Evaluation Report

Eurolyser HbA1c Test Kit on

Smart and Cube Analyser

Locations

Location 1: Klinisk kemi University Hospital MAS Malmö
Operator: Dr. Benny Larsson
Date: June/July 2007

Location 2: Eurolyser Diagnostica GmbH
Operator: M. Gruber
Date: March/December 2013

Specimens

Malmö 2007: The specimens used for analysis were taken from the daily routine for sampling at the university laboratory in Malmö/Sweden.
Salzburg 2013: The specimens used for analysis were taken from the daily routine for sampling at a local hospital in Salzburg.

Equipment

- Eurolyser smart 700/340; serial number: Aa0118; provided by Mr. Werner Rademacher, ILS Sweden;
- Eurolyser HbA1c test kit; Ref. Code ST0110; LOT 710702-2;
- Eurolyser HbA1c test kit; Ref. Code ST0110; LOT 0613-1;
- Mono S;
- BioRad Variant Turbo;
- Menarini HA-8180;

Report revision

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Date: March 2014

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1. Introduction and scope

Designed for the quantitative determination of HbA1c in the point of care field the Eurolyser HbA1c test is easy to process and provides quick and reliable results in combination with the Eurolyser smart or CUBE Analyser.

The following evaluation of Eurolyser HbA1c test kit is valid for Eurolyser smart and CUBE Analysers.

Intended use:

The Eurolyser HbA1c test kit is for the quantitative in vitro determination of haemoglobin A1c in human blood on smart or CUBE laboratory photometer.

Principle:

The Eurolyser HbA1c analysis is based on the interaction of antigen and antibody to directly determine the HbA1c concentration in whole blood. Total haemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse antihuman HbA1c monoclonal antibody is added (R2), latex-HbA1c-mouse anti human HbA1c antibody complex is formed. Agglutination is formed when goat antimouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed on to the surface of latex particles. The amount of agglutination is measured as absorbance at 700 nm wavelength. The HbA1c value is obtained from a calibration curve which is stored on a RFID card.

The smart 700/340 Analyser is equipped with 2 photometers. Photometer 1 works with a wavelength of 700 nm. The Eurolyser HbA1c analysis is performed on the 700 nm wavelength photometer. The Eurolyser CUBE Analyser is equipped with a 700 nm wavelength photometer. Optical unit (photometer) and result calculation are technically equal in smart and CUBE Analyser.

2. Comparison Studies

Malmö (2007)

This comparison study has been performed at the “Klinisk kemi University Hospital MAS Malmö“ by Dr. Benny Larsson. It is based on the correlation between the results of HbA1c percentage (NGSP) levels measurements obtained on Eurolyser smart Analyser and two reference systems, the Mono S and the BioRad Variant Turbo.

