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Mini Agarose Gel Electrophoresis of Hyaluronan (HA)*

Matrix Biology Institute, Edgewater, NJ USA
Standard Operating Procedure

1. Purpose

- 1.1. The purpose of this SOP is to describe a reliable, reproducible method for determining the polydispersity of hyaluronan using an agarose gel electrophoresis method.

2. Scope

- 2.1. This SOP describes the gel electrophoresis method. There are six important issues to consider related to the use of hyaluronan in order to be certain that the average molecular weights and the calculated parameters of polydispersity are indeed correct:
 - 2.1.1. The counter ion of the hyaluronan must be Na, because the standards are Na salts. If another counter ion is used, the standards must have the same.
 - 2.1.2. Hyaluronan cannot be a proteoglycan, meaning that it is covalently-bound to a special protein molecule. Hylan A is an example of such a proteoglycan of hyaluronan and it is used in therapeutics. Hylan A has only very small amounts of protein (<0.1% of the polysaccharide content). The presence of this protein does not interfere with the carboxylic acid group. Consequently the charge density of the molecule is unaltered and the molecule migrates in the electrical field is the same as that of the standards. Other hyaluronan proteoglycans, especially those that interfere with the carboxylic acid group, may change the migration and the standards are not applicable.

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- 2.1.3. The presence of proteins that are not attached but mixed in such as serum proteins in a tissue fluid (liquid vitreous) and tissue extracts may affect the staining of the gel. Serum proteins below a certain amount (in our experience < 70µg/mL) will not interfere with this process but when the protein concentration is high enough, it will interfere with the staining process. In this case, proteolytic enzyme digestion of the sample before electrophoresis is necessary. The proteolytic digestion at 37°C or below does not cause degradation of the pure hyaluronan molecule of any size. It is important to consider that when “enzyme-activating molecules” are added to the end to the digestion mixture, they may have hyaluronan-degrading activity.
- 2.1.4. The presence of pure sulfated glycosaminoglycans does not interfere with the migration and staining of hyaluronan. Chondroitin sulfate and sulfated hyaluronan do generally migrate faster than hyaluronan but do not interfere with the migration of hyaluronan. Very low avg. molecular weight hyaluronans, when mixed with sulfated glucosaminoglycans may interfere, if they migrate at the same rate because they stain similarly.
- 2.1.5. The migration of hyaluronan is dependent on the charge density of the molecule and on their volume, mass and conformation. There will be a difference in migration of molecules in random coil or in denser conformational form. The volume of the random coil will also define the migration. It is therefore important to allow sufficient time for the molecules to “relax” in its final concentration. This can be achieved by appropriately mixing the molecules after dilution. This is most important for molecules having a molecular weight above 6 million (peak).
- 2.1.6. The pH of the buffer in the “migration bath” must remain constant therefore an excess of the buffer is used to ensure it does not become exhausted.

3. Responsibilities

- 3.1. Any personnel performing the Determination of polydispersity of HA by Mini Agarose Gel Electrophoresis of Hyaluronan (HA) must be trained according to this procedure.

4. Materials

- 4.1. Horizontal Electrophoresis Systems, Minigel system, Fisher Biotech 7 x 10 cm, (10 well comb 1.5mm thick), Fisher Scientific FB-SB-710 or equivalent
- 4.2. Horizontal gel chamber, Owl Scientific model # A3 or equivalent
- 4.3. Power source-Occupier 500-z, 120V, 60Hz or equivalent
- 4.4. Umax Imagescanner (Amersham Biosciences catalog # 18113445, Powerlook III or equivalent), MagicScan software version 4.5, ImageMaster™ Labscan version 3.01, Photographic Step Tablet no. 2 (21 steps, density range approximately 0.05 to 3.05) Kodak catalog # 152 3398 or equivalent), Instruction manuals for this equipment.
- 4.5. Microwave oven
- 4.6. Magnetic stirrer and magnetic stir bars

