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EurA1c: the European HbA1cTrial to investigate the performance of HbA1c assays in 2166 laboratories across 17 countries and 24 manufacturers using the IFCC Model for Quality Targets

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Running head:

EurA1c, the European HbA1c trial in 2166 laboratories

Keywords

HbA1c, Diabetes, EQA/PT trial, Model Quality Targets, IFCC

Abbreviations

IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; RMP, reference measurement procedure; IDF, International Diabetes Federation; EASD, European Association for the Study of Diabetes; ADA, American Diabetes Association; QTmodel, IFCC Model for Quality Targets; C-EUBD, Committee Education in the Use of Biomarkers in Diabetes; EQA, external quality assessment; PT, proficiency testing; EurA1c, European HbA1c trial; fresh whole blood (WB); lyophilized hemolysate (LH); NGSP, National Glycohemoglobin Standardization Program; BLCV, between laboratory coefficient of variation.

Abstract

BACKGROUND: A major objective of the IFCC Committee on Education and Use of Biomarkers in Diabetes is to generate awareness and improvement of HbA1c assays through evaluation of the performance in countries and manufacturers.

METHODS: Fresh whole blood and lyophilized hemolysate specimens manufactured from the same pool were used by 17 EQA organizers to evaluate analytical performance of 2166 laboratories. Results were evaluated per country, per manufacturer, and per manufacturer and country combined according to criteria of the IFCC model for Quality targets.

RESULTS: At the country level with fresh whole blood specimens, 6 countries met the IFCC criterion, 2 did not, and 2 were borderline. With lyophilized hemolysates, 5 countries met the criterion, 2 did not, and 3 were borderline. At the manufacturer level using fresh whole blood specimens, 13 manufacturers met the criterion, 8 did not, and 3 were borderline. Using lyophilized hemolysates, 7 manufacturers met the criterion, 6 did not, and 3 were borderline. In both country and manufacturer groups the major contribution to total error derived from between laboratory variation. There were no substantial differences in performance between groups using fresh whole blood or lyophilized hemolysate samples.

CONCLUSION: The state of the art is that 1 out of 20 laboratories does not meet the IFCC criterion but there are substantial differences between country and between manufacturer groups. Efforts to further improve quality should focus on reducing

between laboratory variation. With some limitations, fresh whole blood and well-defined lyophilized specimens are suitable for purpose.

Introduction

HbA1c is a key parameter in the monitoring of diabetic control as well as in the screening and diagnosis of Type 2 diabetes (1,2). The high clinical relevance of HbA1c testing necessitates high quality measurement. This is well recognized by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), which has a long standing program to improve HbA1c testing:

- The development of the IFCC Reference Measurement Procedure (RMP) by the IFCC Working Group on Standardization of HbA1c (3). The working group embedded the RMP in a sustainable global network of 15 approved network laboratories (4).
- A consensus statement from the International Diabetes Federation (IDF), European Association for the Study of Diabetes (EASD), American Diabetes Association (ADA) and the IFCC which recognized the RMP as the only valid analytical anchor to standardize HbA1c (5).
- Subsequent to the working group an IFCC Task Force on Implementation of HbA1c Standardization developed a model for Quality Targets (QTmodel) (6).
- More recently the IFCC Committee on Education and Use of Biomarkers in Diabetes (C-EUBD) has a focus on education around the use of biomarkers for diabetes, encompassing both the analytical and clinical utility of HbA1c (7).

External Quality Assessment (EQA)/Proficiency Testing (PT) is a powerful educational tool to monitor quality which, by identifying poor performing laboratories and test systems, can be used as a tool to improve quality. Smaller scale studies initially in Italy and later in a multinational project in Germany, Belgium and the Netherlands were used as a basis for the design of the current study by the C-EUBD (8,9). 17 EQA organizers in Europe agreed to participate in the European HbA1c Trial (EurA1c). Half of the EQA organizers preferred to use fresh whole blood samples (WB) and the other half lyophilized hemolysates (LH). This paper relates to the EQA results in both matrices and are considered per country, per manufacturer and per manufacturer and country combined.

