510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k063498

B. Purpose for Submission:
New Device

C. Measurand:
Alpha-1-Antitrypsin Phenotype

D. Type of Test:
Qualitative, isofocusing

E. Applicant:
SEBIA, Inc.

F. Proprietary and Established Names:
HYDRAGEL 18 A1AT ISOFOCUSING Kit

G. Regulatory Information:
1. Regulation section:
   21 CFR § 866.5130 Alpha-1-antitrypsin immunological test system
   21 CFR § 862.1660, Quality Control Material (Assayed and Unassayed)
2. Classification:
   Class II, Device
   Class I, Quality Control Material
3. Product codes:
   OBZ, Alpha-1-antitrypsin, qualitative, phenotype
   JJX, Single (Specified) Analyte Controls (Assayed and Unassayed)
4. Panel:
   Immunology 82
   Chemistry 75

H. Intended Use:
1. Intended use(s):
   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 labeled anti-Alpha-1 antitrypsin antiserum enhanced the detection and identification of the different phenotypes. For *in vitro* diagnostic use only.

   The A1AT Controls are designed for the migration control of the human Alpha-1 antitrypsin isoforms pattern obtained with HYDRAGEL 18 A1AT ISOFOCUSING* isoelectric-focusing procedure. The A1AT Controls should be used as human sera. For *in vitro* diagnostic use only.

2. <u>Indication(s) for use:</u>
   Same as Intended Use.
3. <u>Special conditions for use statement(s)</u>:
   The device is for prescription use only.
4. <u>Special instrument requirements:</u>
   HYDRASYS System PN 1211, with option HYDRASYS FOCUSING PN 1235, or PN 1212

I. Device Description:

The HYDRAGEL 18 A1AT ISOFOCUSING kit includes: 10 agarose gels (ready-to-use); 3 mL vial ethylene glycol solution (ready-to-use); 50 mL vial anodic solution (ready-to-use); 50 mL vial cathodic solution (ready-to-use); 10 packs of 2 strips each; 85 mL vial sample diluent CSF/A1AT (ready-to-use); 0.4 mL vial anti-A1AT-PER antiserum (stock solution); 3 mL vial antiserum diluent (ready-to-use); two 70 mL vials Rehydrating solution (ready-to-use); two 20 mL vials TTF3 solvent (ready-to-use); two 0.5 mL vials TTF3 (stock solution); 1 pack of 10 (18 teeth) applicators (ready-to-use); 1 pack of 2 buffer containers (ready-to-use); 1 pack of 10 single trough antiserum segments (ready-to-use); 1 pack of 10 thin filter papers ; 3 packs of 10 each thick filter papers.

The A1AT Controls pack contains three lyophilized vials of three phenotypes: one Normal A1AT Control vial (MM) and two Pathological A1AT Control vials (MZ and SZ). The A1AT Control pack is sold separately.

J. Substantial Equivalence Information:

1. <u>Predicate device name(s)</u>:
   Polyacrylamide gel isoelectric focusing
2. <u>Predicate 510(k) number(s):</u>
   Pre-amendment method
3. <u>Comparison with predicate:</u>

Similarities		
Item	New Device	Predicate Device
Intended use/ Indication for use	For the detection and identification of A1AT phenotyping in human serum by isoelectric focusing as an aid in A1AT deficiency	Same
Sample type	Serum	Same
Results	Qualitative interpretation of patterns of A1AT phenotypic stained fractions after electrophoretic migration	Same
Technology	Gel electrophoretic migration	Same

Differences		
Item	Device	Predicate
Methodology	Isoelectric focusing and Immunofixation	Isoelectric focusing
Gel	Agarose	Polyacrylamide
Equipment	Semi-automated HYDRASYS electrophoresis apparatus	Standard isoelectric focusing apparatus
A1AT Antisera	Antibody specificity to A1AT	No antisera
A1AT Antisera enhancement	Peroxidase enzyme	None
Migration	20ºC for about 1 hour (total 510 Vh)	Premigration 30 minutes 1200 V for 3 hours
Antiserum application/incubation	10 minutes at 20ºC	None

Gel treatment	A. At 20ºC:
	  Blotting #.1: 3 min
	  Gel rehydration #1: 5 min.
	  Blotting #2: 3 min
	  Gel rehydration #2: 5 min.
	B. At 30ºC:
	  TTF3 incubation: 10 min.
	  Blotting #3: 3 min.
	C. At 50ºC:
	  Drying: 3 min.
	D. Washing & final drying: 18 min.
		Fixation with 12.5% trichloracetic acid; washing with ethanol & acetic acid solution; Coomassie Blue staining; Destaining

Sample volume	10 μL	2.5 μL
Sample dilution	1:10 with Sebia diluent	None
Lowest detectible Limit	5 mg/dL	None specified

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

None provided.

L. Test Principle:

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: isoelectrofocusing on agarose gel to fractionate the proteins in the serum samples and immunofixation with enzyme (peroxidase)-labelled anti-AlAT antiserum to detect the different phenotypes of AlAT. The semi-automated HYDRASYS system performs all the steps needed to obtain gels ready for interpretation.

M. Performance Characteristics (if/when applicable):
1. <u>Analytical performance</u>:
   a. *Precision/Reproducibility*:
      The within-run gel reproducibility was determined by testing eight samples fifteen or eighteen times each on a single gel of 15 or 18 tracks. Two gels of the same lot number were used in this study. Two normal serum samples with M1 and M1M2 phenotypes and six pathological serum samples with MS, SS, MZ, ZZ, MX phenotypes reproduced the corresponding phenotype correctly.

