Human α1-Antitrypsin Kit for use on SPAPLUS®

For *in vitro* diagnostic use

Product Code: NK034.S

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FDA (USA) Information
Analyte name: α1-Antitrypsin
Complexity Cat: Moderate

CE

1 INTENDED USE

The Human α1-antitrypsin Kit for use on SPAPLUS is designed for the quantitative *in-vitro* determination of α1-antitrypsin in human serum using the SPAPLUS turbidimetric analyser. The measurement of α1-antitrypsin aids in the diagnosis of several conditions including adult cirrhosis of the liver. In addition, α1-antitrypsin deficiency has been associated with pulmonary emphysema. This test should be used in conjunction with other laboratory and clinical findings.

2 SUMMARY AND EXPLANATION

α1-antitrypsin is a serine protease inhibitor, acting principally on neutrophil elastase, thereby protecting the lung from degradation by this enzyme. Reduced serum levels are associated with liver disease and also occur in early childhood, old age and with hereditary deficiency. This can cause an imbalance between the neutrophil elastase in the lung and the anti-elastases that are responsible for protecting the lung and can lead to the clinical condition emphysema (refs 1-2).

3 PRINCIPLE

The determination of soluble antigen concentration by turbidimetric methods involves the reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed a portion of the light is transmitted and focused onto a photodiode by an optical lens system. The amount of transmitted light is indirectly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

4 REAGENTS

4.1 Human α1-antitrypsin antiserum: Sheep antiserum which is mono-specific for human α1-antitrypsin and is supplied in stabilised liquid form. It contains 0.099% sodium azide, 0.1 % EACA, 0.1% EDTA and 0.01% benzamidine as preservatives.

4.2 Calibrator and controls: These consist of pooled human serum and are supplied in stabilised liquid form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. Value assignments are traceable to DA470k international reference material.

4.3 Reaction buffer: Containing 0.099% sodium azide as a preservative.

5 CAUTION

All donors of human serum supplied in this kit have been serum tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either cleared by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material, including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained personnel for the purposes stated in the Intended Use. Strict adherence to these instructions is essential at all times. Results are likely to be invalid if parameters other than those stated in these instructions are used.

Reagents from different batch numbers of kits are **NOT** interchangeable. If large numbers of tests are performed care should be taken to ensure that all the reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kit should be stored at 2-8°C and can be used until the expiry date shown on the kit box label. DO NOT FREEZE. The human *α1-Antitrypsin Antiserum, Reaction Buffer*, calibrators and controls may be stored for up to three months after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator. *Human α1-Antitrypsin Antiserum* and *α1-Antitrypsin Reaction Buffer* may be stored, uncapped, on the SPAPLUS analyser for up to 30 days, provided the temperature is maintained between 8-12°C.

7 SPECIMEN COLLECTION AND PREPARATION

Use fresh or deep frozen serum samples.
Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum may be stored at 2-8°C for up to 48 hours prior to assay, or for prolonged storage kept at -20°C or below. Repeated freezing and thawing should be avoided. Microbially contaminated, haemolysed and lipaemic serum and samples containing particulate matter should not be used.

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 100 Tests *Human α1-Antitrypsin Antiserum SPAPLUS*
8.1.2 1 x *Human α1-Antitrypsin SPAPLUS Calibrator set 1-6* (6 x 1.0mL)
8.1.3 2 x 1.5mL *Human α1-Antitrypsin SPAPLUS High Control*
8.1.4 2 x 1.5mL *Human α1-Antitrypsin SPAPLUS Low Control*
8.1.5 1 x 100 Tests *α1-Antitrypsin Reaction Buffer SPAPLUS*

8.2 Materials required but not provided

8.2.1 Equipment for collection and preparation of test samples e.g. sample tubes, centrifuge etc.
8.2.2 A fully operational and equipped SPAPLUS analyser.
8.2.3 Current analyser operating instructions: SPAPLUS Reference Guide, Insert Code FIN012.
8.2.4 Sample Diluent (99: Dil 1) Product Code: SN080.S

8.3 Reagent preparation

Before loading, gently mix by inversion ensuring no foam or bubbles are generated or remain on the surface as these may interfere with reagent aspiration.

