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Comparison between Analyte Results Obtained by two Very Common HPLC Systems Used in Haemoglobinopathies: Evaluation Done at Metropolis a Referral Laboratory in Kolkata, India

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Abstract **Original Research Article**

Thalassemia is a heterogenous group of disorders caused by inherited mutations that decrease the synthesis of adult hemoglobin, HbA, (α2β2). The two α chains in HbA are encoded by an identical pair of α globin genes on chromosome 16 while the β chain are encoded by single β globin gene on chromosome 11. B thalassemia is caused by deficient synthesis of β chain, whereas α thalassemia is caused by deficient synthesis of α chain. Alkaline hemoglobin electrophoresis is a common first step in confirmation of hemoglobinopathies. Electrophoresis is based on the separation of hemoglobin molecules in an electric field primarily because of differences in total molecular charge. HPLC and capillary electrophoresis are gaining in popularity because these methods are more automated, the instruments are more user friendly, and they can be used to confirm hemoglobin variants observed with electrophoresis. The two most common instrumentations used for identification of Hb electrophoresis are Bio-Rad D10 and Tosoh G11. This study is used to find out the comparison of Haemoglobin electrophoresis between Bio-Rad and Tosoh.

Keywords: Hemoglobin Electrophoresis, Bio-Rad D10, Tosoh G11, HbA2, HbF.

BACKGROUND

The Metropolis lab tested Haemoglobin electrophoresis for more than 7yrs in Kolkata for diagnosis of Abnormal Haemoglobinopathies. The detection of the Haemoglobin fraction HbA2 and HbF are very important parameters for reaching diagnostic results. In This study we compared the results obtained for the hemoglobin fraction HbA2 & HbF by the Bio-Rad D10 & Tosoh G 11 Using cation exchange HPLC method.

MATERIALS AND METHOD

50 random samples were taken irrespective of Age and Gender for study. These samples were analyzed as routine by the Bio-Rad D 10 and successfully they were examined by the Tosoh G 11. We used Coefficient of Correlation, R^2 and Average Bias to compare the HbA2 & HbF obtained Values.

Calculation done for HbF in both Bio-Rad D10 (Method A) and Tosoh G11 (Method B)

Tab. 1

S. No.	Method A	Method B	Difference (B-A)	Mean (M)	% Difference	Within 2SD	Within % Diff. Goal
1	**2.2**	**2.2**	0.0	2.2	0.00	Yes	Yes
2	**1.1**	**1.1**	0.0	1.1	0.00	Yes	Yes
3	**0.8**	**0.9**	0.1	0.9	12.50	Yes	Yes
4	**0.8**	**0.8**	0.0	0.8	0.00	Yes	Yes
5	**4.2**	**4.5**	0.3	4.4	7.14	Yes	Yes
6	**1.6**	**1.6**	0.0	1.6	0.00	Yes	Yes
7	**1.7**	**1.7**	0.0	1.7	0.00	Yes	Yes
8	**1.4**	**1.4**	0.0	1.4	0.00	Yes	Yes
9	**1.6**	**1.6**	0.0	1.6	0.00	Yes	Yes

