

Hemoglobin A1c
Development Update

Scripps Laboratories

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- Scripps Laboratories has developed a new methodology for measuring percent glycated hemoglobin
 - The new technology addresses the large, and growing, worldwide diabetes epidemic
 - Scripps has shown that the technology can be adapted to a simple strip format
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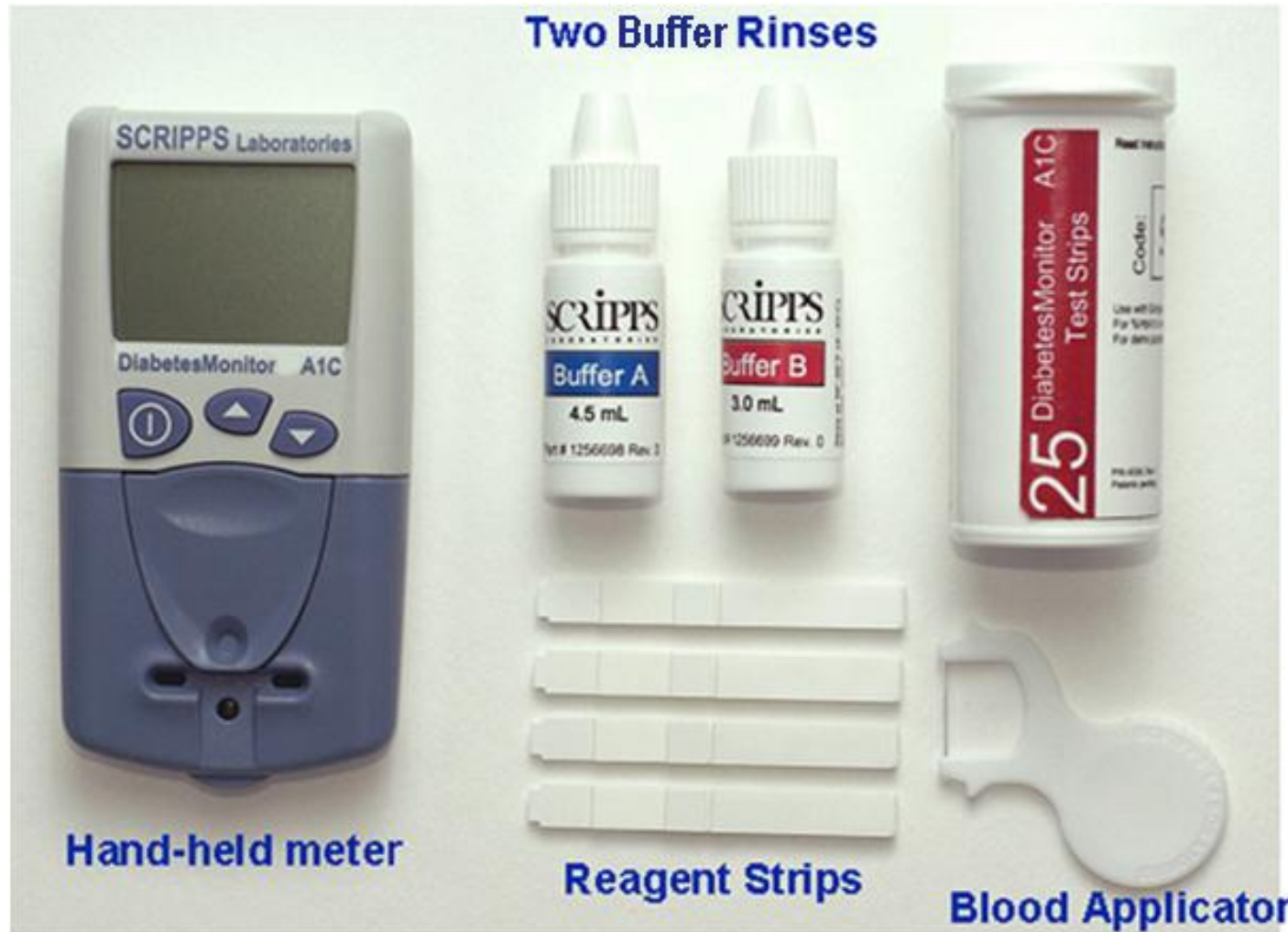
- The strip was purposefully developed to use low cost methods of manufacturing – a significant competitive advantage
 - Patent coverage of glycated hemoglobin technology:
US – 7,195,923 and 7,695,973
Foreign – Canada, China, Germany, France, Great Britain, Israel, India, Italy, Japan, Mexico, South Africa
 - Development stage – proof of concept has been demonstrated
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BUSINESS GOALS