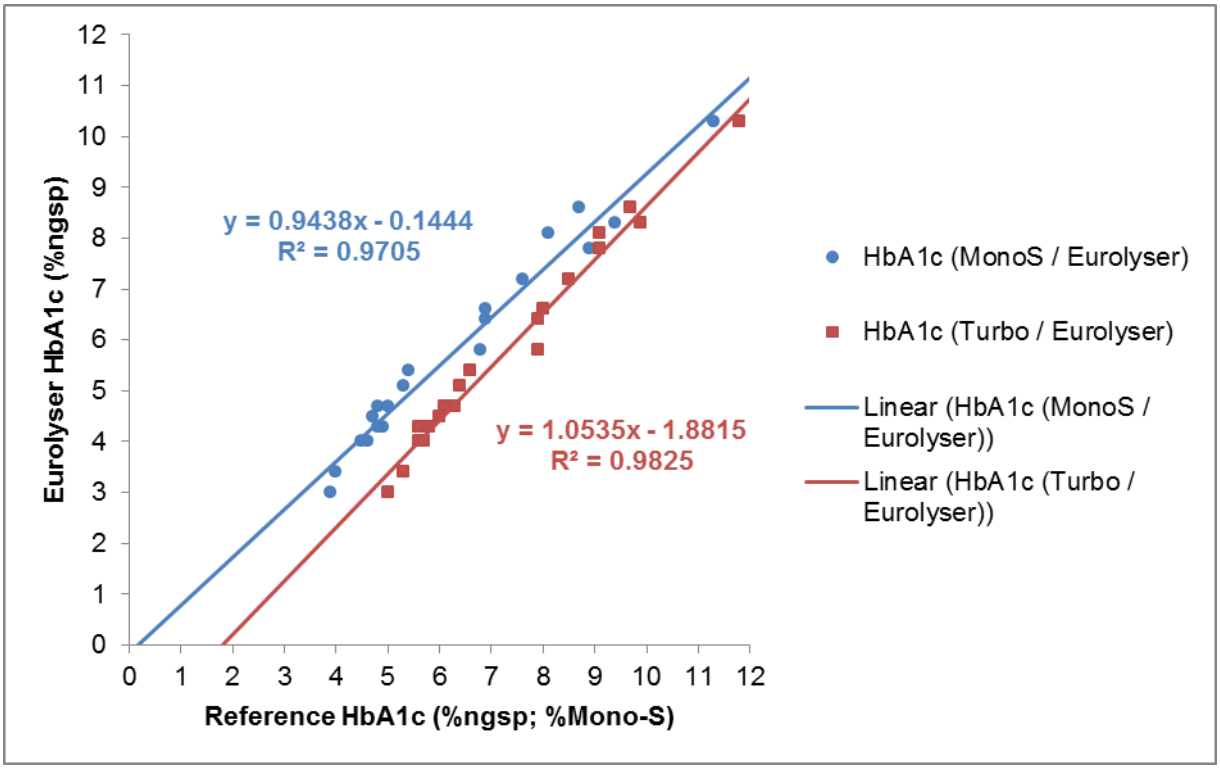
> The Mono S method is a chromatographic method based on the separation of glycated from non-glycated hemoglobin by ion exchange.
>
> The Bio-Rad VARIANT TURBO HbA1c system is intended for the percent determination of haemoglobin A1c in human whole blood using ion-exchange high-performance liquid chromatography (HPLC).

20 patient samples (EDTA whole blood), well allotted over the measurement range of Eurolyser HbA1c test kit (LOT 710702-2), have been analysed with Eurolyser smart 700/340, Mono S and Variant Turbo.

The acceptance criterion for this comparison study is a coefficient of determination **$R^2 > 0.9$** obtained from linear regression between Eurolyser HbA1c and a reference system.

Linear regression

Date	**2007-06-29**		
Parameter	**HbA1c**		
Instrument	**Eurolyser**	**MonoS**	**Turbo**
Unit	**%ngsp**	**%Mono-S**	**%ngsp**
Sample 1	8.1	8.1	9.1
Sample 2	4.7	5.0	6.3
Sample 3	4.5	4.7	6.0
Sample 4	8.6	8.7	9.7
Sample 5	5.8	6.8	7.9
Sample 6	6.4	6.9	7.9
Sample 7	6.6	6.9	8.0
Sample 8	5.1	5.3	6.4
Sample 9	4.3	4.9	5.8
Sample 10	4.7	4.8	6.1
Sample 11	5.4	5.4	6.6
Sample 12	4.3	4.8	5.6
Sample 13	4.0	4.5	5.6
Sample 14	7.2	7.6	8.5
Sample 15	4.0	4.6	5.7
Sample 16	3.0	3.9	5.0
Sample 17	10.3	11.3	11.8
Sample 18	7.8	8.9	9.1
Sample 19	8.3	9.4	9.9
Sample 20	3.4	4.0	5.3



The result for the correlation between Mono S and Eurolyser HbA1c is the linear regression function y(Eurolyser HbA1c) = 0.9438x(Mono S) – 0.1444 and a **R^2 = 0.9705**. Note that the Eurolyser HbA1c was lower than the Mono S though Eurolyser HbA1c was calibrated according to NGSP.

The result for the correlation between Variant Turbo and Eurolyser HbA1c is the linear regression function y(Eurolyser HbA1c) = 1.0535x(Turbo) – 1.8815 and a **R^2 = 0.9825**. Note that the Eurolyser HbA1c was lower than the Variant Turbo. Calibrators which are used for Eurolyser HbA1c are based on a different matrix than the Variant Turbo's.

Salzburg (2013)

This comparison study has been performed using samples from a local hospital in Salzburg. It is based on the correlation between the results of HbA1c percentage (NGSP) levels measurements obtained on Eurolyser smart Analyser and a reference system, the Menarini HA-8180.