- 4.7. Pyrex Erlenmeyer flasks, 125mL, 50mL
- 4.8. Volumetric flasks, 2L, 1L, 50mL
- 4.9. Thermometer probe
- 4.10. Micro-centrifuge tubes, 1.5mL, Fisher Scientific catalog # 02-681-238 or equivalent
- 4.11. Staining tray
- 4.12. 2.5L amber bottles, VWR catalog # 15900192 or equivalent
- 4.13. Pipettes: P1000, P200, P20, M250
- 4.14. 200 µl gel loading pipette tips. Fisher # 02-707-181 or equivalent
- 4.15. Graduated cylinders: 200mL, 500mL, 1000mL
- 4.16. Parafilm® or equivalent
- 4.17. Whatman® filter paper #1, 27 cm, VWR catalog # 28540-207 or equivalent
- 4.18. Glass or plastic funnel to fit filters from step 6.1.1 and 6.1.4.
- 4.19. Heavy duty aluminum foil
- 4.20. Transparency film for plain paper copiers
- 4.21. Pronase protease, *Streptomyces griseus*, (for synovial fluids or > 70 µg/ml serum protein content) EMD Biosciences Inc. catalog # 53702 or equivalent
- 4.22. Certified Molecular Biology Agarose, Bio-Rad #161-3101 or equivalent
- 4.23. Trizma base, reagent grade, Sigma catalog # T-1503 or equivalent
- 4.24. Trizma hydrochloride, reagent grade, Sigma catalog # T-3253 or equivalent
- 4.25. Sodium acetate trihydrate, ACS grade, Sigma catalog # S-8625 or equivalent
- 4.26. Ethylenediaminetetraacetic acid (EDTA), Disodium salt, dihydrate, Sigma catalog # E-4884 or equivalent
- 4.27. Sucrose, Sigma ultra, catalog # S-7903 or equivalent
- 4.28. Hyalose HA standards. Hyalose, L.L.C., catalog #s: HYA-LoLad-20, HYA-HiLad-20, HYA-MGLAD-20 (For SuperMegaLadder, lot SML200604; for MegaLadder, lot ML200505) or equivalent.

Select HA standards in the test range, normally using MW ladders such as LoLadder (27,000-495,000), HiLadder (0.5-1.5 million), MegaLadder (1.5-6.1 million) or SuperMegaLadder (2-8 million) or individual Hyalose standards may be used if applicable. When standards arrive, room to warm temperature and aliquot into appropriately labeled microcentrifuge tubes according to the volume used per run per standard. For example, Loladder is typically run at 5 µL volume so fill a set of appropriately labeled tubes with 5 µL each for that standard and freeze and store in the cryofreezer. Use one tube per standard ladder per run. Volumes needed per run for SuperMegaLadder, 8 µL volume; MegaLadder, 8 µL volume; HiLadder, 5 µL volume and LoLadder, 5 µL volume. Volumes can be adjusted if needed.

Note: If standards are in powder form, perform the following before aliquoting:

Dissolve standard powder to 5µg/ 100µL for each MW. Centrifuge the tube for a few seconds. Carefully open and add sterile de-ionized water directly to bottom of tube. Allow 2 hours at 4° C for sample rehydration and then mix well before use.

- 4.29. Stains-all, electrophoretic grade, MP Biomedicals, LLC # 195175 or equivalent. Store refrigerated.
- 4.30. Ethanol 200 proof, Fisher catalog # AC615090040 or equivalent
- 4.31. Deionized water
- 4.32. General lab equipment

5. Definitions

- 5.1. Appropriately labeled - Label must clearly describe the contents, and when applicable, the preparation date, the expiration date, the preparer's initials, and any relevant information.

6. Procedures

6.1. Reagents

- 6.1.1. 10X Tris-Acetate Buffer (10x TAE)- for 1 or 2 L preparations depending on usage in a 2 month period.

