SPIFE® Touch A1AT Procedure

INTENDED 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 antibody.

For In Vitro Diagnostic Use. RX Only.

SUMMARY

Alpha-1 Antitrypsin deficiency, a hereditary disorder leading to emphysema and liver disease, has a debilitating impact on the quality of life of afflicted individuals. The early detection, diagnosis and treatment of individuals possessing pathological Alpha-1 Antitrypsin phenotypes are paramount in providing proper care. The polymorphic gene for Alpha-1 Antitrypsin presents different alleles designated by letters (A-Z) based on electrophoretic mobility. For example, M denotes normal and Z denotes deficiency. The SPIFE Touch A1AT method offers ease of interpretation, excellent resolution, reagent conservation and rapid turnaround.

PRINCIPLE

The SPIFE Touch A1AT assay is performed in two major stages. Stage 1 consists of separating A1AT isoforms in an electrophoretic field through an agarose medium. Each isoform migrates a specific distance from the application point based on the A1AT isoform's isoelectric point. Once the A1AT protein reaches the pH corresponding to its isoelectric point, the electrophoresis portion of the procedure is complete. Stage 2 is comprised of immunofixation using an A1AT antibody to detect the different phenotypes of A1AT. Visualization is achieved using acid violet protein stain.

REAGENTS

1. **SPIFE A1AT Gel**
   Ingredients: Each gel contains agarose (1.5%) and separators with a preservative.
   Preparation for Use: The gels are ready for use as packaged.
   Storage and Stability: The gels should be stored horizontally at room temperature (15 to 30°C) and are stable until the expiration date indicated on the package. The gels must be stored in the protective packaging in which they are shipped. **DO NOT REFRIGERATE OR FREEZE.**
   Signs of Deterioration: Any of the following conditions may indicate deterioration of the gel: (1) crystalline appearance indicating the agarose has been frozen, (2) cracking and peeling indicating drying of the agarose, (3) bacterial growth indicating contamination.
2. **Anode Solution**
   Ingredients: Contains 0.3 M acetic acid.
   CAUTION: IRRITANT. DO NOT INGEST.
   Preparation for Use: The solution is ready for use as packaged.
   Storage and Stability: The solution should be stored at room temperature (15 to 30°C), and is stable until the expiration date indicated on the label.
   Signs of Deterioration: Discard product if it appears cloudy or contains particulates.
3. **Cathode Solution**
   Ingredients: Contains 1 M NaOH.
   WARNING: CORROSIVE - Causes burns. **DO NOT PIPETTE BY MOUTH**. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention immediately.
   Preparation for Use: The solution is ready for use as packaged.
   Storage and Stability: The solution should be stored at room temperature (15 to 30°C), and is stable until the expiration date indicated on the label.
   Signs of Deterioration: Discard product if it appears cloudy or contains particulates.
4. **Acid Violet Stain**
   Ingredients: The stain is comprised of Acid Violet stain.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.
   Preparation for Use: Dissolve the dry stain in 1 L of 10% acetic acid and mix thoroughly. Fill the SPIFE stain vat.
   Storage and Stability: The dry stain should be stored at 15 to 30°C and is stable until the expiration date indicated on the package. The stain solution is stable for six months when stored at 15 to 30°C in a closed container.
   Signs of Deterioration: The diluted stain should be a homogeneous mixture, free of precipitate. The stain must be replaced after processing ten gels to avoid contamination.
5. **Tris-Buffered Saline**
   Ingredients: The powder contains a Tris base with Tris HCl and sodium chloride.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE.
   Preparation for Use: Dissolve the powder from one package in 8 L of deionized water and mix thoroughly.
   Storage and Stability: Store the dry powder at 15 to 30°C until the expiration date indicated on the label. The buffer solution should be stored at 15 to 30°C.
   Signs of Deterioration: The buffer solution should be discarded if it shows signs of bacterial contamination.
6. **Antibody to Human Alpha-1 Antitrypsin**
   Ingredients: The vials in the kit contain the purified immunoglobin fraction of serum from rabbits immunized with Alpha-1 Antitrypsin.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. To prevent the formation of toxic vapors, do not mix with acidic solutions. When discarding, always flush sink with copious amounts of water. This will prevent the formation of metallic azides which, when highly concentrated in metal plumbing, are potentially explosive. In addition to purging pipes with water, plumbing should occasionally be decontaminated with 10% NaOH.
   Preparation for Use: The antibody is ready for use as packaged.
   Storage and Stability: The antibody should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.
   Signs of Deterioration: Extremely cloudy antibody may be indicative of bacterial contamination.

