HYDRAGEL HR K20
Ref. 3001
INTENDED USE

The HYDRAGEL HR K20 kit is designed for multi-fractionation of proteins from human sera or other biological fluids, such as urine and cerebrospinal fluid (CSF), by electrophoresis on alkaline buffered (pH 8.6) agarose gels. The separated proteins are stained with acid violet solution (qualitative analysis of serum, urine or CSF, or quantitative analysis of serum) or with amidoblack solution (quantitative analysis of serum). The excess of stain is removed with an acidic solution. The electrophoretic separations are evaluated visually for protein pattern abnormalities. Densitometry provides accurate relative quantification of individual zones.

Each agarose gel is intended to run 7 samples in the HYDRAGEL HR K20 kit.

For In Vitro Diagnostic Use.

PRINCIPLE OF THE TEST

Protein electrophoresis is a well established technique routinely used in clinical laboratories for screening of serum and other biological fluids for protein abnormalities.

It is based on the principle of zone electrophoresis performed on a suitable support medium. Agarose has been developed into a versatile and effective support medium. Although most clinical laboratories are satisfied with the "traditional" five-zone pattern of serum proteins, high resolution techniques can yield additional diagnostic information.

With HYDRAGEL HR K20 procedure, serum, urine or cerebrospinal fluid proteins separate into about ten fractions. Each fraction contains one or more proteins.

The composition of the gel, the electrophoretic conditions and the choice of the stain (acid violet or amidoblack) allow an excellent resolution and a high sensitivity particularly in the gamma-zone.

The stained separations are evaluated visually for pattern abnormalities. The visual observations can be complemented by densitometry to obtain semi-quantitative, relative values of the individual or combined protein fractions.

REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL HR K20 KIT

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN 3001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose Gels (ready to use)</td>
<td>10 gels</td>
</tr>
<tr>
<td>Tris-Barbital Buffer (stock solution)</td>
<td>3 vials, 100 mL each</td>
</tr>
<tr>
<td>Acid Violet Stain (stock solution)</td>
<td>1 vial, 75 mL</td>
</tr>
<tr>
<td>Destaining Solution (stock solution)</td>
<td>1 vial, 100 mL</td>
</tr>
<tr>
<td>Applicators 7 teeth (ready to use)</td>
<td>1 pack of 10</td>
</tr>
<tr>
<td>Filter Papers - Thin</td>
<td>1 pack of 10</td>
</tr>
</tbody>
</table>

FOR OPTIMAL RESULTS

All reagents from the same kit must be always used together and according to the package insert instructions.

PLEASE READ THE PACKAGE INSERT CAREFULLY.

1. AGAROSE GELS

Preparation

Agarose gels are ready to use. Each gel contains: agarose, 0.8 g/dL ; Tris-barbital buffer pH 8.6 ± 0.1 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: Agarose gels contain 0.18 % barbital. Do not ingest! If ingested, consult physician immediately!

Use

Support medium for protein electrophoresis from serum, CSF or urine samples.

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 or gel package label. (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. TRIS-BARBITAL BUFFER

Preparation

Each vial of the stock buffer solution to be diluted up to 1 liter with distilled or deionized water. After dilution, the working solution contains: Tris-barbital pH 8.5 ± 0.3 ; sodium azide ; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: Each vial of the stock buffer contains 0.92 % barbital, 5.15 % sodium barbital and 0.10 % sodium azide. Do not ingest! Very toxic if swallowed. If ingested, consult physician immediately! Prevent contact with acids, lead or copper, as these are known to form explosive or toxic compounds with sodium azide. Always flush with a large quantity of water when disposing. After contact with skin, wash immediately with plenty of water.

Use

Electrophoresis buffer.