Scripps has demonstrated proof of concept for this technology and desires to license it to companies interested in developing it into a finished product.

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- To show proof of concept, Scripps adapted the technology to a simple strip format for the separation and measurement of glycated and non-glycated hemoglobin
 - Measurements are made using a glucometer type reflectance meter using an LED at 430nm
 - A sample transfer device for the application of blood to the strip was designed (US patent 7,763,473)
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PROOF OF CONCEPT SYSTEM



ASSAY PROCEDURE



0 min

Sample blood from
fingerstick

~2 min

Place strip into meter
Add Buffer A, then blood

**Meter measures total
hemoglobin**

Add Buffer B

~4 min

**Meter measures glycated
hemoglobin, and displays
%HbA_{1c}**

ASSAY PROCEDURE cont'd.

Step Actions	Results
1. Add buffer A (acidic pH)	Buffer flows into membrane and meter measures the reference reflectance
2. Add blood sample	a. Red blood cells lyse b. Positively charged Hb and GHb bind ionically to ionized carboxyl groups. c. Excess sample is rinsed through to the sink d. Boronate groups do not bind (GHb).
3. Measure reflectance (430nm)	The reflectance is used to calculate the concentration of total hemoglobin bound to the membrane.
4. Add Buffer B (basic pH)	a. Hb and GHb lose their charge and release from the carboxyl groups b. The boronate groups bind GHb c. Hb is rinsed through the strip to the sink
5. Measure reflectance (430nm)	The reflectance is used to calculate the concentration of GHb bound to the membrane
6. Calculate %A1c	The ratio of total to glycated Hb concentrations is calculated and normalized to the reference method

ADVANTAGES OF SCRIPPS' TECHNOLOGY

- Proprietary boronate affinity method
 - Low cost system is possible
 - Small sample size: 2-3 μL fingerstick
 - No sample pre-treatment
 - Capillary and venous blood samples
 - Blood transfer device for fingerstick sampling
 - Short assay time: ~5 minutes
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ADVANTAGES OF SCRIPPS' TECHNOLOGY *cont'd.*

- Good stability at room temperature
 - Excellent correlation to reference methods
 - Very good precision – measurement of both glycated and total hemoglobin at the same site on the strip corrects for variations in strip components and manufacture
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LINEARITY

reference %HbA1c	measured %HbA1c	
	mean	cv
5.0	4.7	2.5%
6.6	6.7	1.8%
8.2	8.4	1.4%
9.8	9.7	3.4%
11.5	11.6	2.4%
13.1	13.1	2.5%
14.7	14.5	3.0%
16.3	16.3	3.2%

Precision is excellent over assay range

PRECISION: VENOUS BLOOD

%HbA1c						
Day 1	4.6	4.8	4.5	4.7	4.6	Average
	4.7	4.7	4.6	4.6	0.086	SD
	4.5	4.7	4.7	4.6	1.8	%CV
	4.7	4.6	4.6	4.6		
Day 2	4.6	4.8	4.6	4.8	4.7	Average
	4.8	4.8	4.7	4.7	0.090	SD
	4.6	4.8	4.8	4.6	1.9	%CV
	4.7	4.6	4.7	4.6		