INSTRUMENT

A SPIFE Touch analyzer must be used to apply samples, electrophorese, wash, stain, destain and dry the gels. Refer to the appropriate Operator's Manuals for detailed instructions.

SPECIMEN COLLECTION AND HANDLING

Specimen: Fresh serum is the specimen of choice. Evaporation of uncovered specimens may cause inaccurate results.

Storage and Stability: If storage is necessary, covered samples may be stored for up to 14 days at 2-8°C or 21 days frozen. A single freeze/thaw cycle is recommended as additional cycles many cause inaccurate results.

PROCEDURE

Materials Provided: The following materials needed for the procedure are contained in the SPIFE A1AT kit (Cat. No. 3432). Individual items are not available.

SPIFE A1AT Gels (10)
Acid Violet Stain (1 vial)
Tris-Buffered Saline (2 pkgs)
A1AT Blotter C (10)
A1AT Blotter D (40)
A1AT Electrode Wicks (30)
A1AT Wick Blotters (60)
Serrated Applicator Blades, 20 sample (20)
Anode Solution (1 vial)
Cathode Solution (1 vial)
A1AT Antibody (2 pkgs)

Materials provided by Helena Laboratories but not contained in the kit above:

Item	Cat. No.
SPIFE Touch Analyzer	1068
SPIFE Reagent Spreaders	3706
SPIFE IEF Sample Tray	1347
SPIFE IEF Tungsten Electrodes and Adapters	3702
A1AT Controls (MM, MS, MZ)	3435
SPIFE Disposable Cups (Deep Well)	3360
SPIFE Rigid Antisera Template IFE-15	3352
REP Prep	3100
Gel Block Remover	1115
Applicator Blade Weights	3387
TITAN Gel Isoenzyme Incubation Chamber	4062

Materials and Supplies Needed but not Supplied:

Methanol

Glacial Acetic Acid: Used to prepare the A1AT Destain and the 10% acetic acid needed for stain preparation.

A1AT Destain: 7 parts deionized water, 2 parts methanol and 1 part glacial acetic acid. Fill the SPIFE destain vat.

STEP-BY-STEP METHOD

I. Instrument Parameters

Using the instructions provided in the Operator's Manual, set up parameters as follows:

	Separator Unit
Load Sample 1	Prompt: None Time: 0:01 Temperature: 21°C Speed: 1
Pause 1	Prompt: None Time: 0:01 Temperature: 21°C

Load Sample 2
Prompt: None
Time: 0:01
Temperature: 21°C
Speed: 1

Pause 2
Prompt: None
Time: 0:01
Temperature: 21°C

Load Sample 3
Prompt: None
Time: 0:01
Temperature: 21°C
Speed: 1

Pause 3
Prompt: None
Time: 0:01
Temperature: 21°C

Load Sample 4
Prompt: None
Time: 0:01
Temperature: 21°C
Speed: 1

Pause 4
Prompt: None
Time: 0:01
Temperature: 21°C

Load Sample 5
Prompt: None
Time: 0:01
Temperature: 21°C
Speed: 1

Pause 5
Prompt: None
Time: 0:01
Temperature: 21°C

Load Sample 6
Prompt: None
Time: 0:01
Temperature: 21°C
Speed: 1

Pause 6
Prompt: None
Time: 0:01
Temperature: 21°C

Load Sample 7
Prompt: None
Time: 0:30
Temperature: 21°C
Speed: 1

Apply Sample 1
Prompt: None
Time: 1:00
Temperature: 21°C
Speed: 1
Location: 2

Load Sample 8
Prompt: None
Time: 0:30
Temperature: 21°C
Speed: 1

Apply Sample 2
Prompt: None
Time: 1:00
Temperature: 21°C
Speed: 1
Location: 2

Electrophoresis 1
Prompt: To Continue
Time: 30:00
Temperature: 16°C
Voltage: 400 V
mA: 50 mA

