

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k123256

B. Purpose for Submission:

New device

C. Measurand:

Human alpha-1 antitrypsin (AAT)

D. Type of Test:

Quantitative, turbidimetric assay

E. Applicant:

The Binding Site

F. Proprietary and Established Names:

Human α 1-Antitrypsin Kit for use on SPA_{PLUS}

G. Regulatory Information:

1. Regulation section:

21CFR §866.5130 *Alpha*-1 antitrypsin immunological test system

2. Classification:

Class II

3. Product code:

DEM- alpha-1 antitrypsin, antigen antiserum, control

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

The Human α 1-antitrypsin Kit for use on SPAPLUS is designed for the quantitative *in vitro* determination of α 1- antitrypsin in human serum using the SPAPLUS turbidimetric analyzer. The measurement of α 1- antitrypsin aids in the diagnosis of several conditions including adult cirrhosis of the liver. In addition, α 1 -antitrypsin deficiency has been associated with pulmonary emphysema. This test should be used in conjunction with other laboratory and clinical findings.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

For use on SPA_{PLUS} Analyzers (k040958) only

I. Device Description:

Materials Provided:

1. 1 x 100 Tests Human alpha-1 antitrypsin Antiserum SPA_{PLUS}
2. 1 x Human alpha-1 antitrypsin SPA_{PLUS} Calibrator set 1-6 (6 x 1.0 mL)
3. 1.5mL Human alpha-1 antitrypsin SPA_{PLUS} High Control
4. 1.5mL Human alpha-1 antitrypsin SPA_{PLUS} Low Control
5. 1 x 100 Tests Human alpha-1 antitrypsin Reaction Buffer SPA_{PLUS}
6. Antitrypsin Antiserum: Sheep polyclonal IgG antiserum, which is monospecific for alpha-1 antitrypsin and is supplied in stabilized liquid form with preservatives.
7. Calibrator and Controls: Pooled human serum, supplied in stabilized liquid form with preservatives.
8. Reaction Buffer: Saline buffer containing preservative.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Siemens N Antiserum to Human α_1 -Antitrypsin

2. Predicate 510(k) number(s):

k053072

3. Comparison with predicate:

| Similarities | | |
|---------------------|---|-----------|
| Item | Device | Predicate |
| Intended Use | Quantification of human alpha 1 antitrypsin | Same |
| Indications for Use | The measurement of alpha-1 antitrypsin aids in the diagnosis of several conditions including adult cirrhosis of the liver. In addition, alpha-1 antitrypsin deficiency has been associated with pulmonary emphysema. This test should be used in conjunction with other laboratory and clinical findings. | Same |

| Differences | | |
|---------------------|--|--|
| Item | Device | Predicate |
| Method | Turbidimetry | Nephelometry |
| Instruments | SPA _{PLUS} Analyser | BN Systems |
| Specimen Type | Serum | Serum (also heparinized and EDTA plasma) |
| Antibody | Sheep polyclonal, monospecific anti-human alpha-1 antitrypsin IgG | Rabbit anti-human alpha-1 antitrypsin (polyclonal) |
| Measuring range | 0.35 – 5.0 g/L (1/10 dilution) | 0.16 – 5.20 g/L |
| Open vial stability | 3 months | 4 weeks |
| On-board stability | 30 days | 5 days at 8 hours per day for 5ml vial |
| Controls | Two; high and low | Provided separately |
| Calibrators | 6 levels: 0.035 g/L, 0.090 g/L, 0.150 g/L, 0.280 g/L, 0.390 g/L, 0.5 g/L | Provided separately |
| Traceability | DA470k | DA470 |

K. Standard/Guidance Document Referenced (if applicable):

1. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (EP05-A2)
2. Method Comparison and Bias Estimation using Patient Samples (EP09-A2).

L. Test Principle:

The determination of soluble antigen concentration by turbidimetric methods involves the reaction of the test sample with specific antiserum to form a suspension of insoluble complexes. When light is passed through the suspension, the light is scattered and only a percentage of the light is transmitted to and focused on a photodiode. The amount of transmitted light measured by the diode is indirectly proportional to the concentration of the specific protein in the test sample.

Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The precision study was carried out in accordance with CLSI (EP05-A2) Evaluation of Precision Performance of Quantitative Measurement Methods. Precision was evaluated using three samples of pooled sera that together had analyte levels that spanned the measuring range of the assay (0.35 – 5.0 g/L). The 21 day precision study was performed by running the sera samples in duplicate (within-run analysis), two runs per day (between-run analysis) over 21 days (between-day) using three reagent lots (Batches 1, 2 and 3) and five instruments (SPA_{PLUS} 1, 6, 7, 8 and 12).

Repeatability

| Sample | Mean (g/L) | Within-Run | | Between-Run | | Between-Day | | Total | |
|------------|------------|------------|-----|-------------|-----|-------------|-----|-------|-----|
| | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| High Level | 4.99 | 0.07 | 1.4 | 0.20 | 4.0 | 0.27 | 5.4 | 0.35 | 6.9 |
| Mid Level | 0.97 | 0.01 | 1.5 | 0.03 | 2.7 | 0.05 | 5.4 | 0.06 | 6.3 |
| Low Level | 0.64 | 0.01 | 1.4 | 0.02 | 2.8 | 0.04 | 5.8 | 0.04 | 6.6 |

Reproducibility

| Sample | Mean (g/L) | # Runs | # Days | Within-Run | | Between-Run | | Between-Instrument | | Between-Batch | | Total | |
|------------|------------|--------|--------|------------|-----|-------------|-----|--------------------|-----|---------------|-----|-------|-----|
| | | | | SD | CV% | SD | CV% | SD | CV% | SD | CV% | SD | CV% |
| High Level | 4.99 | 2 | 21 | 0.07 | 1.4 | 0.20 | 4.0 | 0.12 | 2.4 | 0.21 | 4.2 | 0.35 | 6.9 |
| Mid Level | 0.97 | 2 | 21 | 0.01 | 1.5 | 0.03 | 2.7 | 0.04 | 3.7 | 0.03 | 3.5 | 0.06 | 6.3 |
| Low Level | 0.64 | 2 | 21 | 0.01 | 1.4 | 0.02 | 2.8 | 0.02 | 3.5 | 0.02 | 2.9 | 0.04 | 6.6 |

b. *Linearity/assay reportable range:*

One user assessed the linearity of a pool of high concentration samples using one lot of reagent on one analyser.

The high pool was prepared from pooled clinical serum samples with a high concentration of AAT and adjusted by addition of purified and concentrated AAT. A low pool was prepared from pooled clinical serum samples with a low AAT concentration. A dilution series was prepared by blending the respective high pool and low pool (described above), to produce a total of 14 concentrations that covered the measuring range of the assay. Three replicates of each level of the dilution series were run and the mean calculated.

Linearity was evaluated by calculating the percentage recovery at each concentration in the dilution series, and the %CV of each of the 3 replicates. The acceptance criteria for the study was a mean recovery of between 80 – 120% and a CV of <8%. Linearity was demonstrated at the concentrations within the claimed measuring range.

The approximate measuring range of the human alpha-1 antitrypsin assay is 0.35 – 5.0 g/L.

Hook Effect:

A hook effect study was performed and demonstrated that serum AAT concentrations up to 14.20 g/L would not result in false positive results.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

1. Traceability:

The controls and calibrators are traceable to ERM-DA470k.

i. Calibrator Set and Internal Reference (IR) fluid production and value assignment:

Human serum with AAT concentrations above the top calibrator was obtained and a provisional value was assigned by running against the predicate assay.

The provisional assigned value was used to prepare the bulk calibrator set to the target values. The final assignment was carried out by running five calibration curves using the bulk calibrator set and running a dilution series of four concentrations of DA470k in triplicate against each curve. The deviation of the mean DA470k result from the assigned value was used to adjust the values of the calibrator set. The final assigned values of the calibrator set must be within $\pm 10\%$ of the target values.

ii. Internal Reference fluid (IR) production and value transfer

The IR fluid is pooled human serum. The final AAT concentration in the IR fluid was assigned by comparing it to DA470k using a value transfer procedure, in which a calibration curve was prepared and both DA470k and the IR fluid were analyzed. The concentration of AAT in the IR fluid

was determined from the ratio of slopes of the regression lines. The final value of the IR fluid was therefore assigned to international standard DA470k.

