Procedure for A1AT Phenotyping

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Procedure for A1AT Phenotyping – Performing the Test

Specimen Requirement

The following are the specimen requirements for the procedure.

Specimen	Required Volume	Minimum Volume	Storage	Stability
Serum (preferred sample)	50µl	1 mL	Store at 2-8˚C	14 days at 2-8˚C

Unacceptable Specimen: Refer to **Rejection/Acceptance Criteria for Samples** for guidelines on rejecting unacceptable specimens for **A1AT Phenotyping.**

Materials Needed

Equipment	Reagents	Supplies
Sebia Hydrasys Isofocusing unit, (PN 1235,1212) Scanner Vortex mixer Centrifuge	Hydrasys Destain solution E-Pure water / Di H_2O Antisera (A1AT) Antisera diluent Rehydrating solution TTF3 and TTF3 solvent Wash solution (optional) Agarose gels A1AT Controls 30% Hydrogen Peroxide	5mL, 7mL pipettes and tips 4,10,20,100,200 µl pipettes and tips Wet Storage Chamber Template Guide Bar Dynamic Mask 18 wells antisera segments ENZ template 4 12 x 75mm glass tubes Accessory Kit Waste container

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Procedure for A1AT Phenotyping – Performing the Test, continued

Preparing A1AT samples & controls

Step	Action
1.	• Take serum samples from refrigerator and allow them to come to room temperature
2.	• Prepare a 1:10 dilution of serum samples using the Sample Diluent. • 10 µl patient sample + 90 µl Sample Diluent ***Note: (DO NOT DILUTE SEBIA CONTROLS)***
3.	• Vortex samples thoroughly prior to application.
4.	• Place one applicator comb on a flat surface with the well numbers in the right-side-up position. • Apply 10 µl of the 1:10 diluted patient samples in each patient well of the 18 well applicator combs. Positions 2-8 = patient samples Positions 10-17 = patient samples • Apply 10 µl of the prepared Sebia Controls (MM, MZ, MS) in Position 1 = MM Position 9 = MZ Position 18 = MS (Figure 1) ***Note: Load applicator within 2 Minutes.***
5.	• Place the applicator into the wet chamber with the teeth up (handle it by the plastic tooth protection frame). • Let the samples diffuse into the teeth for a minimum of 5 minutes after the last sample application. *(Note: The applicator is stable for up to 8 hours in wet chamber at 2-8^{o} C)*

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Procedure for A1AT Phenotyping – Performing the Test, continued

How to initialize the instrument

Step	Action
1.	Turn on the instrument using the **switch** on the right side of the instrument. Once initialized, the following screen should appear on the front panel of instrument: **A1AT FOCUSING / 20° C POS: 1 / 1. SELECT MIGRATION / 2. SERVICE PROGRAM (for migration side). WASH ISOENZ/GEL / AMID=3 VIOLET=5 / 3. SELECT STAINING / 4. REAGENT LINES (for staining side).** Or, choose **Menu**, and use arrow key to select the desired method. Choose NO. **1**, and **44** ENTER (for migration side) Choose NO. **3** and **11** ENTER (for staining side)
2.	• Press the square button on the Hydrasys instrument to obtain the high voltage required for isoelectrofocusing; • The voltage mode switch should become RED
3.	• Open the lid of the migration module and carefully raise the electrode and applicator carrier. ***WARNING**: Never close the lid while the carriers are raised. As this may cause damage to the instrument.*

How to prepare Buffer Strips

Step	Action
1.	Using the Buffer Strip trays provided, prepare buffer strips **5 minutes** prior to use.
2.	• Using a sharpie pen, label the buffer troughs accordingly: **Blue trough** → Cathode → Clear solution **Grey trough** → Anode → Red solution
3.	• Pipette 5 mL anodic solution (Red) into grey trough • Pipette 5 mL cathodic solution (Clear) into blue trough ***Note: DO NOT allow solutions to come into contact with each other***

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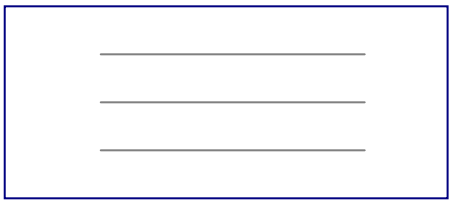
4.	• Open a pack of sponges provided, handling them by the plastic ends • Place one sponge in each trough to saturate in buffer solution • Using a pipette tip press sponge down into solution to absorb buffer evenly along entire strip
5.	• Use the saturated strips without any delay *Note: Saturate strips just before use to avoid carbonation*

How to prepare A1AT gels and instrument

Step	Action
1.	Lift instrument lid and pipette 300 µl of ethylene glycol solution to the migration plate in 3 even strips: (100 µl each strip) 
2.	• Lift carrier up and apply RED sponge on BOTTOM electrode and CLEAR sponge on TOP electrode. (Figure 2)
3.	• Carefully remove A1AT gel from case and **wipe back with tissue paper**
4.	• With thin blotter paper, VERY quickly blot gel (<3 seconds) **from bottom to top motion**
5.	• Place gel on instrument migration plate by: • Line up gel to lower lip and sides of the instrument migration plate and roll gel down, avoiding bubbles. (Figure 3)
6.	• Lower both carriers down. In this position the buffer strips **DO NOT touch the gel.** • **DO NOT** force the carriers all the way down!
7.	• Remove the applicator from the WET chamber. Handle it by the protection frame.
8.	• Snap off the applicator teeth's protection frame. • Place application comb into **POSITION 1** *Note: The numbers printed on applicator must face operator.* (Figure 4)