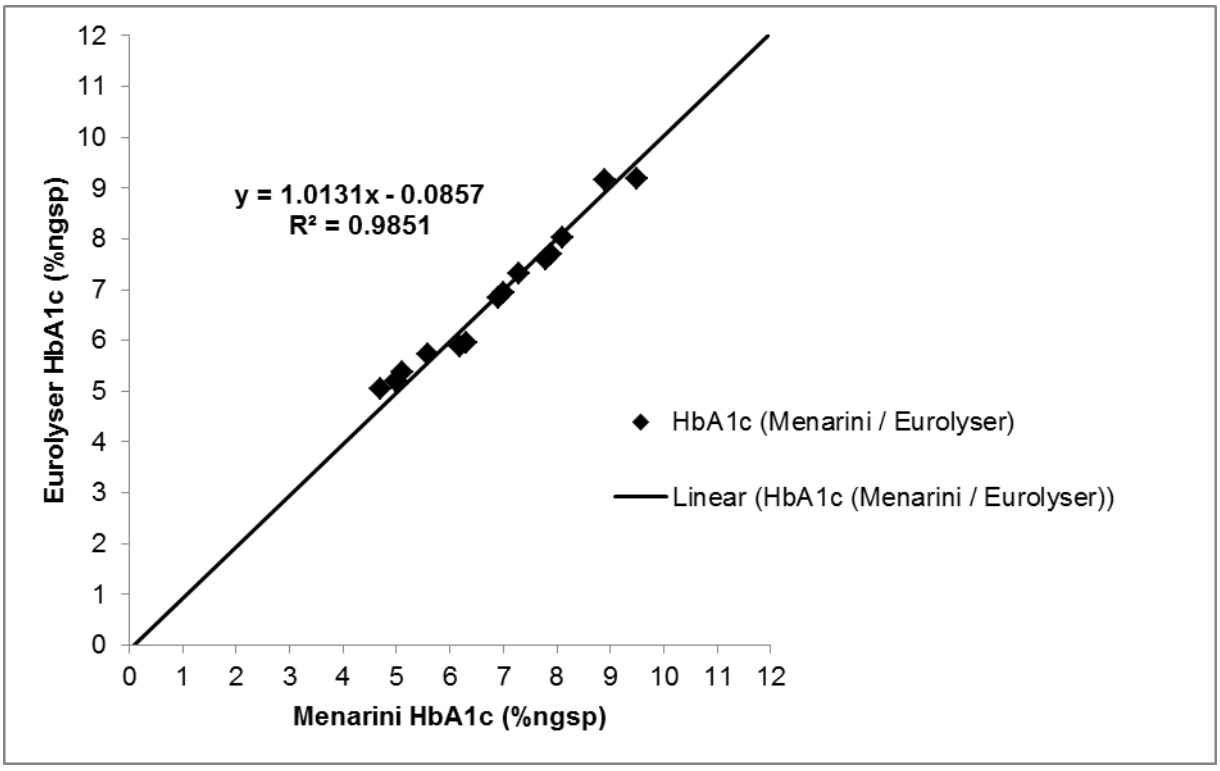
> The principle of Menarini HA-8180 is the reversed-phase cation exchange chromatography of whole blood samples.

15 patient samples (EDTA whole blood), well allotted over the measurement range of Eurolyser HbA1c test kit (LOT 0613-1), have been analysed with Eurolyser smart 700/340 and Menarini HA-8180.

The acceptance criterion for this comparison study is a coefficient of determination **R^2 > 0.9** obtained from linear regression between Eurolyser HbA1c and the reference system.

Linear regression

Date	2013-03-19	
Parameter	HbA1c	
Instrument	Eurolyser	Menarini
Unit	%ngsp	%ngsp
Sample 1	5.1	4.7
Sample 2	5.2	5.0
Sample 3	5.4	5.1
Sample 4	5.7	5.6
Sample 5	5.9	6.2
Sample 6	6.0	6.3
Sample 7	6.8	6.9
Sample 8	6.9	7.0
Sample 9	7.3	7.3
Sample 10	7.6	7.8
Sample 11	7.7	7.9
Sample 12	8.0	8.1
Sample 13	9.1	8.9
Sample 14	9.2	9.5
Sample 15	13.4	12.9



The result for the correlation between Menarini and Eurolyser HbA1c is the linear regression function y(Eurolyser HbA1c) = 1.0131x(Menarini) – 0.0857 and a **R^2 = 0.9851**.

