C-EUBD
Committee Education in the
Use of Biomarkers in Diabetes

Report EurA1c 2016

HbA1c Trial
European EQA organisers

Definite Version, 19 July 2017

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I Introduction and Overview of Results

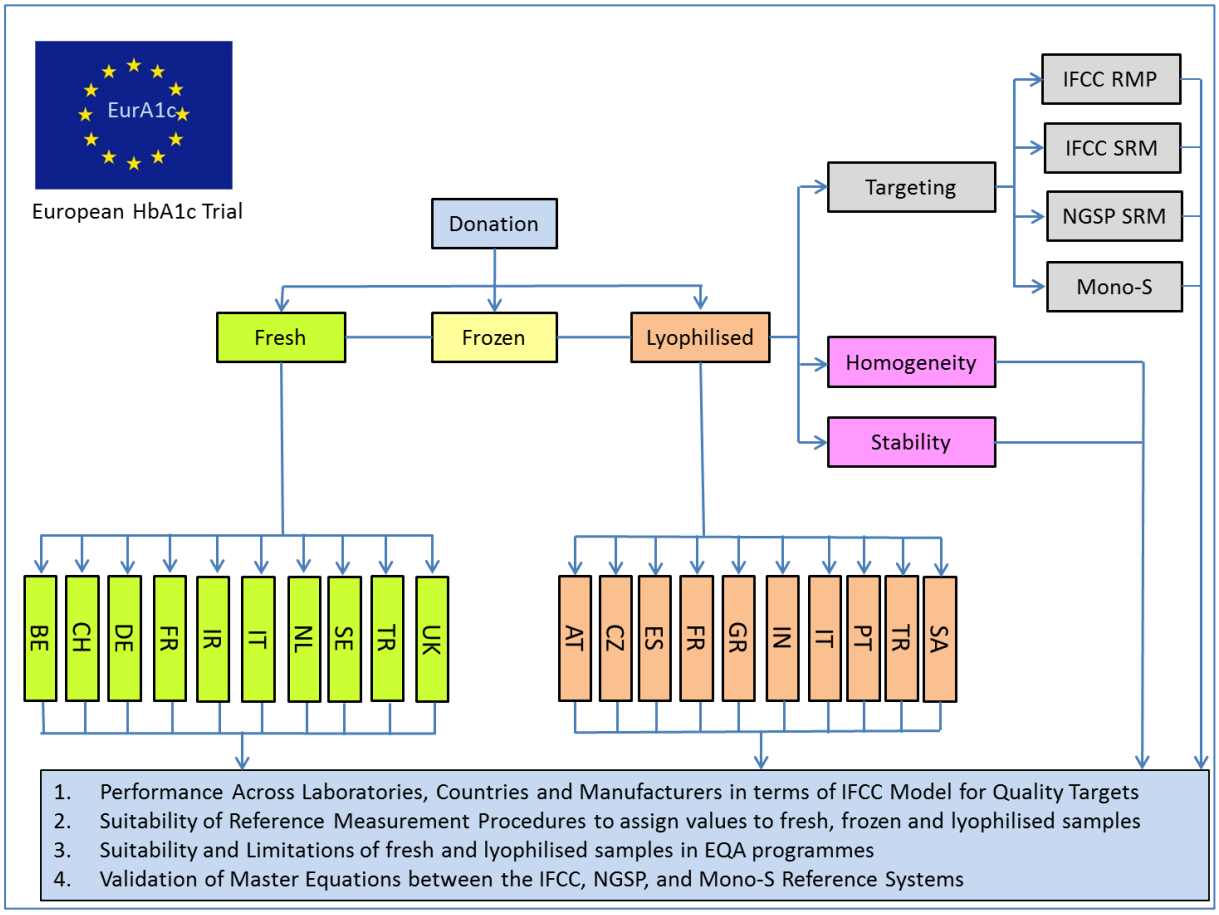
Introduction

In May 2016 seventeen EQA organisers decided to participate in the "EurA1c" project. The design is shown in figure 1. As indicated by the colours in the figure, four major topics are investigated:

- EQA fresh whole blood (green)
- EQA lyophilised (amber)
- Targeting (grey)
- Homogeneity and Stability (pink)

10 EQA organisers used fresh whole blood samples and 10 organisers used lyophilised samples (3 organisations used both fresh and lyophilised samples). In October 2016 the fresh whole blood samples were sent to the participants. From November 2016 up to April 2017 the lyophilised samples were assayed by the participants. This report is dealing with the results.

Figure 1. Design EurA1c Trial 2016



Confidentiality and Ownership

The results of the EurA1c project are owned by all EQA organisers. Previously we agreed that reports are confident and will not be communicated with participants and other third parties until there is the definite report (thus now; starting from 19 July 2017 you are free to communicate). We also agreed that results will be presented during EuroMedLab and the Satellite Symposium by (and on behalf of all organisers and the IFCC-EUBD) Cas Weykamp in June 2017. Finally a paper will be written with all EQA organisers as authors.

The time schedule is:

4 May:	Draft report sent to all who are involved in EurA1c 2016
13 June:	Presentation of results by Cas Weykamp at EuroMedLab (Session “Implementing and Maintaining Standardisation in Laboratory Medicine – Making the pieces work together to improve patient care and public health”)
17 June:	Presentation of results by Cas Weykamp at the EuroMedLab Satellite Symposium on Diabetes in Sounio (near Athens)
30 June:	Deadline for comments and remarks
19 July:	Final report sent to all who are involved
19 July:	Invitation to participate in EurA1c 2017 sent to all who are involved in EurA1c 2016 and additional EQA Organisers who are interested.
1 September:	Start writing paper

Value Assignment
To assign target values the two samples of the EurA1c project have been assayed with the approved IFCC Reference Measurement Procedure by 5 approved IFCC Network Laboratories.
For EurA1c 2016-1 the assigned value is 42.3 mmol/mol (expanded uncertainty 0.7 mmol/mol) and for EurA1c 2016-2 the assigned value is 57.9 mmol/mol (expanded uncertainty 0.9 mmol/mol).

Outliers
Outliers have been removed before calculation of the mean and between laboratory CV. Instead of using statistical criteria we only considered “blunders” as outliers. Criteria were results with a difference greater than 25% of the target values. In our opinion these results are a relevant picture of “real life”. In this way 45 results (1.0% of all results) have been excluded from the database.

Methods
In respect to methods, information given by the participants was not always clear. 123 Laboratories did not report a method at all. The majority of Siemens point-of-care instruments did not indicate whether they used a DCA 2000 or Vantage, reason why we combined their data in one group. For Roche part of the labs indicated the generation of the test kit and part the instrument used; therefore we combined all Roche results to one group. For Menarini/ARKRAY users it was also not clear whether the 8160VP or TP was used so we also combined these instruments in one group. The same treatment was applied to the various types of Bio-Rad Variant results.

Units
In some cases results were reported in NGSP units. We converted them to IFCC units using the Master Equation (NGSP = 0.0915IFCC + 2.15) prior to calculation of means, SDs and comparisons.

Summary Results
Table 1 shows the summary of results. The participating EQA organisers are ranked per country in alphabetical order. Results are given for the fresh whole blood and lyophilised hemolysate samples.