Electrophoresis 2
Prompt: None
Time: 30:00
Temperature: 16°C
Voltage: 850 V
mA: 50 mA

Apply Reagent
Prompt: To Continue
Temperature: 20°C
Cycles: 2

Absorb
Prompt: None
Time: 10:00
Temperature: 30°C

Blot 1
Prompt: Install Blotter
Time: 5:00
Temperature: 30°C

Blot 2
Prompt: Install Blotter
Time: 3:00
Temperature: 30°C

Blot 3
Prompt: Install Blotter
Time: 3:00
Temperature: 30°C

Blot 4
Prompt: Install Blotter
Time: 3:00
Temperature: 30°C

Dry
Prompt: Remove Blotter
Time: 9:00
Temperature: 60°C

End

Stainer Unit

Wash 1
Prompt: Plate Out, Gel Holder In
Time: 0:30
Recirculation: Off
Valve: 1
Fill, Drain

Wash 2
Prompt: Plate In, Gel Holder In
Time: 10:00
Recirculation: Off
Valve: 1
Fill, Drain

Wash 3
Prompt: Plate In, Gel Holder In
Time: 5:00
Recirculation: Off
Valve: 1
Fill, Drain

Wash 4
Prompt: Plate In, Gel Holder In
Time: 5:00
Recirculation: Off
Valve: 1
Fill, Drain

Stain
Prompt: Plate In, Gel Holder In
Time: 5:00
Recirculation: Off
Valve: 5
Fill, Drain

Destain 1
Prompt: None
Time: 1:00
Recirculation: Off
Valve: 2
Fill, Drain

Destain 2
Prompt: None
Time: 1:00
Recirculation: Off
Valve: 2
Fill, Drain

Destain 3
Prompt: None
Time: 1:00
Recirculation: Off
Valve: 2
Fill, Drain

Destain 4
Prompt: None
Time: 1:00
Recirculation: Off
Valve: 2
Fill, Drain

Destain 5
Prompt: None
Time: 1:00
Recirculation: Off
Valve: 2
Fill, Drain

Wash 5	Prompt: None Time: 0:30 Recirculation: Off Valve: 7 Fill, Drain
Dry	Prompt: None Time: 5:00 Temperature: 63°C
End	

II. Sample Preparation

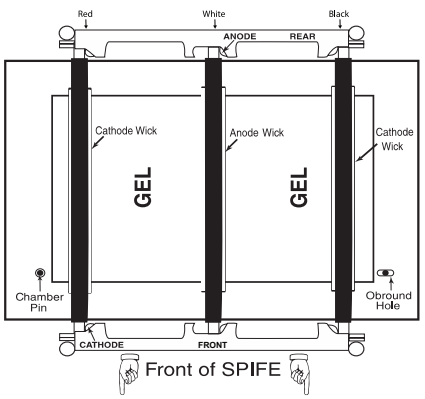
1. Remove two Serrated Applicator Blades from the packaging. Place the blades into the vertical slots in the Applicator Assembly identified as 1 and 16. Press on the end of each blade so that it slides to the back of the slot.
   NOTE: The Serrated Applicator Blades will only fit into the slots one way; do not try to force the blades into the slots.
2. Place a Blade Weight on top of each Serrated Applicator Blade. When placing the weight on the blades, position the weight with the thick side to the right.
3. Slide Disposable Sample Cup strips into appropriate cup tray.
4. Pipette 80 µL of patient serum or control into Disposable Sample Cups. Cover the tray until ready to use.
   NOTE: All three A1AT Controls (MM, MZ and MS) must be run on each row as markers.
5. Place the cup tray with samples on the SPIFE Touch. Align the holes in the tray with the pins on the instrument.