The IR fluid is used to assign the final AAT concentration in the calibrators and controls according to the following procedure.

ii. Control Assignment

Bulk fluid was assigned a provisional value. The final value was assigned to the control using the calibrator set and reagents designated for this kit. The control was tested 5 times on 5 different calibration curves and the median result was the control final assigned value.

2. Stability: Stability studies were performed and all results were within the sponsor's acceptable control limits of $\pm 15\%$ of the assigned value for the time frames indicated below.

- i. *Real-time stability*: The data produced to date supports stability of the kits under the recommended storage of 2 – 8°C for 12 months.
- ii. *Open vial stability*: The open vial study results show that the reagents are stable after opening for up to 3 months when stored between testing at 2-8°C.
- iii. *On-board stability*: The reagents are stable up to 30 days on-board the SPAPLUS, provided the temperature is maintained between 8-12°C.

3. Comparison of Diluents

A study was performed to assess whether there was bias in AAT measurements stemming from the matrix in which the sample was diluted. No significant difference was found between the recovery of AAT from samples diluted in stripped serum and samples diluted in instrument buffer.

d. *Detection limit*:

1. The Limit of Blank and Limit of Detection

The Limit of Blank (LoB) study was carried out by evaluating one run of 60 replicates of a serum pool that was depleted of AAT. The concentration of AAT remaining in the depleted pool was measured using the predicate assay and was determined to be < 0.04 g/L, which is the lowest concentration that can be measured on that assay. The LoB was determined by non-parametric methods, consisting of sorting the results of the run from low to high by g/L and averaging the values in the 57th and 58th replicates. LoB was determined to be 0.009 g/L.

The Limit of Detection (LoD) was calculated according to the formula $\text{LoB} + (1.645 \times \text{SD})$, using the SD determined by the LoQ study. The LoD was calculated to be 0.021 g/L.

2. Limit of Quantitation

Limit of Quantitation (LoQ) is the lowest concentration at which the analyte can be quantified within predefined goals for bias and imprecision. The LoQ for this assay was defined as the lower limit of the analytical measuring range (0.35 g/L).

Verification of the LoQ was carried out using 5 serum samples prepared individually by dilution of normal serum with serum that was depleted of AAT. The analyte levels in the 5 samples were confirmed to be approximately $\pm 10\%$ of the bottom of the measuring range (0.35 g/L) with the predicate assay. These samples were tested in 5 runs over 5 days, 12 replicates per run, for a total of 60 measurements. Total error was calculated from the bias and pooled estimate of precision (SD).

The predefined maximum acceptable total error is $\pm 10\%$ of the concentration of the lower limit of the reference range of the normal population. It was calculated by bias - 2SDs and was determined to be 0.049 g/L, which met the acceptance criterion.

d. Analytical specificity:

1. Interference testing at the medical decision point

The following substances were spiked into serum containing AAT near the reference range concentration (0.9 g/L) in order to test for interference: chyle (1500 formazine turbidity units), bilirubin (200 mg/L), hemoglobin (5.0 g/L), acetaminophen (200 mg/L), ibuprofen (500 mg/L), warfarin (10 mg/L), dexamethasone (0.6 mg/L), albuterol (0.4 mg/L), theophylline (40 mg/L), and triamcinolone (0.6 mg/L). Minimal interference effects ($<10\%$) were detected.

Interference testing at pathological analyte concentrations

In a separate study, the following substances were spiked into serum containing AAT in the pathological concentration (0.3 g/L): chyle (1500 formazine turbidity units), bilirubin (200 mg/L) and hemoglobin (5.0 g/L). Minimal interference ($<10\%$) was detected. In this study only, these samples were tested at a 1:1 sample dilution instead of the standard 1:10 dilution to attempt to detect the maximum potential bias due matrix effects was detected.

