



# NATIONAL LABORATORY HANDBOOK

## Laboratory Testing for Alpha-1 Antitrypsin Deficiency (AATD)

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## Authors

Dr. Tomás Carroll, Chief Scientist, Alpha-1 Foundation Ireland, Senior Lecturer, RCSI. [tcarroll@rcsi.ie](mailto:tcarroll@rcsi.ie)

Dr. Mary Keogan Consultant Immunologist, Beaumont Hospital, & Clinical Lead, National Clinical Programme for Pathology. [mariekeogan@beaumont.ie](mailto:mariekeogan@beaumont.ie)

Professor Gerry McElvaney, Consultant Respiratory Physician, Beaumont Hospital, Professor of Medicine, RCSI, and clinical lead at the National Centre of Expertise for AATD, Beaumont Hospital. [gmcElvaney@rcsi.ie](mailto:gmcElvaney@rcsi.ie)

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## Scope

The aim of this guideline is to provide clear guidelines and indications for testing for alpha-1 antitrypsin deficiency that can be used by clinicians and clinical laboratories, including circumstances where testing is not required. These guidelines apply to adult, non-pregnant patients and paediatric patients with cryptogenic liver disease where alpha-1 antitrypsin deficiency is suspected. In the absence of liver disease in childhood, paediatric testing is not recommended. However, in cases where a strong family history of AATD exists, it is recommended that both parents undergo testing first before deciding whether to pursue testing of paediatric patients.

## Key Recommendations for Clinical Users

Laboratory testing for alpha-1 antitrypsin deficiency should be reserved for specific patient groups with indications for testing as described below.

Following the identification of affected individuals, referrals can be made to the National Centre of Expertise for AATD at Beaumont Hospital for comprehensive clinical assessment.

Additionally, patient advice, information and support services are provided by Alpha-1 Foundation Ireland (see [www.alpha1.ie](http://www.alpha1.ie) for details).

## Key Recommendations for Laboratories

The first line test for investigating alpha-1 antitrypsin deficiency is the quantification by immunoassay of alpha-1 antitrypsin (AAT), usually performed by turbidimetry or nephelometry.

Abnormally low AAT should be commented upon with a recommendation for further testing by phenotyping. Specialist AAT phenotyping is provided by Alpha-1 Foundation Ireland at the

National Centre of Expertise for AATD at Beaumont Hospital. There are at least 100 genetic variants known to cause AAT deficiency and AAT phenotyping can identify the vast majority of pathological variants.

In rare cases, further genetic analysis may be required to identify rare or novel mutations responsible for the observed AAT deficiency (for example Null (Q0) mutations).<sup>(1)</sup>

A low or absent alpha-1 globulin fraction on routine serum protein electrophoresis (SPE) can also indicate severe AAT deficiency and should be commented upon to expedite diagnosis. Serum can be stored to facilitate reflex testing of alpha-1 antitrypsin for samples with a low alpha-1 globulin result. In fact, the association of an absent alpha-1 globulin band on SPE with a hereditary form of pulmonary emphysema is how the condition was originally discovered.<sup>(2)</sup>

### **Background & Epidemiology**

The abundant serum protein alpha-1 antitrypsin (AAT) is a key antiprotease which helps to protect the fragile alveolar tissue of the lungs. AAT also has anti-inflammatory and immune modulation properties. Production of circulating AAT is chiefly by the liver and the normal plasma concentration of AAT is approximately 1.5 g/L (range 1.0 – 2.0 g/L) with a half-life of 4 – 5 days.<sup>(3)</sup> AAT is an acute phase protein and plasma levels can increase by 200 – 500% during infection or inflammation.<sup>(4, 5)</sup>

Alpha-1 antitrypsin deficiency (AATD) is a genetic disorder characterised by low serum AAT and confers risk for lung, liver and rarely skin disease. The polymorphic SERPINA1 gene encodes the AAT protein and the most common harmful mutations in Ireland are Z and S. The Z mutation (Glu342Lys, rs28929474) leads to AAT accumulation within liver cells and a pronounced serum deficiency. 1 in 25 people in Ireland carry the Z mutation with an estimated 2,000 ZZ homozygotes at risk due to severe deficiency.<sup>(6)</sup> 1 in 10 carry the S mutation (Glu264Val, rs17580) which causes a mild serum deficiency. However, the S mutation is of clinical significance when co-inherited with another severe deficiency mutation (e.g. Z) and this is borne out by the large numbers of SZ individuals found in lung disease cohorts. Severe AATD is classically an individual homozygous for the Z mutation (phenotype ZZ) who has an 85% reduction in circulating serum AAT. The high risk of lung disease associated with severe AATD is well understood. In addition, recent research shows that people with mild AATD (phenotype MZ) who smoke have increased risk for chronic obstructive pulmonary disease (COPD).<sup>(7)</sup>

Importantly, a diagnosis of AATD can present the healthcare professional and the affected individual with a unique opportunity for early medical intervention, specific treatments, and lifestyle modification (e.g. smoking cessation), leading to the prevention or postponement of lung disease. Family or cascade screening is strongly recommended as it can identify additional at risk individuals.