III. Gel Preparation

1. Remove the gel from the protective packaging and discard overlay.
2. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
3. Place the left edge of the gel over the REP Prep aligning the round hole on the left pin of the chamber. Gently lay the gel down on the REP Prep, starting from the left and ending on the right side, fitting the obround hole over the right pin. Use lint-free tissue to wipe around the edges of the plastic gel backing, especially next to electrode posts, to remove excess REP Prep. Make sure no bubbles remain under the gel.
4. Place an A1AT Blotter C on the gel. Gently blot the entire surface of the gel using slight fingertip pressure on the blotter. Remove the blotter.

IV. Sample Application and Electrophoresis

1. Place a vial of A1AT antibody into each outer hole of the reagent bar, ensuring that the vials are pushed down as far as they can go. Close the chamber lid.
2. Remove three A1AT Electrode Wicks from the packaging. Soak one wick in the Anode Solution and two wicks in the Cathode Solution. Ensure the anode and cathode solutions do not mix. A TITAN Gel Isoenzyme Incubation Chamber (Cat. No. 4062) can be used for this purpose. Label one side of the dish cathode and the other side anode.
3. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed.
4. The SPIFE Touch will apply samples to the gel and then beep. Open the chamber lid. Dispose of blades and cups as biohazardous waste.
5. Remove the Electrode Wick from the Anode Solution, place it between two A1AT Wick Blotters and blot almost dry.
6. Position the anode A1AT Electrode Wick on the gel surface at the center of the chamber. Gloves should be changed before handling the cathode wicks.
7. Remove the two A1AT Electrode Wicks from the Cathode Solution, and place them between two A1AT Wick Blotters and blot almost dry.
8. Place one cathode A1AT Electrode Wick on the gel surface near the outer edge of the gel on each side. Align the outer edge of the wick with the edge of the copper floor. The wicks should be placed so that the electrodes will be centered over the wicks.
9. Insert the SPIFE IEF Electrode Adapter (Cat. No. 3702) marked Front between the two magnetic posts located at the front of the chamber floor. Insert the IEF Electrode Adapter marked Rear between the two magnetic posts located at the back of the chamber floor.
10. Clean the electrodes with deionized water before and after each use. Wipe with a lint-free tissue.
11. Place the Tungsten Electrode color-coded red into the slots created by the adapter on the left side of the plate on the cathode wick. Place the Tungsten Electrode color-coded black into the slots on the right side of the plate on the cathode wick. Place the Tungsten Electrode color-coded white into the slots at the middle of the plate on the anode wick. Ensure all three electrodes are seated firmly against the electrode wicks and making contact with the adapter electrode posts.
    NOTE: Each color-coded Tungsten Electrode must be placed in the same location on the gel every time it is used. Changing the location may affect pattern integrity.
12. Close the chamber lid and press **CONTINUE** to begin electrophoresis. The instrument will beep when electrophoresis is complete.