8.4 Test procedure

The user should be familiar with the operation of the SPAPLUS analyser before attempting to carry out the test procedures. The analyser should be prepared for use according to the manufacturer's instructions and the assay protocol entered as described below.
For full details of analyser operation refer to the SPAPLUS Reference Guide (FIN012) supplied with the analyser.

8.4.1 Test parameters

Assay parameters are entered into item number 40.

Item Name 40 A1AT
DATA INFORMATION
Units g/L
Decimals 3
ANALYSIS
Type End ▼
Main W.Length 1 340 ▼
Sub W.Length ▼
Method
CORR.
SLOPE INTER
Y = 1 X + 0
CALIBRATION
Type Spline1 ▼
Standard
1 # 4 #
2 # 5 #
3 # 6 #
Auto Fill
NORMAL RANGE

	MALE LOW	MALE HIGH	FEMALE LOW	FEMALE HIGH
Serum	[ ]	[ ]	[ ]	[ ]
Urine	[ ]	[ ]	[ ]	[ ]
Plasma	[ ]	[ ]	[ ]	[ ]
CSF	[ ]	[ ]	[ ]	[ ]
Dialysis	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]

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Item Name 40 A1AT
ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 10
REAGENT1 VOL 160 µL
REAGENT2 VOL 60
Third mix ● **OFF** ○ **ON**
R1 Blank ● **Water – Blank**
MONITOR
0 LEVEL SPAN 1
SPAN 3.0
DATA PROCESS
READ

	START	END
MAIN	53	54
SUB	30	31

ABSORBANCE LIMIT
LOW -3.0
HIGH 3.0
FACTOR
Blank correction * ○ ON ● OFF
ENDPOINT LIMIT 2.0
LINEAR CHECK (%) 0
Reaction Check
CHECK POINT
LOW -3
HIGH 3
DILUTION
Diluent ● 99: Dil 1 ○ 100: Dil 2
Pre Dilution Rate 10 ▼
Auto Rerun Dilution Rate High ▼
Auto Rerun Dilution Rate Low ▼
PROZONE CHECK
0

	START	END	LIMIT (%)	Min dOD	
FIRST	[ ]	[ ]			
SECOND	[ ]	[ ]	[ ]	○ Low	● High
THIRD	[ ]	[ ]	[ ]	○ Low	● High

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*Automatically calculated

Item Name 40 A1AT
Auto Rerun SW
○ On ● Off
Auto Rerun Range (Result)

	Lower		Higher	
	○ On	● Off	○ On	○ ● Off
Serum	Cal 1 #		Cal 6 #	
Urine				
Plasma				
CSF				
Dialysis				
Other				

Auto Rerun Condition (Absorbance)
Absorbance Range
Lower ○ On ● Off
Higher ○ On ● Off
Prozone Range ○ On ● Off
Bottle Size (ml)

24 Items		36 Items	
Reagent1	60	Reagent1	0
Reagent2 R1	17	Reagent2 R1	0
Reagent2 R2	7	Reagent2 R2	0

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The calibrator (Standard #) values are found in the Quality Control Certificate (SIN243.QC). Calibrator values on **Page 1** should be entered in ascending order, i.e. the lowest value first. The analyser will automatically calculate and enter the correct measuring ranges on item pages 3 and 4 providing the Autofill button is pressed after typing the value for calibrator 6 on page 1. View Item parameter pages 3and 4 to ensure correct value entry.
* The Blank correction factor is automatically calculated by the instrument.

8.5 Measuring range

The approximate measuring range of the human α1-antitrypsin assay at standard 1/10 sample dilution is 0.35 – 5.00g/L. Values below the approximate measuring range are reported to the user as <0.35g/L.

9 QUALITY CONTROL

9.1 At least two levels of appropriate control material should be tested a minimum of once a day. In addition, controls should be tested after calibration, with each new lot of reagent and after specific maintenance or troubleshooting steps described in the SPAPLUS Operation Manual.

9.2 Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure. Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to the local technical support organisation.

9.3 The concentrations of the controls provided are stated on the accompanying QC certificate (SIN243.QC). Sample results obtained should only be accepted if the control results are within ±15% of the concentration(s) stated.