Table 1. Results of EurA1c 2016

Country	Fresh Whole Blood			Lyophilised Hemolysate		
	n	Mean Bias in mmol/mol	Between Laboratory CV	n	Mean Bias in mmol/mol	Between Laboratory CV
Austria				107	-1.0	5.3%
Belgium	139	+0.4	3.2%			
Czech Republic				70	-0.4	5.3%
France	135	+0.3	3.6%	132	-0.8	4.6%
Germany	652	-0.2	4.8%			
Greece				73	0.0	6.4%
International*				54	-0.4	4.9%
Ireland	30	+0.2	3.0%			
Italy	84	+0.8	4.5%	48	-0.2	3.1%
Netherlands	136	+0.2	3.4%			
Portugal				43	-0.5	3.8%
South Africa				2	-1.2	4.1%
Spain				76	-0.5	3.3%
Sweden	117	0.0	3.4%			
Switzerland	29	+0.4	5.8%			
Turkey	48	0.0	7.2%	45	-0.2	5.2%
United Kingdom	148	+0.6	3.5%			
Overall	1517	+0.2	4.4%	649	-0.5	4.9%

** Individual laboratories of a number of countries*

In total 2166 laboratories participated in EurA1c 2016: 1517 with fresh whole blood samples and 649 with lyophilised hemolysates. The results are very encouraging. The mean bias of all countries in the fresh whole blood programme is +0.2 mmol/mol and in the lyophilised hemolysate programme -0.5 mmol/mol. In none of the countries the mean bias exceeds 1 mmol/mol. The between laboratory CV is also quite satisfying. The mean CV in both programmes is 4.4 and 4.9% respectively. There are differences per country.

Differentiation Results
Table 1 shows a summary. Results can also be differentiated by sample and by specific manufacturers or even further by performances of manufacturers per country. This is done in part II (fresh whole blood) and part III (lyophilised hemolysates).

II Results EQA Fresh Whole Blood samples

Table 2 shows the results per country for each sample. Tables 3 and 4 show the results per manufacturer for manufacturers with 6 or more participants (table 3) and those with 5 or less participants (table 4).

Table 2. Results per Country for Fresh Whole Blood

Country	EurA1c 2016-1 Target 42.3 mmol/mol				EurA1c 2016-2 Target 57.9 mmol/mol				Mean 2 Samples	
	n	Mean	Bias	CV %	n	Mean	Bias	CV%	Bias	CV%
Belgium	139	42.4	+0.1	3.2	139	58.6	+0.7	3.1	+0.4	3.2
France	135	42.4	+0.1	3.6	135	58.4	+0.5	3.5	+0.3	3.6
Germany	652	41.8	-0.5	5.2	652	58.0	+0.1	4.3	-0.2	4.8
Ireland	30	42.0	-0.3	3.1	29	58.7	+0.8	2.9	+0.2	3.0
Italy	82	42.8	+0.5	4.7	84	59.1	+1.2	4.3	+0.8	4.5
Netherlands	136	42.2	-0.1	3.5	136	58.5	+0.6	3.3	+0.2	3.4
Sweden	115	42.0	-0.3	3.7	117	58.2	+0.3	3.1	0.0	3.4
Switzerland	29	42.5	+0.2	6.5	29	58.5	+0.6	5.1	+0.4	5.8
Turkey	48	41.8	-0.5	6.9	48	58.5	+0.6	7.4	0.0	7.2
UK	148	42.7	+0.4	3.6	148	58.8	+0.9	3.4	+0.6	3.5
Overall	1514	42.2	-0.1	4.6	1517	58.3	+0.4	4.2	+0.2	4.4

Table 3. Results per Manufacturer for Fresh Whole Blood (n>5)

Manufacturer	EurA1c 2016-1 Target 42.3 mmol/mol				EurA1c 2016-2 Target 57.9 mmol/mol				Mean 2 Samples	
	n	Mean	Bias	CV %	n	Mean	Bias	CV%	Bias	CV%
Abbott Architect Enzymatic	21	41.7	-0.6	1.5	21	58.3	+0.4	1.7	-0.1	1.6
Abbott Architect Immuno	6	41.3	-1.0	2.0	6	55.3	-2.6	6.1	-1.8	4.0
Abbott Other	6	43.6	+1.3	5.1	6	60.4	+2.3	4.0	+1.9	4.6
Alere Afinion	76	41.1	-1.2	3.5	74	57.7	-0.2	3.2	-0.7	3.4
Beckman Coulter AU	26	41.4	-0.9	5.6	26	57.6	-0.3	5.5	-0.6	5.6
Beckman Coulter UC DxC	15	40.7	-1.6	3.9	15	57.4	-0.5	3.1	-1.0	3.5
Bio-Rad D10	53	43.0	+0.7	4.6	53	58.8	+0.9	5.1	+0.8	4.8
Bio-Rad D 100	11	41.4	-0.9	1.6	11	57.1	-0.8	2.1	-0.8	1.8
Bio-Rad Variant	86	42.9	+0.6	4.1	86	59.1	+1.2	3.8	+0.9	4.0
Medinor	6	38.2	-4.1	12.1	6	52.6	-5.3	17.1	-4.7	14.6
Menarini HA-8160	91	42.5	+0.2	3.7	91	58.6	+0.7	3.1	+0.4	3.4
Menarini HA-8180	82	42.3	0.0	3.0	82	58.6	+0.7	2.9	+0.4	3.0
Not Known	123	42.2	-0.1	5.5	123	58.1	+0.2	5.1	0.0	5.3
Roche	288	40.9	-1.4	4.7	287	57.5	-0.4	4.1	-0.9	4.4
Sebia Capillarys 2	57	41.4	-0.9	2.9	57	57.9	0.0	2.2	-0.4	2.6
Sebia Capillarys 3	8	41.9	-0.4	2.6	8	58.2	+0.3	2.0	0.0	2.3
Sebia Minicap	10	41.2	-1.1	2.8	11	57.3	-0.6	2.2	-0.8	2.5
Siemens Advia	15	45.1	+2.8	5.6	15	62.1	+4.2	3.9	+3.5	4.8
Siemens DCA/Vantage	158	42.8	+0.5	3.4	160	58.6	+0.7	3.7	+0.6	3.6
Siemens Dimension	47	43.1	+0.8	4.3	46	57.0	-0.9	3.7	0.0	4.0
Siemens Other	13	42.0	-0.3	4.9	14	57.6	-0.3	3.6	-0.3	4.2
Tosoh G7	27	42.9	+0.6	5.1	27	59.5	+1.6	6.2	+1.1	5.6
Tosoh G8	234	43.0	+0.7	2.8	234	59.2	+1.3	2.4	+1.0	2.6
Trinity Premier Hb9210	27	42.8	+0.5	3.5	27	59.7	+1.8	4.0	+1.2	3.8

Table 4. Results per Manufacturer for Fresh Whole Blood (n < 6)

Manufacturer	EurA1c 2016-1 Target 42.3 mmol/mol				EurA1c 2016-2 Target 57.9 mmol/mol				Mean 2 Samples	
	n	Mean	Bias	CV %	n	Mean	Bias	CV%	Bias	CV%
Beckman Coulter other	2	40.5	-1.8	1.9	2	55.0	-2.9	7.8	-2.4	4.9
Beckman Coulter CE	1	40.6	-1.7		1	57.5	-0.4		-1.0	
Biokit ILAB 600	1	41.0	-1.3		1	51.0	-6.9		-4.1	
Bio-Rad other	1	42.8	+0.5		1	58.4	+0.5		+0.5	
Chromsystems Dionex	1	42.5	+0.2		1	64.3	+6.4		+3.3	
Erba XL 1000	1	42.0	-0.3		1	57.0	-0.9		-0.6	
Hitado	2	41.8	-0.5	0.8	2	54.7	-3.2	0.9	-1.9	0.8
ISE Hemo One	1	40.6	-1.7		1	59.8	+1.9		+0.1	
Menarini HA-8140	1	43.0	+0.7		1	51.0	-6.9		-3.1	
Menarini other	3	42.7	+0.4	1.5	3	59.6	+1.7	2.4	+1.0	2.0
Mono S	1	41.7	-0.6		1	56.5	-1.4		-1.0	
Sysmex	1	44.0	+1.7		1	60.0	+2.1		+1.9	
Trinity Tri-Stat	1	44.0	+1.7		1	61.0	+3.1		+2.4	

The results in tables 2 and 3 are very consistent: for each of the samples low biases per country and per manufacturer are achieved. Also quite acceptable are the between laboratory CVs. From tables 3 and 4 it can be seen that significant biases are most frequently seen for manufacturers with few participants. The bias of all countries is within the limits of uncertainty of the assigned value and this is true for most manufacturers. This allows the statement that countries and all major manufacturers are well standardized. Unfortunately quite a number (n=123) laboratories did not specify their method. These laboratories are in the group “Not Known”