For 2 L preparation:

Into a 2L volumetric flask, that is 3/4 filled with deionized water, add:

- 36.30g Tris Base (\pm 0.05 g)
- 78.40g Tris HCl (\pm 0.05 g)
- 13.60g Sodium Acetate (\pm 0.05 g)
- 6.40g EDTA Dihydrate (\pm 0.05 g)

Into a 1L volumetric flask, that is 3/4 filled with deionized water, add:

For 1L preparation:

- 18.15g Tris Base (\pm 0.02 g)
- 39.20g Tris HCl (\pm 0.02 g)
- 6.80 Sodium Acetate (\pm 0.02 g)
- 3.20 g EDTA Dihydrate (\pm 0.02 g)

Cap volumetric flask and invert to mix until all solids are dissolved. Adjust volume to the 2L line on the volumetric flask using deionized water. Cap flask and invert to mix. Check pH and adjust to 7.90 ± 0.05 , if necessary. Filter entire contents using Whatman® #1 filter paper into clean, dry storage bottle. Store refrigerated in an appropriately labeled 2L bottle. Expires in 2 months. Must be at room temperature before use. Record pertinent information in a log book.

- 6.1.2. 1x TAE- Add 225 ml of 10xTAE, using a graduated cylinder, into an appropriately labeled bottle. Add 2025 mL of deionized water, using a graduated cylinder, into the bottle. Cap bottle and invert to mix. Label bottle appropriately. Store at room temperature. Expires in 1 week.

Sucrose Buffer- 2M Sucrose in 1xTAE- Weigh $34.20g \pm 0.05g$ sucrose and transfer to a 50 mL volumetric flask. Fill volumetric flask to line with 1xTAE. Cap flask and invert to dissolve. Transfer to appropriate storage container. Fill a microcentrifuge tube with a portion of the buffer and label appropriately. Use this for daily runs. Replace when about $\frac{1}{2}$ empty with new filled tube. Store refrigerated. Expires in 12 months. Must be at room temperature before use. Record pertinent information in a logbook.

- 6.1.3. 0.005% Stains-All Solution- For a 2 L amount of Stains-All, add 1 L of ethanol and 1 L of deionized water, using a graduated cylinder, into an appropriately labeled 2.5 L dark brown bottle. Weigh $0.100g \pm 0.005g$ Stains-All, then add the Stains-All to the 50% ethanol. Wrap bottle completely with aluminum foil. Add a magnetic stir bar to the bottle and stir the solution at least 4 hours on a magnetic stirrer. Smaller amounts of stain can be prepared if only a few runs will be performed in a one week period. Before using for gel staining for each run, place on stirrer and mix during the run for at least 3 hours then gravity filter about 200mL of solution, enough for one gel staining, through Whatman® #1 filter paper into another appropriately labeled 500mL bottle wrapped in aluminum foil. Cover funnel and sample with aluminum foil during filtration. Label must include the word "filtered". Store at room temperature covered with foil to prevent light penetration until use. Use entire contents for staining gel. Cover entire bottle of unfiltered stain with foil to prevent light penetration and store in dark place. Unfiltered solution expires in one week. Record pertinent information in a log book.

- 6.1.4. 10% Ethanol- Add 250 mL of ethanol and 2250 mL of deionized water using a graduated cylinder into a 2.5 L bottle. Cap bottle and invert to mix. Store at room temperature in an appropriately labeled 2.5L bottle. Expires in 3 months.

6.2. Gel apparatus assembly

- 6.2.1. Visually inspect the gel tray, casting chamber, comb, etc. for signs of dirt. Water spots are okay. Assemble apparatus according to manufacturer's instructions. Use a bubble level to insure the unit is level.