3. Imprecision “within-run”

The imprecision “within-run” of Eurolyser HbA1c has been obtained through 10 measurements of one specimen.

Operator	**BL**
Date	**2007-06-26**
Parameter	**HbA1c**
Unit	**%ngsp**
Meas 1	5.7
Meas 2	5.6
Meas 3	5.6
Meas 4	5.6
Meas 5	5.7
Meas 6	5.5
Meas 7	5.7
Meas 8	5.7
Meas 9	5.6
Meas 10	5.7
Mean	5.6
Std	0.070
%CV	**1.2**

As degree of the imprecision „within-run“, the percentage of the coefficient of variation is 1.2 %.

4. Imprecision “day-to-day” / Reproducibility

The imprecision “day-to-day” of Eurolyser HbA1c has been obtained through the measurement of several specimens at different known concentrations of HbA1c on 5 consecutive days. The following results can be used in order to evaluate the reproducibility.

Summary	**2007-07-02**	**to**	**2007-07-06**
Level	**Low**	**Mid**	**High**
Unit	**%ngsp**	**%ngsp**	**%ngsp**
Day 1 - 1/3	4.2	7.5	9.7
Day 1 - 2/3	4.4	7.5	9.5
Day 1 - 3/3	4.2	7.5	9.6
Day 2 - 1/3	4.1	7.2	9.2
Day 2 - 2/3	4.5	7.3	9.6
Day 2 - 3/3	4.4	7.3	9.5
Day 3 - 1/3	4.3	6.8	9.7
Day 3 - 2/3	4.3	7.1	9.0
Day 3 - 3/3	4.0	7.3	9.4
Day 4 - 1/3	4.0	7.3	9.2
Day 4 - 2/3	3.8	7.5	9.1
Day 4 - 3/3	4.4	7.4	9.3
Day 5 - 1/3	4.5	7.3	9.0
Day 5 - 2/3	3.9	7.4	9.2
Day 5 - 3/3	4.1	7.4	9.4
Mean	4.2	7.3	9.4
Std	0.22	0.19	0.24
%CV	**5.2**	**2.5**	**2.5**

The results for the imprecision “day-to-day” of Eurolyser HbA1c are as follows at three different levels:

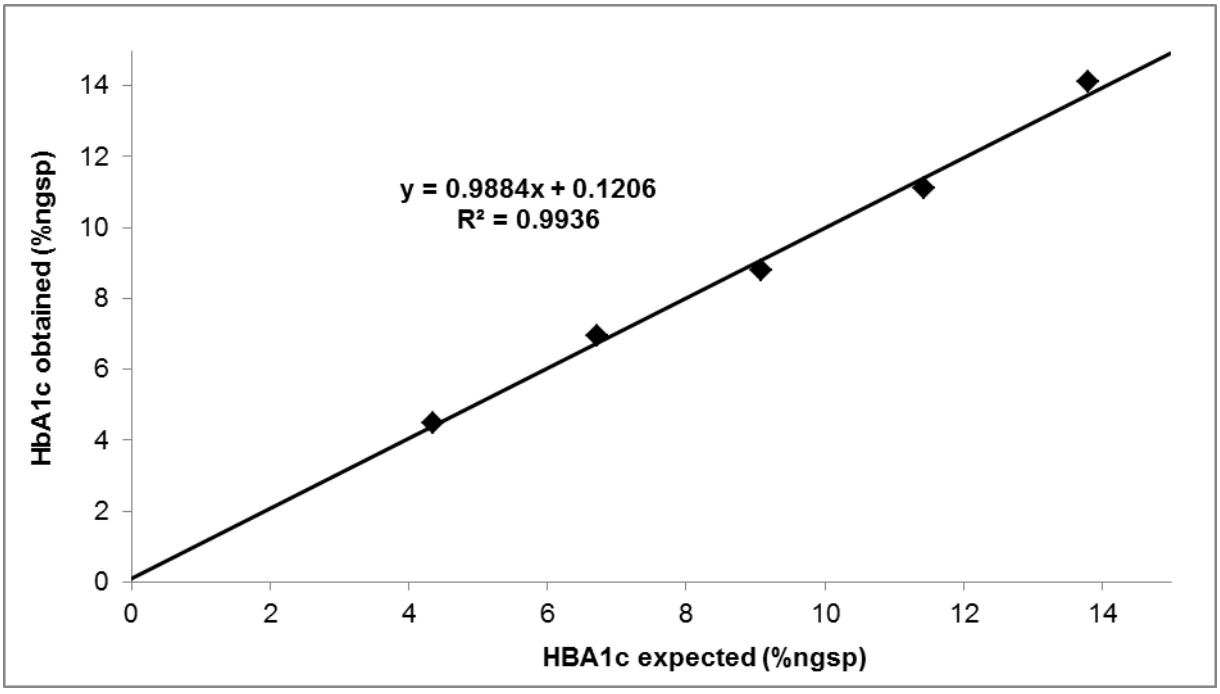
Low (mean: 4.2 %ngsp; n = 15)**:**	**CV = 5.2 %**
Mid (mean: 7.3 %ngsp; n = 15)**:**	**CV = 2.5 %**
High (mean: 9.4 %ngsp; n = 15)**:**	**CV = 2.5 %**