Table 5 on the next page shows even more detailed results: performance is split per manufacturer, per country. Included are only manufacturers meeting 2 criteria: at least 6 participants per country and at least two countries with 6 participants. We marked high biases (>3 mmol/mol) and high between laboratory CVs >6%)

Table 5. Fresh Whole Blood Results per Manufacturer and Country

Method	n	HbA1c Low		HbA1c High		Mean	
		Bias	CV	Bias	CV	Bias	CV
Abbott Enzymatic							
Overall	21	-0.6	1.5	+0.4	1.7	-0.1	1.6
DE	8	-0.8	1.9	+0.4	2.6	-0.2	2.2
FR	7	-0.3	1.1	+0.4	0.7	0.0	0.9
Alere Afinion							
Overall	76	-1.2	3.5	-0.2	3.2	-0.7	3.4
DE	27	-0.8	2.3	+0.4	2.1	-0.2	2.2
SE	32	-1.3	3.8	-0.6	2.9	-0.9	3.4
Bio-Rad D10							
Overall	53	+0.7	4.6	+0.9	5.1	+0.8	4.8
DE	31	+0.4	3.9	+0.9	3.4	+0.6	3.6
FR	9	+1.5	5.9	+1.7	5.6	+1.6	5.8
Bio-Rad Variant							
Overall	86	+0.6	4.1	+1.2	3.8	+0.9	4.0
DE	34	+0.3	5.1	+0.7	4.1	+0.5	4.6
FR	19	+0.8	3.2	+1.5	2.6	+1.2	2.9
NL	6	+0.9	2.7	+3.9	5.2	+2.4	3.9
SE	13	-0.2	2.5	+0.5	2.3	+0.2	2.4
Menarini/ARKRAY HA-8160							
Overall	91	+0.2	3.7	+0.7	3.1	+0.4	3.4
BE	31	+0.4	3.4	+1.1	3.3	+0.7	3.4
DE	8	+0.6	1.3	+1.1	1.2	+0.8	1.2
IE	7	-0.6	2.3	+0.7	1.7	0.0	2.0
IT	21	+0.3	4.8	+0.6	3.5	+0.4	4.2
NL	20	-0.1	3.3	+0.3	3.0	+0.1	3.2
Menarini/ARKRAY HA-8180							
Overall	82	0.0	3.0	+0.7	2.9	+0.4	3.0
BE	32	+0.1	2.7	+0.9	2.2	+0.5	2.4
IT	11	+0.1	2.6	+0.6	2.6	+0.3	2.6
NL	17	-0.1	2.6	+0.8	3.2	+0.3	2.9
UK	13	-0.5	4.7	+0.2	4.3	-0.2	4.5
Roche							
Overall	288	-1.4	4.7	-0.4	4.1	-0.9	4.4
CH	8	+0.7	10.2	+0.1	5.8	+0.4	8.0
DE	210	-1.6	4.5	-0.4	4.1	-1.0	4.3
FR	8	-1.0	4.1	-0.8	3.1	-0.9	3.6
NL	26	-1.5	2.8	-0.8	2.6	-1.1	2.7
SE	8	-1.7	2.9	-0.9	2.5	-1.3	2.7
TR	11	-1.2	6.3	+1.0	7.2	-0.1	6.8
UK	7	-1.1	3.4	-0.5	1.9	-0.8	2.7
Sebia Capillarys 2							
Overall	57	-0.9	2.9	0.0	2.2	-0.4	2.6
BE	11	-1.1	2.8	+0.6	2.6	-0.2	2.7
DE	7	-1.0	1.2	-0.8	1.9	-0.9	1.6
FR	25	-0.8	3.0	-0.1	2.0	-0.4	2.5
UK	6	-0.8	3.0	+0.1	2.2	-0.4	2.6
Siemens DCA/Vantage							
Overall	158	+0.5	3.4	+0.7	3.7	+0.6	3.6
DE	36	-0.2	3.9	-0.3	4.1	-0.2	4.0
IE	10	+0.1	3.2	+1.3	3.8	+0.7	3.5
NL	15	+0.2	3.5	+0.6	3.3	+0.4	3.4
SE	47	+0.6	3.0	+1.1	3.3	+0.8	3.2
UK	52	+0.9	2.8	+0.8	3.6	+0.8	3.2
Tosoh G8							
Overall	234	+0.7	2.8	+1.3	2.4	+1.0	2.6
BE	40	+0.6	2.3	+0.9	3.0	+0.8	2.6
DE	44	+0.7	3.3	+1.0	2.4	+0.8	2.8
FR	31	+0.3	1.9	+0.8	1.4	+0.6	1.6
IT	19	+1.5	3.4	+2.3	3.4	+1.9	3.4
NL	34	+0.9	2.6	+1.6	2.0	+1.2	2.3
SE	11	+0.2	2.9	+0.6	1.8	+0.4	2.3
TR	9	+1.2	4.6	+1.4	2.0	+1.3	3.3
UK	43	+0.9	1.8	+1.8	1.7	+1.4	1.8
Trinity Premier Hb9210							
Overall	27	+0.5	3.5	+1.8	4.0	+1.2	3.8
FR	10	+1.3	5.5	+3.6	3.9	+2.4	4.7
UK	8	+0.3	2.3	+1.4	2.0	+0.8	2.2

III Results EQA Lyophilised Hemolysate samples

Table 6 shows the results per country for each sample. Tables 7 and 8 show the results per manufacturer for manufacturers with 6 or more participants (table 7) and 5 or less participants (table 8).

Table 6. Results per Country for Lyophilised Hemolysate

Country	EurA1c 2016-1 Target 42.3 mmol/mol				EurA1c 2016-2 Target 57.9 mmol/mol				Mean 2 Samples	
	n	Mean	Bias	CV %	n	Mean	Bias	CV%	Bias	CV%
International*	49	42.1	-0.2	5.7	54	57.3	-0.6	4.2	-0.4	4.9
Czech Republic	70	42.0	-0.3	5.7	70	57.4	-0.5	4.9	-0.4	5.3
France	132	41.7	-0.6	5.3	132	56.9	-1.0	3.9	-0.8	4.6
Italy	48	42.1	-0.2	3.3	48	57.5	-0.4	2.9	-0.2	3.1
Turkey	44	42.1	-0.2	5.4	45	57.6	-0.3	4.9	-0.2	5.2
Greece	65	42.4	+0.1	6.3	73	57.7	-0.2	6.4	0.0	6.4
Austria	107	41.5	-0.8	5.9	107	56.7	-1.2	4.7	-1.0	5.3
Portugal	40	42.1	-0.2	3.0	43	57.1	-0.8	4.7	-0.5	3.8
Spain	76	41.9	-0.4	3.8	75	57.2	-0.7	2.8	-0.5	3.3
South Africa	2	40.0	-2.3	7.1	2	57.7	-0.2	1.2	-1.2	4.1
Overall	633	41.9	-0.4	5.2	649	57.2	-0.7	4.5	-0.5	4.9

** Individual laboratories of a number of countries*

Table 7. Results per Manufacturer for Lyophilised Hemolysate (n>5)