6.3. Casting and preparing the gel

- 6.3.1. Add 36mL deionized water into the bottom of a 125mL flask, careful not to wet the sides of the flask.
- 6.3.2. For samples of molecular weight 200,000 and above, prepare a 0.5 % agarose gel. Weigh $0.20g \pm 0.005g$ agarose onto weigh paper then add it to the 36 ml water in

the 125mL flask. For samples of molecular weight 200,000 and below, prepare a 1.0% agarose gel, weigh $0.40\text{g} \pm 0.005\text{g}$ agarose onto weigh paper then add it to the 36 ml water in the 125mL flask.

- 6.3.3. Add 4 ml of the 10xTAE buffer into a separate 50mL flask.
 - 6.3.4. Add 4 ml of the 10x TAE buffer into a separate container and dilute with 36mL water to be used to cover the gel after solidified in 6.3.11.
 - 6.3.5. Place the flask containing the agarose into the microwave oven. Run on high setting for approximately 1 minute, watching flask contents for bubbles. Caution: Flask will be hot! Use mitten or equivalent protection to prevent burning your hand. When bubbles appear, remove flask from microwave and gently swirl. Return to microwave and heat until bubbles form again. Remove and swirl for a few seconds.
 - 6.3.6. Repeat heating and swirling until agarose is dissolved. When dissolved, set the flask aside.
 - 6.3.7. Place the flask containing the 10x TAE into microwave for 6-8 seconds to warm.
 - 6.3.8. Add the 10xTAE to the flask containing the dissolved agarose. Add a stir bar then cover the opening with aluminum foil. Add a thermometer probe to the flask to monitor the temperature.
 - 6.3.9. Place on the magnetic stirrer. Stir gently for approximately 10 minutes until the temperature is $50^{\circ}\text{C} \pm 5^{\circ}\text{C}$.
 - 6.3.10. Pour the agarose solution into the assembled chamber with the comb in place. Tap the comb to dislodge any trapped bubbles. Immediately remove any other bubbles or any debris that may be floating in un-gelled fluid with the tip of a disposable pipette. Loosely place aluminum foil cover on the gel caster or apparatus.
 - 6.3.11. Allow the agarose solution to cool and gel for at least 20 minutes. Remove the foil cover from the apparatus. Slowly pour 40mL of the 1xTAE buffer prepared in 6.3.4 onto the top of the gel. Loosely place the cover on the apparatus. Allow the gel to set overnight. May be stored up to 3 days at room temperature before use.
 - 6.3.12. Place the Owl unit so that the connections for the leads are to your left. Remove the foil cover from the chamber. Pour off the TAE buffer that was on the gel. Lift out the inner gel chamber. Center the inner gel chamber in the Owl unit so that the gel is centered from side to side and at the front of the dish against the edge of the center piece. Verify that the top of the gel where the wells are located is placed on the side where the black negative wire is found (closest to you). Check the bubble level and adjust if needed so unit is level.
 - 6.3.13. **Add 1x TAE (approximately 2250mL)** into the Owl Unit, filling both sides, and covering the gel with 1x TAE buffer to about 3 mm above gel. Try not to get the buffer under the gel. Remove the comb. See section 6.5 for loading and running the gel.
 - 6.3.14. Rinse the comb, gel tray and chamber with deionized water and air dry.
- 6.4. Sample and Standard Preparation

Note 1: The final 0.27mg/mL concentration can be adjusted to optimize visibility in the gel.

Note 2: The following instructions are based on the knowledge of the HA and protein concentrations. If the HA concentration is unknown, a series of dilutions may be necessary until the appropriate degree of visibility of the HA after staining is achieved. If the protein content is unknown, the samples may be run without Pronase treatment. If there is a visible decrease in the staining of the HA in the 1-2M region during de-staining, then protein is affecting the de-staining process and the Pronase treatment is necessary.