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9.	• Close lid **and** • Make sure voltage button on instrument is **"RED"** before proceeding with run. • If not, then press square voltage button on instrument until it is RED
10.	• Press **GO→** (Application 30 seconds) (Migration 1hour)

Preparing & applying A1AT antiserum

Step	Action
1.	Antiserum should be prepared **during migration step**
2.	In a disposable glass test tube pipette: **40 µl** A1AT antisera + **300 µl** antisera diluent
3.	Vortex antisera mixture and **keep protected from light** ie) cover with foil or store in a dark cabinet / drawer
	Assemble antisera segment and segment holder together a few minutes before the end of migration step. 1. Set-up antiserum segment on segment holder (figure 6) • Tilt antiserum segment at a 45° angle and position it against the plastic springs of the segment holder • Pull the segment and pivot it until it snaps into the notches of the segment holder
	*When instrument voltage is approximately **498 volts***, then Pipette 20 µl diluted A1AT antisera into all 15 segment wells (figure 8)
6.	When the beep sounds signaling end of incubation, the screen will display "↓ **AS** "
7.	Open instrument lid (the message stops flashing)
8.	Remove the sample applicator (from migration step) and discard

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9.	Raise both carriers, remove the buffered strips by their plastic ends and discard Remove both carriers
10.	Clean electrodes by wiping them carefully with soft wet tissue
11.	Leave the gel in place in the migration module
12.	Set up dynamic mask for antiserum application onto the gel as follows: (figure 7&9)

1.	Place dynamic mask on instrument surface.
2.	Position the mask guide on the anchoring clip
3.	Hold the dynamic mask by the tab and position it into the guide with notches aligned with the marks.
4.	Lower the dynamic mask onto the plate of the instrument
5.	Place antisera segment and holder assembly onto dynamic mask
6.	Make sure the segment holder is at the lowest point on the mask guide, facing the operator
7.	Hold the segment holder by the handle on its right and press on the **central pressure point** such that the antiserum segment contacts the gel.
8.	**Release the pressure**, then the reagent will spread under the entire segment. (figure 10)

13.	Immediately, using the segment holder handle; slide the segment holder up / down **2 times very slowly,** counting 5 seconds up/down each time. (figure 11) A1AT antisera will be applied on gel for 10 minutes

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14.	Leave the dynamic mask in the instrument chamber with the antiserum segment at the lowest point on the mask guide.
15.	Close the lid of the migration module Immediately press **"GO" →** on the instrument and message on screen will display "[INCUBATION]" (10min)

Operating Hydrasys for A1AT phenotyping

Step	Action
1.	When the beep sounds signaling end of incubation, open lid and remove the dynamic mask
2.	The screen will display "↓ **PAP** " (apply thick filter paper on gel, smooth side down)
3.	Press blotter paper firmly over gel, secure edge gently, then close instrument lid and Press **"GO" →** on the instrument and message on screen will display "[BLOTTING]" (3min)
4.	When the beep sounds and screen displays : **"↑ PAP. + ↓ REHYD1"** (remove paper, apply rehydration solution) Install **ENZ template 4** (figure 12)
5.	Close instrument lid
6.	Apply **7 mL** of rehydration solution carefully through template hole, ensuring the solution is evenly spread in the space under the template (figure 13) then press **"GO" →** on the instrument **HYDRATION 1** (5min)
7.	When beep sounds and screen displays : **"↑ REHYD1 + ↓ PAP"** (remove rehydration solution) Then, remove template
8.	Apply heavy blotter over gel, smooth side down; secure edge gently, then close instrument lid and Press **"GO" →** (3 min)
9.	When beep sounds and screen displays : **"↑ PAP + ↓ REHYD2"** (remove paper) and Install **ENZ template 4** (figure 12)

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10.	Apply **7 mL** of rehydration solution carefully through template hole, ensuring the solution is evenly spread in the space under the template (figure 13) Close instrument lid, then press **"GO" →** on the instrument **HYDRATION2** (5 min) *(Note: During this incubation prepare visualization reagent)*
11.	After incubation, a beep sounds and screen displays: **"↑ REHYD2 + ↓ TTF3"** (remove rehydration solution and apply the visualization solution) Leave template in place- **DO NOT REMOVE**

Preparing Visualization reagent

Step	Action
1.	Visualization reagent should be prepared just before use In a disposable glass test tube pipette: 1. **4 mL** TTF3 solvent + **100 µl** TTF3, then 2. Vortex and keep protected from light (cover with foil or in dark cabinet)
2.	When beep sounds and screen displays: **"↑ REHYD2 + ↓ TTF3" (**remove rehydrating solution) ***Note: Do not remove template. Do not blot gel.***
3.	Take the tube of TTF3 solution (previously prepared in step 1) and Add to it exactly (wipe tip) **4 µl** H2O2 (30%) and vortex
3.	Apply **3.5 mL** of TTF3 prepared solution carefully through template hole, ensuring the solution is evenly spread in the space under the template (figure 13) Then close instrument lid and Press **"GO" " →.** (Visualization step- 10 min)