Storage, stability and signs of deterioration

Store stock buffer solution at room temperature or refrigerated. Stock solution is stable for several years, at least until the expiration date indicated on the kit package or buffer vial labels. Diluted buffer solution is stable for one year at room temperature in a closed bottle. Discard diluted buffer if it changes its appearance, e.g., becomes cloudy due to microbial contamination.
3. ACID VIOLET STAIN
Preparation
The vial of the stock acid violet stain to be diluted up to 300 mL with distilled or deionized water.
After dilution, the working stain solution contains: acid solution pH ≈ 2 ; acid violet, 0.2 g/dL ; ethylene-glycol, 3.25 % ; additives, nonhazardous at concentrations used, necessary for optimum performance.
WARNING: Harmful if swallowed.

Use
For staining gels with electrophoretic protein separations.

Storage, stability and signs of deterioration
Store both stock and working stain solutions at room temperature or refrigerated in closed containers to prevent evaporation. Stock stain solution is stable until the expiration date indicated on the kit package or stain vial labels. Working stain solution is stable for 6 months.

4. DESTAINING SOLUTION
Preparation
The vial of stock destaining solution to be diluted up to 100 liters with distilled or deionized water. It is convenient to dilute only 1 mL of the stock solution to 1 liter. After dilution, the working destaining solution contains: citric acid, 0.05 g/dL.

Use
For destaining, that is removal of excess and background stain from the gels.

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.

Do not add any sodium azide.

To prevent microbial proliferation in the diluted destaining solution to be stored more than one week, add 5 µL/dL of ProClin 300.

Working destaining solution added with ProClin is stable in a closed bottle at room temperature or refrigerated until the expiration date indicated on the kit package or destaining solution vial labels.

5. APPLICATORS
Use
Precut, single use applicators for sample application.

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

6. FILTER PAPERS - THIN
Use
Single use absorbent 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.

REAGENTS REQUIRED BUT NOT SUPPLIED

1. SALINE
Preparation
Make 0.15 M (0.9 g/dL) NaCl solution in distilled or deionized water.

Use
To dilute samples.

Storage, stability and signs of deterioration
Store at room temperature or refrigerated. Discard after 3 months or if it changes its appearance, e.g., becomes cloudy due to microbial contamination. For longer storage periods, add sodium azide, 0.1 g/dL.

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

Use
To dilute viscous or turbid samples, e.g., sera containing cryoglobulin or cryogel.

Storage, stability and signs of deterioration
Store at room temperature or refrigerated. It is stable until the expiration date indicated on the Fluidil vial label. Fluidil must be free of precipitate.

3. FIXATIVE SOLUTION (optional)
Preparation
At least 15 minutes before use, prepare a solution containing (vol. / vol.): 60 % ethanol ; 10 % acetic acid and 30 % distilled or deionized water. Mix well.

Use
To fix electrophoretic protein separations in agarose gel plates.

Storage, stability and signs of deterioration
Store fixative solution at room temperature tightly capped to prevent evaporation. Discard after 3 months. Do not fix more than 4 gels in each 150 mL of fixative solution.
4. AMIDOBLEACK STAIN (SEBIA, PN 4554) (optional)

Preparation
The amidoblack concentrated stain is a visqueous 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, we advise you to 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.

WARNING: Harmful if swallowed.

Use
For staining gels after serum proteins electrophoresis.

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.

Store the stock staining solution diluent at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or destaining solution vial labels. DO NOT FREEZE.

EQUIPMENT AND ACCESSORIES REQUIRED BUT NOT SUPPLIED
1. Power supply: GD 61 D SEBIA, PN 1300 ; GD 251 D SEBIA, PN 1301 ; MG 300 SEBIA, PN 1302 or MG 500 SEBIA, PN 1303.
2. HYDRAGEL K20 APPLICATOR SEBIA, PN 1409, containing the HYDRAGEL K20 applicator carrier.
3. Wet Storage Chamber, PN 1270.
4. Electrophoresis chamber K20 SEBIA, PN 1400.
5. Tanks and Gel Holders for processing of gel plates: HYDRAGEL K20 Accessory Kit SEBIA, PN 1420.
6. Pipettes: 10 µL and 200 µL.
7. Incubator-Dryer: IS 80 SEBIA, PN 1430.
8. Densitometer / flat-bed scanner able to scan 82 x 51 mm gel plates at 570 nm (yellow filter) : HYRYS SEBIA, DVSE SEBIA or PHORESIS software for flat-bed scanner. Refer to manufacturer’s instructions for operation and calibration procedures.
9. Control materials, such as Control Serum, SEBIA PN 4785.

