YALE-NEW HAVEN HOSPITAL	TITLE: **Protein Electrophoresis, Serum and Urine using the HYDRAGEL β1+β2 assay kit from sebia®** **Soft Code: SPEP, UPEP, TUPEP**		**DEPT OF LAB MEDICINE** **Immunology, Flow Cytometry, and Molecular Diagnostics Laboratories Policy and Procedure Manual** **DOCUMENT # IMM 206** Page 1 of 17
WRITTEN BY: Virgilio Macalalad Penny Smith	**EFFECTIVE DATE:** March 21, 2013	**REVISION:** New	**SUPERCEDES:** **Serum and Urine Protein Electrophoresis by SPIFE SPE VIS System(Doc# CC-3003)**

I. Intended Use

Protein electrophoresis is a well-established technique routinely used in clinical laboratories for screening of serum and other body fluids for protein abnormalities. The HYDRAGEL β1-β2 kits are intended for separation of proteins in human serum and urine by electrophoresis on alkaline buffered (pH 8.5) agarose gels. The kits are used in conjunction with the sebia® HYDRASYS and HYDRASYS2 electrophoresis systems and the sebia® ASSIST liquid handler.

II. Introduction

In disease states, both the total protein and the ratio of the individual protein fractions may change independently of one another. The concentration of these fractions and the electrophoretic pattern may be characteristic of diseases such as monoclonal gammopathies, A1AT deficiency disease, nephrotic syndrome, and inflammatory processes associated with infection, liver disease, and autoimmune diseases. In multiple myeloma, the increase being due to the presence of markedly elevated levels of myeloma proteins (monoclonal immunoglobulins). In nephrotic syndrome, albumin maybe lost in the urine as a result of leakage of the albumin molecules through the damage kidney.

A characteristic monoclonal band (M-spike) is often found on protein electrophoresis (PEL) in the gamma-globulin region and more rarely in the beta or alpha-2 regions. The finding of an M-spike, restricted migration, or hypogammaglobulinemic pattern is suggestive of a possible monoclonal protein The M-spike can be quantitated by protein electrophoresis and identified by additional testing such as Immunofixation electrophoresis. A qualitatively normal but elevated gamma fraction (polyclonal hypergammaglobulinemia) is consistent with infection, liver disease, or autoimmune disease, while a depressed gamma fraction (hypogammaglobulinemia) is consistent with immune deficiency and can also is associated with primary amyloidosis or nephrotic syndrome. In the hereditary deficiency of a protein (eg, agammaglobulinemia, alpha-1-antitrypsin (A1AT) deficiency, hypoalbuminemia), the affected fraction is faint or absent.

In urine, the total protein concentration, the electrophoretic pattern, and the presence of a monoclonal immunoglobulin light chain may be characteristic of monoclonal

gammopathies such as multiple myeloma, primary systemic amyloidosis, and light chain deposition disease. The presence of a monoclonal light chain M-spike may be consistent with a diagnosis of MM or macroglobulinemia. The presence of a small amount of monoclonal light chain and proteinuria which is predominantly albumin is consistent with amyloidosis (AL) or light chain deposition disease (LCDD).

III. Principle of the Assay

Electrophoresis refers to the migration of charged solutes or particles in an electric field. The normal serum proteins will separate into six major fractions albumin, alpha 1, alpha2, beta 1, beta 2 and gamma. Each zone contains one or more serum proteins. The urine protein patterns resemble those of serum. The separated proteins are stained with amidoblack. The electrophoregrams are interpreted visually for pattern abnormalities. Densitometry provides accurate, relative quantification of individual zones as well as an M-spike if present.

IV. Specimen Requirements:

Serum

The test should be performed on serum only (from red top tube). Separate serum by centrifugation, 3000 rpm for 15 minutes. Serum aliquots can be stored at 2-8°C for up to 7 days (**NOTE**: the beta-2 fraction, C3 complement, disappears after 3 days) or at below -20°C for up to one month. Do not perform the test on grossly hemolyzed (hemoglobin > 350 mg/dL) specimens.