5. Linearity Study

Salzburg (2013)

Five samples have been used to obtain linearity data. The acceptance criterion is a linear regression with a coefficient of determination **$R^2 > 0.9$** between expected and recovered (obtained) values.
The dilution was prepared by diluting 2 samples (low and high) with a concentration of 4.35 %ngsp and 13.8 %ngsp. The linearity set was made according to Clinical Laboratory Standards Institute NCCLS EP-6A as follows:

Dilution	HbA1c expected	Eurolyser HbA1c obtained	HbA1c error	Recovery
-	%ngsp	%ngsp; mean: n = 4	%ngsp	%
Sample low 1:0	4.35	4.50	0.150	103.4
3:1 (SL:SH)	6.71	6.95	0.238	103.5
1:1 (SL:SH)	9.08	8.80	-0.275	97.0
1:3 (SL:SH)	11.44	11.10	-0.338	97.0
Sample high 1:0	13.80	14.10	0.300	102.2



The result for the correlation between expected and recovered (obtained) values for Eurolyser HbA1c is the linear regression function y(obtained) = 0.9884x(expected) + 0.1206 and a **$R^2 = 0.9936$**.

6. Interferences of the Eurolyser HbA1c Assay

Bilirubin to 50mg/dl, ascorbic acid to 50mg/dl, triglycerides to 2000mg/dl, carbamylated Hb to 7.5mmol/L and acetylated Hb to 5.0mmol/L do not interfere in this assay.

It has been reported that results may be inconsistent in patients who have the following conditions: Opiate addiction, lead-poisoning, alcoholism, ingestion of large doses of aspirin.

It has been reported that elevated levels of HbF may lead to underestimation of HbA1c. Also, it has been reported that labile intermediates (Schiff base) do not interfere with HbA1c determination by immunoassay.

Uremia does not interfere with HbA1c determination by immunoassay.

It has been determined that haemoglobin variants HbA2, HbC and HbS do not interfere with this method.

Other very rare variants of haemoglobin (e.g. HbE) have not been assessed.

7. Limitations of the Eurolyser HbA1c Assay

This assay should not be used for the diagnosis of diabetes mellitus.

It has been reported that labile intermediates (Schiff base) are not detected by immunoassay.

8. Long Term Stability – Shelf-Life Experiment

Salzburg (finished 2013)

The test kit was tested in accordance to CLSI EP-25A. The test kits used for this study were chosen from the same lot. The test kits were stored according to storage conditions at 2 to 8 °C as well as given increased temperatures (details noted).

The study was set up to assess measurand drift over time. The duration of the study was limited to 9 months. There were P = 9 test points per test series roughly equally spaced over the duration of each study.

It is known that the product increases apparent measurand content over time. An increase of up to 20% was deemed acceptable (response ≤ 1.2). Data from studies are presented below as the mean % recovery in decimal units (response) for each test point (day). Samples were tested on the measurement systems of Eurolyser smart and CUBE Analyser.