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4.	When beep sounds and screen displays: **"↑ TTF3 + ↓ PAP"** (remove visualization solution, apply blotter paper)
5.	Apply heavy blotter over gel, smooth side down; secure edge gently, then close instrument lid and Press **"GO" →** (3 min)
6.	When beep sounds and screen displays: **"↑ PAP."** (remove filter paper) Remove filter paper and leave gel in place Close instrument lid, and Press **"GO" →** on the instrument **"[DRYING]"**(3 min)
7.	When beep sounds, open instrument cover and remove dried gel

Performing Gel Wash

Step	Action
1.	Place gel into gel frame holder and place into instrument staining compartment by: 1. Open gel holder by laying it flat 2. Position the gel (with gel side facing up) into the grooves of the two rods and close the holder. (figure 14)
2.	Place the gel holder into the gel processing / staining module
3.	Select program **(11)-WASH ISOENZ/GEL** from menu (20 min) Press **"START"** *Note: If the staining chamber has been used with a stain program prior to A1AT run, then clean the chamber with the **"WASH CHAMBER"** program prior to use.*
4.	When instrument beeps, signaling wash cycle is complete, then Remove the gel holder from the compartment, open the clips and remove dried gel

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Non-Controlled Documents

The following non-controlled documents support this procedure.

- References:
  - Sebia Hydragel 18 A1AT Isofocusing Quick Reference Guide
  - Sebia Hydragel 18
  - Sebia HYRYS 2 Instruction Manual
  - Sebia Hydragel 18 A1AT Isofocusing Kit & Control package inserts
  - Sebia Hydragel 18 A1AT Isofocusing Figures 1-14

- Records:
  - Preventive Maintenance for Hydrasys
  - A1AT Phenotyping Inventory

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Procedure for A1AT Phenotyping – Interpreting & Reporting

Reviewing patterns

Step	Action
1	• Review the **MM**, **MZ** & **MS** control results to check for acceptable separation of the allele isoforms. • Corrective action must be taken when the quality control results do not appear as they should. • Refer to **Procedure for Quality Control** for corrective actions.

Calculating

There is no calculation for this assay.

Quality Control Notes

- ***As part of Quality Control, all A1AT Phenotyping reports are reviewed and if discrepancies are found all paper work and transcription of results are rechecked. If necessary, repeat testing of specimen will be done.***
- ***Corrected reports are only done after the above mentioned parameters are met and necessary changes are made on the report which is then reviewed by the management and called to the physician.***

Interpreting Patterns

Refer to the following criteria for consideration when interpreting results of A1AT Phenotyping.

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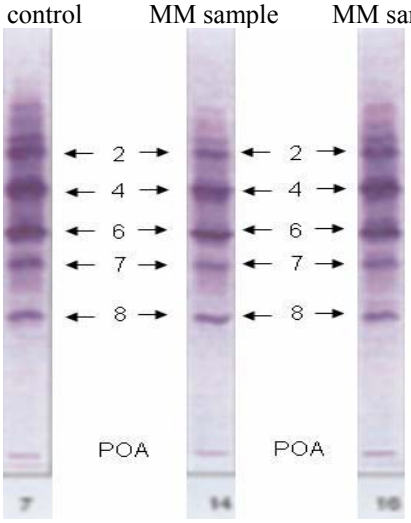
Procedure for A1AT Phenotyping – Interpreting & Reporting, *Continued*

MM phenotype

• The **M alleles** (M1 to M6) are the most common and are defined as "**normal variants**" because they are associated with normal serum alpha-1 antitrypsin protein levels.
• A homozygous **MM** pattern consists of two major bands (bands **4** and **6**) and three minor bands (bands **2**, **7**, and **8**)
  • All bands are named in order of migration:
  Band **2** is the most **anodal**
  Band **8** is the most **cathodal**
• Band **4** is the **most prominent** band in MM phenotype

Interpreting Patterns

MM phenotype
+ Anode MM control MM sample MM sample    - Cathode

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Procedure for A1AT Phenotyping – Interpreting & Reporting,
Continued

MM Phenotype interpretation
• Use bands **2**,**4**, & **6** in your MM control as "anchors" to confirm the presence of "**M**" phenotype • Bands **4** & **6** in your MM control and samples **should align** (have the same position on the gel) **perfectly**

If	Then
1. Bands **4** & **6** in **MM** control and samples <u>align perfectly</u> **and** <u>no difference present</u> between control and patient sample	• Report patient sample as **MM** phenotype
1. Bands **2**, **4**, & **6** are present and align, **But** sample also <u>contains bands that differ</u> from those in the MM control	• Patient has **heterozygous M** phenotype • CLS will need to determine the other allele before reporting (ie, MS, MZ, M?)