Manufacturer	EurA1c 2016-1 Target 42.3 mmol/mol				EurA1c 2016-2 Target 57.9 mmol/mol				Mean 2 Samples	
	n	Mean	Bias	CV %	n	Mean	Bias	CV%	Bias	CV%
Abbott Architect Enzymatic	24	38.5	-3.8	5.2	24	53.6	-4.3	6.8	-4.0	6.0
Beckman Coulter AU	7	43.7	+1.4	7.9	6	59.7	+1.8	5.0	+1.6	6.5
Bio-Rad D10	37	41.4	-0.9	5.5	36	56.4	-1.5	4.9	-1.2	5.2
Bio-Rad D 100	16	42.4	+0.1	2.1	19	57.1	-0.8	1.7	-0.3	1.9
Bio-Rad Variant	38	43.8	+1.5	5.8	40	59.1	+1.2	3.9	+1.3	4.8
Menarini HA-8160	87	42.0	-0.3	2.9	92	57.0	-0.9	3.0	-0.6	2.9
Menarini HA-8180	72	41.7	-0.6	3.9	72	57.0	-0.9	3.1	-0.7	3.5
Not Known	14	41.6	-0.7	8.7	18	57.0	-0.9	7.4	-0.8	8.1
Roche	100	41.9	-0.4	5.2	103	58.0	+0.1	4.7	-0.1	4.9
Sebia Capillarys 2	45	40.8	-1.5	2.5	46	56.5	-1.4	2.5	-1.4	2.5
Sebia Capillarys 3	9	41.3	-1.0	2.6	9	56.2	-1.7	1.6	-1.3	2.1
Siemens DCA/Vantage	6	46.4	+4.1	3.9	5	61.9	+4.0	3.3	+4.0	3.6
Siemens Dimension	17	44.0	+1.7	5.1	18	57.0	-0.9	4.2	+0.4	4.7
Tosoh G7	33	42.4	+0.1	4.8	34	57.0	-0.9	4.7	-0.4	4.7
Tosoh G8	85	41.8	-0.5	4.1	83	56.9	-1.0	3.8	-0.7	3.9
Trinity Premier Hb9210	16	41.5	-0.8	4.2	16	57.0	-0.9	3.2	-0.8	3.7

The results in tables 6 and 7 are consistent: for each of the samples low biases per country and per manufacturer are achieved. Also quite acceptable are the between laboratory CVs. From table 8 it can be seen that significant biases are only seen for manufacturers with few participants. It can be concluded that all countries and most major manufacturers are well standardized.

Table 8. Results per Manufacturer for Lyophilised Hemolysate (n < 6)

Manufacturer	EurA1c 2016-1 Target 42.3 mmol/mol				EurA1c 2016-2 Target 57.9 mmol/mol				Mean 2 Samples	
	n	Mean	Bias	CV %	n	Mean	Bias	CV%	Bias	CV%
Abbot Immuno Assays	1	45.0	+2.7		1	60.0	+1.1		+1.9	
Abbott Other	2	43.0	-0.7	0	2	59.6	+1.7	5.69	+0.5	2.8
Beckman C. P/ACE MDQ	1	41.0	-1.3		2	57.0	-0.9	0	-1.1	
Beckman Coulter UC DxC	1	43.2	+0.9		2	62.4	+4.5	0.94	+2.7	0.5
Bio Rad other	2	44.0	+1.7	0	2	59.5	+1.6	1.19	+1.6	0.6
Ceragem Labona Check	1	44.0	+1.7							
Medinor					1	63.0	+5.1			
Menarini HA-8140	1	40.0	-2.3		1	56.0	-1.9			
Menarini other	3	40.0	-2.3	2.5	3	55.0	-2.9	1.82	-2.6	2.2
Mindray	1	41.6	-0.7		1	59.7	+1.8		+0.5	
Sebia Minicap	5	42.2	-0.1	5.5	5	58.0	+0.1	6.00	0.0	5.7
Sekisui	1	36.0	-6.3		1	55.0	-2.9		-4.6	
Siemens Advia	2	45.5	+3.2	10.9	2	59.5	+1.6	10.70	+2.4	10.8
Siemens Other	1	43.0	+0.7		1	59.0	+1.1		+0.9	
Tosoh Other	4	43.7	+1.4	6.3	4	58.8	+0.9	5.13	+1.1	5.7
Trinity Ultra2	1	42.0	-0.3							

Table 9 on the next page shows even more detailed results: performance is split per manufacturer, per country. Included are only manufacturers with 6 or more participants in at least 2 countries. High biases (>3 mmol/mol) and high between laboratory CVs >6%) are marked. The high negative bias of Austrian labs using the Abbott enzymatic assay is remarkable and should be investigated.

Table 9. Lyophilised Hemolysate Results per Manufacturer and Country

Method	n	HbA1c Low		HbA1c High		Mean	
		Bias	CV	Bias	CV	Bias	CV
Abbott Enzymatic							
Overall	24	-3.8	5.2	-4.3	6.8	-4.0	6.0
AT	11	-5.5	2.9	-6.0	2.2	-5.8	2.6
FR	7	-2.2	3.0	-2.6	1.8	-2.4	2.4
Bio-Rad D10							
Overall	37	-0.9	5.5	-1.5	4.9	-1.2	5.2
CZ	12	-0.8	5.6	-1.4	4.6	-1.1	5.1
FR	7	-1.5	4.2	-2.1	3.2	-1.8	3.7
PT	6	+0.2	1.9	+1.1	5.2	+0.6	3.6
Menarini/ARKRAY HA-8160							
Overall	92	-0.3	2.9	-0.9	3.0	-0.6	2.9
AT	13	-0.3	1.4	-1.7	1.9	-1.0	1.6
CZ	8	0.0	4.6	+0.5	2.6	+0.2	3.6
ES	15	-0.2	1.3	-0.7	1.4	-0.4	1.4
GR	10	-1.2	3.3	-1.9	3.7	-1.6	3.5
IT	13	-0.5	3.3	-0.5	3.0	-0.5	3.2
PT	30	-0.2	3.3	-0.8	3.3	-0.5	3.3
Menarini/ARKRAY HA-8180							
Overall	72	-0.6	3.9	-0.9	3.2	-0.7	3.5
AT	17	-1.0	3.8	-1.3	3.2	-1.1	3.5
ES	25	-0.3	3.3	-0.4	2.1	-0.4	2.7
INT*	10	+0.7	1.6	-0.5	2.0	+0.1	1.8
IT	11	0.0	2.6	-0.1	2.4	0.0	2.5
Roche							
Overall	103	-0.4	5.2	+0.1	4.7	-0.1	4.9
AT	40	-0.3	4.6	-0.2	3.6	-0.2	4.1
CZ	6	+0.8	7.2	+2.1	6.2	+1.4	6.7
ES	6	-0.1	4.4	+0.9	3.9	+0.4	4.2
FR	8	-2.3	6.3	-1.1	4.1	-1.7	5.2
GR	23	-0.3	5.6	-0.2	5.8	-0.2	5.7
TR	10	+0.6	4.7	+1.4	5.6	+1.0	5.2
Sebia Capillarys 2							
Overall	46	-1.5	2.5	-1.4	2.5	-1.4	2.5
ES	6	-1.2	1.9	-2.0	1.6	-1.6	1.8
FR	23	-1.5	2.6	-1.5	2.2	-1.5	2.4
INT*	10	-1.5	3.2	-0.8	3.5	-1.2	3.4
Tosoh G7							
Overall	34	+0.1	4.8	-0.9	4.7	-0.4	4.7
CZ	13	-0.7	5.2	-1.7	3.8	-1.2	4.5
FR	6	+0.7	4.7	-1.6	3.5	-0.4	4.1
GR	6	+1.0	4.5	+1.3	6.9	+1.1	5.7
Tosoh G8							
Overall	85	-0.5	4.1	-1.0	3.8	-0.7	3.9
AT	6	+1.5	3.4	+0.9	2.0	+1.2	2.7
CZ	10	+0.5	4.5	+0.2	3.8	+0.3	4.2
ES	6	-0.9	1.5	-1.9	1.5	-1.4	1.5
FR	31	-1.3	3.1	-2.0	1.9	-1.6	2.5
INT*	8	-0.4	4.3	-1.6	5.3	-1.0	4.8
IT	9	-0.3	3.4	-0.2	2.7	-0.2	3.0
TR	13	-0.8	4.9	-1.0	4.3	-0.9	4.6

** Group of Individual laboratories of a number of countries*

IV Value Assignment (Targeting)

Preambule

This is a difficult part of the study with a lot of considerations and without simple straight forward results and explanations. Therefore it may be confusing.