Standard Aliquot volume = 350 uL
Minimum Aliquot volume = 100 uL
Grossly Hemolyzed: Reject if Total Protein is hemolyzed (conc >350 mg/dL)
Stability: 7 days refrigerated, 1 month at frozen (-20°C)

If necessary treat serum samples with Fluidil in the following cases:
a) Serum samples with hindered diffusion through the sample applicator teeth, for example, viscous or turbid sera after storage at 2 to 8 °C or after freezing (particularly those containing cryoglobulin or cryogel).
b) Serum samples with polymerized Ig M.
c) Serum samples giving low intensity electrophoretic pattern.
In such cases, add 25 µL Fluidil to 75 µL serum and vortex for 15 seconds. Then follow the standard procedure.

Urine

A 24 hour specimen is preferred but random urines are acceptable. Specimens should be collected without the addition of preservatives. Urine should be centrifuged at 3000 rpm for 15 minutes. Analysis is performed on samples concentrated to a total protein concentration of approximately 15 - 20 g/L using ProChem BJP20 concentrators. Use the chart below for concentration guidelines.

Total Protein (g/L)	Volume to be concentrated (mL)	Concentration
>4	N/A	Neat
2.0-4.0	6	5 X
1.0-2.0	6	10X
0.2 - 1.0	6	25X
<0.2	6	50X

Take out urine with a 9-inch glass fastener pipette into a conical vial. Note total protein and concentration factor (___X) on the worksheet.

Standard Aliquot volume = 10 mL
Minimum Aliquot volume = 3 – 6 mL (depending on protein conc.)
Stability: 7 days refrigerated, 1 month at frozen (-20°C)

V. Materials:

For optimal results, all reagents from the same kit must be always used together..

A. REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 30 ß1-ß2 KITS

For optimal results, all reagents from the same kit must be always used together

Agarose gels (ready to use)	10 gels
Buffered Strips (ready to use)	10 packs of 2 each
Staining solution diluent (stock solution)	1 vial, 60 ml
Amidoblack Stain (stock solution)	1 vial, 20 ml
Applicators (ready to use)	2 packs of 10 (15 teeth)
Filter papers	1 pack of 10

1. Agarose gels

Used as support medium for electrophoresis.

Agarose gels are ready to use. Each gel contains: agarose, 0.8 g/dL ; Tris-barbital buffer pH 8.5 ± 0.1.

Storage, stability and signs of deterioration

Store the gels horizontally in the original protective packaging at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). They are stable until the expiration date indicated on

the kit package and the gel package labels. (The arrow on the front of the kit box must be pointing upwards). Avoid storage close to a window or to a heat source. Avoid important variation of temperature during storage. DO NOT FREEZE.

Discard when:
(i) crystals or precipitate form on the gel surface or the gel texture becomes very soft (all these result from freezing the gel),
(ii) bacterial or mold growth is indicated,
(iii) abnormal quantity of liquid is present in the gel box (as a result of buffer exudation from the gel due to improper storage conditions).

2. Buffered strips
Function as electrophoresis buffer reservoir and ensure contact between the gel and electrodes.

Buffered sponge strips are ready to use. Each contains: Tris-barbital buffer pH 8.5 ± 0.3 ; sodium azide ; additives, nonhazardous at concentrations used, necessary for optimum performance.
Storage, stability and signs of deterioration
Store the buffered strips horizontally in the original protective packaging at room temperature or refrigerated. (The arrow on the front of the kit box must be pointing upwards). They are stable until the expiration date indicated on the kit package or buffered strips package label. DO NOT FREEZE. Discard buffered strips if the package is opened and the strips dry out.

3. Staining solution diluent
Use for the preparation of the amidoblack staining solution.

The stock staining solution diluent must be used as described in below under Amidoblack stain.
Storage, stability and signs of deterioration
Store the stock staining solution diluent at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or staining solution diluent vial labels. DO NOT FREEZE. Do not add any sodium azide.

4. Amidoblack stain
Use for staining gels with electrophoretic protein separations.

Preparation
The amidoblack concentrated stain is a visquous solution which may gelify. The integrity of the stock staining solution is not altered by the increase in viscosity or solidification. In all cases, to obtain a perfect reconstitution of the stain, please respect the following procedure:
1. Add 15 mL of stain diluent to the concentrated amidoblack vial.
2. Close carefully the vial.