Materials and Methods

Study Design

The EurA1c study design is shown in Fig.1. From two pools of fresh whole blood (yellow), batches of WB (green) and LH EQA specimens were prepared. Specimens were shipped in bulk to the EQA organizers who forwarded them to their participants. Results were collected and evaluated (blue). The study also included frozen whole blood specimens (the common sample in IFCC and NGSP certification). Homogeneity and stability were tested according to ISO 13528 and results met the criteria. Frozen samples, homogeneity, stability and targeting (all grey) were beyond the scope of this paper and will not be addressed.

Sample preparation and assigned values

250 mL donations of whole blood were collected into EDTA from diabetic and non-diabetic volunteers and used to make two pools (EurA1c-1 and EurA1c-2). Target values were assigned with the RMP by 5 approved network laboratories; each laboratory measured the samples in fourfold. The assigned value for EurA1c-1 was 42.3 (6.02%) with an expanded uncertainty of 0.7 mmol/mol (0.06%). The assigned value for EurA1c-2 was 57.9 mmol/mol (7.45%) with an expanded uncertainty of 0.9 mmol/mol (0.08%). From each pool WB and LH specimens were made. Throughout the paper IFCC- and National Glycohemoglobin Standardization Program (NGSP) units will be referred to with results in NGSP units in brackets.

Logistics

In order to process the samples in a timely manner, donations were collected on day one, on day two the WB samples were shipped by courier to the respective EQA organizers at ambient temperature. On day three the EQA organizers distributed the samples to participants, again at ambient temperature, who analyzed the samples on day four or five. An exception to this was the samples for Italy which were shipped direct to the participating laboratories on cool packs. LH samples were manufactured the day after blood donation; shipment and analysis was between November 2016 and April 2017.

Data Collection and Evaluation

EQA organizers collated the results from their participants and forwarded them to the IFCC network coordinator. The number of laboratories (2166) is the number of submitted datasets. The number of manufacturers (24) is the number of platforms that could be evaluated reasonably (N>5) according to the QTmodel. Mean values,

between laboratory CVs and bias were calculated, after removal of outliers, defined as a value outside the target ± 25%. Outliers amounted to 1% of all results. Commonly these outliers were due to mix-up of samples or decimal errors. IFCC results were converted to NGSP units with the Master Equation (NGSP = 0.0915IFCC + 2.15). (10)

The bias is defined as {(M1-T1) + (M2 –T2)}/2 in which M1 and M2 are the mean measured HbA1c concentrations in samples 1 and 2, and T1 and T2 are the target values of samples 1 and 2 assigned with the RMP. The between laboratory CV (BLCV) is defined as the mean of the BLCV in samples 1 and 2. Note that the BLCV in IFCC and NGSP units differs substantially; for explanation see Ref 11.

Manufacturers/Instruments

The study aimed to capture all manufacturer details but unfortunately registration was different per EQA organizer. For Siemens point-of-care users the DCA 2000 and Vantage instruments were combined to one group. The Menarini/ARKRAY 8160 VP and TP instruments formed a single group as did the various types of Bio-Rad Variant. There was a considerable variation in reporting method type for Roche methods, therefore these were combined into one group as they all used the same method principle.137 laboratories did not report their instrument at all; results of this group were included in the calculation per country and in the result per manufacturer they are considered as a separate group.