Whole blood can be used directly, without pre-treatment

MULTI-DAY PRECISION

Normal Sample						
Day	Mean	CV		Day	Mean	CV
1	4.9	4.1%		11	4.8	2.2%
2	5.0	2.9%		12	5.0	4.3%
3	5.0	2.5%		13	5.0	1.8%
4	5.1	4.9%		14	5.1	3.9%
5	5.3	2.1%		15	5.0	3.0%
6	5.2	2.4%		16	5.0	1.9%
7	4.8	3.1%		17	5.2	2.5%
8	5.1	3.3%		18	5.3	3.3%
9	5.3	1.8%		19	5.4	3.6%
10	4.9	2.5%		20	5.4	1.5%

MULTI-DAY PRECISION

Elevated Sample						
Day	Mean	CV		Day	Mean	CV
1	11.2	3.2%		11	11.2	2.2%
2	11.2	2.4%		12	11.5	3.2%
3	11.3	3.7%		13	11.4	0.7%
4	11.0	3.2%		14	10.8	2.2%
5	11.1	2.2%		15	11.4	4.1%
6	11.1	1.3%		16	11.5	3.6%
7	11.1	1.8%		17	11.1	2.7%
8	11.1	2.0%		18	11.1	3.9%
9	11.1	2.7%		19	11.2	2.2%
10	11.2	3.3%		20	11.2	1.1%

MULTI-DAY PRECISION

	Normal	Elevated
Days	20	20
Reps/day	8	8
Mean %HbA1c	5.1	11.2
Total SD	0.221	0.328
Within day SD	0.153	0.308
Between day SD	0.160	0.114
Total CV	4.4%	2.8%
Within day CV	3.0%	2.7%
Between day CV	3.1%	1.0%
P (CV \leq 4.0%)	0.170	1.000