10 LIMITATIONS

10.1 Turbidimetric assays are not suitable for measurement of highly lipaemic or haemolysed samples or samples containing high levels of circulating immune complexes (CICs) due to the unpredictable degree of non-specific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.

10.2 This assay has not been validated using paediatric samples, samples taken from pregnant females or individuals being administered oestrogen.

10.3 Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to supplier.

10.4 Diagnosis cannot be made and treatment must not be given on the basis of α1-antitrypsin measurements alone. High results must be interpreted with caution. Clinical history and other laboratory findings must be taken into account.

10.5 Variation in reagent temperature may affect results. Ensure that reagents are transferred directly from the refrigerator to the refrigerated reagent compartment of the analyser – do not allow to warm to room temperature.

10.6 High levels of α1-antitrypsin may be present in patients with acute inflammation.

11 EXPECTED VALUES

Adult serum range

The literature (ref 3) reference interval of α1-antitrypsin in serum is 0.9 – 2.0 g/L. This was verified in a small study of normal healthy blood donors to be 0.85-1.94 g/L.

Each laboratory should establish its own reference range.

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A study was performed following CLSI *Evaluation of Precision Performance of Clinical Quantitative Measurement Methods; Approved Guideline* (CLSI Document EP5-A2). The study was performed over 21 working days, with two runs per day. One user assessed three different samples using three different reagent lots on five analysers.

Human α1-antitrypsin Precision Summary									
	Mean	Within run		Between run		Between day		Total	
	(g/L)	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	4.96	0.06	1.3	0.26	5.3	0.21	4.3	0.34	6.9
Serum 2	0.97	0.01	1.5	0.03	2.7	0.05	5.4	0.06	6.3
Serum 3	0.64	0.01	1.4	0.02	2.8	0.04	5.8	0.04	6.6

12.2 Comparison

A correlation study was performed on 151 samples (using a variety of normal and clinical sera) using this kit on a SPAPLUS and an alternative commercially available human α1-antitrypsin assay. The study demonstrated excellent agreement with the following Passing & Bablok fit:

y = 0.99x - 0.08 (g/L) (y = SPAPLUS human α1-antitrypsin; x = alternative assay)

correlation coefficient r = 0.988 (calculated by linear regression)

12.3 Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantification (LoQ)

The LoB was calculated as 0.009g/L from analysis of 60 replicates of a pooled α1-antitrypsin depleted serum sample on 1 run, over 1 day, using 1 instrument and 1 operator.

The LoD was calculated as 0.021g/L.

The LoQ for this assay is defined as the bottom of the measuring range (0.35 g/L). The LoQ was validated by running 5 serum samples over 5 runs on 5 days, using 1 instrument and 1 operator. The total error was within the pre-determined acceptance criteria.

12.4 Linearity

The assay has been demonstrated to be linear throughout the analytical measuring range (within 10% of the expected values).

One user evaluated linearity using one lot of reagent, on one analyser. A regression equation of y = 1.008x + 0.040 (y = measured α1-antitrypsin concentration, x = theoretical concentration) was obtained over the range of 0.164-6.195g/L.

12.5 Interference

2 samples were tested to check for interference: one with an analyte concentration at the lower limit of the reference interval (0.9 g/L) and one with an analyte concentration in the clinical range (0.6 g/L). Both samples were tested at the standard 1/10 sample dilution.

	Concentration of interferent tested	
	0.9g/L sample concentration	0.6g/L sample concentration
Chyle	1500 formazine turbidity units	
Bilirubin	200mg/L	
Haemoglobin	5.0g/L	
Acetaminophen	200mg/L	200mg/L
Ibuprofen	500mg/L	500mg/L
Warfarin	10mg/L	10mg/L
Dexamethasone	0.6mg/L	0.6mg/L
Albuterol	0.4mg/L	0.4mg/L
Theophylline	40mg/L	40mg/L
Triamcinolone	0.6mg/L	0.6mg/L

Both samples demonstrated minimal interference (<10%) when tested with the above interferents.

12.6 Antigen excess

No antigen excess was observed up to a concentration of 5.5 g/L.

Further testing demonstrated that the assay will not incorrectly report deficient results (i.e. ≤ 0.9 g/L) in high samples up to a concentration of 14.2 g/L.

13 BIBLIOGRAPHY

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