IFCC Model for Quality Targets

EurA1c results were evaluated according to the criteria of the QTmodel. Although previously described in the literature a short explanation of the model follows to

facilitate the reader in understanding the Figures (6,12).The QTmodel is based on the concept of total error which takes into account the principal sources of analytical error: bias and imprecision. Performance criteria are derived from sigma metrics. Bias is plotted on the vertical axis with scaling in IFCC units (mmol/mol) and NGSP units (% in parentheses). Imprecision, expressed as the Coefficient of Variation (CV) is plotted on the horizontal axis. The criterion was set at 5 mmol/mol (0.46%) at the 2 sigma level and applies to HbA1c concentrations around 50 mmol/mol (6.7%). In the graph this criterion is shown as the line drawn from 5 mmol/mol (0.46%) on the vertical axis to 5.0% (3.4%) on the horizontal axis. A performance within the triangle meets the criterion. When HbA1c is used for diagnosis more stringent criteria might be desirable. Therefore more challenging criteria are defined at total allowable errors of 3.3, 2.2 and 1.1 mmol/mol (0.3-0.2 and 0.1%), represented by the bronze, silver and gold triangles in the QTmodel. The QTmodel can be applied at the level of a single laboratory (precision is the within laboratory CV) or for groups of laboratories (precision is the between laboratory CV). The latter is used in this paper to evaluate the performance of specific country/manufacturer groups.

Results

Preamble

The EurA1c Trial revealed many data: results of 2166 laboratories provided by 17 EQA organizers and measured with assays of 41 different manufacturers. In addition there were two matrices and according to the consensus statement results have to be reported in IFCC- and NGSP units. The multiple and detailed data necessitated

choices on what and how to present results. Condensed EQA results in WB and LH are included in the main body of the paper and the detailed data are systematically presented in the supplemental data section.

Results by Country

Table 1 shows the results ranked alphabetically by country. The first two columns show the names and countries of the EQA organizers. Then there are sections with results in WB and LH. For each matrix there are columns for the number of laboratories (n), bias and between laboratory CV in IFCC- and NGSP units respectively. Results are the mean of both samples EurA1c-1 and EurA1c-2.

Results by Manufacturer

Table 2 shows the results ranked alphabetically by manufacturer. The first column shows the manufacturer. Then there are sections with results in WB and LH. For each matrix there are columns for the number of laboratories (n), bias and between laboratory CV in IFCC- and NGSP units. Results are the mean of both samples EurA1c-1 and EurA1c-2.

Performance of each Country in the QTmodel

Fig. 2A and B show the performances by country in WB and LH in the framework of the criteria of the QTmodel. The plotted bias and BLCV were taken from table 1.

Performance of each Manufacturer in the QTmodel

Fig. 2C and D show the performance of each manufacturer in WB and LH in the framework of the criteria of the QT model. The plotted bias and BLCV were taken from table 2.

Manufacturer performance by Country in the QTmodel

Fig. 3 shows the performance of each manufacturer by country within the framework of the QTmodel. Scaling is omitted to simplify. There were more than 200 manufacturer/country combinations, therefore only data for combinations with at least 5 laboratories per manufacturer in a country are calculated. This resulted in 79 such combinations (data in tables 4,8,12,16 of the supplemental data). Fig. 3 shows the QTmodel plots for manufacturers with at least 6 laboratories using their test in at least 4 countries. Four manufacturers are included for both WB and LH and three for WB only.

Detailed results in supplemental data

Detailed results are provided in the supplemental data. Table 3 shows how the data are systematically differentiated and organized in the 16 supplemental tables. For example: Supplemental table 2 shows the results per sample in IFCC units in WB for manufacturers with more than 5 laboratories using their assay.

Discussion

Overall performance

The last line of table 1 shows the overall performance of all participating laboratories. In the group of laboratories that used WB the mean overall bias of 1517 laboratories was +0.2 mmol/mol (+0.02%) and the BLCV was 4.4% (3.0%). In the group of laboratories that used LH the mean bias of 649 laboratories was -0.5 mmol/mol (-0.05%) and the BLCV was 4.9% (3.2%). These data are plotted in the QTmodel in Fig. 2 A and B (black stars). It can be seen that the performance in WB is borderline within and in LH borderline outside the criterion. The overall performance data can be interpreted as, in both matrices, approximately 95% of the laboratories meet the criterion of a total allowable error below 5 mmol/mol (0.46%). The position of the black stars is close to the horizontal and not to the vertical axis, implying that the major contribution to the total error is derived from the BLCV rather than bias. A similar performance pattern has been reported by the College of American pathologists (CAP) survey in the US (13).