V. Immunofixation, Staining, Destaining and Drying

1. When electrophoresis is complete, open the chamber lid and remove the electrodes and adapters. Carefully remove and discard the electrode wicks.
2. Place a SPIFE Reagent Spreader (glass rod) across each end of the gel inside the magnetic posts. Close the chamber lid and press **CONTINUE** to apply the antibody and begin absorption.
3. When the beeper sounds, open the chamber lid and remove the SPIFE Reagent Spreaders. Place an A1AT Blotter D on the surface of the gel, smooth side down. Place the Antisera Template on top of the A1AT Blotter D. Close the chamber lid and press **CONTINUE**. Blot 1 will be timed for 5 minutes.
4. While the gel is blotting, use the arrows under **STAINER UNIT** to select the appropriate test. Press **START** and choose Wash 1 to rinse the stainer chamber.
5. When the beeper sounds on the Separator side, open the chamber lid and remove the Antisera Template and blotter. With the front of the Gel Holder facing the operator, attach the gel to the holder by placing the round hole over the left pin and the obround hole over the right pin. The gel should face away from the operator. Place the Gel Holder with attached gel into the stainer chamber with the front of the Gel Holder facing the operator. Press **CONTINUE** to begin Wash 2. The instrument will wash the gel for 10 minutes and beep.
6. When the beeper sounds, remove the gel from the Gel Holder and return it to the separator floor using REP Prep under the gel. Place an A1AT Blotter D on the surface of the gel, smooth side down. Place the Antisera Template on top of the A1AT Blotter D. Close the chamber lid and press **CONTINUE** on the Separator side. Blot 2 will be timed for 3 minutes.
7. When the beeper sounds on the Separator side, open the chamber lid and remove the Antisera Template and blotter. With the front of the Gel Holder facing the operator, attach the gel to the holder by placing the round hole over the left pin and the obround hole over the right pin. The gel should face away from the operator. Place the Gel Holder with attached gel into the stainer chamber with the front of the Gel Holder facing the operator. Press **CONTINUE** to begin Wash 3. The instrument will wash the gel for 5 minutes and beep.
8. When the beeper sounds, remove the gel from the Gel Holder and return it to the separator floor using REP Prep under the gel. Place an A1AT Blotter D on the surface of the gel, smooth side down. Place the Antisera Template on top of the A1AT Blotter D. Close the chamber lid and press **CONTINUE** on the Separator side. Blot 3 will be timed for 3 minutes.
9. When the beeper sounds on the Separator side, open the chamber lid and remove the Antisera Template and blotter. With the front of the Gel Holder facing the operator, attach the gel to the holder by placing the round hole over the left pin and the obround hole over the right pin. The gel should face away from the operator. Place the Gel Holder with attached gel into the stainer chamber with the front of the Gel Holder facing the operator. Press **CONTINUE** to begin Wash 4. The instrument will wash the gel for 5 minutes and beep.
10. When the beeper sounds, remove the gel from the Gel Holder and return it to the separator floor using REP Prep under the gel. Using the Gel Block Remover, remove and discard the gel that extends beyond the copper floor. Use a lint-free tissue to wipe around the edges of the gel backing to remove excess moisture. Place an A1AT Blotter D on the surface of the gel, smooth side down. Place the Antisera Template on top of the A1AT Blotter D. Close the chamber lid and press **CONTINUE** on the Separator side. Blot 4 will be timed for 3 minutes. When the beeper sounds, open the chamber lid and remove the Antisera Template and blotter.
11. Lay one SPIFE Reagent Spreader across each end of the gel to prevent curling during the drying step. Close the chamber lid and press **CONTINUE**. The gel will be pre-dried in the electrophoresis chamber.
    NOTE: The Antisera Template should be cleaned with a mild biocidal detergent. Rinse with deionized water and wipe completely dry.
12. When the beeper sounds on the Separator side, open the chamber lid and remove the gel from the electrophoresis chamber. With the front of the Gel Holder facing the operator, attach the gel to the holder by placing the round hole over the left pin and the obround hole over the right pin. The gel should face away from the operator. Place the Gel Holder with attached gel into the stainer chamber with the front of the Gel Holder facing the operator.
13. Press **CONTINUE** to begin the staining process. The instrument will stain, destain and dry the gel.
14. When the gel has completed the process, the instrument will beep. Remove the gel from the Gel Holder to view the bands.

Stability of the End Product: The completed, stained and dried immunofixation gel is stable for an indefinite period of time if protected from humidity and light.

Quality Control: The A1AT Control panel (Cat. No. 3435) contains three controls representing three A1AT phenotypes: MM, MS, and MZ. The control panel is recommended for use on each row as a qualitative control for migration. The control panel indicates the migration of the M, S, and Z protein variant banding patterns and assists in interpretation of patient samples. The controls are to be run undiluted and as a patient sample. Further information may be found on the A1AT Controls assay sheet.

