 activity is crucial. This is best done before exposure to drugs that interfere with the purine salvage pathway or ADA enzyme function.

Clinical Report:

The report will provide ADA2 enzyme activity levels in three primary clinical contexts:

1. Deficiency of ADA2 enzyme activity (DADA2)
2. Carrier status for DADA2
3. Increased ADA2 enzyme activity consistent with hyperinflammation

The report will include a reference interval, derived from DADA2 patients and carriers (Pui Lee team, 2025, In Press, Arthritis & Rheumatology; Pui Lee et al, 2020, doi: 10.1016/j.jaci.2019.12.908) as well as from pediatric and adult healthy controls (internally established). A relevant comment on clinical significance will be included in the report.

If you have any additional questions about **Adenosine Deaminase 2 Enzyme Activity Assay**, please refer to the Laboratory Test Directory or call Client Services at 614-722-5477.