Per Country

In table 1 the performance data is split by country. In WB blood the bias ranges from 0.0 mmol/mol (0.0%) in Sweden and Turkey to +0.8 mmol/mol (+0.08%) in Italy. The BLCV ranges from 3.0% (2.0%) in Ireland to 7.2% (4.8%) in Turkey. In LH bias ranges from 0.0 mmol/mol (0.0%) in Greece to -1.2 mmol/mol (-0.11%) in South Africa (2 laboratories). The between laboratory CV ranges from 3.1% (2.1%) in Italy to 6.4% (4.2%) in Greece. The data are plotted in the QTmodel in Fig 2 A and B. There are substantial differences in performance per country. The best performing countries are approaching the bronze performance criterion line (Ireland in WB; Italy in LH) whereas other countries are outside the 2 sigma criterion (Turkey and Switzerland in WB; Greece and Austria in LH). In other words: the total error in the

best performing countries (approximately 3 mmol/mol; 0.27%) is half of the total error in countries with the poorest performance.

A remarkable phenomenon was observed in Austria: the 11 laboratories using the Abbott enzymatic test had an excellent BLCV of 2.6% (1.8%) but a high bias of -5.8 mmol/mol (-0.53%; tables 8 and 16 supplemental data). This led to suspicion of a matrix effect with the LH samples. However no difference in results with WB or LH was seen when samples were measured with the Abbott enzymatic test at the IFCC Reference laboratory (results not shown). This suggests a specific standardization issue with the Abbott assay in Austria. When the results of the 11 labs were omitted from of the calculations for Austria, bias and BLCV dropped substantially and the overall Austrian performance moved from outside the criterion (AT in Fig. 2B) to within the criterion (A* in Fig. 2B).

The data points representing the respective countries are all close to the horizontal and distant from the vertical axis. Thus, like for the overall performance, traceability to the IFCC RMP is achieved in all countries and remaining total error stems mainly from between laboratory variation.

Per Manufacturer

Detailed results per manufacturer are in the supplemental data and divided into results of manufacturers with 6 or more laboratories (n = 40; tables 2,6,10,14) and manufacturers with less than 6 laboratories (n = 29; tables 3,7,11,15). In the small groups relevant conclusions can not be made and therefore only the condensed data of manufacturers with 6 or more laboratories are shown in Table 2 and Fig. 2. Fig. 2 C and D show that there are substantial differences between manufacturers.

Excellent performance is seen with WB (A) and LH (H) but there are also poor performers (S and K in WB; T and A in LH). In general the data points for the manufacturers are quite close to the horizontal and distant from the vertical axis. From this it can be concluded that the majority of manufacturers achieved traceability to the IFCC RMP. Total error at the manufacturer level mainly came from between laboratory variation.

By Manufacturer and by Country

Fig. 3 shows the performance per manufacturer per country for manufacturers with at least 6 laboratories using their test in at least 4 countries. For some manufacturers the data points are close to each other which implies that performance in the countries is similar (Fig. 3G and 3F). However for Roche using WB there are differences between the countries: good results in Sweden, the Netherlands and UK and quite poor in Switzerland and Turkey (Fig. 3C2). Differences between countries for the assay of a manufacturer can be laboratory based and be related to maintenance, rigidity of quality management or training/motivation of the staff. They can also be manufacturer based and be related to training and education of the customers and batch-to-batch management of calibrators and reagents. Like in the previous sections the major contribution to total error derived from between laboratory variation.