The conundrum of the man with three watches

Once upon a time there was a man who had his first appointment with a woman in a prestigious restaurant. He was very nervous about being in time. He felt that being too early or too late would be a disaster. To be on the safe side he bought three watches. When the appointment came near he saw that the watches showed different times. Which of the three should he believe? He could not decide and in the end he did not go to the appointment at all …….

In EurA1c we are in a similar position although we have 39 watches instead of 3. The value of a sample is assigned with 5 IFCC Network labs and 8 IFCC SRLs. Apart from that this is done in three matrixes: fresh whole blood, frozen whole blood and lyophilised hemolysate. Thus (5+8) x 3 = 39. What should we believe as the true value? And why? There are several aspects to be considered:

a. Each assigned value has an uncertainty. Differences in value assignment are small and mostly within statistical limits or just outside.
b. There is doubt on proper sample handling of frozen whole blood and lyophilised hemolysate with the IFCC RMP.
c. There is doubt whether differences could be due to a matrix effect.
d. There is doubt on full stability of fresh whole blood in all situations; can be different from laboratory to laboratory.
e. A remarkable phenomenon is that lysis of erythrocytes proceeds faster in the refrigerator than at room temperature (impact on POCT instruments).
f. There are contradicting results. Example: in EurA1c 2016-2 the assigned value with the IFCC SRLs is 1.0 mmol higher than with IFCC RMP (statistically just significant) but this is not confirmed in EurA1c 2016-1
g. Could it be that in lyophylised hemolysates HbA1c is really lower than in the fresh whole blood where it was made from? It is speculated that when erythrocytes are washed, glucose drops to zero and that either the Schiff Base does not proceed to stable HbA1c or that there may even be a reverse reaction of HbA1c.
h. Quite a number of SRLs use the Tosoh G8 and without frequent maintenance results in fresh whole blood have a positive bias (hemolysates have not).

It is impossible to unravel the contribution of each of these aspects (if there is a contribution at all) to the differences in assigned values (if there are differences at all). To prevent that we “miss our appointment“ with all of you we decided to choose the mean value of 5 IFCC network laboratories in fresh whole blood as the best estimate of the true value. In future we will try to come to a better approach. A first step is that there will be a SOP for value assignment of frozen whole blood and lyophilised hemolysates with the IFCC RMP. In addition we will repeat all measurements in the next EurA1c to see if the differences are consistent.

Assigned values of EurA1c Samples.

The IFCC Reference Measurement Procedure (IFCC RMP) has been developed and evaluated for the measurement of HbA1c in fresh whole blood (Ref 1). In the EurA1c initiative the 2 fresh whole blood samples have been assayed in fourfold by five approved IFCC network laboratories. The mean value of those five network laboratories is considered the true (assigned) HbA1c concentration in mmol/mol with an expanded uncertainty calculated according to the guideline of the IFCC Network of Reference Laboratories.

Assigned Values
EurA1c 2016-1: 42.3 mmol/mol (expanded uncertainty at k=2 = 0.7 mmol/mol)
EurA1c 2016-2: 57.9 mmol/mol (expanded uncertainty at k=2 = 0.9 mmol/mol)

Consideration of Reference Systems other than the IFCC RMP and other sample matrixes than fresh whole blood.
As stated above the IFCC RMP has been developed for the measurement of HbA1c in fresh whole blood. However, values can also be assigned with a) IFCC SRLs (these are robust routine methods calibrated to the IFCC RMP), b) NGSP SRLs, and c) Mono S. Also value assignment can be done in samples with a different matrix: a) lyophilised hemolysate, and b) frozen whole blood.
One can ask the following questions:

1. Are values assigned with the IFCC SRL system reliable (thus equivalent to values assigned with the IFCC RMP)?
2. Are values assigned with the NGSP SRL system convertible to IFCC units (thus is the Master Equation IFCC NGSP still true)?
3. Are values assigned with the Mono S system convertible to IFCC units (thus is the Master Equation IFCC Mono S still true)?
4. Is value assignment in lyophilised and frozen samples with each of the methods within the respective reference systems reliable?

EurA1c was designed in such a way that these questions can be considered and possibly answered: from the same fresh whole blood pool three sample types have been manufactured: fresh whole blood, lyophilised hemolysate and frozen whole blood. Samples of these three matrixes have been assayed with the IFCC RMP (n = 5 labs), with IFCC SRLs (n=8), with NGSP SRLs (n=3), and with Mono S (n=1). This is summarised in table 10.

*Table 10. Design to investigate comparability of Reference Systems in fresh whole blood, lyophilised hemolysated and frozen whole blood**

Sample Matrix	IFCC RMP	IFCC SRL	NGSP SRL	Mono S
Fresh Whole Blood	True Value	?	?	?
Lyophilised Hemolysate	?	?	?	?
Frozen Whole Blood	?	?	?	?

** To investigate comparability all results are compared with the value assigned with the IFCC RMP in fresh whole blood*

Summary Raw Data

The raw data are summarised in table 11. In the first column samples are ranked according to HbA1c concentration and matrix. On top the samples with the low HbA1c concentration (Low HbA1c) with the matrixes fresh whole blood (Fresh = EurA1c 2016-1), lyophilised hemolysate (Lyoph = EurA1c 2016-3) and frozen whole blood (Frozen = EurA1c 2016-5). Following are the samples with the high HbA1c concentration (High HbA1c) with the same three matrixes. In the next columns are the results of the 17 individual reference methods, ranked per reference system. Explanation of the abbreviations is below the table. Results of IFCC RMP and IFCC SRLs are in mmol/mol. Results of NGSP and Mono S are in %. Each lab assayed the respective samples in fourfold and the results are the mean of these 4 measurements.

Table 11. Results Reference Measurement Systems, IFCC in mmol/mol and NGSP/Mono S in %

Sample	IFCC RMP					IFCC SRL								NGSP SRL			MonS
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17
	MS		CE			IEC			Affin		Immu	Enzy	Capp	IEC		Affin	IEC
	MS	MS	CE	CE	CE	G8	G8	8180	9210	9210	TQ	Abb	Seb	G8	G8	Ultra	MonS
Low HbA1c																	
Fresh (EurA1c 2016-1)	42.9	41.6	42.8	41.4	42.9	43.4	42.9	42.2	42.5	42.4	41.7	42.3	42.7	6.10	6.18	5.90	5.02
Lyoph (EurA1c 2016-3)	40.7	41.5	42.5	38.9	42.2	43.1	42.5	42.7	41.9	42.0	43.9	42.6	42.6	6.10	6.02	6.02	5.03
Frozen (EurA1c 2016-5)	40.4	41.6	42.6	41.6	42.9	43.3	42.5	42.0	41.8	42.0	42.0	41.7	42.3	6.10	6.12	6.02	5.04
High HbA1c																	
Fresh (EurA1c 2016-2)	57.4	57.5	58.8	57.8	57.8	59.7	58.5	58.7	59.3	59.8	57.4	58.4	59.1	7.52	7.70	7.42	6.42
Lyoph (EurA1c 2016-4)	55.8	56.5	58.2	54.1	56.6	58.2	57.3	58.4	57.5	58.2	58.9	57.4	57.4	7.48	7.52	7.55	6.35
Frozen (EurA1c 2016-6)	56.6	57.6	58.7	61.0	58.5	59.5	58.0	58.3	58.3	58.8	57.2	57.4	58.1	7.55	7.68	7.52	6.45

- *Abbreviation of Reference Systems: IFCC RMP = IFCC Reference Measurement Procedure; IFCC SRL = IFCC Secondary Reference Laboratories; NGSP = National Glycohemoglobin Standardisation Program Secondary Reference Laboratories; MonS = Pharmacia Mono S, the Swedish Reference System.*
- *Abbreviation of Method Principles: MS is IFCC RMP with Mass Spectrometry detection; CE = IFCC RMP with Capillary Electrophoresis detection; IEC = Ion Exchange Chromatography; Affin = Affinity Chromatography; Immu = Immunochemical method; Enzy = Enzymatic method, Capp = Capillary Electrophoresis.*
- *Abbreviation of Methods: G8 = Tosoh G8; 8180 = ARKRAY/Menarini HA 8180V; 9210 = Trinity Biotech Premier Hb9210; TQ = Roche Tina Quant Gen 3 on Cobas C513; Abb = Abbott Architect C4000 Enzymatic; Seb = Sebia Capillarys 2 Flex Piercing; Ultra = Trinity Biotech Ultra; MonS = Pharmacia Mono S.*

All Results in IFCC Units and Outlier Test

Due to the difference in units it is difficult to compare the results of NGSP and Mono S with IFCC RMP and IFCC SRL. To facilitate comparison, table 12 shows results of NGSP and Mono S systems converted to IFCC units with the respective Master Equations (Ref 1). In addition the colours indicate the respective reference systems. Explanation of abbreviations is as in table 11.

