I. MIXING STUDY FOR LINEARITY VARIANT II AND D10:

A. Requires a normal Hgb AA specimen and a specimen or prepared hemolysate of a known abnormal Hgb (S,C,E, and F)("Hgb X")

B. Prepare dilutions for each sample according to the following procedure.

C. Analyze on HPLC and D10.

D. Calculate expected % Hb X vs measured %Hb X utilizing Excel, Linearity Template.

E. Enter the expected and measured Hb X into an Excel spreadsheet, graph. R sq value for linear regression should be >0.9.

F. Print the simple regressions graphs and file in the Linearity notebook.

G. The Hb S, A2, and C the expected and measured results should agree within 20%.

H. The Hb F expected and measured results should agree within 30%.

II. INSTRUCTIONS:

A. $-(ax / (a x+by)) \cdot$ (measured %HbX) = expected %HbX(formula preset in program)

1. a = vol Hb X
2. b = vol Hb A
3. x = area of Hb X peak in 1000µL·dilution and y = areas of Hb A peak in 1000 µl A dilution

B. Instructions for the Linearity assay for the Variant II and D10 instruments:
(Every 6 months)

1. Fill a 10 ml red top tube with 6 ml of fresh wash solution. (**Use the wash specific to each instrument**) Add 30 µl of sample.
(For %S, C, and EE, hemolysates stored in -70C)
(For %F find a recent whole blood sample with %F>50%)
(If abnormal hgb sample has too low a Hct add extra µl so area count will be within instrument tolerance range)

2. Fill a 10 ml red top tube with 10 ml of wash add 50 µl of the AA patient (normal patient should have a Hct >37%)

3. Need 5 tubes (12 x 75) Label each tube using the dilution factors below for example:
SS 50/950 - Add 50µl of the red tube SS to 950 µl of the red tube AA in a 12 x 75 tube marked SS 50/950.

Patient X	**Patient AA**
50 µl	**950 µl**
200 µl	**800 µl**
400 µl	**600 µl**
600 µl	**400 µl**
800 µl	**200 µl**

4. Fill one micro vial with 100% of Patient AA from the red top tube.

5. Fill one micro vial with 100% of Patient X from the red top tube.

6. Label each micro vial with the dilution and type of X Hb eg: 50/950 SS. using a transfer pipette fill each micro vial with the correct 1 ml solution.

7. The order of micro vials after the control and calibrators are:
   a. **100%** HbAA
   b. 50/950 Hb X
   c. 200/800
   d. 400/600
   e. 600/400
   f. 800/200
   g. **100%** Hb X
   h. **00%** Hb AA to prevent carry over especially after CC.

8. Variant II change the demographics as follows:
   a. 50/950 FF then print the samples and the 100% AA.

9. D10 enter the sample numbers with the dilution factors ie:
   a. 50/950 FF and print the samples and the first 100% AA.

10. Access the templates for the Variant II and D10 linearity:

a. Enter the 100% value for the abnormal Hgb X.
b. Enter the TOTAL AREA COUNT for the 100% X sample
c. Enter the TOTAL AREA COUNT for the 100% Hgb AA sample
d.. Under MEASURED enter the % of abnormal Hgb found on each dilution.
e. The expected %Hg X then will be calculated. Compare this answer to the measured value.
f. When all results are entered print the data page.
g. Measured and expected are entered into an Excel spreadsheet as follows.

III. LINEARITY AND REGRESSION PROCEDURE:

A. In Excel enter results into two columns expected and measured
1. Highlight information
2. Go to Chart Wizard
3. Line graph
4. Next
5. Next
6. Chart Title: Linearity % Hgb(S,C,E,or F), instrument and date
7. For X axis: dilution
8. For Y axis: % Hgb(S,C,E,or F)
9. Choose Data Table, choose "show data table"
10. Next
11. Finish
12. Right click on a "measured" point on the graph, chose "add a trendline"
13. Choose: Options
    a. Display equation on chart
    b. Display R sq value on chart (should be >.90)
    c. OK
    e. Print and place in linearity notebook, along with supporting documents.

IV. HISTORY:

H-1 This procedure was written by Paula Morris on 10-2009.
H-2 This procedure was revised by S. Richardson 3/12/12.