

This Procedure retrieved from the Matrix Biology Institute website at www.matrixbiologyinstitute.org, HA Procedures.

Statistical Analysis Method for Determining the Polydispersity and Statistical Parameters of Hyaluronan (HA) by Mini Agarose Gel Electrophoresis*

Matrix Biology Institute, Edgewater, NJ USA
Standard Operating Procedure

1. Purpose

- 1.1. The purpose of this SOP is to determine polydispersity parameters and statistically analyze the molecular weight distribution of the hyaluronan component of various materials after using the “Mini Agarose Gel Electrophoresis of Hyaluronan (HA)” method.

2. Scope

- 2.1. Any personnel testing samples using the “Mini Agarose Gel Electrophoresis of Hyaluronan (HA)” Method.

3. Responsibilities

- 3.1. Any personnel testing samples using the “Mini Agarose Gel Electrophoresis of Hyaluronan (HA)” Method must be trained according to this procedure.

4. Materials

- 4.1. Excel Spreadsheet “Gel Analysis spreadsheet” completed for the mini gel being analyzed.
- 4.2. Working copy of the Excel Spreadsheet “Statistical Analysis”.

5. Definitions (not applicable)

6. Procedures

- 6.1. Open a working copy of the Statistical Analysis spreadsheet. Save with Minigel number, sample descriptions and date if applicable. Refer to the Instruction Form for Transferring Gel Electrophoresis Data to Gel Analysis Spreadsheet page 3 and 4 of this

**Developed by: David Gibbs, PhD; Julie Heeter; Endre A Balazs, MD*

References: Evans, M., Hastings, N., Peacock, B., Statistical Distributions, Wiley-Interscience, 3rd edition, 2000.

Rudin, A., Elements of Polymer Science & Engineering: An Introductory Text and Reference for Engineers and Chemists, Academic Press, 2nd edition, 1998.

procedure and the Work Form for Transferring Gel Electrophoresis Data to Gel Analysis Spreadsheet, page 5 of this procedure. Make a copy of these pages.

- 6.2. Follow directions using the Instruction Form and fill in the Work Form until all data is calculated except Mode and polydispersity index.
- 6.3. For each sample, copy and paste as values the MW data (column A) and the corresponding weight fraction data (column C) and the number fraction data (column M) into a separate spreadsheet. Graph MW vs weight fraction and number fraction as xy scatter, no data points visible, in a separate spreadsheet in the same file. They may be made separately or as one graph. Choose the point of highest value on the peak and left click on the spot to get the MW value and record as the Mode or Peak value for each graph on the Work Form. For Polydispersity Index, divide the Weight average MW by the Number average MW.

Instruction Form for transferring gel electrophoresis data to Gel Analysis Spreadsheet

Open a completed MW distribution excel spreadsheet that you wish to analyze.

Select the best data range that incorporates data points relevant to the MW distribution from this Spreadsheet from column C. Leave out raw data points that bounce from 0, go up a few points and go back down to 0 as this is "noise". Copy and paste as values the MW data into column A of the Statistical Analysis excel spreadsheet. Record the highest MW and the lowest MW of the distribution on the work form. Copy and paste corresponding raw data into column B. Observe last row occupied in both columns on the Gel Analysis spreadsheet and record row # in cell B1. Record gel run number in cell A1. Follow instructions below.

<u>Column</u>	<u>Instructions</u>
Column A	Delete all data below last used row # to row #1233
Column B	Delete all data below last used row # to row #1233
Column B	Change B1234 formula to =SUM(B3:B"x") where "x" = last used row #
Column C	Change C3 formula to = B3/"x" where "x" = B1234 value
Column C	Copy and paste B3 formula to rest of column to row # 1233
<i>Check</i>	C1234 = 1
Column D	Change D1234 formula to =SUM(D3:D"x") where "x" = last used row #
Column H	Change H3 formula =A3 - "x" where "x" = E1234 value
Column H	Copy and paste H3 formula to rest of lane to row #1233
<i>Check</i>	I1235 = I1238
Column I	Change I1234 formula to =SUM(I3:I"x") where "x" = last used row #
Column L	Change L1234 formula to =SUM(L3:L"x") where "x" = last used row #
Column M	Change M1234 formula to =SUM(M3:M"x") where "x" = last used row #
Column M	Change M3 formula =L3/"x" where "x" = L1234 value
Column M	Copy and paste M3 formula to rest of column to row # 1233
<i>Check</i>	M1234 = 1
Column N	Change N3 formula to =A3 - "x" where "x" = D1235 value
column N	Copy N3 formula to rest of lane to row # 1233
Column O	Change O1234 formula to =SUM(O3:O"x") where "x" = last used row #
<i>Check</i>	O1235 = O1238
Column P	Change P1234 formula to =SUM(P3:P"x") where "x" = last used row #
Column Q	Change Q1234 formula to =SUM(Q3:Q"x") where "x" = last used row #
Column R	Copy C3 value into R3 cell
<i>Check</i>	R "last used row # = 1
Column S	Copy M3 value into S3 cell

Check S "last used row #" = 1

Column c Graph MW vs wf. Find the highest value and right click on the spot.
Record the corresponding MW on the work form.

Column M Graph MW vs nf. Find the highest value and right click on the spot.
Record the corresponding MW on the work form.

PD index Divide the weight average MW by the number average MW. Record on work form.

Work Form for transferring gel electrophoresis data to Analysis Spreadsheet

Sample id #, description: _____

Run # _____

Date _____

Initials _____

Last cell row # used: _____

MW range: _____

column B sum = _____

L1234 # = _____

<u>Cell #</u>	<u>MW parameter</u>	<u>value</u>
D1235	avg. M_n	
E1234	avg. M_w	
I1238	Std dev (w)	
O1238	Std dev (n)	
J1235	Skewness (w)	
P1235	Skewness (n)	
K1235	Kurtosis (w)	
Q1235	Kurtosis (n)	
R (@0.50000)	Median (w)	
S (@0.50000)	Median (n)	
C, highest value	Mode (w)	
M, highest value	Mode (n)	
E1234/D1235	PDI, polydispersity index	