



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

I Background Information:

A 510(k) Number

K213396

B Applicant

Helena Laboratories, Corp.

C Proprietary and Established Names

SPIFE A1AT Kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
OBZ	Class II	21 CFR 866.5130 - Alpha-1-Antitrypsin Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New Assay

B Measurand:

Alpha-1 Antitrypsin (A1AT)

C Type of Test:

Qualitative, isoelectric focusing

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The SPIFE A1AT kit is designed for the qualitative detection and identification of the different phenotypes of Alpha-1 Antitrypsin (A1AT). Phenotyping results in conjunction with clinical findings and other laboratory assays aid in the diagnosis of Alpha-1 Antitrypsin deficiency. The analysis is performed on human sera separated into electrophoretic patterns ready for qualitative analysis. The procedure includes isoelectrofocusing on agarose gel, performed on the semi automatic SPIFE Touch system followed by immunofixation with anti-Alpha-1 Antitrypsin antiserum. For in vitro diagnostic use only.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For In Vitro Diagnostic Use Only

D Special Instrument Requirements:

SPIFE Touch System

IV Device/System Characteristics:

A Device Description:

The SPIFE A1AT kit includes: 10 SPIFE A1AT agarose gels, one vial of acid violet stain, two packs of Tris buffered saline, 10 vials of A1AT Blotter C, 40 vials of A1AT Blotter D, 30 A1AT electrode wicks, 60 A1AT wick blotters, 20 serrated applicator blades, one vial of anode solution, one vial of cathode solution and two packs of A1AT antibody.

The A1AT Controls are required for the assay and are sold separately. The SPIFE A1AT Control panel contains three vials of lyophilized serum representing three phenotypes: one Normal A1AT Control vial (MM) and two Pathological A1AT Control vials (MZ and MS).

B Principle of Operation:

A1AT is a polymorphic glycoprotein found in normal human serum. It belongs to a group of proteins which inhibit a variety of human proteases including trypsin, cathepsin G, neutrophil elastase, etc. A1AT is mainly produced in hepatic cells and by immune system. The gene that encodes A1AT is located at a single locus on chromosome 14 and has three alleles designated by letters with M for normal, Z and S for deficiency, and null. Individuals with ZZ, SZ, MZ or null phenotypes show increased risks of disease which may vary in patients exhibiting the same phenotype. A1AT deficiency is a genetic disorder first described in patients with pulmonary emphysema.

The assay is carried out in two stages: During stage 1 of the procedure, A1AT isoforms are separated by an electric current in an agarose medium. Each isoform migrates a specific distance from the application point based on the isoform's isoelectric point. Once the A1AT protein is

focused, the electrophoresis portion of the procedure is complete. Stage 2 is comprised of immunofixation using an A1AT anti-serum to detect the different phenotypes of A1AT. Visualization is achieved using acid violet protein stain. The semi-automated SPIFE Touch performs all the steps needed for A1AT phenotype interpretation.

V Substantial Equivalence Information:

A Predicate Device Name(s):

HYDRAGEL 18 A1AT ISOFOCUSING Kit

B Predicate 510(k) Number(s):

K063498

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K213396</u>	<u>K063498</u>
Device Trade Name	SPIFE A1AT Kit	HYDRAGEL 18 A1AT ISOFOCUSING Kit
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The SPIFE A1AT kit is designed for the qualitative detection and identification of the different phenotypes of Alpha-1 Antitrypsin (A1AT). Phenotyping results in conjunction with clinical findings and other laboratory assays aid in the diagnosis of Alpha-1 Antitrypsin deficiency. The analysis is performed on human sera separated into electrophoretic patterns ready for qualitative analysis. The procedure includes isoelectrofocusing on agarose gel, performed on the semi automatic SPIFE Touch system followed by immunofixation with anti-Alpha-1 Antitrypsin antiserum. For <i>in vitro</i> diagnostic use only.	The HYDRAGEL 18 A1AT ISOFOCUSING kit is designed for the qualitative detection and identification of the different phenotypes of Alpha-1 antitrypsin (A1AT). Phenotyping results in conjunction with clinical findings and other laboratory assays aid in the diagnosis of Alpha-1 antitrypsin deficiency. The analysis is performed on human sera separated into electrophoretic patterns ready for qualitative analysis. The procedure includes isoelectrofocusing on agarose gel, performed on the semi-automatic HYDRASYS system, followed by immunofixation with anti-Alpha-1 antitrypsin antiserum. The use of enzyme labelled anti-Alpha-1 antitrypsin antiserum enhanced the detection and identification of the different phenotypes. For <i>in vitro</i> diagnostic use only.