INTERFERENCES

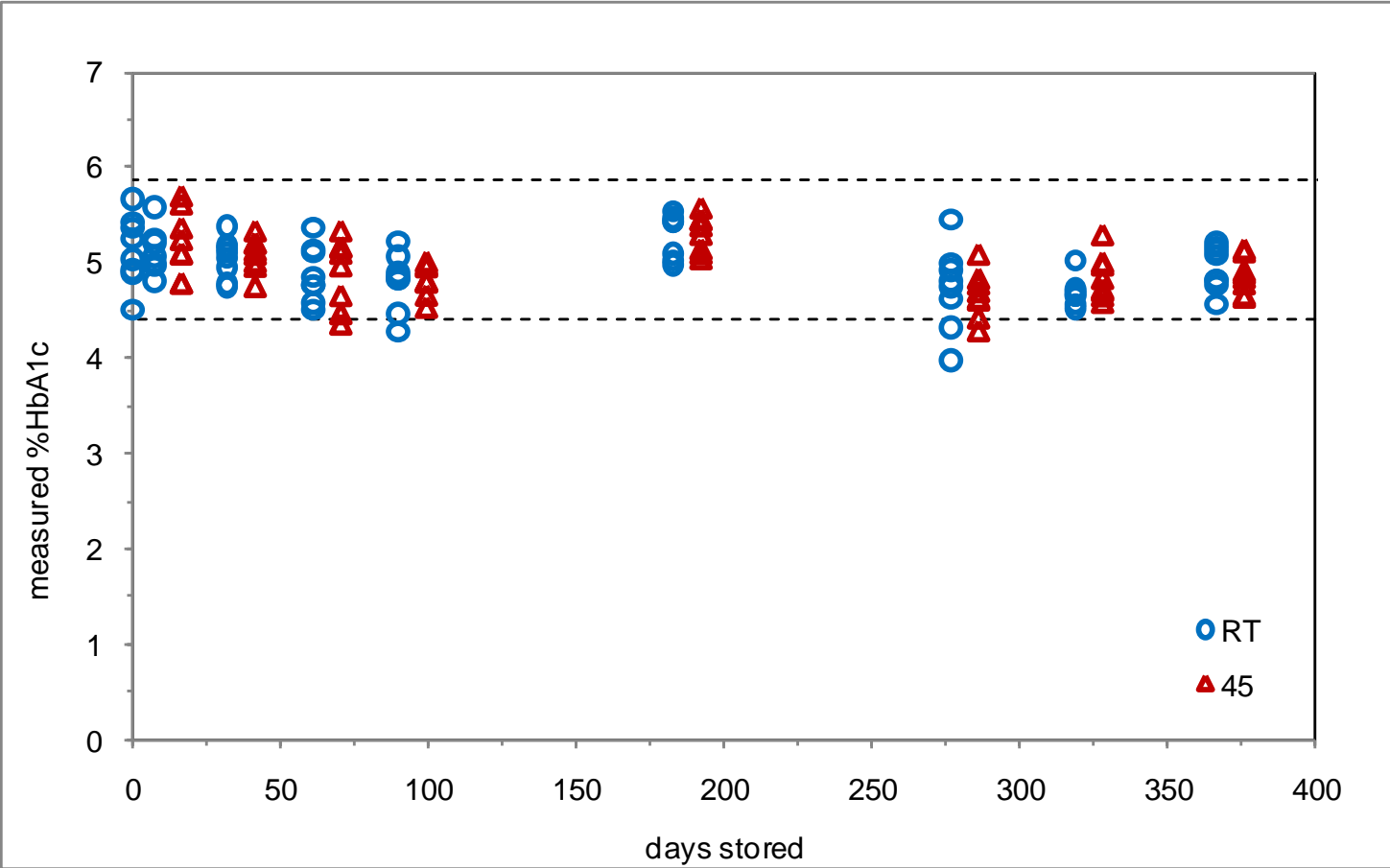
- Stable-A1c is measured – labile A1c (L-A1c) is not measured.
 - No sample pre-treatment – blood is transferred directly from finger to strip
 - Boronate affinity methods are not influenced by hemoglobin variants
 - Preliminary results show that Hb AE, AD, AC and AS do not alter the results
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INTERFERENCES - 2