SAMPLES FOR ANALYSIS
Samples for qualitative analysis with HYDRAGEL HR K20 procedure may be serum, urine or cerebrospinal fluid (CSF).

Serum proteins may also be quantified after staining with acid violet or amidoblack stain.

Sample collection and storage
Fresh samples are recommended for analysis. They must be collected according to established procedures used in clinical laboratory testing. If needed, store samples at 2 to 8 °C for up to one week. Frozen samples are stable for at least one month. Freezing serum and CSF samples with sodium azide, 0.02 g/dL improves the storage stability. Freezing urine samples with HEPES 0.1 M (pH 6.75) and sodium azide, 0.02 g/dL improves the storage stability.

Thawed samples may give slight application marks due to protein or lipoprotein denaturation.

CAUTION: Do not use boric acid as a preservative.

Sample preparation for qualitative analysis (with acid violet staining)

Serum
Apply undiluted serum samples. Upon storage at 2 to 8 °C or after freezing, some sera (particularly those containing cryoglobulin or cryogel) may become viscous or develop turbidity. Such sera might present application problems due to hindered diffusion through the sample applicator teeth. In such case, add 25 µL Fluidi to 75 µL serum and vortex for 15 seconds. Then follow the standard procedure.

CSF or serum / CSF pairs
Apply samples with a total protein concentration of about 0.6 to 1.0 g/dL.

IMPORTANT: Serum and CSF sample pairs must be adjusted to have identical total protein concentration.

Unconcentrated urines
Analysis is performed on unconcentrated urine. Concentrate urine if higher sensitivity is needed.

NOTE: Diffusion of urine samples into the applicator tips may be hindered when the urine (neat or concentrated) is turbid. It is recommended to remove the particulates by centrifugation (e.g., 10 minutes at 3,000 rpm) or filtration (e.g., 0.45 µm syringe filter).

IMPORTANT: Some urines contain a high salt concentration. This may cause gel deformation during migration and consequently, distortion of the migration profiles. If such a distortion makes interpretation inaccurate, the urine should be dialyzed to remove the salts.
Serum preparation for semi-quantitative analysis (with amidoblack or acid violet staining)

**Amidoblack staining**
Use undiluted serum samples.

**Acid violet staining**
Use serum samples previously diluted 4 times (1 vol. / 3 vol.) in saline. Then follow the standard procedure.

Sample to avoid
Do not use hemolyzed serum samples. Hemolysis increases alpha-2 and beta zones.
Avoid plasma samples. Fibrinogen gives a band close to the application point which might be mistaken for a monoclonal immunoglobulin.

**PROCEDURE**

I. MIGRATION STEP

1. Place the HYDRAGEL K20 applicator carrier on a flat surface (Fig. 1) and raise the part of the applicator carrier with the numbered notches.
2. Pool 120 µL distilled or deionized water on the lower third of the frame printed on the HYDRAGEL K20 applicator carrier.
3. Unpack the HYDRAGEL agarose gel plate.
4. 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.
5. Place the gel plate (the gel side up) with its edge against the stop at the bottom of the printed frame (Fig. 2).
6. Bend the gel and lower it down onto the water pool (Fig. 2). 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 the applicator carrier with the numbered notches down to the intermediate position with the switch in high position.
8. Place one applicator on a flat surface with the well numbers in the right-side-up position (Fig. 3).
9. Apply 10 µL of sample into the applicator wells. Load the applicator within 2 minutes.
   - Use the applicator without any delay.
   - For later use (up to 8 hours), place the applicator into the wet storage chamber with the teeth up [handle it by the plastic tooth protection frame], keep the entire chamber under refrigeration and set-up the gel plate onto the HYDRAGEL K20 applicator carrier just before use. See wet chamber package insert for further details.
10. Snap off the applicator teeth’s protection frame.
11. Place the sample applicator into position No. 5 on the applicator carrier.
   **IMPORTANT:** The numbers printed on the sample applicator must face the operator (Fig. 4).
12. Lower the applicator carrier with the switch so that the applicator contacts the gel surface for the time specified below. **DO NOT FORCE THE CARRIERS ALL THE WAY DOWN.**
   - Serum analysis: Let the sample applicator in contact with the gel for 30 seconds.
   - CSF / serum pairs analysis and concentrated urines: Let the sample applicator in contact with the gel for 2 minutes.
   - Unconcentrated urines: Let the sample applicator in contact with the gel for 7 minutes and 30 seconds.
13. After sample application, turn the switch to rise up the applicator, remove the applicator and discard.
14. Put the gel into an appropriate electrophoresis chamber, according to the polarity indicated on the gel, the lower side of the gel on the cathodic side. When using SEBIA K20 chamber, place the HYDRAGEL on the bridge with the gel side facing down; the gel should dip about 1 cm into the buffer on each side.
   **See K20 chamber package insert for further details.**
15. Plug the chamber to the power supply.