S. No.	Method A	Method B	Difference (B-A)	Mean (M)	% Difference	Within 2SD	Within % Diff. Goal
10	**1.7**	**1.8**	0.1	1.8	5.88	Yes	Yes
11	**1.2**	**1.3**	0.1	1.3	8.33	Yes	Yes
12	**1.9**	**1.9**	0.0	1.9	0.00	Yes	Yes
13	**6.6**	**7.1**	0.5	6.9	7.58	Yes	Yes
14	**7.7**	**8.2**	0.5	8.0	6.49	Yes	Yes
15	**17**	**18.3**	1.3	17.7	7.65	No	Yes
16	**7**	**7.9**	0.9	7.5	12.86	Yes	Yes
17	**4.3**	**4.5**	0.2	4.4	4.65	Yes	Yes
18	**9**	**10.3**	1.3	9.7	14.44	No	Yes
19	**11.3**	**12.5**	1.2	11.9	10.62	No	Yes
20	**5.5**	**5.9**	0.4	5.7	7.27	Yes	Yes
21	**6.8**	**7**	0.2	6.9	2.94	Yes	Yes
22	**0.8**	**0.8**	0.0	0.8	0.00	Yes	Yes
23	**0.8**	**0.9**	0.1	0.9	12.50	Yes	Yes
24	**0.8**	**0.8**	0.0	0.8	0.00	Yes	Yes
25	**0.8**	**0.8**	0.0	0.8	0.00	Yes	Yes
26	**11.2**	**12.6**	1.4	11.9	12.50	No	Yes
27	**0.8**	**0.9**	0.1	0.9	12.50	Yes	Yes
28	**0.8**	**0.9**	0.1	0.9	12.50	Yes	Yes
29	**11.2**	**12.6**	1.4	11.9	12.50	No	Yes
30	**1**	**1.1**	0.1	1.1	10.00	Yes	Yes
31	**1.1**	**1.1**	0.0	1.1	0.00	Yes	Yes
32	**0.9**	**0.9**	0.0	0.9	0.00	Yes	Yes
33	**1.1**	**1.1**	0.0	1.1	0.00	Yes	Yes
34	**1.2**	**1.2**	0.0	1.2	0.00	Yes	Yes
35	**0.9**	**0.9**	0.0	0.9	0.00	Yes	Yes
36	**1.3**	**1.2**	-0.1	1.3	-7.69	Yes	Yes
37	**0.9**	**0.9**	0.0	0.9	0.00	Yes	Yes
38	**0.8**	**0.8**	0.0	0.8	0.00	Yes	Yes
39	**0.8**	**0.8**	0.0	0.8	0.00	Yes	Yes
40	**0.9**	**0.9**	0.0	0.9	0.00	Yes	Yes
41	**0.8**	**0.9**	0.1	0.9	12.50	Yes	Yes
42	**0.8**	**0.9**	0.1	0.9	12.50	Yes	Yes
43	**11.2**	**12.6**	1.4	11.9	12.50	No	Yes
44	**1**	**1.1**	0.1	1.1	10.00	Yes	Yes
45	**1.1**	**1.1**	0.0	1.1	0.00	Yes	Yes
46	**1.9**	**1.9**	0.0	1.9	0.00	Yes	Yes
47	**6.6**	**7.1**	0.5	6.9	7.58	Yes	Yes
48	**7.7**	**8.2**	0.5	8.0	6.49	Yes	Yes
49	**17**	**18.3**	1.3	17.7	7.65	No	Yes
50	**7**	**7.9**	0.9	7.5	12.86	Yes	Yes

Calculation done for HbA2 in both Bio-Rad D10 (Method A) and Tosoh G11 (Method B)

Tab. 2

S. No.	Method A	Method B	Difference (B-A)	Mean (M)	% Difference	Within 2SD	Within % Diff. Goal
1	**2.2**	**2.2**	0.0	2.2	0.00	Yes	Yes
2	**1.1**	**1.1**	0.0	1.1	0.00	Yes	Yes
3	**0.8**	**0.9**	0.1	0.9	12.50	Yes	Yes
4	**0.8**	**0.8**	0.0	0.8	0.00	Yes	Yes
5	**4.2**	**4.5**	0.3	4.4	7.14	Yes	Yes
6	**1.6**	**1.6**	0.0	1.6	0.00	Yes	Yes
7	**1.7**	**1.7**	0.0	1.7	0.00	Yes	Yes
8	**1.4**	**1.4**	0.0	1.4	0.00	Yes	Yes
9	**1.6**	**1.6**	0.0	1.6	0.00	Yes	Yes
10	**1.7**	**1.8**	0.1	1.8	5.88	Yes	Yes
11	**1.2**	**1.3**	0.1	1.3	8.33	Yes	Yes
12	**1.9**	**1.9**	0.0	1.9	0.00	Yes	Yes
13	**6.6**	**7.1**	0.5	6.9	7.58	Yes	Yes
14	**7.7**	**8.2**	0.5	8.0	6.49	Yes	Yes