Sample Type	Serum	Same
Results	Qualitative interpretation of patterns of A1AT phenotypic stained fractions after electrophoretic migration	Same
Technology	Gel electrophoretic migration	Same
Methodology	Isoelectric focusing and Immunofixation	Same
Gel	Agarose	Same
General Device Characteristic Differences		
Test per Gel	40	18
Sample Volume	80 µL	10 µL
Sample Preparation	No dilution	1:10 with Sebia diluent
Lowest Detectable Limit	6 mg/dL	5 mg/dL
Instrument	Semi-automated SPIFE electrophoresis apparatus	Semi-automated HYDRASYS electrophoresis apparatus
Staining/Visualization	Acid violet	Peroxidase enzyme
Migration	ELP 1: 16°C/30 min/400V ELP 2: 16°C/30 min/850V	20°C for about 1 hour (total 510 V)

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition CLSI EP07-A3, Interference Testing in Clinical Chemistry – Third Edition
- CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
- CLSI EP09c 3rd Edition Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- CLSI EP07-A3, Interference Testing in Clinical Chemistry – Third Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

a. Operator-to-Operator Precision

One lot of A1AT gel, one lot of A1AT antisera, one lot of A1AT controls and 14 samples of various A1AT phenotypes (2xMM, 3xMZ, 3xMS, 3xSS, 3xZZ) were run on a single SPIFE Touch instrument. Three operators ran one gel per day for three days resulting into a total of nine gels. Each gel had two replicates of the MM, MS and MZ controls and single replicate of each patient sample per row (2 rows/gel). A total of 36 replicates for controls and 18 replicates for each patient sample were collected and all samples were correctly identified for their corresponding phenotypes.

b. Instrument-to-Instrument Precision

One lot of A1AT gel, one lot of A1AT antisera, one lot of A1AT controls and 14 samples of various A1AT phenotypes (2xMM, 3xMZ, 3xMS, 3xSS, 3xZZ) were run on a three SPIFE Touch instruments for three days (one gel per day per instrument) by three independent operators resulting into a total of nine gels. Each plate had two replicates of the MM, MS and MZ controls and single replicate of each patient sample per row (2 rows/gel). A total of 36 replicates for controls and 18 replicates for each patient sample were collected and all samples were correctly identified for their corresponding phenotypes.

c. Gel Lot-to-Lot Precision

Three lots of A1AT gels, one lot of A1AT antisera, one lot of A1AT controls and 14 samples of various A1AT phenotypes (2xMM, 3xMZ, 3xMS, 3xSS, 3xZZ) were utilized. Three gels (each from different lot) were run for three days (3 gels/day) on three instruments by three independent operators, resulting a total of nine gels. Each plate had two replicates of the MM, MS and MZ controls and single replicate of each patient sample per row (2 rows/gel). 36 replicates for controls and 18 replicates for each patient sample were collected in total, and all samples were correctly identified for their corresponding phenotypes.

d. Antibody Lot-to-Lot Precision

One lot of A1AT gel, three lots of A1AT antisera, one lot of A1AT controls and 14 samples of various A1AT phenotypes (2xMM, 3xMZ, 3xMS, 3xSS, 3xZZ) were utilized. The tests were run for three days (3 gels/day, each gel/lot of A1AT antisera) on three instruments by three independent operators, resulting a total of nine gels. Each plate had two replicates of the MM, MS and MZ controls and single replicate of each patient sample per row (2 rows/gel). A total of 36 replicates for controls and 18 replicates for each patient sample were collected, and all samples were correctly identified for their corresponding phenotypes.