| SEBIA K20 |
|-------------------|-------------------|
| **MIGRATION CONDITIONS** | **SERUM AND CSF/URINE** |
| Volume of buffer per compartment | 150 mL | 150 mL |
| Total buffer volume | 300 mL | 300 mL |
| Migration time | 40 minutes | 30 minutes |
| Constant voltage | 80 V | 90 V |
| Initial current (per gel) | 9 ± 1 mA | 9 ± 1 mA |

16. After migration, unplug the chamber and remove the gel plate.

II. FIXATION

Process gels according to one of the following procedures.

**Hot air fixation (recommended only with SEBIA IS 80 Incubator-Dryer):**
Dry the gel completely in the incubator-dryer at 80 °C (for 10 minutes minimum).

**Fixation with fixative solution:**
1. Place the gel into a gel holder (supplied with SEBIA HYDRAGEL K20 Accessory Kit) for further processing.
2. Fill one tank (supplied with SEBIA HYDRAGEL K20 Accessory Kit) with 150 mL of fixative solution.
3. Immerse the gel in the fixative solution for 15 minutes.
4. Remove the gel and dry it with hot 80 °C air flow.
   **IMPORTANT:** The gel must be perfectly dry.
III. STAINING - DESTAINING
1. Immerse the dried and cooled gel in the staining solution (acid violet or amidoblack solution) for 10 minutes.
2. Destain in three successive baths of destaining solution until the background is completely colorless and clear.
3. Soak up excess liquid on the gel surface with a tissue paper and dry the gel with hot 80 °C air. If needed, clean the back side (the plastic support side) of the dry film with a wet tissue paper.

IV. INTERPRETATION - SCANNING
Interpret the separations visually for pattern abnormalities. The visual observations may be completed by densitometry at 570 nm or with a yellow filter to obtain semi-quantitative, relative values for the individual or combined protein fractions.

RESULTS

Quality Control
It is advised to include a normal sample or control serum (Control serum, SEBIA, PN 4785) into each run of samples.

Values (quantitative analysis)
Densitometer scanning (at 570 nm) of stained electrophoregrams yields relative concentrations (percentages) of individual protein zones.

Normal values (mean ± 2 SD) for individual major electrophoretic serum protein zones using HYDRAGEL HR K20 procedure have been established from a healthy population of 225 adults (men and women):

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>HYRYS</th>
<th>PHORESIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>56.2 - 69.0</td>
<td>57.5 - 67.9</td>
</tr>
<tr>
<td>Alpha-1 globulins</td>
<td>1.2 - 3.2</td>
<td>1.0 - 3.8</td>
</tr>
<tr>
<td>Alpha-2 globulins</td>
<td>7.8 - 13.4</td>
<td>7.2 - 12.8</td>
</tr>
<tr>
<td>Beta globulins</td>
<td>7.4 - 13.4</td>
<td>7.6 - 13.6</td>
</tr>
<tr>
<td>Gamma globulins</td>
<td>9.8 - 18.6</td>
<td>10.3 - 18.3</td>
</tr>
</tbody>
</table>

It is recommended each laboratory establishes its own normal values.