3. Shake very vigorously the vial during approximately 5 seconds.
4. Pour this solution in the container for staining solution processing.
5. Repeat this step twice, three times if necessary.
6. Pour the remaining diluent in the container and complete the volume to 300 mL with distilled or deionized water.
7. Mix contents of stain cubitainer well for 5 to 10 minutes.
The staining solution is ready to use.
NOTE : An incomplete reconstitution of the stain will lead to an under-evaluation of albumin fraction (low percentage or white hole inside the fraction).
After dilution, the working staining solution contains: acid solution pH ≈ 2 ; amidoblack, 0.4 g/dL ; ethylene-glycol, 6.7 % ; additives, nonhazardous at concentrations used, necessary for optimum performance.
IMPORTANT : The staining solution is designed to stain only 10 gels. Change the solution after 10 staining steps.

Storage, stability and signs of deterioration
Store both stock and working staining solutions at room temperature or refrigerated in closed containers to prevent evaporation. Stock staining solution is stable until the expiration date indicated on the kit package or staining vial labels. **Working staining solution is stable for 1 month.** Its stability may be extended for 3 months if the working solution is refrigerated. The closed container must be stored refrigerated immediately after each use. Do not store the working staining solution close to a heat source.

5. Applicators
Precut, single use applicators for sample application.

Storage
Store the applicators in a dry place at room temperature or refrigerated.

6. Filter papers
Precut, single use, thin absorbent paper pads for blotting excessive moisture off the gel surface before sample application.

Storage
Store the thin filter papers in a dry place at room temperature or refrigerated.

B. REAGENTS REQUIRED BUT NOT SUPPLIED

1. Destaining solution
Use to remove excess and background stain from the gels.

Preparation
Dilute 5 mL of the stock solution to 5 liters.

Storage, stability and signs of deterioration

Store the stock destaining solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or destaining solution vial labels. **Working destaining solution is stable for one week at room temperature** in a closed bottle. Discard working destaining solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

2. HYDRASYS WASH SOLUTION

It serves for cleaning of the HYDRASYS Staining Compartment. Use periodically, e.g., if the instrument is used daily, wash the staining compartment weekly.

Preparation

Each vial of the stock HYDRASYS Wash Solution (SEBIA, PN 4541, 10 vials, 80 mL each) to be diluted up to 5 liters with distilled or deionized water. After dilution, the working wash solution contains: alkaline buffer pH 8.8 ± 0.3 ; sodium azide.

Storage, stability and signs of deterioration

Store the stock and working wash solutions in closed containers at room temperature or refrigerated. They are stable until the expiration date indicated on the wash solution vial label. Discard working wash solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

3. FLUIDIL

Use to dilute samples with hindered diffusion through the sample applicator teeth (for example, viscous or turbid samples, e.g., sera containing cryoglobulin or cryogel, samples with polymerized Ig M, ...) or giving low intensity electrophoretic pattern.

Preparation

Fluidil (SEBIA, PN 4587, 5 mL) is ready to use.

Storage, stability and signs of deterioration

Store at room temperature. It is stable until the expiration date indicated on the Fluidil vial label. Fluidil must be free of precipitate.

4. URINE CONCENTRATORS

ProChem BJP20, Cat# BJP20/100

Ready for Use, Stable until the expiration date on the box.

C. EQUIPMENT AND ACCESSORIES REQUIRED

1. Sebia Hydrasis System
2. Sebia ASSIST (refer to Doc# IMM 198)
3. Epson V700 Scanner
3. Wet Storage Chamber

4. Container Kit supplied with HYDRASYS.
5. Pipettes: 10 µL and 200 µL.

VI. Assay Procedure - Electrophoresis

The HYDRASYS system is a semi-automated multi-parameter instrument. The automated steps include processing of HYDRAGEL agarose gels in the following sequence: sample application, electrophoretic migration, drying, staining, destaining and final drying. The manual steps include handling samples and gels, and setting up the instrument for operation.