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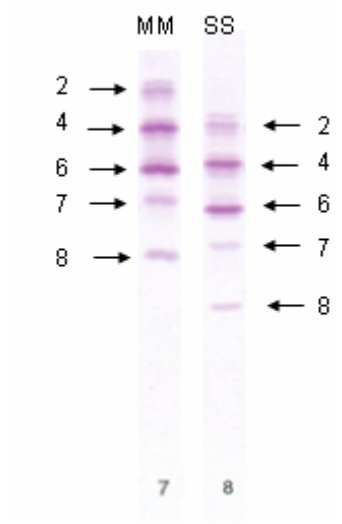
Procedure for A1AT Phenotyping – Interpreting & Reporting, *Continued*

S variants
• A homozygous **SS** pattern consists of two major bands (bands **4** and **6**) and three minor bands (bands **2, 7** and **8**) • However, **SS** variant is "**slower**" than a homozygous MM pattern   ◦ All bands are shifted cathodaly (downwards) in SS when compared to corresponding bands in MM phenotype • Bands **4** & **6** are the **most prominent** bands in SS phenotype

Interpreting Patterns, continued

SS phenotype
+ Anode MM sample SS sample    - Cathode

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Procedure for A1AT Phenotyping – Interpreting & Reporting,
Continued

SS Phenotype interpretation

- On **SS** sample, bands **2** & **4** align well with M4 & M6 (bands 4 & 6 of MM phenotype)
- ***Important:*** Homozygous **SS** does not have bands corresponding to band **2** in MM phenotype
- Band **8** in **SS** variant does not have a matching band in MM phenotype and is the most cathodal band of all common variants

If	Then
1. Bands **2** & **4** in sample aligns well with M4 & M6 bands (MM control) 2. **and** there is no corresponding **2** band to M2 (MM control 3. **and** band **8** is present and more cathodal than M8 (MM control)	• Report patient sample as **SS** phenotype
1. Band **8** is present and more cathodal than M8 (MM control) 2. **But** sample also contains bands that do not correspond with homozygous SS phenotype	• Patient has **heterozygous S** phenotype • CLS will need to determine the other allele before reporting (ie, MS, SZ, etc.)

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Procedure for A1AT Phenotyping – Interpreting & Reporting, *Continued*

Interpreting Patterns, continued

Z variants

- A homozygous **ZZ** variant is the **slowest** of all common variants
- Theoretically, ZZ variants consist of five bands called **2, 4, 6, 7,** & **8**
- Bands **7 & 8** in ZZ variant are not visible on the gel due to their low concentration

Practically, **ZZ** variant will be manifested by <u>three visible bands</u>:

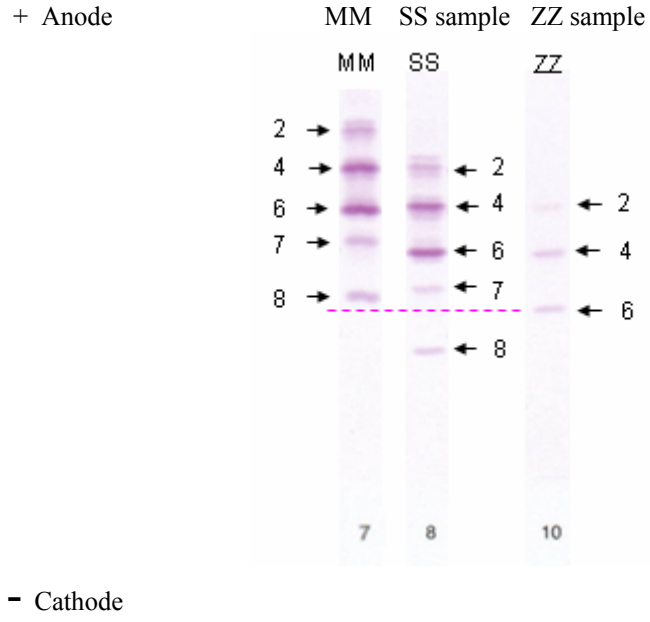
a) Band **2** in Z variant is the most anodal band in the pattern. It is slightly cathodal to MM band 6 and SS band 4

b) Band **4** in Z variant has the same migration location as S6 and therefore will superimpose

c) Band **6** in Z variant is cathodal to MM band 8 and is represented by dotted line below

Interpreting Patterns, continued

ZZ phenotype



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Procedure for A1AT Phenotyping – Interpreting & Reporting,

Continued

ZZ Phenotype interpretation

- Bands **7 & 8** in ZZ variant are not visible on the gel due to their low concentration
- ZZ displays only three clearly visible bands and is very light in comparison to the other two variants
- ZZ has almost no alpha-1 antitrypsin activity, and is associated with low serum AAT levels

If	Then
1. Pattern displays <u>only three</u> clearly visible bands 2. **and** there is no corresponding **2 & 4** bands to M2 & M4 (MM control) 3. **and** band **6** is present and slightly more cathodal than M8 (MM control)	• Report patient sample as **ZZ** phenotype
1. Band **6** is present and slightly more cathodal than M8 (MM control) 2. **But** sample also contains bands that <u>do not</u> correspond with homozygous ZZ phenotype	• Patient has **heterozygous Z** phenotype • CLS will need to determine the other allele before reporting (ie, MZ, SZ, etc.)