Interpretation
Interpretation is qualitative. As an aid in interpretation see BIBLIOGRAPHY.

SERUM PROTEINS
The interpretation is made by comparing the electrophoregrams of the clinical sample and a normal control. In the case of an increased, decreased or additional fraction, it is usually necessary to confirm the observed changes by other tests such as quantification of specific proteins, immunoelectrophoresis, or immunofixation. Among the very numerous plasma proteins, only about fifteen exist in a sufficient quantity to contribute to the staining intensity of the bands observed on HYDRAGEL 7 HR gels. The value of HYDRAGEL HR K20 procedure is primarily in the detection of faint monoclonal or oligoclonal bands.

CEREBROSPINAL FLUID PROTEINS
The cerebrospinal fluid (CSF) is a biological liquid with a low protein concentration; in a normal CSF, it is about 30 ± 15 mg/dL. The majority of CSF proteins originate in serum from where they are filtered and transported through the blood - CSF barrier. For analysis using HYDRAGEL HR K20 procedure, the CSF must be concentrated up to about 0.6 to 1.0 g/dL.

A normal CSF pattern shows the following order of zones:
- Prealbumin, or transthyretin, the fastest fraction;
- Albumin, the prevalent fraction representing about 75 % of the total proteins;
- Alpha-1 zone, consisting primarily to alpha-1 antitrypsin;
- Alpha-2 zone, contains primarily high molecular weight proteins; it is usually very weak since such proteins cannot pass through the blood - CSF barrier;
- Beta fraction, containing transferrin;
- Beta-2 zone, containing primarily carbohydrate (sialic acid) deficient "CSF specific" transferrin also called the Tau protein;
- Gamma zone, contains essentially Ig G, and sometimes Ig A and Ig M.

An increase of immunoglobulins synthesized within the central nervous system (intrathecal synthesis of Ig's) has been reported in association with various disorders of the central nervous system including demyelinating, inflammatory and infectious disorders and auto-immune reactions. The intrathecal Ig CSF synthesis is often found associated with reduced heterogeneity of the Ig's which manifests itself as "oligoclonal banding" discerned in high resolution electrophoretic patterns.

A distinct characteristic of intrathecal Ig CSF synthesis is the presence of oligoclonal Ig bands in CSF and absence of such banding in the serum.

Therefore, it is necessary:
- To analyze the CSF & serum pairs collected from the same patient at the same time while excluding any treatment which could affect the sample's concentration of immunoglobulins.
- To apply identical quantities of proteins (about 1 g/dL) with each of the 2 samples.
- To subject any abnormal band detected in the gamma zone of CSF, without any corresponding fraction in the serum, to immunofixation analysis. The immunological confirmation of the Ig character of such CSF band is necessary since bands other than immunoglobulins may be found in the gamma zone (e.g., tau fraction, carbonic anhydrase, post gamma, or CRP). The latter bands do not have the same diagnostic significance as the "true", immunoglobulin type oligoclonal bands.
URINE
Urine electrophoresis is performed in order to detect monoclonal components and other urinary proteins. HYDRAGEL HR K20 procedure is used as a screening test for urinary protein. It allows the detection of monoclonal components (which must be confirmed and identified by immunofixation), and the detection of proteins of tubular, glomerular or mixed origin (which must be characterized with suitable methods, such as molecular sieving in SDS buffer, nephelometric immunoassay or immunofixation with appropriate antibodies).

CONFIRMATORY TESTS
When compared to a normal pattern, increased, decreased or additional fractions are observed, confirmatory tests might be needed, such as identification and quantification of specific proteins by immunochemical procedures. SEBIA offers a number of specialized tests for example :
- Monoclonal proteins in serum: HYDRAGEL IF K20 SEBIA (PN 3031) and HYDRAGEL DOUBLE IF K20 SEBIA (PN 3036),
- Bence Jones proteins in urine: HYDRAGEL BENCE JONES K20 SEBIA (PN 3038),
- Characterization of tubular or glomerular damage (molecular sieving in SDS buffer): HYDRAGEL PROTEINURIE K20 (PN 3004).