Target criteria:

Levels	Target	Lower limit (LL)	Upper limit (UL)	LL	UL
-	%ngsp	%ngsp	%ngsp	%	%
Level1	5.7	4.57	6.83	-20	20
Level2	10.5	8.9	12.1	-15	15

Temperature levels:

Levels	°C	°K
T norm.	8 (2 - 8)	281.15
T accel.	25	298.15
T stress.	37	310.15

Raw data:

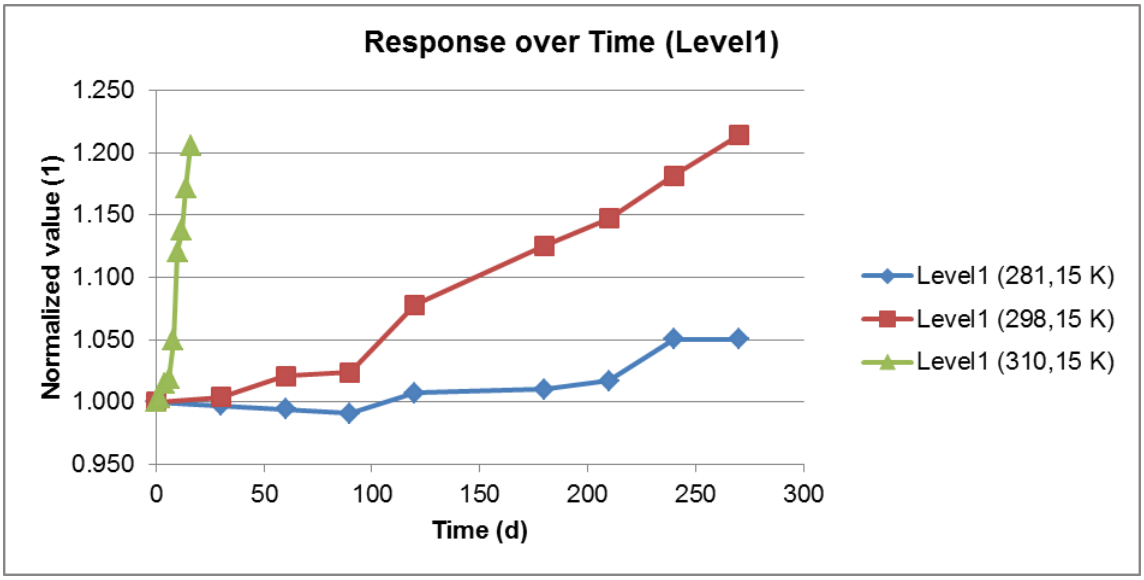
Day	Level1	Level1	Level2	Level2
-	stored at 8 °C	stored at 25 °C	stored at 8 °C	stored at 25 °C
d	%ngsp	%ngsp	%ngsp	%ngsp
0	5.7	5.7	10.5	10.5
30	5.7	5.7	10.7	10.8
60	5.7	5.8	10.6	10.8
90	5.6	5.8	10.6	11.1
120	5.7	6.1	10.8	11.3
180	5.8	6.4	10.8	11.1
210	5.8	6.5	10.8	12.1
240	6.0	6.7	10.7	13.1
270	6.0	6.9	10.6	13.1

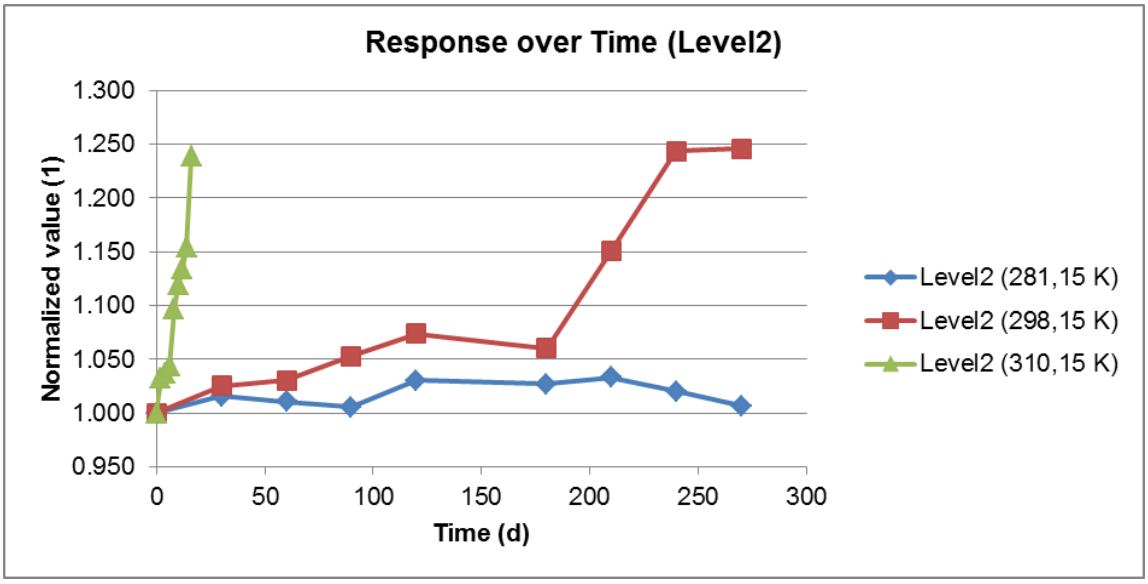
Day	Level1	Level2
-	stored at 37 °C	stored at 37 °C
d	%ngsp	%ngsp
0	5.7	10.5
2	5.7	10.8
4	5.8	10.9
6	5.8	11.0
8	6.0	11.5
10	6.4	11.7
12	6.5	11.9
14	6.7	12.1
16	6.9	13.0