The Q-test (Ref 2) was used to identify outliers. Most extreme results (potential outliers considered at the level of a reference system) are the 38.9 (lab 4; EurA1c 2016-3), the 54.1 (Lab 4; EurA1c 2016-4), the 61.0 (Lab 4; EurA1c 2016-6), the 60.7 (Lab 15; EurA1c 2016-2), and the 60.4 (Lab 15; EurA1c 2016-6). However none of these suspicious results are statistical outliers and therefore they are maintained in the database.

*Table 12. Results Reference Measurement Systems of NGSP and Mono S converted to IFCC units with Master Equations**

Sample	IFCC RMP					IFCC SRL								NGSP SRL			MonS
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 16	Lab 17
	MS		CE			IEC			Affin		Immu	Enzy	Capp	IEC		Affin	IEC
	MS	MS	CE	CE	CE	G8	G8	8180	9210	9210	TQ	Abb	Seb	G8	G8	Ultra	MonS
Low HbA1c																	
Fresh (EurA1c 2016-1)	42.9	41.6	42.8	41.4	42.9	43.4	42.9	42.2	42.5	42.4	41.7	42.3	42.7	43.2	44.0	41.0	41.8
Lyoph (EurA1c 2016-3)	40.7	41.5	42.5	38.9	42.2	43.1	42.5	42.7	41.9	42.0	43.9	42.6	42.6	43.2	42.4	42.4	41.9
Frozen (EurA1c 2016-5)	40.4	41.6	42.6	41.6	42.9	43.3	42.5	42.0	41.8	42.0	42.0	41.7	42.3	43.2	43.4	42.4	42.0
High HbA1c																	
Fresh (EurA1c 2016-2)	57.4	57.5	58.8	57.8	57.8	59.7	58.5	58.7	59.3	59.8	57.4	58.4	59.1	58.7	60.7	57.6	56.5
Lyoph (EurA1c 2016-4)	55.8	56.5	58.2	54.1	56.6	58.2	57.3	58.4	57.5	58.2	58.9	57.4	57.4	58.3	58.7	59.0	55.7
Frozen (EurA1c 2016-6)	56.6	57.6	58.7	61.0	58.5	59.5	58.0	58.3	58.3	58.8	57.2	57.4	58.1	59.0	60.4	58.7	56.8

** Master Equation NGSP to IFCC: IFCC = 10.93xNGSP – 23.5 mmol/mol*
Master Equation Mono S to IFCC: IFCC = 10.45xMono S – 10.62 mmol/mol

Consideration of the impact of the Sample Matrix on the respective methods

In table 13 results are reorganised to analytical principles. For the IFCC RMP the Mass Spectrometry and Capillary Electrophoresis detection are presented in yellow and green respectively. The Ion Exchange Chromatography methods are grouped and given an amber colour. Affinity Chromatography is blue. The three analytical principles represented by only one lab (immunochemistry, enzymatic assay, capillary electrophoresis) have no colour.

To facilitate consideration of matrix effects of lyophilisation and freezing, the differences between the results in the lyophilised hemolysate and the frozen whole blood is calculated in table 14. Example: in table 13 it can be seen that lab 1 measures 42.9 mmol/mol in the fresh whole blood sample with low HbA1c (EurA1c 2016-1) and 40.7 mmol/mol in the lyophilised hemolysate sample with low HbA1c (EurA1c 2016-3). Then the impact of lyophilisation is the difference in outcome between lyophilised and fresh sample = 40.7 - 42.9 = -2.2 mmol/mol. This -2.2 is shown in table 14 (column Lab1; line EurA1c 2016-3).

Table 13. Lyophilisation and Freezing in relation to Value Assignment with the respective Reference Systems: HbA1c concentrations in mmol/mol

Sample	IFCC RMP					SRLs											
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 14	Lab 15	Lab 8	Lab 17	Lab 9	Lab 10	Lab 16	Lab 11	Lab 12	Lab 13
	MS		CE			Ion Exchange Chromatography						Affinity			Immu	Enzy	Capp
	MS	MS	CE	CE	CE	G8	G8	G8	G8	8180	MonS	9210	9210	Ultra	TQ	Abb	Seb
Low HbA1c																	
Fresh (EurA1c 2016-1)	42.9	41.6	42.8	41.4	42.9	43.4	42.9	43.2	44.0	42.2	41.8	42.5	42.4	41.0	41.7	42.3	42.7
Lyoph (EurA1c 2016-3)	40.7	41.5	42.5	38.9	42.2	43.1	42.5	43.2	42.4	42.7	41.9	41.9	42.0	42.4	43.9	42.6	42.6
Frozen (EurA1c2016-5)	40.4	41.6	42.6	41.6	42.9	43.3	42.5	43.2	43.4	42.0	42.0	41.8	42.0	42.4	42.0	41.7	42.3
High HbA1c																	
Fresh (EurA1c 2016-2)	57.4	57.5	58.8	57.8	57.8	59.7	58.5	58.7	60.7	58.7	56.5	59.3	59.8	57.6	57.4	58.4	59.1
Lyoph (EurA1c 2016-4)	55.8	56.5	58.2	54.1	56.6	58.2	57.3	58.3	58.7	58.4	55.7	57.5	58.2	59.0	58.9	57.4	57.4
Frozen (EurA1c 2016-6)	56.6	57.6	58.7	61.0	58.5	59.5	58.0	59.0	60.4	58.3	56.8	58.3	58.8	58.7	57.2	57.4	58.1

Table 14. Lyophilisation and Freezing in relation to Value Assignment with the respective Reference Systems: Difference between lyophilised/frozen samples and fresh whole blood samples with the same method in the same lab in mmol/mol.

Sample	IFCC RMP					SRLs											
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 14	Lab 15	Lab 8	Lab 17	Lab 9	Lab 10	Lab 16	Lab 11	Lab 12	Lab 13
	MS		CE			Ion Exchange Chromatography						Affinity			Immu	Enzy	Capp
	MS	MS	CE	CE	CE	G8	G8	G8	G8	8180	MonS	9210	9210	Ultra	TQ	Abb	Seb
Low HbA1c																	
Fresh (EurA1c 2016-1)																	
Lyoph (EurA1c 2016-3)	-2.2	-0.1	-0.3	-2.5	-0.7	-0.3	-0.4	0.0	-1.8	+0.5	+0.1	-0.6	-0.4	+1.4	+2.2	+0.3	-0.1
Frozen (EurA1c2016-5)	-2.5	0.0	-0.2	+0.2	0.0	-0.1	-0.4	0.0	-0.6	-0.2	+0.2	-0.7	-0.4	+1.4	+0.3	-0.6	-0.4
High HbA1c																	
Fresh (EurA1c 2016-2)																	
Lyoph (EurA1c 2016-4)	-1.6	-1.0	-0.6	-3.7	-1.2	-1.5	-1.2	-0.4	-2.0	-0.3	-0.8	-1.8	-1.6	+1.4	+1.5	-1.0	-1.7
Frozen (EurA1c 2016-6)	-0.8	+0.1	-0.1	+3.2	+0.7	-0.2	-0.5	+0.3	-0.3	-0.4	+0.3	-1.0	-1.0	+1.1	-0.2	-1.0	-1.0