S. No.	Method A	Method B	Difference (B-A)	Mean (M)	% Difference	Within 2SD	Within % Diff. Goal
15	**17**	**18.3**	1.3	17.7	7.65	No	Yes
16	**7**	**7.9**	0.9	7.5	12.86	Yes	Yes
17	**4.3**	**4.5**	0.2	4.4	4.65	Yes	Yes
18	**9**	**10.3**	1.3	9.7	14.44	No	Yes
19	**11.3**	**12.5**	1.2	11.9	10.62	No	Yes
20	**5.5**	**5.9**	0.4	5.7	7.27	Yes	Yes
21	**6.8**	**7**	0.2	6.9	2.94	Yes	Yes
22	**0.8**	**0.8**	0.0	0.8	0.00	Yes	Yes
23	**0.8**	**0.9**	0.1	0.9	12.50	Yes	Yes
24	**0.8**	**0.8**	0.0	0.8	0.00	Yes	Yes
25	**0.8**	**0.8**	0.0	0.8	0.00	Yes	Yes
26	**11.2**	**12.6**	1.4	11.9	12.50	No	Yes
27	**0.8**	**0.9**	0.1	0.9	12.50	Yes	Yes
28	**0.8**	**0.9**	0.1	0.9	12.50	Yes	Yes
29	**11.2**	**12.6**	1.4	11.9	12.50	No	Yes
30	**1**	**1.1**	0.1	1.1	10.00	Yes	Yes
31	**1.1**	**1.1**	0.0	1.1	0.00	Yes	Yes
32	**0.9**	**0.9**	0.0	0.9	0.00	Yes	Yes
33	**1.1**	**1.1**	0.0	1.1	0.00	Yes	Yes
34	**1.2**	**1.2**	0.0	1.2	0.00	Yes	Yes
35	**0.9**	**0.9**	0.0	0.9	0.00	Yes	Yes
36	**1.3**	**1.2**	-0.1	1.3	-7.69	Yes	Yes
37	**0.9**	**0.9**	0.0	0.9	0.00	Yes	Yes
38	**0.8**	**0.8**	0.0	0.8	0.00	Yes	Yes
39	**0.8**	**0.8**	0.0	0.8	0.00	Yes	Yes
40	**0.9**	**0.9**	0.0	0.9	0.00	Yes	Yes
41	**0.8**	**0.9**	0.1	0.9	12.50	Yes	Yes
42	**0.8**	**0.9**	0.1	0.9	12.50	Yes	Yes
43	**11.2**	**12.6**	1.4	11.9	12.50	No	Yes
44	**1**	**1.1**	0.1	1.1	10.00	Yes	Yes
45	**1.1**	**1.1**	0.0	1.1	0.00	Yes	Yes
46	**1.9**	**1.9**	0.0	1.9	0.00	Yes	Yes
47	**6.6**	**7.1**	0.5	6.9	7.58	Yes	Yes
48	**7.7**	**8.2**	0.5	8.0	6.49	Yes	Yes
49	**17**	**18.3**	1.3	17.7	7.65	No	Yes
50	**7**	**7.9**	0.9	7.5	12.86	Yes	Yes

RESULTS

The analysis of the results obtained by the two methods for both HbA2 in Bio-Rad D10 and Tosoh G11.

Tab. 3

Statistical Analysis			
No of Samples	**50**	**Acceptable Limit**	**Inference**
Coefficient of Correlation - R	**0.990**	**> 0.975**	PASSED
*** R^2**	**0.981**	**> 0.950**	PASSED
Average Bias	**6.893**	**15.00**	PASSED
*** R^2 is significant only when sample size is >/= 20**			
	Outliers	**% Outliers**	**% Values within goal**
2SD Goal	**12**	**24%**	**76%**
% Difference Goal	**3**	**6%**	**94%**