A. MIGRATION SET UP

1. Switch on HYDRASYS instrument.
2. Place two applicators for HYDRAGEL ß1-ß2 15/30 (30 samples), on a flat surface with the well numbers in the right-side-up position. Apply 10 µL of neat serum or concentrated urine sample in each well. Load each applicator within 2 minutes. Place the applicator(s) into the wet storage chamber with the teeth up. Hold it by the plastic tooth protection frame. Let the samples diffuse into the teeth for 5 minutes after the last sample application. For later use (up to 8 hours), keep the entire chamber under refrigeration.
3. Open the lid of the Migration Module and raise the electrode and applicator carriers. **WARNING: Never close the lid while the carriers are raised!**
4. Select "15/30 B1-B2" for HYDRAGEL ß1-ß2 15/30 migration program from the instrument menu (left side of the keyboard).
5. Remove buffered strips from the package; handle them by the plastic ends. Engage the punched ends of the strip's plastic backing to the pins on the electrode carrier; the strip's plastic backing must face the carrier.
6. Unpack the HYDRAGEL plate. Roll quickly and uniformly one thin filter paper onto the gel surface to absorb the excess of liquid. Remove the paper immediately. **WARNING: Do not leave the filter paper for a too long contact with the gel to avoid its dehydration.** Pool 200 µL of distilled water for HYDRAGEL ß1-ß2 15/30, on the lower third of the frame printed on the Temperature Control Plate of the migration module. Place the gel plate (the gel side up) with its edge against the stop at the bottom of the printed frame. Bend the gel and ease it down onto the water pool. Ensure that no air bubbles are trapped, water is spread underneath the entire gel plate and the gel is lined up with the printed frame.
7. Lower both carriers down. In this position the buffered strips do not touch the gel. DO NOT FORCE THE CARRIERS ALL THE WAY DOWN.
8. Remove the applicator(s) from the wet chamber. Handle it by the protection frame. Snap off the applicator teeth's protection frame. For 30 samples analysis, place the two applicators each into position No 3 and 9. **IMPORTANT:** *The numbers printed on the applicator(s) must face the operator.*
9. Close the lid of the migration module.
10. Start the procedure immediately by pressing the green arrow "START" key on the left side of the keyboard. **IMPORTANT:** *Make sure that the ventilation air inlet on the right side of the instrument is not blocked.*

B. MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS

• The two carriers are lowered so that buffered strips and applicator(s) contact the gel surface.
• Sample applicator carrier rises up.
• Migration is carried out under 20 W constant for HYDRAGEL ß1-ß2 15/30, at 20 °C controlled by Peltier effect, until 36 Vh have accumulated, for about 7 minutes.
• The electrode carrier rises to disconnect the electrodes.
• The temperature of the control plate rises to 65 °C for 10 minutes to dry the gel.
• The control plate is cooled down ; when it reaches 50 °C, an audible beep signals that the migration module lid unlocks. The plate temperature remains at 50 °C until the lid is opened. Then, the temperature keeps decreasing until it reaches 20 °C (in less than 5 minutes) after which a new migration run may start. **NOTE:** *The migration module lid remains closed during all migration steps.*

C. GEL PROCESSING SET-UP

1. Open the lid.
2. Remove the applicator(s) and discard.
3. Raise both carriers, remove the buffered strips by their plastic ends and discard.
4. Remove the dried gel film for further processing.
5. After each use, wipe the electrodes and the temperature control plate with a soft wet tissue.
6. Open the Gel Holder. Lay it flat and position the dried gel (with gel side facing up) into the grooves of the two rods and close the holder. Make sure that the film is correctly positioned inside the holder.
7. Place the gel holder into the Gel Processing / Staining Module.

IMPORTANT: *Before starting the gel processing / staining program, check the following: the staining container is filled with 300 mL of staining solution ; the destaining container contains at least 1 liter of destaining solution ; the waste container is empty.*

8. Select "PROTEIN(E)/ß1-ß2" staining program from the instrument menu and start the run by pressing the "START" key (green arrow on the right side of the keyboard). During staining, destaining and drying steps, the compartment remains locked. After cooling step, an audible beep signals that the compartment unlocks (the ventilation is maintained until the gel holder is removed).

D. GEL PROCESSING COMPLETION

1. Remove the gel holder from the compartment, open it and remove the dried gel.

NOTE : After gel staining / destaining and before densitometry / scanning, a gel may be put through an additional wash step, if needed, to further clarify the gel background and to remove any residual stain that may appear as blue spots. Wash the gel using the "WASH ISOENZ/GEL" program.

2. If needed, clean the back side (the plastic support side) of the dry film with a damp soft paper.
3. Scan using a densitometer / scanner at 570 nm or with a yellow filter.

NOTE : The lengths of electrophoretic migrations may be slightly different with gels containing 2 or 3 analysis rows, without any adverse effects on performanc

VII. Phoresis Software/Scanner

The PHORESIS Software/Scanner allows the operator to scan, view, edit, search and print patient results.