      The between-run gel reproducibility was determined by testing fifteen samples and three controls twelve times on two lots of gels. Seven normal serum samples (2 M1M2, 4 MM, 1M1M3); eight pathological samples (2 MS, 1 SS, 1 MZ, 1 ZZ, 1 SZ, 1 MX, 1 MI [I allele is rare and associated with decreased A1AT levels]); and three controls (1 normal MM and 2 pathological MZ and SZ) reproduced the corresponding phenotype correctly.

      The within-run and between-run antiserum reproducibility studies were determined by testing fifteen samples and three controls on four different lots of antiserum. The 18 samples were applied in 18 tracks of 4 gels. Nine normal samples with MM phenotype; six pathological samples with 2 SS, 2 M2Z, 1 MP, 1 M undetermined variant; and three controls with 1 normal MM and 2 pathological MZ and SZ reproduced the corresponding phenotype correctly.

      The within-run and between-run control reproducibility studies were determined by testing each of the three controls (MM, MZ, SZ phenotypes) six times in four gels for two days. All three controls reproduced the corresponding phenotype correctly.
   b. *Linearity/assay reportable range:*
      Not applicable.
   c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
      No reference standards and method available.
      Stability: The expiration date claims for the products are as follows:

Product	Temperature	Expiration Date Claim
A1AT Antiserum	2-8 $^{\circ}$C	2 years
Control: Lyophilized	2-8 $^{\circ}$C	3 years
Control: Reconstituted	2-8 $^{\circ}$C	1 week
Control: Reconstituted	- 20 $^{\circ}$C	6 months

Expected values: Qualitative Interpretation of normal pattern for A1AT 'MM' phenotype fraction.

d. *Detection limit:*
The detection limit was determined by testing diluted A1AT (concentration of 27 mg/dL with pathological ZZ phenotype) down to 5 mg/dL. The ZZ phenotype was detectable at 5 mg/dL.

e. *Analytical specificity:*
Interference study: Two studies were performed. For each interfering substance, four concentrations were tested. Study 1 consisted of aliquots from one normal serum pool sample (normal MM phenotype) and three controls (1 normal MM and 2 pathological MZ and SZ). One set of these aliquots was spiked with hemoglobin up to 0.35 g/dL and another set with bilirubin up to 0.20 mg/mL. Study 2 consisted of aliquots from six normal MM serum and three controls (1 normal MM and 2 pathological MZ and SZ). One set of these aliquots were spiked with total cholesterol up to 6.9 mmol/L and another with triglycerides up to 8.0 mmol/L. No interference was observed.

f. *Assay cut-off:*
Not applicable.

2. <u>Comparison studies:</u>
a. *Method comparison with predicate device:*
i. Study design: A total of 68 serum samples were tested on HYDRAGEL 18 A1AT ISOFOCUSING kit using HYDRASIS apparatus and on polyacrylamide gels using a standard isoelectric focusing apparatus. Forty-one (41) were pathological serum samples, 4 unclear diagnoses, and 23 were normal serum samples. There was 100% agreement between the two methods. Results of the phenotype interpretation are summarized below:

Phenotype Qualitative Results	N	Agreement
Normal Samples		
MM	22	22
M1M2	1	1
Pathologic Samples		
MZ	14	14
MS	9	9
M2S	1	1
ZZ	9	9
SS	4	4
SZ	4	4
Others (unclear diagnosis)		
IM or FM	1	1
IM	2	2
Heterogeneous (Indeterminate)	1	1
Total	68	68

b. *Matrix comparison:*
   Not applicable.
3. Clinical studies:
   a. *Clinical Sensitivity and specificity:*
      The clinical sensitivity and specificity studies were evaluated on 68 clinically characterized sera from patients with the following diagnosis: 16 congenital deficiency, 15 pulmonary disorders, 8 hepatic disorders, 1 infertility and 1 panniculitis, 23 normals and 4 indeterminate diagnoses. Sensitivity was 100% and specificity was 92%. The overall agreement was 97%.

		Diagnosis			
		Positive	Equivocal*	Negative	Total
HYDRAGEL 18 A1AT ISOFOCUSING kit	Positive	39		2**	41
	Equivocal*		(4)		
	Negative			23	23
	Total	39	(4)	25	64

*Equivocals were excluded from the calculation. The four equivocal samples were from patients with normal A1AT quantitative measurements being ruled out for infertility, pulmonary and hepatic disorder and congenital deficiency. The A1AT phenotypes were IM or FM, IM, IM, and heterogeneous pattern respectively.
**The two false positive samples were from asymptomatic patients, one with a diagnosis of infertility and the other, panniculitis and the A1AT phenotypes were MZ and MS respectively. These two patients (without underlying pulmonary or hepatic disorders) are assumed to be carriers of S and Z deficiency alleles.

      Sensitivity: 100 % (39/39)
      Specificity: 92 % (23/25)
      Overall percent Agreement: 97% (62/64)

   b. *Other clinical supportive data (when a. is not applicable):*
      Not applicable.
4. Clinical cut-off:
   Same as Expected values.
5. Expected values/Reference range:
   Expected values in the normal population should be the presence of MM phenotype fraction. The sponsor has also provided literature on the A1AT concentrations for the different A1AT phenotypes (see table below).

A1AT phenotype	A1AT concentration (mg/dL)
MM	103 - 200
MS	100 - 180
SS	70 - 105
MZ	66 - 120
SZ	45 – 80
ZZ	10 - 40
Null/Null	0

Vidal et al. (Guidelines for the diagnosis and management of α-1-antitrypsin deficiency Arch Bronconeumol 2006

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.