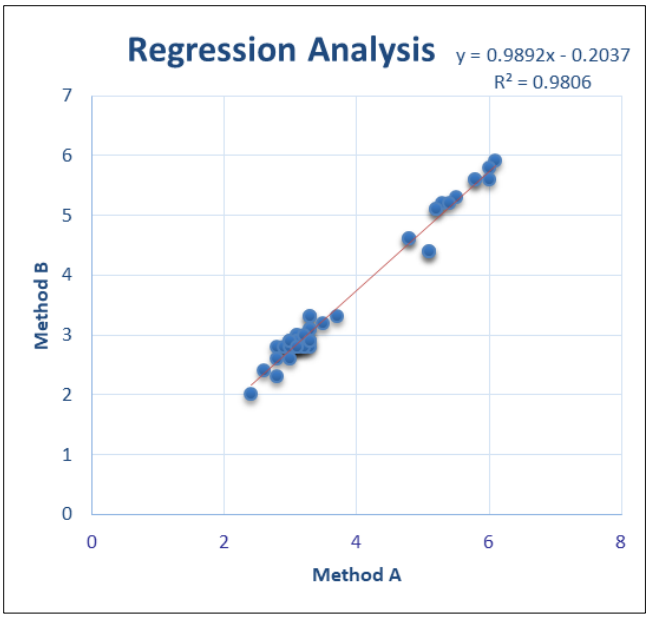

Fig. 1

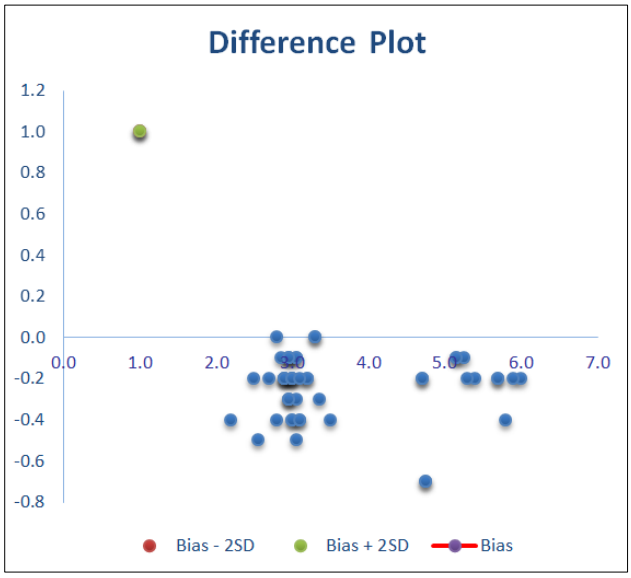

Fig. 2

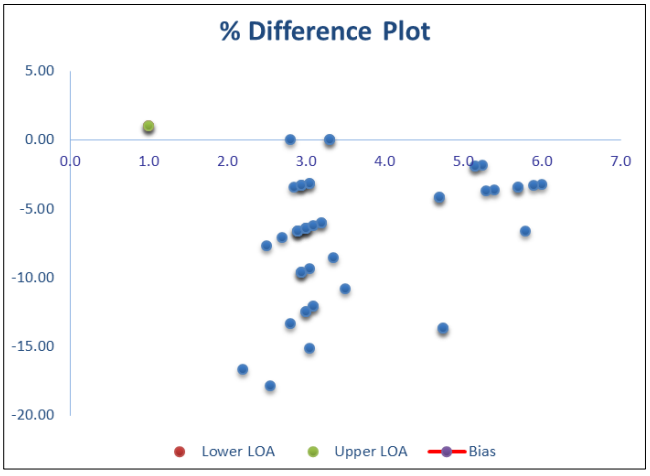

Fig. 3

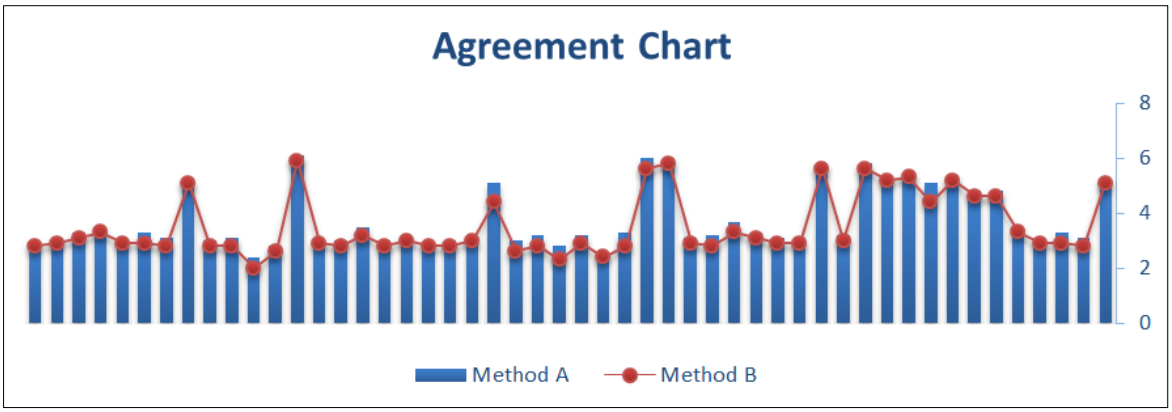

Fig. 4

The analysis of the results obtained by the two methods for both HbF in Bio-Rad D10 and Tosoh G11.

Tab. 4

Statistical Analysis			
No of Samples	**50**	**Acceptable Limit**	**Inference**
Coefficient of Correlation - R	**0.999**	**> 0.975**	PASSED
*** R^2**	**0.999**	**> 0.950**	PASSED
Average Bias	**5.413**	**15.00**	PASSED
*** R^2 is significant only when sample size is >/= 20**			
	Outliers	**% Outliers**	**% Values within goal**
2SD Goal	**7**	**14%**	**86%**
% Difference Goal	**0**	**0%**	**100%**