6.4.1. Pronase digestion for samples containing protein (serum protein) > 70 µg/mL (such as human synovial fluid).

6.4.1.1. For hyaluronan concentration of 0.4mg/mL or higher .

6.4.1.1.1. Dilute samples to a concentration of 0.4mg/mL with 0.15M NaCl. This should be done in advance, especially with high molecular weight samples, to allow time for the molecules to relax. Store the samples refrigerated.

6.4.1.1.2. Prepare a 38mg/mL Pronase solution in 0.15 M NaCl. Do not vortex.

6.4.1.1.3. Pipette 100µL of the sample into an appropriately labeled microcentrifuge tube. Pipette 50 µL of the Pronase solution into the tube. Cap, invert and tap the tubes to mix. Do not vortex. These amounts can be increased proportionally to produce a larger amount of sample, necessary for instance, if the sample is to be run in multiple lanes. The ratio is 50µg Pronase to 1 µg HA. Final HA concentration is 0.26mg/mL.

6.4.1.1.4. Incubate the tubes for 5 hours at 37°C. Occasionally invert and tap tubes to mix.

6.4.1.1.5. Store the samples in cryofreezer or low temperature freezer until ready to use, warm to room temperature before use.

6.4.1.1.6. Centrifuge the samples for 1 minute before use.

6.4.1.1.7. Note: If volumes of available sample are less than 100 µL, volumes may be reduced to accommodate the available volumes: For example, 20 µL sample to 10 µL Pronase.

6.4.1.2. For hyaluronan concentration less than 0.4 mg/mL.

6.4.1.2.1. Prepare a 125 mg/mL Pronase solution in 0.15 M NaCl. Do not vortex.

6.4.1.2.2. Pipette 100µL of the sample into an appropriately labeled micro centrifuge tube. Calculate a ratio of 50µg Pronase to 1µg HA. Then add this amount of Pronase to your sample in the tube.

6.4.1.2.3. Incubate the tubes for 5 hours at 37°C. Occasionally invert and tap the tubes to mix.

6.4.1.2.4. Store samples in cryofreezer until ready to use, warm to room temperature before use.

- 6.4.1.2.5. Centrifuge samples for 1 minute before use.
- 6.4.1.2.6. Note: If volumes of available sample are less than 100 μL , volumes may be reduced to accommodate the available volumes: For example, 20 μL sample to 10 μL Pronase.
- 6.4.2. **Sample preparation for samples with low protein (< 70 $\mu\text{g}/\text{ml}$ serum protein).**
- 6.4.2.1. For approximately 6 million peak molecular weight samples and above, a 1-2mg/mL stock solution diluted to about 2mL or more in 0.15 molar saline (if available sample volume permits) should be prepared at least two days before further dilution. Mix the samples in the cold on a shaker at about 120 rpm, with 7 cc plastic tubes flat on the bottom of the shaker for maximum mixing of fluid. Be certain that the sample is completely homogenous before continuing with further dilution.
- For running concentration, dilute the 1-2mg/mL stock samples to 2mL total volume in 7mL plastic test tubes with 0.15 molar saline to an approximate concentration of 0.2-0.3mg/mL hyaluronan. Mix the samples overnight in the cold on a shaker at about 120 rpm, with tubes flat on the bottom of the shaker for maximum mixing of fluid. Allow samples to warm to room temperature before use.
- 6.4.2.2. For medium molecular weight material or lower (less than 6 million peak molecular weight), a 0.5mg/mL dilution should be prepared in 0.15N saline at least 1 day in advance before running on gel. Store samples refrigerated.
- 6.4.3. Hyaluronan standards
- Use Hyalose standards that encompass the estimated molecular weight distribution range of the test samples (at least 3 standards individually or as a ladder). See step 4.28. If the distribution is broad or there are samples of various MW distributions run on the same gel, 2 ladders may be used in separate wells. **Please note that for samples below 200,000, the standards used are the lower three standards in the Hyalose LoLadder standard ladder namely 214,000, 110,000 and 27,000 or appropriate individual Hyalose standards below 200,000. The standard slope shifts in this region and it is important that the proper standards are used for accurate MW distribution determination.**
- 6.4.4. Standard and sample preparation for loading gel
- 6.4.4.1. Using the air displacement pipette, place samples into appropriately labeled microcentrifuge tubes, 5 μL of each sample or standard (or appropriate volume depending on the standard used if not already in tubes from storage). Then pipette 3 μL of sucrose buffer into each tube and an appropriate amount of water to make a **total volume of 18 μL** . Hyalose standards may be mixed together and placed into one lane, providing final volume is 18 μL and the standards do not interfere with each other on the gel.