### **Who to Test**

Guidelines published by the World Health Organisation (WHO) and separately by the American Thoracic Society (ATS) and European Respiratory Society (ERS) recommend targeted screening programmes for the detection of individuals with AATD.<sup>(8,9)</sup> Targeted testing of first degree relatives of individuals with AATD is recommended as it offers the most realistic prospect of detecting asymptomatic relatives who may be at risk.

The Irish National AATD Targeted Detection Programme began in 2004 and follows the WHO and joint ATS/ERS guidelines (**Table 1**). Funded by the HSE and co-ordinated by Alpha-1 Foundation Ireland, over 700 people with severe AATD and over 3,000 with moderate AATD have been identified to date.

**Table 1:** ATS/ERS recommendations for diagnostic testing for AATD (type A recommendations)

Adults with symptomatic emphysema or COPD (regardless of age or smoking history)
Adults with asthma with airflow obstruction that is incompletely reversible after aggressive treatment with bronchodilators
Asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors (e.g. cigarette smoking, occupational exposure)
Adults with necrotising panniculitis
Siblings of individuals with AATD
Individuals with unexplained liver disease, including neonates, children, and adults, particularly the elderly

In addition to above patient groups, ATS/ERS guidelines also recommend testing should be considered in a number of other scenarios (ATS/ERS type B recommendations). These include early onset emphysema, a family history of emphysema, asymptomatic individuals with persistent airflow obstruction that does not respond to treatment, vasculitis, and unexplained bronchiectasis.

It is particularly important to consider screening of first degree relatives as this will identify further at risk individuals within families, particularly smokers.

#### Who Not to Test

Testing for AATD is **not recommended** in the following circumstances:

Paediatric testing (unless investigating cryptogenic liver disease).

#### Who to Re-Test

Repeat phenotype testing is not recommended. This should be discouraged when requested as the phenotype does not change. However, a notable exception is liver transplantation. A healthcare professional may wish to confirm the new phenotype of the patient as the circulating AAT derives from the donor liver. Case reports exist in the literature of liver transplanted individuals receiving ZZ and SZ livers. Other exceptions include individuals recently transfused with blood products containing plasma and cases where a lab error is suspected. If a laboratory error is suspected, the laboratory should also be informed.

## Specimen and Ordering Information

All requests (electronic and paper) and specimens must adhere to the laboratories standard requirements. In order to comply with accreditation standards, laboratories cannot accept or process samples which do not meet minimum standards.

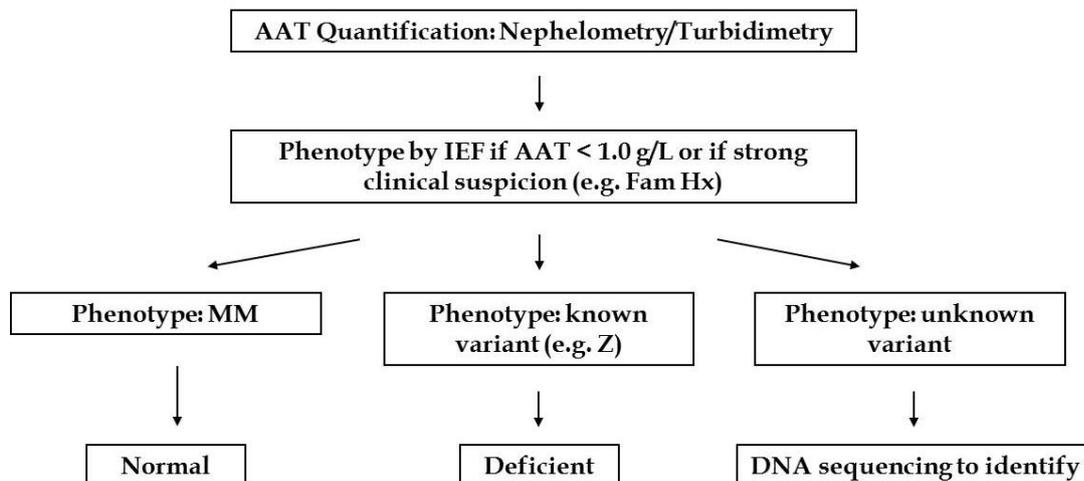
**Blood sample collection:** serum or plasma bottles, minimum of 3ml volume.

**Stability and transport:** The AAT protein is relatively stable once it is not exposed to repeated cycles of freeze-thaw. On arrival samples for AAT analysis should be stored at 4°C for up to 14 days without any effect on IEF assay quality.<sup>(10)</sup> Longer periods of storage [are/may be] possible but IEF results should be carefully interpreted and monitored for degradation.

**Special requirements:** None. There are no special requirements for samples in terms of declaration of medications or fasting.