Normalized data:

Day	Level1	Level1	Level2	Level2
-	stored at 281.15 K	stored at 298.15 K	stored at 281.15 K	stored at 298.15 K
d	1	1	1	1
0	1.000	1.000	1.000	1.000
30	0.997	1.004	1.016	1.025
60	0.994	1.021	1.010	1.030
90	0.991	1.024	1.006	1.053
120	1.007	1.078	1.030	1.074
180	1.010	1.125	1.027	1.060
210	1.017	1.147	1.033	1.151
240	1.050	1.182	1.020	1.244
270	1.050	1.214	1.007	1.246

Day	Level1	Level2
-	stored at 310.15 K	stored at 310.15 K
d	1	1
0	1.000	1.000
2	1.003	1.032
4	1.015	1.036
6	1.018	1.044
8	1.049	1.096
10	1.120	1.119
12	1.137	1.134
14	1.172	1.155
16	1.206	1.238





Calculation of estimated shelf-life:

Day	Level1	Level1	Level2	Level2
-	stored at 281.15 K	stored at 298.15 K	stored at 281.15 K	stored at 298.15 K
d	1	1	1	1
0	0.0000	0.0000	0.0000	0.0000
30	0.0030	-0.0039	-0.0158	-0.0252
60	0.0060	-0.0209	-0.0104	-0.0305
90	0.0091	-0.0238	-0.0055	-0.0528
120	-0.0070	-0.0777	-0.0300	-0.0736
180	-0.0103	-0.1253	-0.0271	-0.0603
210	-0.0166	-0.1468	-0.0328	-0.1506
240	-0.0502	-0.1815	-0.0202	-0.2436
270	-0.0504	-0.2140	-0.0066	-0.2460
k(T)	-0.000203239	-0.000833959	-5.12921E-05	-0.00089016

Day	Level1	Level2
-	stored at 310.15 K	stored at 310.15 K
d	1	1
0	0.0000	0.0000
2	-0.0032	-0.0325
4	-0.0151	-0.0364
6	-0.0183	-0.0440
8	-0.0493	-0.0962
10	-0.1204	-0.1189
12	-0.1373	-0.1340
14	-0.1715	-0.1547
16	-0.2057	-0.2384
k(T)	-0.013951639	-0.013250542

Level1:

Temp. levels	ln(k(T))	1/T
-	1	1/K
T norm.	-8.5011	0.00355682
T accel.	-7.0893	0.003354016
T stress.	-4.2722	0.003224246
Trend T norm.	**-8.7999**	**0.00355682**

Level2:

Temp. levels	ln(k(T))	1/T
-	1	1/K
T norm.	-9.8780	0.00355682
T accel.	-7.0241	0.003354016
T stress.	-4.3237	0.003224246
Trend T norm.	**-10.0145**	**0.00355682**

According to the Arrhenius Equation this results in the following estimated shelf life:

Level	Time
-	d
Level1	1200
Level2	3170

Summary

The results listed in the paragraphs above demonstrate that the test kit is stable for at least 39 *months*, stored at 2 - 8 °C. Nevertheless lot specific expiration dates – according to the data provided by the reagent manufacturers – are used according to the specifications.
Test kits will therefore be labeled with a maximum of ***12 months*** from manufacturing date, when stored at 2 - 8 °C.

9. Abbreviations

CV	…	Coefficient of variation
NGSP	…	National Glycohemoglobin Standardization Program
RFID	…	Radio Frequency Identification
Std	…	Standard deviation of the taken sample