1. Open the PHORESIS software and select "Hydragel β1-β2" working program.
2. Under Worklist drop down menu, select "worklist by table".
3. Under No. column, type the number starting from one depending on the number of samples to be done.
4. Under ID column, type in or scan the specimen barcode accordingly.
5. Under Conc. Column, type in the corresponding total protein values.
6. After all the necessary data were entered, click anywhere to save.
7. Place the stained gel face down on any of the six positions on the scanner. Click on the scanner icon to start scanning.
8. After scanning is complete, close the image and the worklist table.
9. Click on the "Curve mosaic" icon to view all the electrophoregrams.
10. Click on individual electrophoregram to examine, edit and quantify.
11. Use the Normal control as the reference pattern by clicking "Save current curve as reference pattern" icon, then click Yes.
12. Make sure that every electrophoregram has six fractions (blue) and not less or more (pink).
13. If necessary, click on the "Overlay reference pattern" icon (this will bring up the reference pattern as an amber color overlay).
14. If monoclonal peak is present, quantify it by clicking on "quantify peaks" icon, (the cursor will in pencil mode). Then click and drag from the beginning of the monoclonal component to quantify. You must visually check the gel as you go along.
15. For specimens with light to moderate hemolysis, enter a comment reflecting the degree of hemolysis into the Comment field.
16. If the presence of fibrinogen is detected visually, enter "Improperly Clotted Specimen" into the comment field. Fibrinogen, a beta 2 protein, is a visible band between the beta and gamma globulins. If this band is suspected, check the aliquot tube for the presence of fibrin and note as described above.
17. After each and every electrophoregram is scrutinized, click "Save and exit" icon, then click "Print reports" icon to print all reports.

VIII. Quality Control

A. Quality Control Material

1. Normal Control Serum (4785)
2. Hypergamma Control Serum (4787)

The control serum is obtained from a pool of human sera. The serum is in a stabilized lyophilized form. It is designed for the quality control of electrophoretic quantification of human serum proteins on HYDRAGEL. It is used as a marker for

identification of the different serum protein fractions separated by electrophoretic methods. The values must fall within the mean ± 2SD confidence range provided with each batch of the control serum.

Procedure
Reconstitute the lyophilized serum with 1 ml of CLRW water. Allow to stand for 30 minutes and mix gently avoiding formation of foam. Store the lyophilized serum refrigerated (2 to 8 °C). It is stable until the expiration date indicated on the vial labels. Store the reconstituted serum at 2 – 8 °C and use within 7 days due to risk of microbial contamination and denaturation. The reconstituted serum may also be frozen in aliquots and stored at -18 to -22 °C for a maximum of 6 months. **NOTE:** *During transportation, the control serum can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.*

B. Frequency

Both Normal control serum and Hypergamma control serum must be run with **each applicator used.**

C. New lots of Quality Control

1. New lots of control material are pretested until at least 30 data points are collected to determine an in-house control range of +/- 3 standard deviations.
2. If a new lot of control is put into use before 30 points are collected the manufacturer's range will be used until 30 data points are collected.

D. Test Pattern (Densitometry control)

The Test Pattern allows scanner verification in absorbance mode the performance, such as resolution, sensitivity, linearity and accuracy of the densitometer.

The Test Pattern consists of a film with 7 tracks:

- Tracks 1, 2, 3 and 4: resolution and sensitivity test

- Tracks 5, 6, and 7: linearity and accuracy test

Frequency

The Test Pattern should be used once a week to check the densitometer.

Expected Results

Tracks 1, 2, 3, 4

Print the results after scan and measure the distance between the base line and the bottom of the valley between any two peaks.

Track 1	< 5 mm
Track 2	< 5 mm
Track 3	< 5 mm
Track 4	< 5 mm

Tracks 5, 6, 7

Track 5	**% Min**	**% Max**
Band 1	50	58
Band 2	27.5	32.5
Band 3	14	18.5
Track 6	**% Min**	**% Max**
Band 1	55	60
Band 2	27	31
Band 3	12.5	16
Track 7	**% Min**	**% Max**
Band 1	31.5	34.5
Band 2	31.5	34.5
Band 3	31.5	34.5

NOTE: Track 5 is not applicable.

IX. Interpretation of Results

All fractions will be entered and verified in SoftLab by the performing technologist. The Beta 1 and Beta 2 fractions will be added together and reported as total Beta.