## How to Test

The laboratory diagnosis of AATD is usually performed by following two steps; determination of AAT concentration in serum or plasma (quantitative) and identification of allelic variants by phenotyping (qualitative).<sup>(11,12,13)</sup> Quantification of AAT is generally the first investigation (Figure 1). If quantification of AAT reveals a level below the established cut-off (approximately 1.0 g/L or 100 mg/dL) the sample should be reflexed for AAT phenotyping.<sup>(14,15)</sup> If AATD is strongly suspected (for example a positive family history) then AAT level and AAT phenotype can be ordered simultaneously. Genotyping using allele-specific PCR (usually for Z and S variants) and/or direct sequencing of the SERPINA1 gene may be required either as a further investigation of suspected rare or novel mutations or on a complementary basis.



**Figure 1.** Laboratory Diagnosis of AAT Deficiency

## Interpretation of tests

For the interpretation of AAT phenotype results, see Table 2 for a description of the most common AAT phenotypes observed in Ireland. For information on rare or unusual phenotype results, contact Alpha-1 Foundation Ireland on 01-8093871 or [alpha1@rcsi.ie](mailto:alpha1@rcsi.ie).

**Table 2.** Common alpha-1 antitrypsin (AAT) phenotypes (\**phenotype reported when testing is performed on serum – genotype reported when testing is performed on DNA*).

Status	AAT Phenotype/ AAT Genotype*	What does it mean?
Normal	MM	Does not have the disorder – does not have any altered AAT genes.
Carrier	MS	No evidence of increased risk for disease but does carry altered AAT gene.
Carrier	MZ	Mild AAT deficiency – significantly increased risk of lung disease <u>in smokers</u> . Definite but not yet quantified risk of liver disease.
AAT Deficiency	SS	Mild AAT deficiency – presumed increased risk of lung disease <u>in smokers</u> .
AAT Deficiency	SZ	Moderate AAT deficiency – significantly increased risk of lung disease <u>in smokers</u> . Increased risk of liver disease.
AAT Deficiency	ZZ	Severe AAT deficiency – significantly increased risk of lung disease <u>in smokers and ever smokers</u> . Increased risk of liver disease.

Note: There are non-genetic reasons for a low AAT level which include severe liver disease, nephrotic syndrome or protein-losing enteropathy.

## Quality

Testing of AAT levels and phenotype should incorporate appropriate internal quality control, and laboratories should participate in external quality assurance schemes. In line with standard quality management systems, quality issues and non-conformances should be recorded, investigated and communicated directly with the referral lab. The most common quality issues are discrepancies between the AAT level and AAT phenotype as well as poor sample quality.

## Recommendations for National Laboratory Information System (MedLIS)

Repeat phenotype testing should be prevented using a rule. In exceptional circumstances (e.g. testing inconsistent with attributed relationships or post liver transplant), repeat testing should be discussed with the laboratory.

Repeat testing of patients tested in the pre-MedLIS era would be a waste of significant resources. A solution should be developed to incorporate historical results on the MedLIS record.

To streamline workflow and convenience for patients an “Alpha-1 Antitrypsin Deficiency Care Set” should be developed. Text to acknowledge that ordering this care set is testing for a genetic disease should be incorporated. Reason for request can be chosen from a drop-down menu with options of:

- First investigation,
- Family history of AATD,
- Previous low AAT (Result, where and when tested).

Interpretive comments should be added to all AAT levels.

< 0.8 g/L Alpha-1 antitrypsin deficiency likely. AAT phenotyping indicated if not previously tested.

0.8 - 1.0 g/L Alpha-1 antitrypsin deficiency should be considered. Suggest AAT phenotyping if not previously tested.

1.0 - 1.5 g/L Low normal AAT does not exclude alpha-1 antitrypsin if tested during an acute phase response. If CRP elevated, and AAT deficiency suspected, suggest retest when acute phase response has resolved.

MedLIS data should be audited to evaluate adherence to phenotyping recommendation. Measurement of unmet need should be shared with appropriate Clinical Programmes to assist in service planning.

### Information for Patients

AAT phenotyping might be performed because you have been diagnosed with lung or liver disease and your doctor suspects AATD may be responsible. It might also be performed because you have a low blood AAT level or because you have a family history of AATD or lung disease. There is no need to fast before giving a blood sample for this test.

The test result will provide you with information about the AAT genes you have inherited from your parents. This will give you information about your risk of developing disease and can help your healthcare professional provide you with the best treatment plan.

Detailed information is available on:

<https://www.alpha1.ie/>

### Guideline Development Methodology - Consultation Plan and History

The guideline was drafted by the authors and reviewed by the Chemical Pathology Working Group and the Immunology Working Group of the National Clinical Programme for Pathology.

Following incorporation of feedback, the guideline was submitted to the Irish Thoracic Society, the Irish Society for Gastroenterology, and the National Clinical Programme for COPD.

Following incorporation of feedback from these expert groups, the guideline was submitted to the full National Clinical Programme for Pathology Consultation Process.

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