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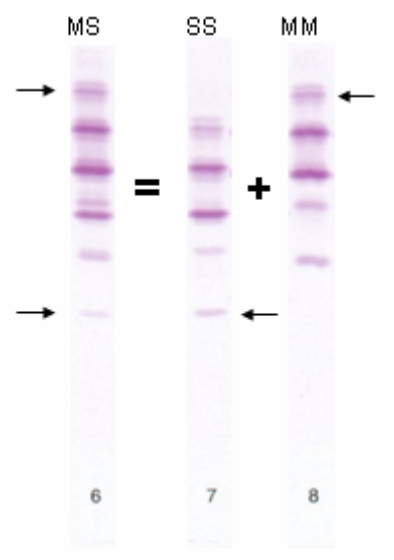
Procedure for A1AT Phenotyping – Interpreting & Reporting, *Continued*

Interpreting Patterns, continued

MS (Heterozygous interpretation)
• **MS** is a fusion product where all bands from both MM & SS are visible • **MS** phenotype will have band **2** from MM (most anodal band) • **MS** phenotype will have an "**S**" band (**band 8** in SS phenotype) that is not seen in MM sample.

Interpreting Patterns, continued

MS phenotype
+ Anode MS SS sample MM sample    - Cathode

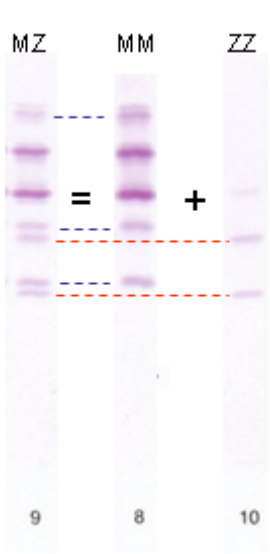
MS Phenotype interpretation	
If	Then
1. Pattern displays band **2** (most anodal band) corresponding to the MM control 2. **and** there is an "**S**" band present (band **8**)	• Report patient sample as **MS** phenotype

Continued on next page

Procedure for A1AT Phenotyping – Interpreting & Reporting, *Continued*

Interpreting Patterns, continued

MZ (Heterozygous interpretation)
• **MZ** variant contains both M & Z bands • **MZ** variant will have band **2** from MM (most anodal band) • Bands **M8** and **Z6** will be clearly separated from each other to form a very distinct "**double**" band (resembling an = sign). The same is true for bands **M7** and **Z4**.

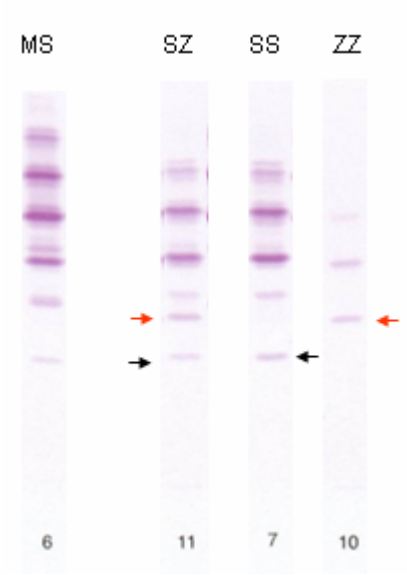
MZ phenotype
+ Anode MZ MM sample ZZ sample    - Cathode

MZ Phenotype interpretation		
	If	Then
	1. Pattern displays band **2** (most anodal band) corresponding to the MM control 2. **and** there are "**Z**" bands present (bands **6 & 4**) forming distinct "**double banding**"	• Report patient sample as **MZ** phenotype

Procedure for A1AT Phenotyping – Interpreting & Reporting, *Continued*

Interpreting Patterns, continued

SZ (Heterozygous interpretation)
• **SZ** variant contains both S & Z bands • **SZ** variant will **NOT** have band **2** from MM (most anodal band) • **S8** band (indicated by black arrows below) and **Z6** band (indicated by red arrows below) will be present in SZ variant

SZ phenotype
+ Anode    - Cathode

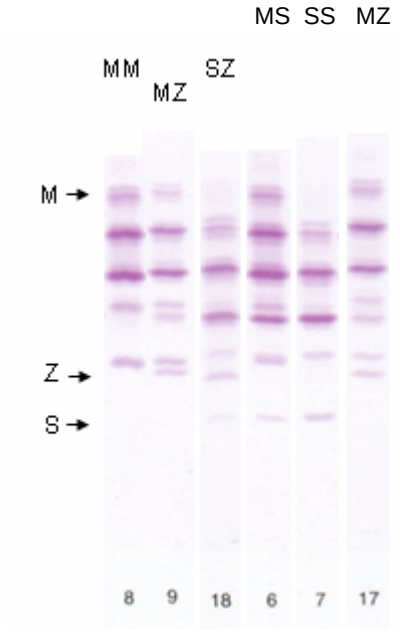
SZ Phenotype interpretation		
	If	Then
	1. Pattern **does not** display band **2** (most anodal band) corresponding to the MM control 2. **and** there are "**Z**" and "**S**" bands present (bands **Z6 & S8**)	• Report patient sample as **SZ** phenotype

Procedure for A1AT Phenotyping – Interpreting & Reporting, *Continued*

Interpreting Controls

Sebia Controls (MM, MZ, MS)
• **MM**, **MZ** and **MS** controls should be included on each gel to ensure acceptable band separation • Note **M2** band on MM and MZ controls (the uppermost anodal band) • Note the **location of Z band** on MZ and SZ controls • Note the **location of S band** on MS control