In a further study, the following substances were spiked into serum containing AAT at another pathological concentration (0.6 g/L): acetaminophen (200 mg/L), ibuprofen (500 mg/L), warfarin (10 mg/L), dexamethasone (0.6 mg/L), albuterol (0.4 mg/L), theophylline (40 mg/L), and triamcinolone (0.6 mg/L). Minimal interference effects ($<10\%$) were detected.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Testing was performed on 151 samples covering a range from 0.46 to 5.0 g/L. The samples were from clinically defined patients, patients selected by genotype, controls from differential diagnosis patients, normal samples, and constructed samples (see table, below). The study was conducted according to CLSI Guideline Method Comparison and Bias Estimation using Patient Samples, EP09-A2. Assays were performed in singlicate according to the package inserts, and no outliers were removed. The study was carried out using Batch 1 of the comparator reagent lot and two batches of predicate reagent on the BNII and SPA_{PLUS} analyzers. The results were analyzed in two ways: the total dataset (151), and a sub-analysis of those disease samples in which the genotype was unknown and without normal samples (69).

| Admission Diagnosis | Number of Samples |
|-----------------------------|-------------------|
| COPD | 20 |
| Liver Disease | 14 |
| Emphysema | 14 |
| Asthma | 20 |
| Known Genotype (deficiency) | 18 |
| Normal Human Serum | 29 |
| Other * | 36 |
| Total | 151 |

*Includes samples with high CRP (these were expected to have high levels of AAT) and constructed samples that were generated by pooling and diluting disease samples to produce samples with concentrations throughout the measuring range

Results:

a. Total Dataset

Passing & Bablok analysis of the total dataset of 151 samples, covering the range of 0.46 to 5.0 g/L, resulted in a regression equation of $y = 0.99x - 0.08$ g/L. The slope was statistically equivalent to 1 (95% confidence intervals 0.97 – 1.02) and the intercept was close to 0 (95% confidence intervals -0.12 – -0.03).

PPA and NPA were calculated comparing the predicate and test device:

| | | Reference Test | | |
|----------|----------------------|---------------------|----------------------|-------|
| | | Positive (<0.9 g/L) | Negative (> 0.9 g/L) | Total |
| TBS test | Positive (<0.9 g/L) | 18 | 5 | 23 |
| | Negative (> 0.9 g/L) | 0 | 128 | 128 |
| | Total | 18 | 133 | 151 |

Percentage positive agreement (18/18) = 100% 95%CI 100% – 100%

Percentage negative agreement (128/133) = 96.2% 95%CI 92.95% - 99.45%

Overall agreement ((18+128)/151) = 96.7% 95%CI 93.85% - 99.55%

FDA analysis of the results excluding the 29 NHS samples yielded the following:

| | | Reference Test | Reference Test | Total |
|-----|----------------------|---------------------|----------------------|-------|
| | | Positive (<0.9 g/L) | Negative (> 0.9 g/L) | |
| TBS | Positive (<0.9 g/L) | 18 | 3 | 21 |
| | Negative (> 0.9 g/L) | 0 | 101 | 101 |
| | Total | 18 | 104 | 122 |

Percentage positive agreement (18/18) = 100% 95%CI 82.42% – 100%

Percentage negative agreement (101/104) = 97.1% 95%CI 91.86% - 99.01%

Overall agreement ((18+101)/119) = 97.54% 95%CI 93.02% -99.16%

- b. Samples from relevant disease states (COPD, liver disease, emphysema and asthma)

Passing & Bablok analysis of 69 samples with relevant admission diagnosis, covering a range of 0.80 to 2.98 g/L, resulted in a regression equation of $y = 1.05x - 0.2$ g/L with 95% CI slope 0.97 – 1.15 and 95% CI intercept -0.35 – 0.90.

- b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable

b. *Clinical specificity:*

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

See expected values section below

5. Expected values/Reference range:

For adults, Tietz, Fundamentals of Clinical Chemistry, 6th edition) cites a reference interval of AAT in serum is 0.9-2.0 g/L. The sponsor has conducted a small study to verify the published reference range with samples from normal healthy blood donors and the reference range was 0.85-1.94 g/L. Each laboratory should establish its own normal range as values may differ depending on the population studied.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.