Fresh Whole Blood and Lyophilized Hemolysate

In the EurA1c trial both WB and LH were used. In principle WB is the ideal sample: it is patient material and thus commutable per definition, but sample stability limits its use. General ageing causes lysis, glucose consumption by erythrocytes (formation of

lactic acid lowers pH), spectral changes (browning) and additional hemoglobin fractions. Glycation (HbA1c formation) may proceed during storage. Ageing processes mean that WB is a dynamic specimen that may change characteristics over time depending on shipment time and temperature, resulting in different properties from laboratory to laboratory over wide geographical areas with differing infrastructures and thus different HbA1c results. Results of Italian laboratories in WB are slightly higher than in other countries. This may reflect differences in shipment temperature (Italian samples were shipped on cool packs). It is not clear if the difference is significant at all, and if yes, if there is a negative impact on shipment on cool packs (increased lysis) or at ambient temperature (ageing). LH does not have stability problems but may have commutability issues. Before lysis plasma is removed and cell-debris is removed. The reconstitution volume is such as that the hemoglobin concentration is equal to WB but matrix changes may have an impact. It is assumed that LH may not be commutable with the Roche assays. However, EurA1c results (bias in WB -0.9 mmol/mol/0.08%; bias in LH -0.1 mmol/mol/0.01%) show only a small difference between the matrices and it is not clear whether this difference derives from non-commutability of LH or from instability of WB blood. Lyophilized material is not suitable for (point of care) methods that can only work with whole blood. Inappropriate reconstitution may also cause complications. It must be taken into account that the manufacture of LH, in general, – and thus the commutability- varies widely per manufacturer (use of cryolyoprotectants and native/artificial HbA1c). In summary: with WB samples there is doubt around sample stability and with LH there is doubt around commutability. The EurA1c trial showed that bias and BLCV are comparable for both matrices. Probably the impact of instability of WB and non-commutability of LH is low but it should be stressed that this

is only true for the specimens and test conditions in this study. With knowledge of the limitations and in the proper setting, both WB and LH are suitable for EQA purposes. EQA organizers balance the advantages and disadvantages of both sample types. In countries with fast and reliable communication links where the whole logistic chain of blood donation, dispensing/packaging of the samples, shipment and measurement in the laboratories is feasible within one working week WB is the specimen of choice. In countries where this is not possible, LH will be used.

State of the art

The EurA1c results show that the present state of art of HbA1c measurement in relation to quality targets can be summarized in one number: a total error of approximately 5 mmol/mol (0.46%). In words: if HbA1c of a patient is measured in one of the 2166 laboratories that participated in the EurA1c trial, it can be expected that 1 out of 20 laboratories will report a result that will differ 5 mmol/mol (0.46%) or more from the true value.

Results have shown that the major contribution to total error was derived from the between laboratory variation. The low bias observed suggests that the main manufacturers have made significant improvements in the calibration of their instruments to align with the consensus statement, which dictates the use of the IFCC RMP. To achieve further improvement, the focus should be on reducing the between laboratory variation. A starting point for such improvement is knowledge of the causes. The EurA1c trial attempted to elicit some of these causes by investigation of a number of factors. Figures 2 and 3 show that there are substantial differences: between countries and between manufacturers. In addition the performance of laboratories using the test of the same manufacturer can be (but is

not always) quite different per country. The data do not allow a clear conclusion on the cause of poor performance. One can speculate that it is a combination of factors. Due to e.g. financial pressure, quality may have a different priority. In case of low priority, the attitude of the laboratories towards quality will be lower.

Clinical considerations

The quality target in the QTmodel is a total allowable error of 5 mmol/mol (0.46%). Thus if the true value is 43 mmol/mol (6.1%) results between 38 and 48 mmol/mol (5.6 to 6.5%) are acceptable. One can argue that an error of 5 mmol/mol (0.46%) is good enough for monitoring of diabetic control, but questionable for diagnosis: the clinical interpretation of 38 or 48 mmol/mol (5.6/6.5%) is quite different. The community of laboratory medicine should aim for a tighter quality goal, closer to the "bronze" target of 3.3 mmol/mol (0.30%) in the QTmodel.