e. Control Lot-to-Lot Precision

One lot of A1AT gel, one lot of A1AT antisera, and three lots of A1AT controls were run for three days (3 gels/day, each gel/lot of A1AT controls) on three instruments by three independent operators, resulting a total of nine gels. Each plate had a minimum of 12 replicates of each of the three A1AT control phenotypes (MM, MS, MZ) resulting in a total of 36 A1AT control replicates per phenotype per lot of control. All samples were correctly identified for their corresponding phenotypes.

f. Within-Run, Between-Run, Between-Day Precision

One lot of A1AT gel, one lot of A1AT antisera, one lot of A1AT controls (MM, MS, MZ) and nine patient samples of various A1AT phenotypes (1xMM, 2xMS, 2xMZ, 2xSS, 2xZZ) were run for three days (three gels per day) on three instruments by three independent operators, resulting a total of nine gels. The controls had single replicates of

the MM, MS and MZ phenotype per row. There are two rows per gel thus each plate had two determinations per gel for of each control phenotype. The patient samples per gel were run as follows: two MM phenotypes in singlicate per row (18 per patient), six MS patient phenotypes in duplicates per row (36 per patient), two MZ patient phenotypes in duplicate per row (36 per patient), one SS phenotype in duplicate per row (36 per patient), two ZZ patient phenotypes in duplicate per row (36 per patient). All samples were correctly identified for their corresponding phenotypes.

2. Linearity:

Not applicable

3. Analytical Specificity/Interference:

Interference was evaluated by spiking six different concentrations of hemoglobin, bilirubin and lipids on each of the MM, MS and MZ A1AT patient samples. Each interferent concentration was tested in duplicate for each A1AT phenotype on a single instrument by a single operator. No interference with the SPIFE Touch A1AT procedure was detected for lipid concentrations up to 30 mg/mL, bilirubin concentrations up to 20 mg/dL and hemoglobin concentrations up to 0.35 g/dL.

4. Assay Reportable Range:

Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Traceability:

There are no reference standards available for this device.

Stability:

a. A1AT plate and A1AT controls stability

The real-time stability of the A1AT plate and the lyophilized A1AT controls were determined over a 17-month period using three lots of A1AT plates and three lots of A1AT controls. Each lot of plate stored at 20–25°C was tested at 0, 6, 11, 12, 13, 15 and 17 months. At each testing point, three lots of A1AT control and three A1AT samples (MM, MS, MZ) were tested on all three A1AT gels. The controls were run as singlet on each row and the patient samples were run in duplicate or triplicate on each row of every plate tested. The migration distances observed on all three A1AT gel lots for patient samples and all three A1AT control lots were significantly different at 17 months than at time zero. When the difference in migration/separation was observed, the possibility arose that there may be an A1AT control or patient sample issue and not an A1AT gel issue. Thus, a gel from a manufactured lot less than 12 months from the date of manufacture was run side by side with A1AT gels from the three lots in the stability study using three lots of A1AT control and patient samples. The A1AT gel less than 12 months from the date of manufacture produced acceptable migration, separation and

resolution indicating the issue observed was not an A1AT control nor patient sample issue. Therefore, the stability claims for A1AT plate and A1AT controls is 12 months and 15 months, respectively at 20–25°C.

b. A1AT Anti-Sera Stability

The real-time stability for A1AT anti-sera was assessed using three lots of anti-sera dispensed in 2020 using one lot of A1AT gel and one lot of A1AT control at 14 months and 17 months subsequent to the date of manufacture. All three lots of anti-sera identified all expected phenotypes for patient and control specimens. The claimed stability for A1AT Anti-sera is 14 months at 2–8°C.