Interpreting Patterns, continued

A1AT phenotype examples
+ Anode  - Cathode

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Procedure for A1AT Phenotyping – Interpreting & Reporting, *Continued*

Interpreting less common variants

Less common & Unusual variants

- There are three known **M** variants: $\mathbf{M_1}$ (the most common and usually referred to as M), $\mathbf{M_2}$ and $\mathbf{M_3}$
- Clinically, there are no reasons to differentiate **M** subtypes, but they can be helpful in genetic studies.
- There are many other variants and can be identified by comparing banding patterns using M4 and M6 bands as "anchors".
- Note: *It requires significant expertise and/or genotyping to positively identify less common variants.*

Encountering Less Common Variants

Encountering Less Common & Unusual Variants

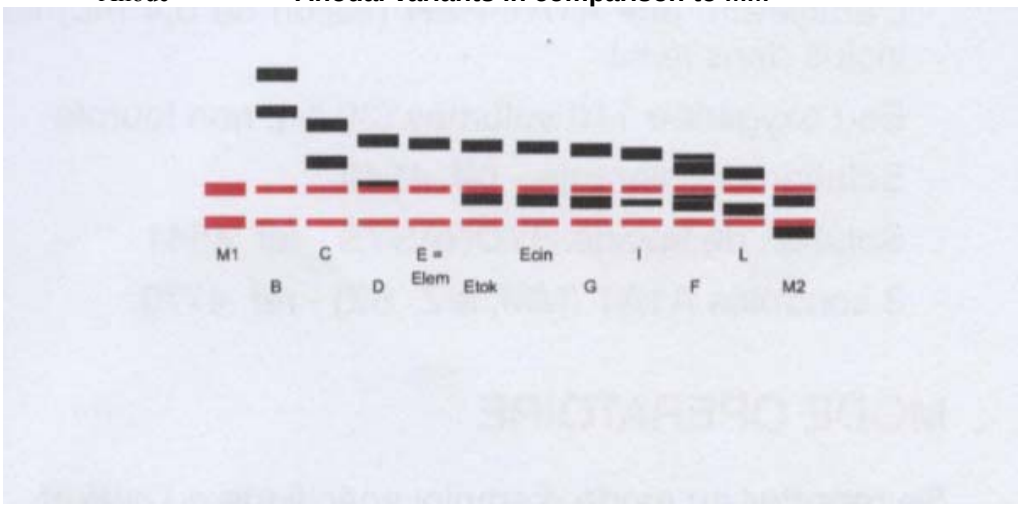
- When a less common or unusual variant is encountered, then:
  1. Consult RRL A1AT atlas collection of IEF gels representing previously identified variants. If no match found in the atlas, then
  2. Send 1 mL serum aliquot to Mayo Medical Clinic Laboratories for *"A1AT Phenotype, Serum"* [26953]. **Request identification of variant**

Interpreting Patterns, continued

Anodal variants

\+ Anode

Anodal variants in comparison to MM



\- Cathode

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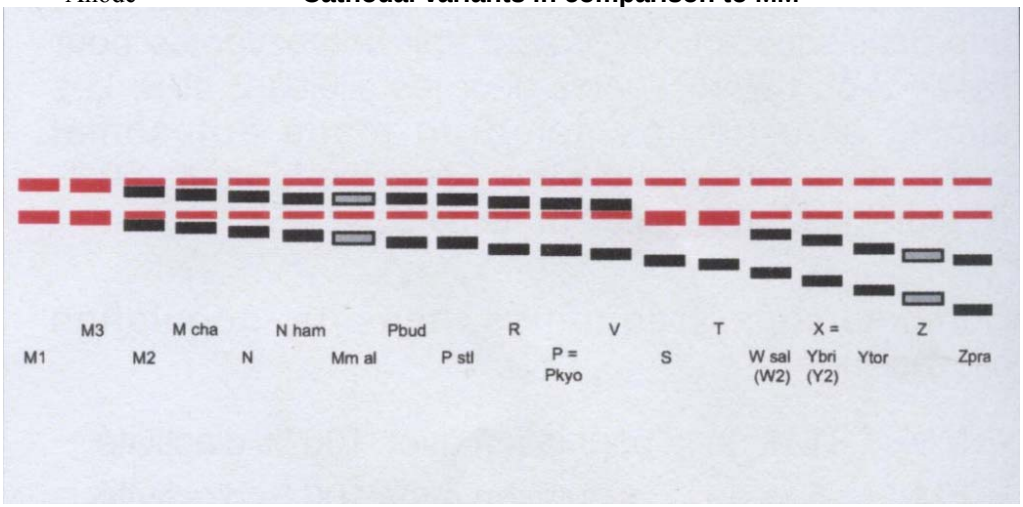
Procedure for A1AT Phenotyping – Interpreting & Reporting, *Continued*

+ Anode

Cathodal variants in comparison to MM



- Cathode

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Procedure for A1AT Phenotyping – Interpreting & Reporting, *Continued*

Reporting A1AT phenotype	**Reporting Controls**	
	1	Review the controls for acceptable isoform band separation
	2	If the controls are not acceptable, troubleshoot the assay.