Discussion
Differences in table 14 are relatively small. Still there are remarkable differences between laboratories operating the same analytical method. Of the two IFCC RMP labs with MS detection (yellow), lab 1 observes much more differences than lab 2. The same phenomenon is seen for the IFCC RMP labs with CE detection: hardly any difference for lab 3 but substantial differences for lab 4. One can speculate that there are matrix effects but given the differences between the individual labs one can also suggest that differences are due to the sample treatment and that sample treatment for lyophilised and frozen whole blood samples within the network should be standardized (this is also confirmed by the between laboratory SD of the network labs: in lyophilised and frozen samples higher than in fresh whole blood; see table 15). In the ion exchange group it is interesting to compare the four G8 labs. Of these, lab 14 observes hardly any differences between the matrixes but lab 15 observes substantial differences for the lyophilised samples. It is known that there is an issue with fresh samples on the Tosoh G8: when maintenance is not done frequently, results in fresh whole blood get higher (this is not seen in lyophilised samples). Thus one can question whether lyophilised samples have a matrix effect for the G8 or whether fresh whole blood samples have a matrix effect when maintenance of the instrument is not perfect. In the affinity group (blue) contradictive results are seen: both Premier Hb9210 labs tend to a negative bias but the Ultra tends to a positive bias. For the "single" methods (white) no comparison within the analytical principle can be made. Of these, immunochemistry tends to a small positive bias in lyophilised samples.

In summary:

a. differences in measured HbA1c in each of the matrixes are small for the respective methods
b. for the IFCC RMP with MS, the IFCC RMP with CE, ion exchange chromatography, and affinity chromatography observed differences require more research.
c. for immunochemistry a small positive effect of lyophilisation seems to be confirmed

Comparison of IFCC RMP and other Reference Systems
From the results above it can be concluded that none of the methods/labs should be excluded when considering the reference systems as a whole. Table 15 summarises the results per reference system in terms of the mean and the SD (SD = within laboratory SD for Mono S; between laboratory SD for all other systems).

To facilitate the discussion on the question "Can values be assigned with other systems than the IFCC RMP?" the difference in mean values of the reference systems and the IFCC RMP result in fresh whole blood is calculated and shown in table 16. In addition the t-value is calculated to estimate whether the difference is significant or not (Ref 3).
Example: With the IFCC RMP the mean result of the five laboratories in the fresh whole blood sample with the low HbA1c (EurA1c 2016-1) is 42.3 mmol/mol with a between laboratory SD of 0.8 mmol/mol. The mean result of the eight IFCC SRLs in this sample is 42.5 mmol/mol with a between laboratory SD of 0.5 mmol/mol. Then the difference is 42.5 - 42.3 = +0.2 mmol/mol. The t-value is derived from this difference, the number of labs (5 and 8) and the pooled SD of both groups is 0.52. The critical value at the 95% confidence interval is 2.20. The 0.52 is far below this 2.20 and therefore the difference between IFCC RMP and IFCC SRL is not significant. Significant differences are indicated in amber. It can be seen that there is a borderline issue for the IFCC RMP in the lyophilised sample with the high HbA1c. But this is not seen in the lyophilised sample with low HbA1c and this makes the impact of lyophilisation questionable. This should be further investigated. For the IFCC SRL methods there is an issue with the fresh whole blood sample with high HbA1c. But also not confirmed in the fresh whole blood sample with the low HbA1c. This might be related to the Tosoh G8. Substantial significance is seen for the Mono S in all samples with high HbA1c (see also next page, under Master Equations).

Master Equations

Quite often the question arises whether the master equations published in 2004 (Ref 1) are still valid. In table 15 the NGSP SRL and Mono S results are converted to IFCC units with these master equations. From the table it can be seen that in the fresh whole blood sample with the low HbA1c concentration (EurA1c 2016-1), the NGSP SRL mean exceeds the IFCC SRL mean with 0.2 mmol/mol (42.7 versus 42.5 mmol/mol); in the fresh whole blood sample with the high HbA1c (EurA1c 2016-2) the difference with the IFCC SRL is 0.1 mmol/mol (58.9 versus 59.0). Thus the mean of the two samples is very close in both samples, a firm confirmation of the validity of the master equation. For Mono S results in the high HbA1c sample are slightly but borderline lower than the IFCC RMP values.

Table 15. Mean results of the Reference Systems

Sample	**IFCC RMP** n = 5		**IFCC SRL** n = 8		**NGSP SRL** n = 3		**Mono S** n = 1	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fresh (EurA1c 2016-1)	42.3	0.8	42.5	0.5	42.7	1.6	41.8	0.2
Lyoph (EurA1c 2016-3)	41.2	1.4	42.7	0.6	42.7	0.5	41.9	0.5
Frozen (EurA1c 2016-5)	41.8	1.0	42.2	0.5	43.0	0.5	42.0	0.6
Fresh (EurA1c 2016-2)	57.9	0.6	58.9	0.8	59.0	1.6	56.5	0.3
Lyoph (EurA1c 2016-4)	56.2	1.5	57.9	0.6	58.7	0.4	55.7	0.6
Frozen (EurA1c 2016-6)	58.5	1.6	58.2	0.7	59.4	0.9	56.8	0.5

*Table 16. Difference in assigned value between a Reference System in any matrix and the assigned value with the IFCC RMP and t-values**

Sample	IFCC RMP (n = 5) versus IFCC RMP (n = 5) Critical t = 2.31		IFCC SRL (n = 8) versus IFCC RMP (n = 5) Critical t = 2.20		NGSP SRL (n = 3) versus IFCC RMP (n = 5) Critical t = 2.45		Mono S* (n = 1) versus IFCC RMP (n = 5) Critical t = 2.78	
	Difference	t	Difference	t	Difference	t	Difference	t
Fresh (EurA1c 2016-1)	0.0	0.00	+0.2	0.52	+0.4	0.43	-0.5	0.78
Lyoph (EurA1c 2016-3)	-1.1	1.23	+0.4	0.99	+0.4	0.81	-0.4	0.54
Frozen (EurA1c 2016-5)	-0.5	0.88	-0.1	0.26	+0.7	1.43	-0.3	0.38
Fresh (EurA1c 2016-2)	0.0	0.00	+1.0	2.46	+1.1	1.24	-1.4	2.77
Lyoph (EurA1c 2016-4)	-1.7	2.36	0.0	0.00	+0.8	1.68	-2.2	3.34
Frozen (EurA1c 2016-6)	+0.6	0.79	+0.3	0.81	+1.5	2.67	-1.1	1.82

** Critical t depends on degrees of freedom (Ref 3)*

Comparison between other Reference Systems

Table 16 shows that IFCC SRL and NGSP SRL tend to be a bit high in relation to the IFCC RMP and that Mono S tends to be low in relation to the IFCC RMP. Thus it is expected that the difference between IFCC SRL/NGSP SRL and Mono S may be substantial. This is calculated systematically for the fresh whole blood samples in table 17. One can ask why the -2.5 for Mono S versus NGSP SRL is not significant (t = 2.19) whereas the -2.4 difference Mono-S versus IFCC SRL is significant (t=3.76). The explanation is that the t-test depends on SDs and n. For NGSP SRL n is low and SD is high and that makes that the difference is not significant. This also illustrates the relative power of statistical tests.

Table 17. Differences and t-values for comparisons between other Reference Systems than IFCC RMP for fresh whole blood samples

Reference Systems	Fresh Whole Blood				
	Low HbA1c (EurA1c 2016-1)		High HbA1c (EurA1c 2016-2)		Critical t-factor
	Difference mmol/mol	t	Difference mmol/mol	t	
(Mono S) - (IFCC SRL)	-0.7	1.73	-2.4	3.76	2.36
(Mono S) - (NGSP SRL)	-0.9	0.59	-2.5	2.19	4.30
(IFCC SRL) - (NGSP SRL)	-0.2	0.25	-0.1	0.12	2.26

Summary.