Note:

5 μ l of 0.2-0.3mg/mL =1-1.5 μ g total (for > 6 million peak molecular weight).

5 μ l of 0.5mg/mL =2.5 μ g total (for < 6 million peak molecular weight)

5 μ l of 0.05 μ g/mL = 0.25 μ g total (for a single MW band of Hyalose HA Select standards)

6.5. Loading and Running the Gel

6.5.1. Using the P20 pipette and gel loading pipette tips, slowly pipette 18 μ L of each sample or standard from 6.4.3 up and down at least four times into tip to mix and then into the bottom of the appropriate well, so that the material is layered under the TAE buffer. Make sure there is a blank lane at least every second sample/standard. Be careful not to create turbulence that may mix the sample with the TAE buffer. Record the lane number (location) of each sample or standard. Place the cover of the owl unit over top so that leads are tightly connected.

6.5.2. Adjust voltage on power source. **For a 0.5% agarose gel, set voltage to 40 volts and run gel for 5 hours (300 minutes). For low molecular weight samples (below 200,000 peak molecular weight), set voltage to 50V and run gel for 3 hrs (180 minutes).** Press “run” to begin current. Power source display can be toggled between time, amperage and voltage using the “select” button.

6.6. Staining the Gel

6.6.1. Press the “stop” button after time point has been reached. Shut off the power source. Remove cover. Immediately perform the following five steps.

6.6.2. Slowly raise the tray containing the gel from the apparatus. Be careful that gel does not slide off tray. Tilt tray slightly to drain off excess TAE buffer.

6.6.3. Slowly slide gel into staining dish. Be careful that gel does not stick to indentation near bottom of tray when sliding gel out.

6.6.4. Slowly add Stains-All Solution to the staining dish to a level approximately 0.5 cm higher than gel, about 250mL in a 6” x 6” plastic container. Remove any bubbles that are trapped under the gel.

6.6.5. Place cover on the staining dish, then cover with aluminum foil and set it in a dark environment, for example, under a large box in a dark cabinet.

6.6.6. Allow gel to remain at room temperature undisturbed for at least 14 hours. May store up to 3 days before de-staining.

6.7. De-staining the Gel

6.7.1. De-stain the gel by removing the Stains-All Solution from the staining dish.

6.7.2. Cover the gel with 10% ethanol solution at least 0.5 cm higher than gel (about 200 mL for a 6” x 6” dish). Replace cover. Allow the gel to remain at room temperature undisturbed in a dark environment, for example, in a dark cabinet or covered with foil, for at least 2 hours.

- 6.7.3. Remove the 10% ethanol solution from the staining dish by carefully pouring off the majority of buffer, then pipette off the remainder. Then cover the gel with new 10% ethanol solution at least 0.5 cm higher than gel. Replace cover, and then cover with aluminum foil. Allow the gel to remain at room temperature undisturbed in a dark environment, for example, in a dark cabinet or covered with foil, for at least 1 hour.
- 6.7.4. Remove the 10% ethanol solution from the staining dish. Then cover the gel with new 10% ethanol solution to just cover the gel. Allow the gel to remain at room temperature, exposed to light; undisturbed for 1-2 hrs or until the background or unbound stain becomes preferentially faded.
- 6.7.5. After 1-1.5 hrs, remove the 10% ethanol solution from the staining dish. Carefully place an appropriate size piece of transparency film under gel so the gel can be moved to the scanner.
- 6.7.6. Refer to 6.9.4 for calibrating the scanner and 6.9.1- 6.9.3 for details on setting up the scanner before placing gel on scanner. Transfer gel to the glass surface of the scanner by carefully sliding the gel off the film onto the scanner. Fill in the wells with excess 10% ethanol using a disposable pipette. Add an additional transparency to the top of the gel by starting at one end and slowly rolling it over entire surface of gel applying gentle pressure to transparency to reduce bubble formation. Additional 10% ethanol may be used in wells or other parts of the gel to remove any bubbles that are visible beneath the gel and between the gel and transparency film cover.