Strengths and Weaknesses

The strengths of EurA1c include the scale, rigour and quality of the study with international oversight from the C-EUBD resulting in an overview of the state of the art of HbA1c measurement with comparisons between countries and manufacturers. However there are also weaknesses. A weak point is that a number of laboratories did not report their method (group "unknown") and that EQA organizers have different definitions of the same method. Striking examples are the tests of Roche: definitions are "instrument type", "generation 2 or 3 reagent", "whole blood/hemolysate mode" or simply "Roche Tina-quant". The approach of the EurA1c evaluation was to consider all Roche results as one group, whilst not ideal the differentiation into all variables would reveal 16 Roche methods which is not ideal either.

Conclusions

A trial like EurA1c with collaboration of many EQA organizers was possible. With some limitations, WB specimens and well-defined LH appeared suitable for purpose. The state of the art was a total error of 5 mmol/mol at the 2 sigma level. Differentiation of the results showed substantial differences between countries and between manufacturers. Traceability to the IFCC RMP was a minor issue; total error derived mainly from between laboratory variation. International studies like EurA1c trigger all parties involved in HbA1c measurement to consider and improve quality and thus to work on better patient care. Therefore the IFCC C-EUBD will continue to organize the trial yearly. EurA1c has Eurocentric roots but participation is open to non-European countries.

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513 *Table 1. EQA/PT organizers in the EurA1c project and summary per country of number of participating labs, bias, and between*
514 *laboratory CV in fresh whole blood and lyophilized hemolysates.*
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Country	Organisation	Fresh Whole Blood			Lyophilized Hemolysate		
		n	IFCC Bias in mmol/mol (Between Lab CV)	NGSP Bias in % (Between Lab CV)	n	IFCC Bias in mmol/mol (Between Lab CV)	NGSP Bias in % (Between Lab CV)
Austria	ÖQUASTA				107	-1.0 (5.3%)	-0.09 (3.6%)
Belgium	WIV-ISP	139	+0.4 (3.2%)	+0.04 (2.1%)			
Czech Republic	SEKK				70	-0.4 (5.3%)	-0.04 (3.6%)
France	Biologie Prospective	135	+0.3 (3.6%)	+0.03 (2.4%)	132	-0.8 (4.6%)	-0.07 (3.1%)
Germany	INSTAND e.V.	652	-0.2 (4.8%)	-0.02 (3.2%)			
Greece	ESEAP				73	0.0 (6.4%)	0.00 (4.2%)
International*	ERL				54	-0.4 (4.9%)	-0.04 (3.3%)
Ireland	IEQAS	30	+0.2 (3.0%)	+0.02 (2.0%)			
Italy	Centro di Ricerca Biomedica	84	+0.8 (4.5%)	+0.08 (3.0%)	48	-0.2 (3.1%)	-0.02 (2.1%)
Netherlands	SKML	136	+0.2 (3.4%)	+0.02 (2.2%)			
Portugal	Inst. Nac. de Saude Dr. Ricardo Jorge				43	-0.5 (3.8%)	-0.05 (2.6%)
South Africa	Tygerberg Hospital				2	-1.2 (4.1%)	-0.11 (2.7%)
Spain	SEQC-ML				76	-0.5 (3.3%)	-0.05 (2.2%)
Sweden	EQUALIS	117	0.0 (3.4%)	0.00 (2.3%)			
Switzerland	MQ	29	+0.4 (5.8%)	+0.04 (3.9%)			
Turkey	TUBITAK UME	48	0.0 (7.2%)	0.00 (4.8%)	45	-0.2 (5.2%)	-0.02 (3.5%)
United Kingdom	WEQAS	148	+0.6 (3.5%)	+0.06 (2.4%)			
Overall		1517	+0.2 (4.4%)	+0.02 (3.0%)	649	-0.5 (4.9%)	-0.05 (3.2%)