Reporting Patient Results	
1	• Interpret A1AT patterns and transcribe results to the worksheet. • Set aside all gels and worksheets for review by Chemistry Services Director *Note: Consultation with Dr. Palmer-Toy is required when reviewing gels / results prior to release.*
2	• Refer to **A1AT Phenotype Interpretation Message and Text Codes Table** to enter results for Worksheet. • Enter the results into the LIS.
3	• Review all worksheet results against the entered results in the LIS prior to reporting

If	Then
• Phenotype result is **MM**, • Phenotype result is **MZ**,	• Enter **A1MM** into LMS • Enter **A1MZ** into LMS

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Procedure for A1AT Phenotyping – Interpreting & Reporting,

Continued

Phenotype LMS code	Message Code
A1MM	Pattern consistent with the **MM** phenotype. However, if the patient has a significant quantitative deficiency of AAT and the appropriate presentation, the patient may have a rare null allele or rare pathologic allele that resembles the normal M allele.
A1MS	Pattern consistent with the **MS** phenotype. The M allele is considered normal. The S allele is a mild deficiency variant associated with a less severe deficiency than the classic Z variant. **MS** heterozygotes are not likely to develop pulmonary or hepatic disease.
A1MZ	Pattern consistent with the **MZ** phenotype. The M allele is considered normal. The Z allele is a deficient variant known to be associated with liver and pulmonary disease. **MZ** heterozygotes may be at increased risk to develop pulmonary disease, particularly if they smoke.
A1SZ	Pattern consistent with the **SZ** phenotype. Both the S & Z alleles are associated with deficiency and these compound heterozygotes are at increased risk to develop liver and pulmonary disease.
A1SS	Pattern consistent with the **SS** phenotype. The S allele is associated with deficiency and homozygotes may be at increased risk to develop liver and pulmonary disease.
A1ZZ	Pattern consistent with the **ZZ** phenotype. The Z allele is responsible for most AAT deficiency. These individuals are at increased risk to develop liver and pulmonary disease.
A1IM	Pattern consistent with the **IM** phenotype. The M allele is considered normal. The I allele is a mild deficiency variant. IM heterozygotes are not likely to develop pulmonary or hepatic disease. The I allele can predispose to emphysema when paired with a Z allele.
A1MP	Pattern consistent with the **MP** phenotype. The M allele is considered normal. The P allele is heterogeneous and may represent either a normal variant or a pathologic, deficiency variant that can predispose to emphysema when paired with a Z allele. Correlation with clinical information and serum alpha-1 antitrypsin level is advised.
A1CM	Pattern consistent with the **CM** phenotype. This phenotype has no known association with clinical disease or pathology.
A1FM	Pattern consistent with the **FM** phenotype. The M allele is considered normal. The F allele is a normal to mild deficiency variant that may have decreased anti-protease activity. FM heterozygotes are not likely to develop pulmonary or hepatic disease. Although not clearly established, the F allele may predispose to emphysema when paired with a Z allele.

A1M?	Pattern consistent with one **M** allele and a variant allele other than S or Z.
A1OTH	Pattern consistent with at least one variant allele other than the most common alleles (M, S or Z).
Always message code attached to result	**MM** is the most common form of alpha-1-antitrypsin (AAT), and is not associated with any deficiency or pathology. Many other phenotypes can occur, most of which are associated with normal serum AAT levels and no pathology.

Continued on next page

Procedure for A1AT Phenotyping – Interpreting & Reporting, *Continued*

Reference Range

Phenotype MM

Controlled Document

The following controlled documents support this procedure.

Title	Number
Procedure	
Rejection/Acceptance Criteria for Samples	LQL 602
Procedure for Quality Control	LQL 605
Reagents, Standards and Controls for A1AT Phenotyping	LQL 674B

Non-Controlled Document

The following non-controlled documents support this procedure.

- Sebia Directional Insert: Hydragel 18 A1AT Isofocusing
- Sebia Directional Insert: A1AT Controls

Continued on next page

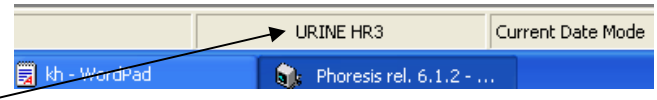
Procedure for A1AT Phenotyping – Gel Scanning

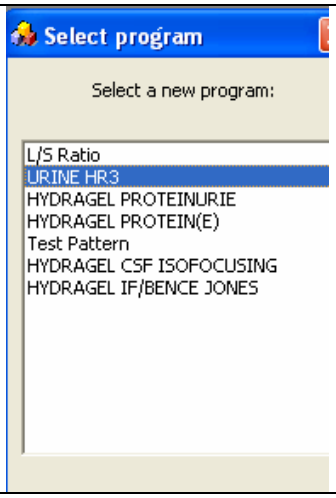
Purpose

To properly scan the A1AT phenotype gels into computer database for storage, filing and creating library references.