Interpretation will be performed by an attending pathologist who will release the results in SoftFlow.

Serum

Fractions and M-components will be reported ing/dL.

Random Urine

Fractions will be reported in g/L but M-components will be reported as percentages.

Timed Urine

Fractions and M-components will be reported as g/TV.

X. Reference Range

Serum Reference Ranges:

Albumin	3.5 – 4.7 g/dL
Alpha-1	0.1 – 0.3 g/dL
Alpha-2	0.6 – 1.0 g/dL
Beta	0.7 – 1.2 g/dL
Gamma	0.7 – 1.5 g/dL

Urine: Reference Range has not been established

XI. Interference and Limitations

• Do not use hemolysed serum samples. Hemolysis increases alpha-2 and beta-zones.
• Avoid plasma samples or improperly clotted serum samples. Fibrinogen, a beta 2 protein, is found in normal plasma but also absent from normal serum. Occasionally, blood drawn from heparinized patients does not fully clot, resulting in a visible fibrinogen band between the beta and gamma globulins.
• Avoid aged, improperly stored urine samples where enzymatic degradation of proteins might occur.
• Some sera have different phenotypes (Haptoglobin, GC Globulin).
• Alpha-1 lipoprotein depends on the concentration and storage of the sample.
Some serum samples may show a small, fairly sharp band corresponding to a component of C3 complement that migrates cathodic to the Beta-2 zone. See MIGRATION PATTERN.

The following comments concern samples stored at 2 to 8 °C or frozen.
In serum, the β2-fraction (C3 complement) disappears after 3 days. B-lipoproteins in fresh serum co-migrate with transferrin in β1-zone; upon freezing of long storage under refrigeration, β-lipoproteins shift anodally to β1/α2-interzone; the older the older the serum, the greater the shift. Similarly, α-lipoproteins move anodally from α2-zone.

XII. YNHH Method Validation Summary

Method Correlation:

Method correlation was performed by comparing results from the Helena SPIFE® assay to the **sebia®** HYDRAGEL □ı□□assay. Serum from 74 patients and urine from 45 patients were used for comparison. Data on each fraction and measurement of monoclonal components were analyzed using EP Evaluator. For comparison the sebia® □ı□□fractions were added together to represent the total Beta fraction. Because the assay may be run on either the Hydrasys or Hydrasys 2 instrument, an instrument

comparison was also performed. Regular regression analysis was performed using the EP Evaluator program. Percent Bias and Total Error were calculated (see below) and acceptability was determined by a Total Error of less than 30%.

SERUM: All fractions had a Total Allowable Error of <30%.

Fraction	Slope	Intercept	Std Err Est	R value	Bias	Y Mean	%Bias	Inter-run precision %CV	% Total Allowable Error	Acceptability <30% Total Error
Albumin	0.973	0.073	0.144	0.9599	-0.025	3.586	0.697	1.200	3.10	Pass
Alpha 1	0.623	0.114	0.033	0.8274	0.032	0.25	12.800	4.700	22.20	Pass
Alpha 2	0.674	0.263	0.064	0.8654	-0.031	0.872	3.555	4.350	12.26	Pass
Beta	0.507	0.463	0.085	0.6987	0.052	0.885	5.876	2.000	9.88	Pass
Gamma	0.934	0.05	0.114	0.9852	-0.027	1.149	2.350	5.100	12.55	Pass
M-component	0.947	0.031	0.097	0.9925	-0.024	1.025	2.341	1.900	6.14	Pass

%Bias = [Bias] / Y Mean X 100

% Total Error = 2X%CV (Inter-run Precision) + %Bias

URINE:

All fractions had a Total Allowable Error of <30% except Alpha 1 and the M-component quantification.