RESULTS

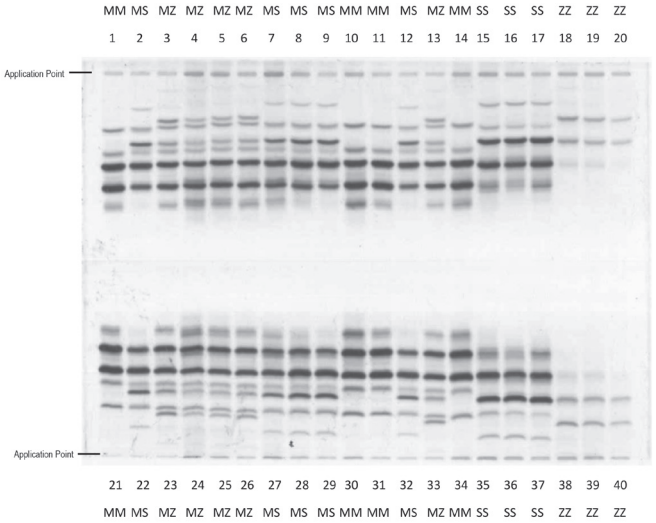


Figure 1 illustrates the electrophoretic mobility of bands on the SPIFE A1AT Gel.

Expected Values

The following table is presented for information purposes only.[1]

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

INTERPRETATION OF RESULTS

Interpretation is qualitative and is used in conjunction with clinical findings, functional activity and genotyping for complete diagnosis of A1AT deficiencies.

LIMITATIONS

No interference with the SPIFE TOUCH A1AT procedure was detected due to sample lipid concentrations of ≤ 30 mg/mL, sample bilirubin concentrations ≤ 20.0 mg/mL and sample hemoglobin concentrations ≤ 0.35 g/dL.

The use of antiserum other than supplied by Helena Laboratories may affect the results. Due to the limitations in resolution and separation inherent to zone electrophoresis, some rare A1AT phenotypes may not be detected with this method. Helena Laboratories stringently qualifies all raw materials in the manufacture of the A1AT assay. Some differences in migration electrophoretic pattern presentation may be observed with different A1AT gel lots. However, this will not negatively impact assay utility.

PERFORMANCE CHARACTERISTICS

Repeatability within gel, between gel and between day

Repeatability was assessed using one lot of A1AT controls (MM, MS, MZ) and 9 patient samples of various A1AT phenotypes (1xMM, 2xMS, 2xMZ, 2xSS, 2xZZ). The tests were run over 3 days with 3 gels/day. Eighteen replicates for controls and 36 replicates for each patient sample were collected.

All repeats gave concordant within gel, between gel and between day results and the patterns corresponded to the A1AT phenotype of each sample tested.

Repeatability gel lot to lot

Repeatability between A1AT gel lots was assessed by running one lot of A1AT Controls and 14 samples of various A1AT phenotypes (2xMM, 3xMZ, 3xMS, 3xSS, 3xZZ) on each of three gel lots. The tests were run over 3 days with 3 gels/day. Thirty-six replicates for controls and 18 replicates for each patient sample were collected.

All repeats gave concordant gel lot to lot results and the patterns corresponded to the A1AT phenotype of each sample tested.

Sensitivity

Serial dilutions were prepared from an A1AT ZZ phenotype sample with a total A1AT concentration of 24 mg/dL. The minimum detection limit of an A1AT isoform was about 6 mg/dL.

Method Comparison

Concordance studies were performed at 3 sites on 357 serum samples between the SPIFE Touch A1AT assay and a commercially available FDA cleared isoelectric focusing method. Thirty normal and 327 different pathological serum samples were run on both platforms. The total A1AT concentrations ranged between ≥20 mg/dL and ≤200 mg/dL. The data are summarized in the following table for Method Comparison.

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

Overall % agreement = 357/357 = 100%

There were no discordant results between the candidate and the comparator device.

Clinical Performance

We tested 172 clinically characterized samples. The clinical performance was assessed using the clinical diagnostic criteria cited in Stoller, et al., 2020.[2] Based on these criteria the clinical sensitivity and specificity were the same as that of the predicate.

BIBLIOGRAPHY

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2. 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. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1519/

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Test System: Analyte Complexity: HIGH

HELENA LABORATORIES

1530 Lindbergh Dr.
Beaumont, Texas USA 77707