	Equipment	Materials
Supplies Needed	• Epson scanner • Desktop PC with Sebia Phoresis software	• A1AT Phenotype gel

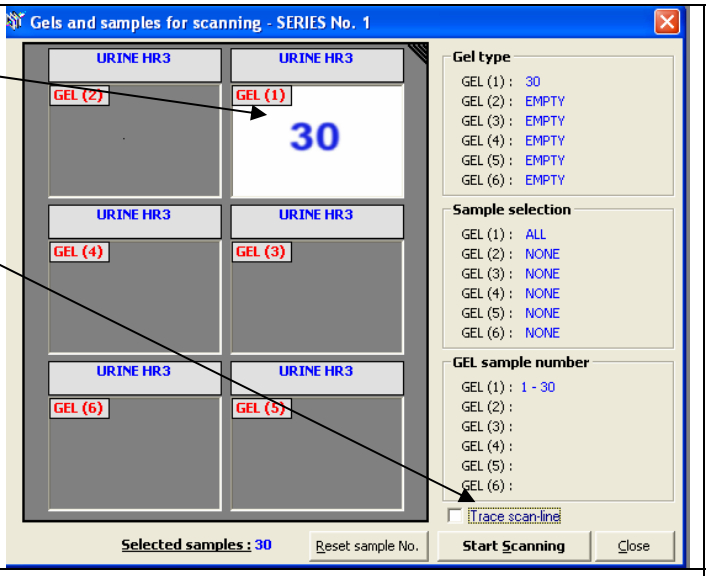
Task 1: Operating Desktop PC & Scanner

	Step	
Performed by CLS or designee	1. Turn on computer	
	2. From desktop, double click on Phoresis software icon	
	3. From **Operator ID** screen prompts, enter the following, then click **OK**	ID: **ADM** Password: **sebia**
	4. In Phoresis program, check the tool bar at the bottom of the screen to make sure the working program is **UrineHR3**	 • If working program is something other than **URINE HR3**, then double click on the program and chose the correct working program from the pull-down screen, then click **OK**.

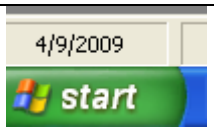
	Selecting working program, continued	

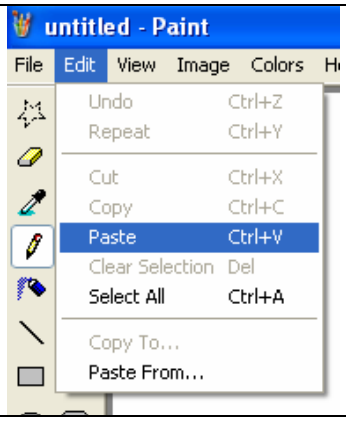
Task 2: Scanning & Filing Gels

	Step	
Performed by CLS or designee	5. Click on **Scanner** icon from top toolbar menu 6. Open scanner lid and place gel **face side down** on one of the scanner template positions	

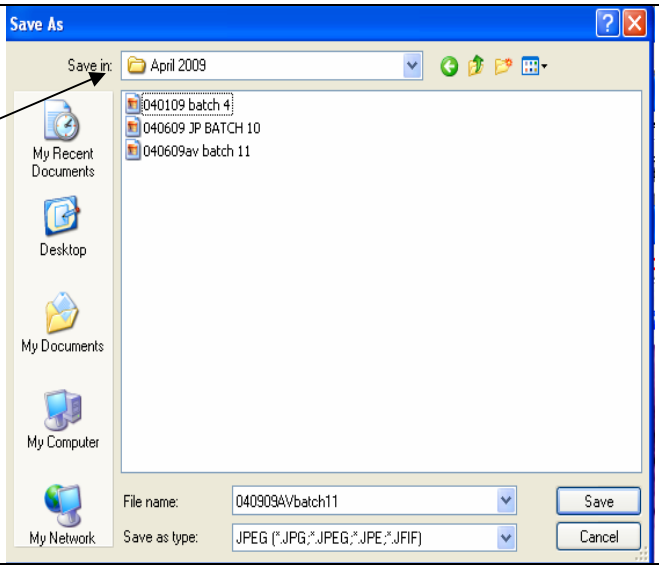
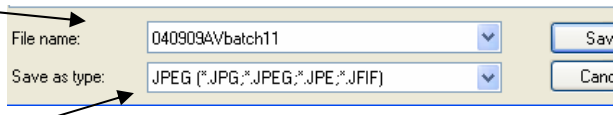
	7. Click on scanner position (Gel 1) and un-check the √ in **Trace scan-line box** 8. Then click on **Start scanning**	
	9. When scanning is complete, view the gel on the screen 10. Select keyboard buttons **Ctrl + Print Scrn** at same time	

Task 2: Scanning & Filing Gels, continued

	Step	
Performed by CLS or designee	11. Select the **Windows start** button from the bottom task bar	
	12. Select the **Paint program** from the Program menu options	

	13. From the task bar, select **Edit** & **Paste**	
	14. To save the gel image, select **File** (from the upper left task bar) then **Save As**	

Task 2: Scanning & Filing Gels, continued

	Step	
Performed by CLS or designee	15. Select the location to save the gels from the pull-down prompts: **Save In:** **A1AT Images** folder, **Year** folder **Month** folder	
	16. Name file by **date**, **initials**, **batch #** (ie, 040909AVbatch11)	

	17. **Save file type** as **JPEG f**rom the pull-down prompt	
	18. Click **Save and Exit**	