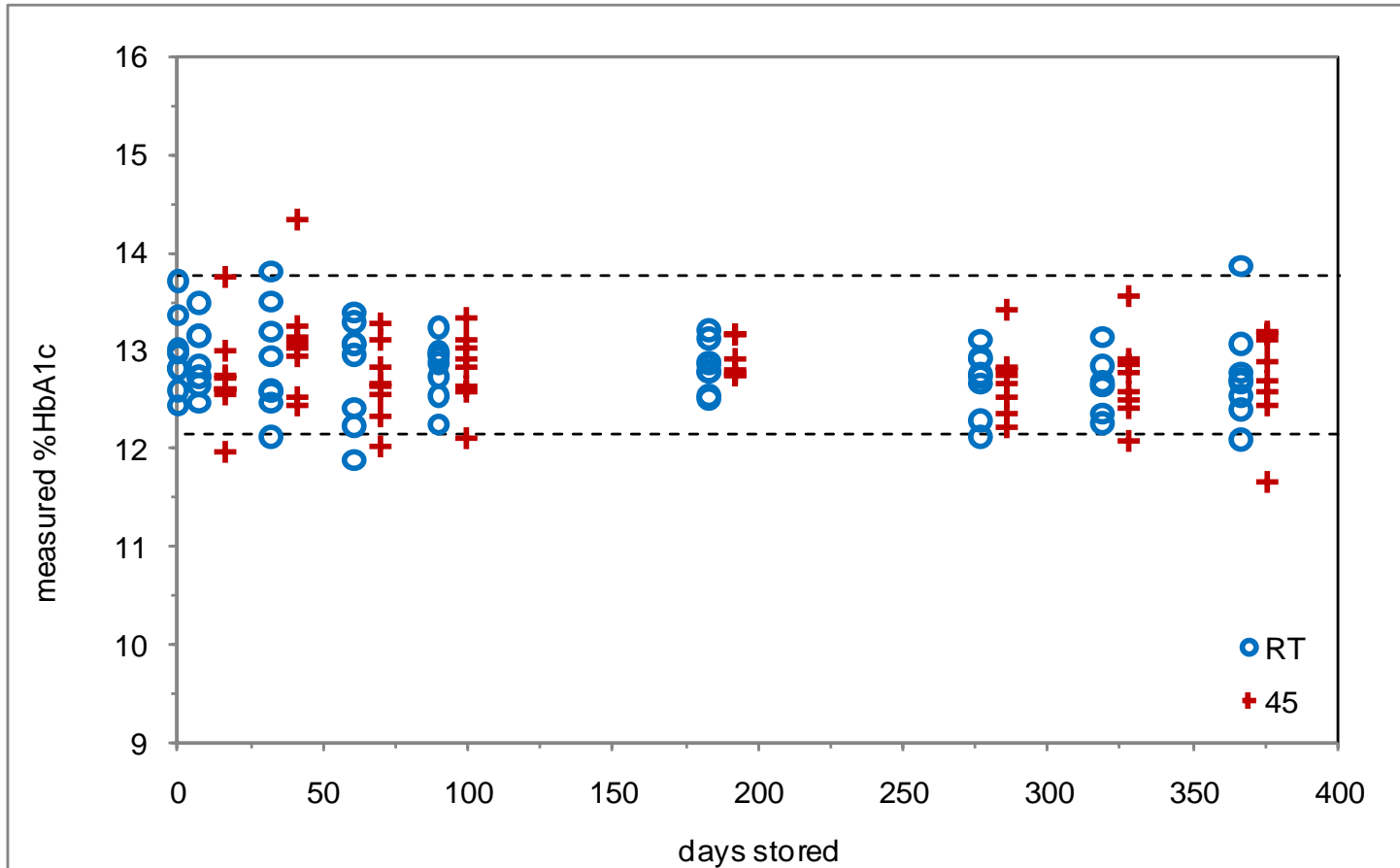
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- Preliminary testing has shown no interference by bilirubin and lipemia.
 - Expect little interferences by other blood components as most will wash through the read area.
 - Compounds must both bind and absorb light at 430 nm in order to interfere
 - Reduced binding is compensated for by measuring the ratio of total and glycated hemoglobin
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- The separation matrix is a chemically modified off-the-shelf membrane - contains no proteins or indicators
 - Expect stability to be excellent: two year shelf life with desiccation
 - Studies indicate good assay strip stability through 365 days at RT and 45°C
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STRIP STABILITY – 5.1% HbA1c