Limitations
In thawed samples, slight application marks caused by denatured proteins may be seen. Do not use hemolyzed and plasma samples. Due to the resolution and sensitivity limits of zone electrophoresis, it is possible that some monoclonal components may not be detected with this method.

Troubleshooting
Call Technical Service of the supplier when the test fails to perform while the instruction for the preparation and storage of materials, and for the procedure were carefully followed. Kit reagent Safety Data Sheets and informations on waste products elimination are available from the Technical Service of the supplier.

PERFORMANCE DATA
Standard materials, sample preparation and procedures were used. All electrophoregrams were evaluated visually.

Qualitative analysis
Results obtained with HYDRAGEL HR K20 procedure indicate a very good reproducibility within and between gels for all the tested aspects and there were no visually detectable differences among the repeats. Pathological and normal serum samples, CSF / serum pairs and urine samples from 116 patients were run using HYDRAGEL HR K20 procedure and on comparable commercially available high resolution gels. The samples were prepared as recommended by the respective procedures. Similarities / dissimilarities of the electrophoretic patterns and the presence of monoclonal or oligoclonal bands were the basis for comparison. The visual, qualitative comparison between these two tests has been based on the number of bands and their location. The results of the two procedures were in agreement in all 116 samples and were consistent with the clinical diagnosis. A pathological serum containing a monoclonal component (identified and quantitated by other means) was serially diluted with a normal serum and the individual dilutions electrophoresed. The sensitivity was determined from the highest serial dilution giving a discernible band upon staining with acid violet and was 0.1 g/dL.

Semi-quantitative analysis
For the semi-quantitative applications, results obtained with HYDRAGEL HR K20 procedure indicate a very good reproducibility within and between gels for all the tested aspects and there were no visually detectable differences among the repeats, the mean CV was 3.8 %. The HYDRAGEL HR K20 procedures with acid violet and amidoblack staining alternatives were compared to another commercially available high resolution gels procedure (traditional high resolution separations of serum proteins and acid violet staining). For the sake of comparison, all electrophoregrams were scanned (yellow filter) as 5-zone separations. Clinical serum samples (n = 56) were run with each procedure and the relative concentrations for each fraction were compared using linear regression analysis, 0.952 being the mean coefficient correlation for all protein fractions with both staining solutions.

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>HYDRAGEL HR K20 (acid violet)</th>
<th>HYDRAGEL HR K20 (amidoblack)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Correlation coefficient</td>
<td>Slope</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.960</td>
<td>0.95</td>
</tr>
<tr>
<td>Alpha-1</td>
<td>0.965</td>
<td>1.02</td>
</tr>
<tr>
<td>Alpha-2</td>
<td>0.920</td>
<td>0.93</td>
</tr>
<tr>
<td>Beta</td>
<td>0.909</td>
<td>0.95</td>
</tr>
<tr>
<td>Gamma</td>
<td>0.985</td>
<td>0.95</td>
</tr>
</tbody>
</table>

A pathological serum containing a monoclonal component (identified and quantitated by other means) was serially diluted with a normal serum and the individual dilutions electrophoresed using HYDRAGEL HR K20 procedure. The sensitivity was determined from the highest serial dilution giving a discernible band upon staining with acid violet and amidoblack. Approximately the same staining sensitivity / intensity was observed when the electrophoresis was performed on neat serum followed by staining with amidoblack and on a serum diluted 1/4 followed by staining with acid violet, and was 0.1 g/dL. The assays were linear to at least 5.8 g/dL albumin and 3.9 g/dL gammaglobulins for either staining procedure.

NOTE: According to the position of the monoclonal and polyclonal background in the gamma zone, the detection limit may vary.
BIBLIOGRAPHY


MIGRATION PATTERNS

![Migration Patterns Diagram](image-url)