The answers to the questions in the beginning of this document summarise the results.

1. Are values assigned with the IFCC SRL system reliable (thus equivalent to values assigned with the IFCC RMP)?
   Yes, though attention should be given to proper maintenance for the Tosoh G8.
2. Are values assigned with the NGSP SRL system convertible to IFCC units (thus is the Master Equation IFCC NGSP still true)?
   Yes.
3. Are values assigned with the Mono S system convertible to IFCC units (thus is the Master Equation IFCC Mono S still true)?
   Questionable; at the higher HbA1c level the master equation might be reconsidered.
4. Is value assignment in lyophilised and frozen samples with each of the methods within the respective reference systems reliable?
   Yes for the IFCC SRL and NGSP SRL system. The IFCC RMP assignment to the lyophilized samples has to be further investigated.

References

1. Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, Hoshino T, John WG, Kobold U, Little R, Mosca A, Mauri P, Paroni R, Susanto F, Takei I, Thienpont L, Umemoto M, Wiedmeyer HM; IFCC Working Group on HbA1c Standardisation. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardisation schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem 2004;50:166-74.
2. Fundamentals of Analytical Chemistry. Holt Rinehart & Winston. London. Second Edition 1970. Chapter 3 Evaluation of Analytical Data; Q test table 3.6 page 45.
3. Fundamentals of Analytical Chemistry. Holt Rinehart & Winston. London. Second Edition 1970. Chapter 3 Evaluation of Analytical Data; t test table 3.5 page 44.

V Homogeneity and Stability

Homogeneity

Homogeneity testing of the samples EurA1c 2016-1, 3 and 5 is performed according to ISO 13528:2005 (Annex B). The results in table 17 show that the samples are homogeneous.

Table 17. Homogeneity test of EurA1c 2016-1, 3, and 5.

Vial	Fresh Whole Blood				Lyophilised				Frozen Whole Blood			
	EurA1c 2016-1				EurA1c 2016-3				EurA1c 2016-5			
	1	2	mean	Δ	1	2	mean	Δ	1	2	mean	Δ
1	42.6	42.8	42.70	0.2	43.0	43.0	43.00	0.0	43.0	42.7	42.85	0.3
2	42.7	42.8	42.75	0.1	42.8	42.8	42.80	0.0	43.0	42.5	42.75	0.5
3	42.7	42.8	42.75	0.1	42.8	43.0	42.90	0.2	42.8	42.5	42.65	0.3
4	42.7	42.8	42.75	0.1	42.5	42.5	42.50	0.0	42.8	42.5	42.65	0.3
5	42.7	42.7	42.70	0.0	42.8	42.8	42.80	0.0	43.0	42.5	42.75	0.5
6	42.8	42.7	42.75	0.1	42.8	42.8	42.80	0.0	42.5	42.5	42.50	0.0
7	42.7	42.8	42.75	0.1	42.5	42.5	42.50	0.0	42.5	42.7	42.60	0.2
8	42.7	42.7	42.70	0.0	42.8	43.0	42.90	0.2	42.5	42.5	42.50	0.0
9	42.6	42.7	42.65	0.1	42.8	42.7	42.75	0.1	42.5	42.5	42.50	0.0
10	42.7	42.6	42.65	0.1	43.0	42.8	42.90	0.2	42.7	43.0	42.85	0.3
11	42.7	42.7	42.70	0.0					42.5	42.5	42.50	0.0
12	42.6	42.7	42.65	0.1					42.5	42.5	42.50	0.0
average			42.7				42.8				42.6	
SD		0.000	0.042	0.071		0.157	0.167	0.081		0.022	0.139	0.194
0.3 x SD_{RL}			0.306				0.306				0.306	
Criterion			-0.306				-0.149				-0.284	
Homogeneity			**Pass**				**Pass**				**Pass**	

Stability

Fresh whole blood samples EurA1c 2016-1 are stored at room temperature and at 2-8°C and measured after storage of 1,2,3,4,7 and 8 days. Frozen samples are stored at -20°C and -84°C and will be measured after 1 and 2 years. Lyophilised samples are stored at 2-8, -20, and -84°C and will be measured after 1 and 2 years storage.
Results of the stability testing of fresh whole blood are shown in table 18 and 19. At 2-8°C samples are stable for at least 8 days. At room temperature there is a decrease of HbA1c on days 7 and 8 for several methods. It was observed that hemolysis proceeded faster at 2-8°C than at room temperature.

Table 18. HbA1c (mean of duplicate) after 1 to 8 days of storage at room temperature

Storage at room temperature						
Method	Day 1	Day 2	Day 3	Day 4	Day 7	Day 8
Menarini/ARKRAY HA8180V	42.2	42.4	42.1	42.0	40.4	39.9
Trinity Premier Hb9210	42.5	42.0	42.6	41.8	40.4	40.3
Sebia Capillarys 2	42.9	41.9	41.8	41.1	40.5	40.8
Roche Tinaquant Gen 3 on Cobas C513	41.9	41.8	41.6	41.5	42.5	42.4
Abbott Architect C4000 (Enz)	42.0	42.1	42.1	42.5	42.9	42.3
Tosoh G8	42.9	43.0	43.2	42.9	42.9	43.2

Table 19. HbA1c (mean of duplicate) after 1 to 8 days of storage at 2-8°C

Storage at 2-8 °C						
Method	Day 1	Day 2	Day 3	Day 4	Day 7	Day 8
Menarini/ARKRAY HA8180V	42.2	42.2	41.1	42.4	42.3	42.2
Trinity Premier Hb9210	42.5	42.3	41.7	42.6	42.3	42.3
Sebia Capillarys 2	42.9	42.4	42.3	41.6	43.3	43.2
Roche Tinaquant Gen 3 on Cobas C513	41.6	42.0	41.7	41.6	42.2	42.1
Abbott Architect C4000 (Enz)	42.4	42.4	42.4	42.5	42.6	42.3
Tosoh G8	43.0	43.0	43.4	43.2	43.2	43.4

Remark
Stability Studies of frozen and lyophilised samples will be done after one- and two years of storage and thus can not be reported now.

VI Preview EurA1c 2017

The project will be continued this year as “EurA1c 2017”. Several remarks and suggestions you made in response to the draft version of this report will be addressed:

- There will be an SOP for value assignment to frozen whole blood and lyophilised hemolysate with the IFCC RMP
- EQA organisers will be asked to specify the methods of all laboratories (thus to limit the labs with method "unknown”)
- We will try to have more relevant HbA1c concentrations: at the decision limit for diagnosis (48 mmol/mol) and at the decision limit for more stringent therapy (64 mmol/mol).
- We will ask EQA organisers to give information on use and conversion of IFCC- and NGSP-units.

The invitation to participate in EurA1c 2017 will be sent on 27 July 2017 together with the final version of this report. Following subscription all details will be arranged with the respective EQA organisers. For your planning however it is useful to know that some dates have been fixed already.

27 July 2017:	Invitation EurA1c 2017 sent
15 September 2017:	Deadline for subscription with estimate of number of required samples
16 Sept - 6 Oct 2017:	All details on e.g. labelling and shipment are arranged
Tuesday 24 October:	Shipment of fresh whole blood samples to the respective EQA organisers
Tuesday 31 October:	Provisional target values sent to the EQA organisers
Nov 2017 - Jan 2018:	Lyophilised samples shipped to the EQA organisers; results to be reported not later than 15 April 2018

VII Organisations and Persons Involved

Country	Organisation	Person
EQA Organisers		
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Mono S Laboratory		
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Oversight Committee (members IFCC C-EUBD)		
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UK	Norfolk University	Emma English
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NL	Queen Beatrix Hospital	Cas Weykamp
Trial Management		
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NL	Coordination	Carla Siebelder
NL	Quality Assurance	Liesbeth Schröer
NL	Data Processing	Irene de Graaf
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