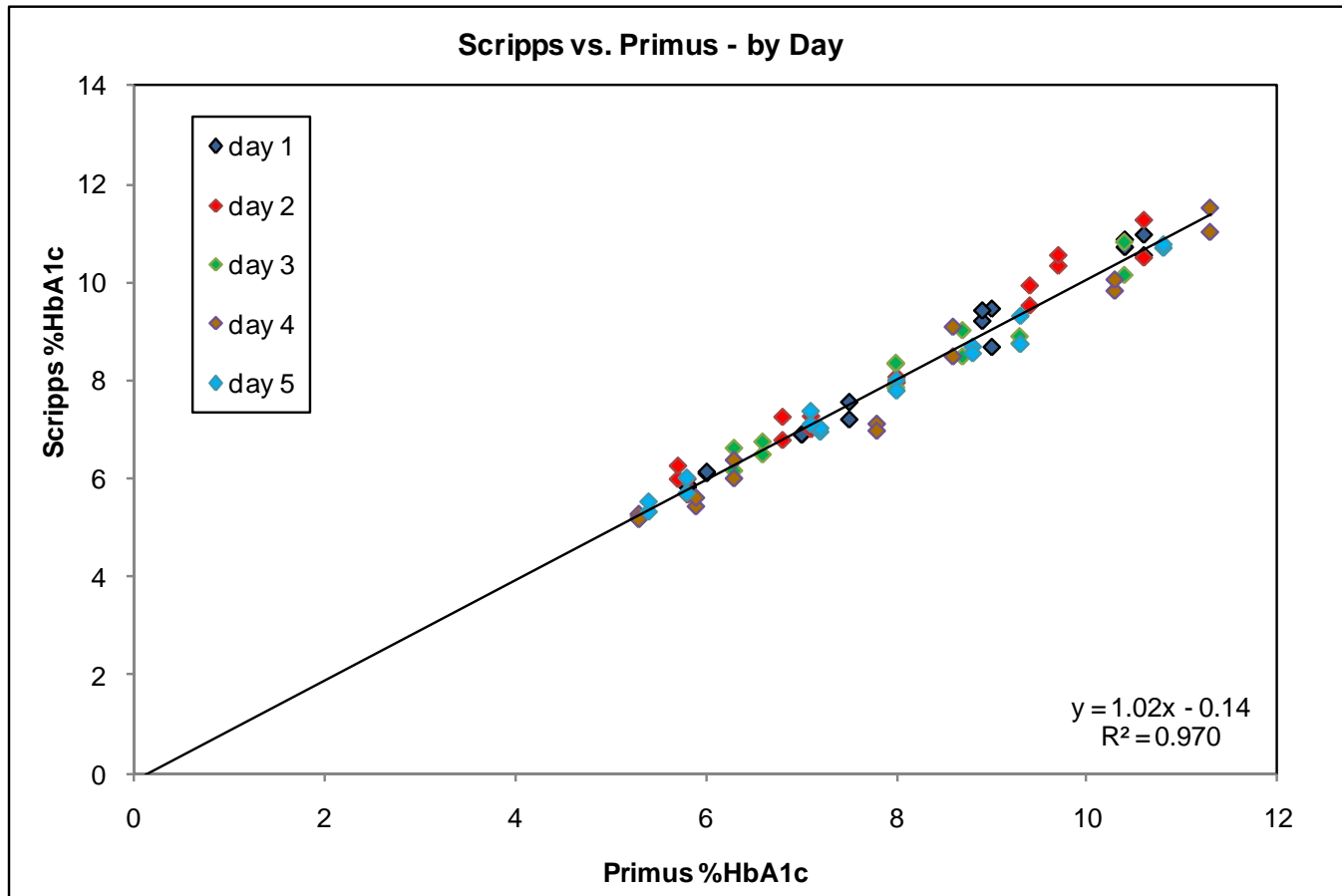
STRIP STABILITY – 13%HbA1c



CLINICAL CORRELATION: METHODOLOGY

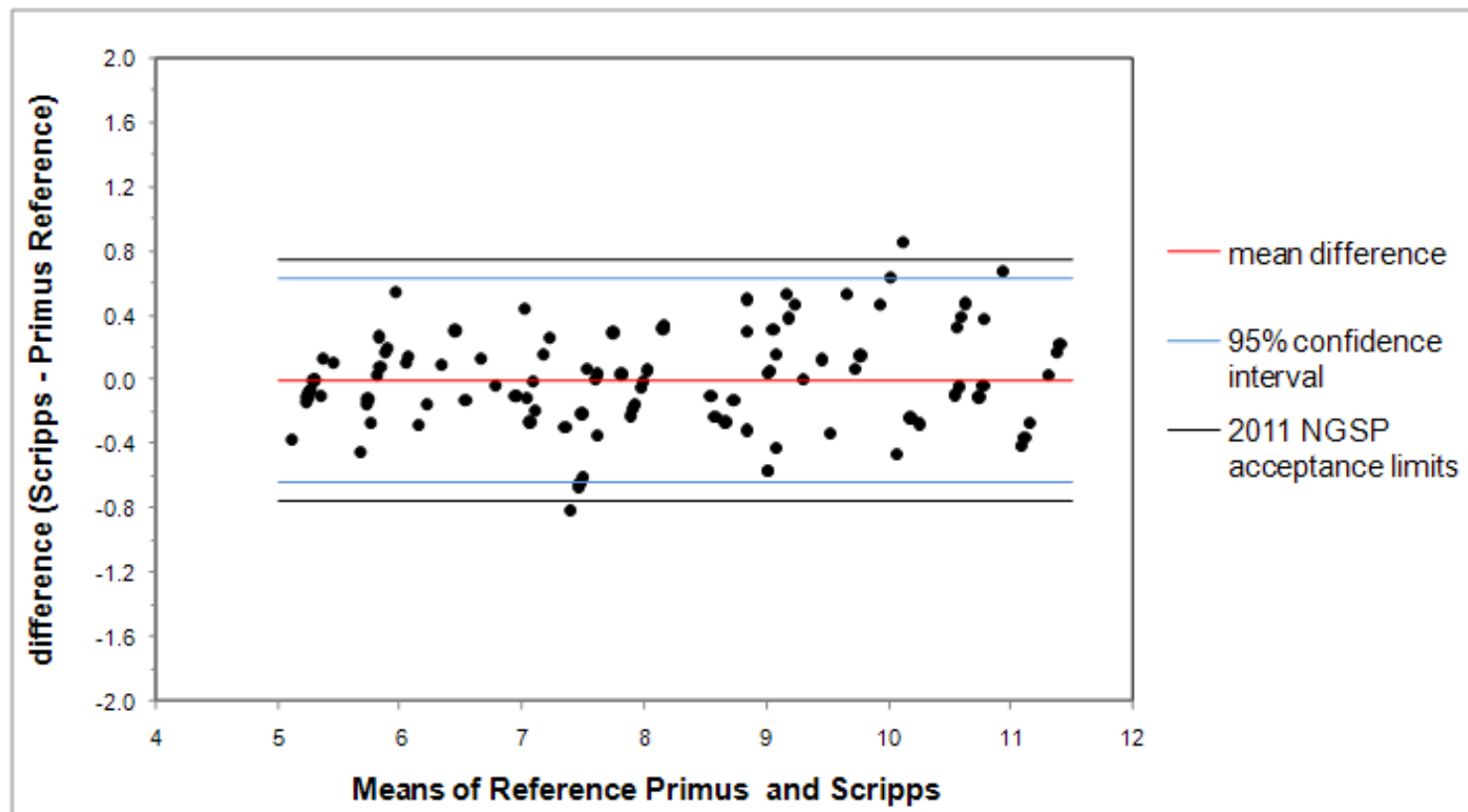
- 40 freshly collected, frozen clinical samples provided by the University of Missouri with reference values
 - NGSP (National Glycohemoglobin Standardization Program)
Secondary Reference Method: Primus: boronate affinity HPLC
 - Assayed by Scripps method in duplicate over 5 days – samples were applied without pretreatment
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CLINICAL CORRELATION: SCRIPPS vs PRIMUS (Affinity)



Linear correlation with little scatter

NGSP ASSESSMENT OF AGREEMENT TO PRIMUS AFFINITY



Confidence Interval	Y - (Mean X)	Acceptable Limit	Pass/Fail
Lower 95%	-0.65	-0.75	Pass
Upper 95%	0.67	+0.75	Pass

Passes the NGSP certification criteria

CLINICAL CORRELATION: SUMMARY

- Scripps assay correlated well to the Primus reference method.
 - The Scripps method passed published NGSP certification criteria for agreement with the Primus method.
 - There were no outliers with either comparison method giving a first indication that the Scripps method is not subject to interferences or matrix effects.
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