Fraction	Slope	Intercept	Std Err Est	R value	Bias	Y Mean	%Bias	Inter-run precision %CV	% Total Error	Accebtability <30% Total Error
Albumin	0.86	0.037	0.083	0.9823	0.005	0.228	2.193	1.200	4.59	Pass
Alpha 1	1.004	0.009	0.025	0.8301	0.009	0.037	24.324	4.700	33.72	Not clinically significant
Alpha 2	0.874	0.014	0.045	0.8609	0.006	0.068	8.824	4.350	17.52	Pass

Beta	0.978	0.008	0.03	0.9628	0.007	0.08	8.750	2.000	12.75	Pass
Gamma	0.862	0.022	0.067	0.9916	-0.013	0.238	5.462	5.100	15.66	Pass
M-component	0.712	-0.046	0.1255	0.8067	-0.2127	0.3653	58.226	1.900	62.03	See explanation below

Both the Total Error on the Alpha 1 fraction and the M-component quantification were >30%. For Alpha 1, the laboratory director has determined that the Total Error of 33.72% is not a clinically significant difference. Regarding the M-component measurement, the laboratory director has determined that the Total Error of 62.03% is due to the difference in methodology. To correct for this difference, urines on patients with previously quantified M-components will be run concurrently on both systems. Re-baselining will take place for approximately 6 months. The SEBIA result will be reported with an interpretation that takes into account the Helena result.

Instrument Correlation: Serum Hydrasys vs Hydrasys 2:

Fraction	Slope	Intercept	Std Err Est	R value	Bias	Y Mean	%Bias	Inter-run precision %CV	% Total Error	Accebtability <30% Total Error
Albumin	0.982	0.044	0.079	0.9885	-0.025	3.708	0.674	1.200	3.07	Pass
Alpha 1	0.922	0.01	0.013	0.9556	-0.008	0.224	14.286	4.700	23.69	Pass
Alpha 2	1	0.011	0.039	0.9408	0.011	0.859	3.609	4.350	12.31	Pass
Beta	1.026	-0.023	0.049	0.9841	0.002	0.98	5.306	2.000	9.31	Pass
Gamma	1.008	0.01	0.072	0.9977	0.019	1.138	2.373	5.100	12.57	Pass
M-component	1.008	0.04	0.36	0.9992	0.12	10.1	0.238	1.900	4.04	Pass

Precision:

Intrarun Precision: Intra-assay performance was evaluated on both the Hydrasys and the Hydrasys 2 instruments by testing Normal and Hypergamma controls 15 times each on a single run. The CV's were calculated for each fraction. The limit of acceptability is CV <5%.

Hydrasys

Analyte	Sample	N	Mean	SD	CV
Abnorm Alb	Precision	15 of 15	60.27	0.56	0.9%
Abnorm Alpha 1	Precision	15 of 15	3.13	0.10	3.3%
Abnorm Alpha 2	Precision	15 of 15	12.21	0.28	2.3%
Abnorm Beta	Precision	15 of 15	12.08	0.24	2.0%
Abnorm Gamma	Precision	15 of 15	12.32	0.24	1.9%
Normal Alb	Precision	15 of 15	63.33	0.57	0.9%
Normal Alpha 1	Precision	15 of 15	3.65	0.15	4.1%
Normal Alpha 2	Precision	15 of 15	12.34	0.28	2.3%
Normal Beta	Precision	15 of 15	13.83	0.16	1.1%
Normal Gamma	Precision	15 of 15	6.85	0.20	3.0%

Hydrasys 2

Analyte	Sample	N	Mean	SD	CV
Hypergam Albumin	Precision	15 of 15	48.480	0.361	0.7%
Hypergam Alpha 1	Precision	15 of 15	2.473	0.122	4.9%
Hypergam Alpha 2	Precision	15 of 15	8.313	0.192	2.3%
Hypergam Beta	Precision	15 of 15	9.853	0.196	2.0%
Hypergam Gamma	Precision	15 of 15	30.880	0.418	1.4%
Normal Albumin	Precision	15 of 15	59.580	0.505	0.8%
Normal Alpha 1	Precision	15 of 15	3.260	0.150	4.6%
Normal Alpha 2	Precision	15 of 15	10.787	0.203	1.9%
Normal Beta	Precision	15 of 15	13.820	0.278	2.0%
Normal Gamma	Precision	15 of 15	12.553	0.416	3.3%

Interrun Precision: Inter-assay performance was evaluated on both the Hydrasys and the Hydrasys 2 instruments by testing the Normal and Hypergamma controls on 4 different runs. The CV's were calculated for each fraction. The limit of acceptability is CV <10%. The Alpha 1 fraction on the abnormal sample had a CV of 14.0% which can be attributed to its low percentage value (mean 0.130).