516 ** Individual laboratories of a number of countries*
517
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Clinical Chemistry

519 *Table 2. Summary per manufacturer of number of participating labs, bias, and between laboratory CV in fresh whole blood and*
520 *lyophilized hemolysates.*
521

Manufacturer	Fresh Whole Blood					Lyophilized Hemolysate				
	n	IFCC Bias in mmol/mol	(Between Lab CV)	NGSP Bias in %	(Between Lab CV)	n	IFCC Bias in mmol/mol	(Between Lab CV)	NGSP Bias in %	(Between Lab CV)
Abbott Architect Enzymatic	21	-0.1	(1.6%)	-0.01	(1.1%)	24	-4.0	(6.0%)	-0.37	(4.0%)
Abbott Architect Immuno	6	-1.8	(4.0%)	-0.16	(2.8%)					
Abbott Other	6	+1.9	(4.6%)	+0.18	(3.0%)					
Alere Afinion	76	-0.7	(3.4%)	-0.06	(2.2%)					
Beckman Coulter AU	26	-0.6	(5.6%)	-0.06	(3.8%)	7	+1.6	(6.5%)	+0.15	(4.4%)
Beckman Coulter UC DxC	15	-1.0	(3.5%)	-0.10	(2.4%)					
Bio-Rad D10	53	+0.8	(4.8%)	+0.07	(3.2%)	37	-1.2	(5.2%)	-0.11	(3.5%)
Bio-Rad D 100	11	-0.8	(1.8%)	-0.08	(1.2%)	16	-0.3	(1.9%)	-0.03	(1.2%)
Bio-Rad Variant	86	+0.9	(4.0%)	+0.08	(2.6%)	38	+1.3	(4.8%)	+0.12	(3.2%)
Medinor	6	-4.7*	(14.6%)	-0.43	(9.9%)					
Menarini HA-8160	91	+0.4	(3.4%)	+0.04	(2.3%)	87	-0.6	(2.9%)	-0.06	(2.0%)
Menarini HA-8180	82	+0.4	(3.0%)	+0.03	(2.0%)	72	-0.7	(3.5%)	-0.06	(2.4%)
Not Known	123	0.0	(5.3%)	0.00	(3.6%)	14	-0.8	(8.1%)	-0.07	(5.4%)
Roche	288	-0.9	(4.4%)	-0.08	(3.0%)	100	-0.1	(4.9%)	-0.01	(3.3%)
Sebia Capillarys 2	57	-0.4	(2.6%)	-0.04	(1.8%)	45	-1.4*	(2.5%)	-0.14	(1.7%)
Sebia Capillarys 3	8	0.0	(2.3%)	0.00	(1.6%)	9	-1.3	(2.1%)	-0.12	(1.4%)
Sebia Minicap	10	-0.8	(2.5%)	-0.08	(1.7%)					
Siemens Advia	15	+3.5*	(4.8%)	+0.32	(3.2%)					
Siemens DCA/Vantage	158	+0.6	(3.6%)	+0.06	(2.4%)	6	+4.0	(3.6%)	+0.38	(2.4%)
Siemens Dimension	47	0.0	(4.0%)	0.00	(2.7%)	17	+0.4	(4.7%)	+0.04	(3.1%)
Siemens Other	13	-0.3	(4.2%)	-0.03	(2.8%)					
Tosoh G7	27	+1.1	(5.6%)	+0.10	(3.8%)	33	-0.4	(4.7%)	-0.04	(3.2%)
Tosoh G8	234	+1.0*	(2.6%)	+0.09	(1.8%)	85	-0.7	(3.9%)	-0.07	(2.6%)
Trinity Premier Hb9210	27	+1.2	(3.8%)	+0.10	(2.5%)	16	-0.8	(3.7%)	-0.08	(2.5%)