6.8. Cleaning the Apparatus

Wash all components of apparatus with water then rinse thoroughly with deionized water. Store the parts so that they will not be scratched.

6.9. Scanning a Gel (See manufacturer's instruction manual for any questions.)

- 6.9.1. Be sure the scanner power is turned on before starting computer; otherwise reboot computer after scanner is turned on.
- 6.9.2. Be sure that the glass surfaces on the scanner are clean and dry. If not, clean and dry the glass surfaces with an appropriate cloth.
- 6.9.3. To scan a gel, click "scan a new image." Use the following setting:

6.9.3.1. Scanner options: Colour optimized for: red source
 Light path: transmissive
 Bits per pixel: 16 bits (65536 greys)
 Do not check "use twain default settings".

6.9.3.2. Initial settings: Brightness: 49.61
 Contrast: 100
 Dots per inch: 600

6.9.4. Scanner Calibration

- 6.9.4.1. Place Photographic Step Tablet on scanner with the lightest region on your right. Preview the scan by clicking the “refresh preview” button. Resize the image box. Scan image.
 - 6.9.4.2. Click intensity calibration from drop down “analysis” menu. Check the following density values for the step wedge: 0.07, 0.28, 0.49, 0.70, 0.91, 1.12, 1.33. Some higher value may be removed if image is too dark. Use at least 5 values.
 - 6.9.4.3. Select “optical density” units.
 - 6.9.4.4. Select “Log Quadratic curve” fit.
 - 6.9.4.5. Do not check the “intersect origin” box.
 - 6.9.4.6. Magnify the area you are working in using the magnifying glass button.
 - 6.9.4.7. Drag the numbered steps, using the left mouse button, into the appropriate spot on the image so that one value lies in the center of each shaded area. Lowest value will be last box on right. Watch the R^2 value on the calibration curve. This value must be at least 0.97.
 - 6.9.4.8. Remove step tablet from the scanner. Go to “mode”, “scanning”.
 - 6.9.5. Place the gel on the scanner as described in 6.7.6. Close the scanner cover, leaving it partially open using a wedge so that the gel does not touch the upper glass.
 - 6.9.6. Preview the scan by clicking the “refresh preview” button. Resize the image box. Preview the scan again. If the preview looks good, click the “scan” button. If not, adjust the settings appropriately and preview the scan again.
 - 6.9.7. Go to the file menu and save the image, using the gel number as the file name then “-next consecutive number”, for example, minigel 001-1.
 - 6.9.8. Repeat steps 6.9.6 through 6.9.7 if a rescan is needed. Use the next consecutive number at end of the file name for any additional images that are to be saved.
- 6.10. Gel Analysis
- 6.10.1. Refer to SOP “Gel Analysis Method for Determining the Distribution of the Molecular Weight of Hyaluronan (HA) by Minigel Agarose Gel Electrophoresis” to analyze the desitometric data and “Statistical Analysis Method for Determining the Polydispersity and Statistical Parameters of Hyaluronan (HA) by Mini Agarose Gel Electrophoresis” to calculate the mean, peak (mode), median, standard deviation, skewness, kurtosis (both weight and number values) and the polydispersity index.