Hydrasys

Analyte	Sample	N	Mean	SD	CV
ALB	Hypergam	4 of 4	2.250	0.045	2.0%
ALB	Normal	4 of 4	3.717	0.050	1.3%
Alpha 1	Hypergam	4 of 4	0.130	0.018	14.0%
Alpha 1	Normal	4 of 4	0.185	0.017	9.4%
Alpha 2	Hypergam	4 of 4	0.402	0.022	5.5%
Alpha 2	Normal	4 of 4	0.675	0.024	3.5%
Beta	Hypergam	4 of 4	0.515	0.040	7.8%
Beta	Normal	4 of 4	0.888	0.030	3.4%
Gamma	Hypergam	4 of 4	1.400	0.055	3.9%
Gamma	Normal	4 of 4	0.835	0.021	2.5%

Hydrasys 2

Analyte	Sample	N	Mean	SD	CV
ALB	Hypergamma	4 of 4	2.205	0.070	3.2%
ALB	Normal	4 of 4	3.645	0.039	1.1%
ALPHA 1	Hypergamma	4 of 4	0.143	0.013	8.8%
ALPHA 1	Normal	4 of 4	0.20	0.00	0.0%
ALPHA 2	Hypergamma	4 of 4	0.422	0.022	5.2%
ALPHA 2	Normal	4 of 4	0.700	0.012	1.6%
BETA	Hypergamma	4 of 4	0.510	0.018	3.6%
BETA	Normal	4 of 4	0.865	0.017	2.0%
GAMMA	Hypergamma	4 of 4	1.417	0.022	1.6%
GAMMA	Normal	4 of 4	0.892	0.046	5.1%

Reference Range Verification

Reference ranges for serum were established for each fraction by performing a reference range study using serum from 129 new YNHH employees. There is no current reference range established for urine.

Serum Reference Ranges:

Albumin	3.5 – 4.7 g/dL
Alpha-1	0.1 – 0.3 g/dL
Alpha-2	0.6 – 1.0 g/dL
Beta	0.7 – 1.2 g/dL
Gamma	0.7 – 1.5 g/dL

Carryover:

Carryover on the sebia® Assist automated pipetting system was evaluated using the EP Evaluator protocol for carryover testing. The Gamma fraction was used for quantification. The carryover test passed.

	Mean	SD
Low-Low	6.80	0.19
High-Low	6.88	0.25

Limit (Low-Low SD X3) = 0.57

Carryover (High-Low mean - Low-Low Mean) = 0.08

Interfering Substance:

Assay performance was evaluated with respect to interference due to hemolysis. No interference with the pattern of protein distribution on the gel was identified with the exception of the known detection of haptoglobin-hemoglobin complex migration in the alpha2-beta interzone. However, due to interference of hemolysis in the chemical assay for total protein, which invalidates the numerical result, quantification of protein fractions in a grossly hemolyzed specimen is precluded. Therefore, protein electrophoresis will not be performed on samples with hemoglobin concentrations greater than 350 mg/dL. In addition, specimens with elevated levels of C-reactive protein (CRP), complement and fibrinogen were analyzed to access possible interference with interpretation of gel patterns. Fibrinogen migrated in the beta-gamma interzone and poorly clotted specimens will be interpreted with a comment recommending resubmission of a repeat specimen. CRP and complement were not detected as independent bands.

XIII. References:

1. HYDRAGEL 30 ß1-ß2 - 2012/03[package insert]. Sebia –USA 2012/03 edition

XIV. Appendix

206	Protein Electrophoresis, Serum and Urine using the HYDRAGEL β1+β2 assay kit from sebia®
206-A	Protein Electrophoresis, Serum and Urine using the HYDRAGEL β1+β2 assay kit from sebia® Check List
206-B	Quick Reference Guide Protein Electrophoresis, Serum and Urine using the HYDRAGEL β1+β2 assay kit from sebia®

Document Author
Virgilio Macalalad
Penny Smith
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Signature Approval
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Document #: IMM 206

Name (Print)	Title	Signature	Date of Review	Revision Page and Section # (Use Procedure Review Log to document staff review)	Issue Date for Training if Applicable	Effective Date for Use
TEODORICO LEE	LAB MANAGER	Teodorico Lee	3/27/2013	NEW		3/21/13
JOHN MCCLASKEY	LAB DIRECTOR		3/21/2013	NEW		3/21/13
BRIAN SMITH	LAB DIRECTOR		3/21/13	New		3/21/13