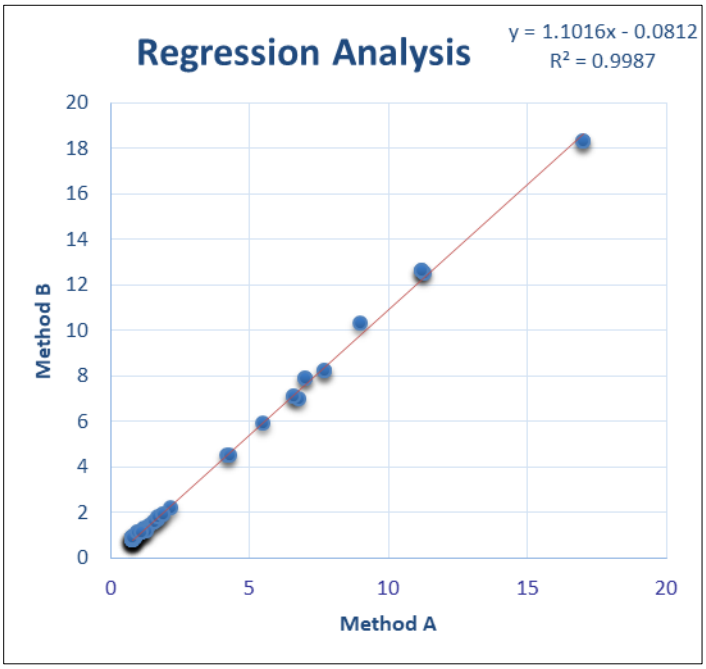

Fig. 5

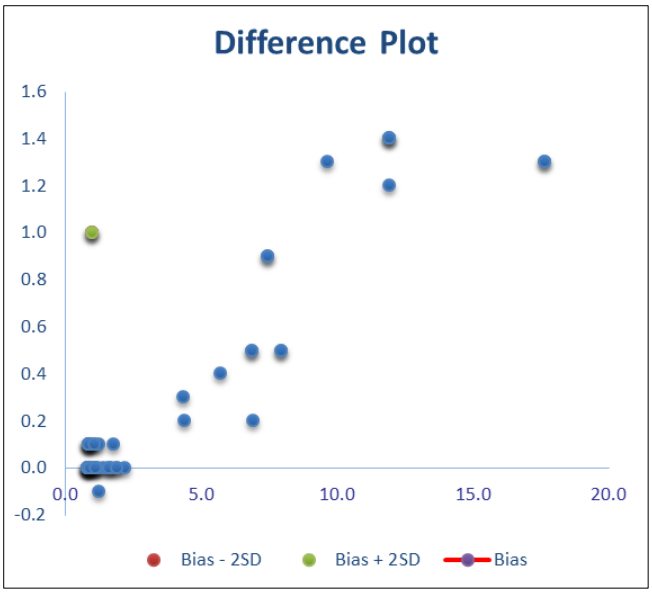

Fig. 6

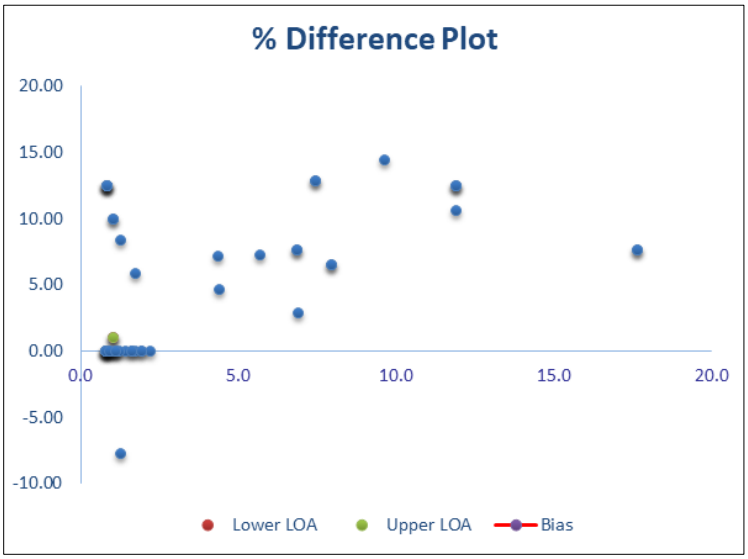

Fig. 7

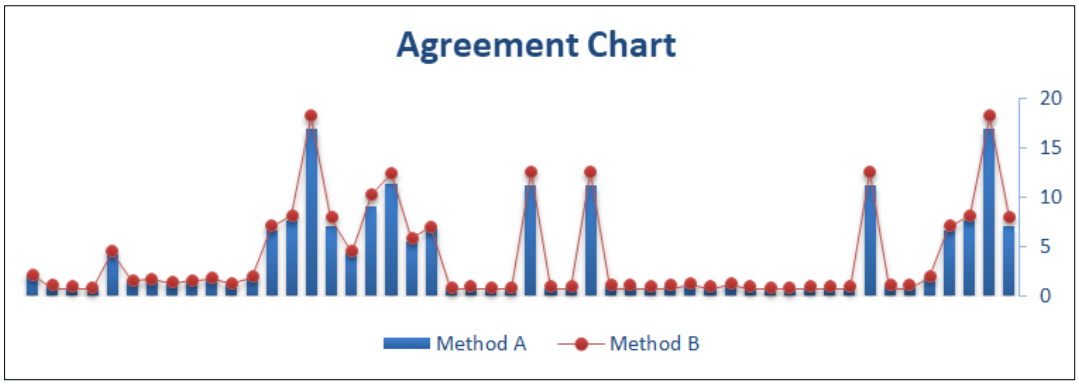

Fig. 8

DISCUSSION

Hemoglobin-related disorders are among the most common inherited genetic disorders in the world. They are posing a serious health burden to the global health system. As per WHO, the highest incidence of hemoglobinopathies is in the Middle East and Indian subcontinent. Screening methods like High-Performance Liquid Chromatography (HPLC) help in determining

values of HbA, HbA2, and HbF and diagnosing hemoglobinopathies at the initial stages. Tosoh G11 is based on the High-Performance Liquid Chromatography (HPLC) principle. Separation is obtained with a cation exchange column based on differences in ionic interactions between hemoglobin components within 5.0 minutes. A step gradient elution is used to separate and assay HbF and HbA2. The three types of G11 β thalassemia elution buffers contain different salt concentration and pH. HbF (%) and HbA_2 **(%)** are reported as a relative percentage of the integrated area of each Hb fraction against the sum of those hemoglobin fractions, after being calibrated using the calibration curve established with G11 HbF & HbA_2 Calibrator set or HbF & HbA_2 Calibrator set 2. Purpose of this study to find out the correlation between HbA_2 and HbF values in two most used instrumentation in eastern part of India where we find out the value of HbA_2 and HbF are correlating with both instrumentations. However final conclusion can only be said when large number of patients will be tested under both instrumentation.

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