c. A1AT Controls Reconstituted Stability

Three lots of A1AT controls were tested for reconstituted stability at 2–8°C using one lot of A1AT gel, one lot of A1AT antisera at the following time points (days): 0, 1, 3, 5, 7, 8, 12, 14 and day 15 post reconstitution. Two vials of each lot of control were analyzed in triplicates. All phenotypes were correctly identified over the 15-day reconstitution time course. A reconstitution stability of 14 days at 2–8°C will be claimed for A1AT controls.

d. Sample Stability

Sample stability was evaluated for two storage conditions: -20 °C and at 2–8 °C. For -20 °C storage condition, three blood samples were drawn from each of individuals with known A1AT phenotypes (MM, MS, MZ) and stored at -20 °C after the samples were analyzed after draw (Time Zero (T₀)). Stability was assessed by testing the samples at 7, 14, 21 and 28 days and compared to the initial analysis. At T₀, each sample was repeated twice on each row of an A1AT gel. At subsequent time points, each sample was repeated four or five times on each row of each A1AT gel. All A1AT phenotypes were correctly identified over 28 days. The results support the claimed sample stability of 21 days at -20 °C.

For 2–8 °C storage condition, three blood samples were drawn from each of individuals with known A1AT phenotypes (MM, MS, MZ) and stored at 2–8 °C after the samples were analyzed after draw (T₀). Stability was assessed by testing the samples at 2, 4, 7, 9, 11, 14 and 15 days and compared to the initial analysis. At T₀, each sample was repeated twice on each row of an A1AT gel. At subsequent time points, each sample was repeated five or six times on each row of each A1AT gel. All A1AT phenotypes were correctly identified over 15 days. The results support the claimed sample stability of 14 days at 2–8 °C.

6. Detection Limit:

Sensitivity was determined by testing an A1AT ZZ phenotype sample diluted with the concentration of 24 mg/dL, 12 mg/dL, 8 mg/dL, 6 mg/dL, 4.8 mg/dL. Each sample dilution was run four times per gel on two separate gels (a total of eight replicates per sample). The ZZ phenotype was identifiable at approximately 6 mg/dL on all eight replicates.

7. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

Concordance between the SPIFE Touch A1AT assay and the predicate were assessed at three sites on 357 serum samples. A total of 30 normal and 327 different pathological serum samples were run on both platforms. The total A1AT concentrations ranged between 20 mg/dL and 242 mg/dL. The results are summarized in the following table:

Phenotype	Site 1		Site 2		Site 3		All Sites	
	N	Agree	N	Agree	N	Agree	N	Agree
MM	10	10	10	10	10	10	30	30
MZ	20	20	20	20	18	18	58	58
MS	20	20	20	20	20	20	60	60
ZZ	20	20	20	20	4	4	44	44
SS	20	20	20	20	-	-	40	40
SZ	20	20	20	20	7	7	47	47
Null	16	16	-	-	-	-	16	16
Other	46	46	5	5	11	11	62	62
Totals	172	172	115	115	70	70	357	357

The overall agreement between the SPIFE A1AT with the predicate is 100% (357/357). There were no discordant results observed between the two methods.

2. Matrix Comparison:

Not applicable

C Clinical Studies:

Clinical performance of SPIFE A1AT Kit was established using 172 clinically characterized samples including 10 MM, 20 MZ, 20 MS, 20 ZZ, 20 SS, 20 SZ, 16 null and 46 rare phenotypes. The same set of samples were also run with the predicate device, HYDRAGEL 18 A1AT ISOFOCUSING Kit. The clinical performance was assessed using the clinical diagnostic criteria cited in Stoller, et.al. (Stoller JK, Hupertz V, Aboussouan LS. Alpha-1 Antitrypsin Deficiency. 2006 Oct 27 [Updated 2020 May 21]. In: Adam MP, Mirzaa GM, Pagon RA, et al., editors. Gene Reviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2022. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1519>).

Based on these criteria, the clinical sensitivity and specificity were the same as that of the predicate.

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Not applicable. The phenotype for the normal healthy subjects is MM using this device.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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