522 * *Significant different from target (p<0.05)*
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Table 3. Overview of Supplemental Data organized according to reporting units, matrix of the samples, and subgroups

	Reporting Units	Matrix of Samples	Subgroups	Supplemental Table no.
All Results	IFCC	Fresh Whole Blood	Per Country	1
			Per Manufacturer (n>5)	2
			Per Manufacturer (n<6)	3
			Per Country per Manufacturer	4
		Lyophilized Hemolysate	Per Country	5
			Per Manufacturer (n>5)	6
			Per Manufacturer (n<6)	7
			Per Country per Manufacturer	8
	NGSP	Fresh Whole Blood	Per Country	9
			Per Manufacturer (n>5)	10
			Per Manufacturer (n<6)	11
			Per Country per Manufacturer	12
		Lyophilized Hemolysate	Per Country	13
			Per Manufacturer (n>5)	14
			Per Manufacturer (n<6)	15
			Per Country per Manufacturer	16

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Figure Legends

Fig.1. Design of the European HbA1c Trial

Donation (yellow) from which fresh whole blood (green) and lyophilized hemolysate (pink) samples are prepared and used in the respective countries (blue). Supporting tests (grey). Countries: Austria (AT), Belgium (BE), Switzerland (CH), Czech Republic (CZ), Germany (DE), Spain (ES), France (FR), Greece (GR), group of individual laboratories in multiple countries (I), Ireland (IE), Italy (IT), the Netherlands (NL), Portugal (PT), Sweden (SE), Turkey (TR), United Kingdom (UK), South Africa (ZA).

Fig 2. Performance per Country (A,B) and per Manufacturer (C,D)

Mean between laboratory CV is on the horizontal axis; mean absolute bias on the vertical axis. The black star represents the overall performance of all laboratories. In Fig. A and B the circles and squares represent the countries (for abbreviations see legend of Fig. 1; A* is Austria without Abbott enzymatic Test users). In Fig. C and D the circles and squares represent the manufacturers: Abbott Architect Enzymatic test (A), Abbott Architect Immunochemical test (B), Abbott test not specified (C), Alere Afinion (D), Beckman Coulter AU systems (E), Beckman Coulter Unicell DxC systems (F), Bio-Rad D10 (G), Bio-Rad D100 (H), Bio-Rad Variant II (J), Medinor (K), Menarini-ARKRAY HA-8160 (L), Menarini-ARKRAY HA-8180 (M), not-specified methods (N), Roche (O), Sebia Capillarys 2 Flex Piercing (P), Sebia Capillarys 3 Tera (Q), Sebia MiniCap (R), Siemens Advia (S), Siemens DCA/Vantage (T), Siemens Dimension (U), Siemens not specified (V), Tosoh G7 (W), Tosoh G8 (X), Trinity Biotech Premier Hb9210 (Z). The bias on the y-axis is absolute; to differentiate

between positive and negative bias, circles represent positive and squares negative bias.

Fig. 3. Performance per Manufacturer per Country

Performance per manufacturer per country in lyophilized and fresh whole blood for a selection of major manufacturers. The black star represents the overall performance of all laboratories in the groups. The circles and squares show the performances per country within the respective manufacturer groups. The bias on the y-axis is absolute; to differentiate between positive and negative bias, circles represent positive and squares negative bias. For scaling, see legend of Fig. 2. For country abbreviations see legend of Fig 1.

European HbA1c Trial

Donation

Fresh

Frozen

Lyophilized

Targeting

IFCC RMP

IFCC SRM

NGSP SRM

Mono S

Homogeneity

Stability

BE

CH

DE

FR

IE

IT

NL

SE

TR

UK

AT

CZ

ES

FR

GR

I

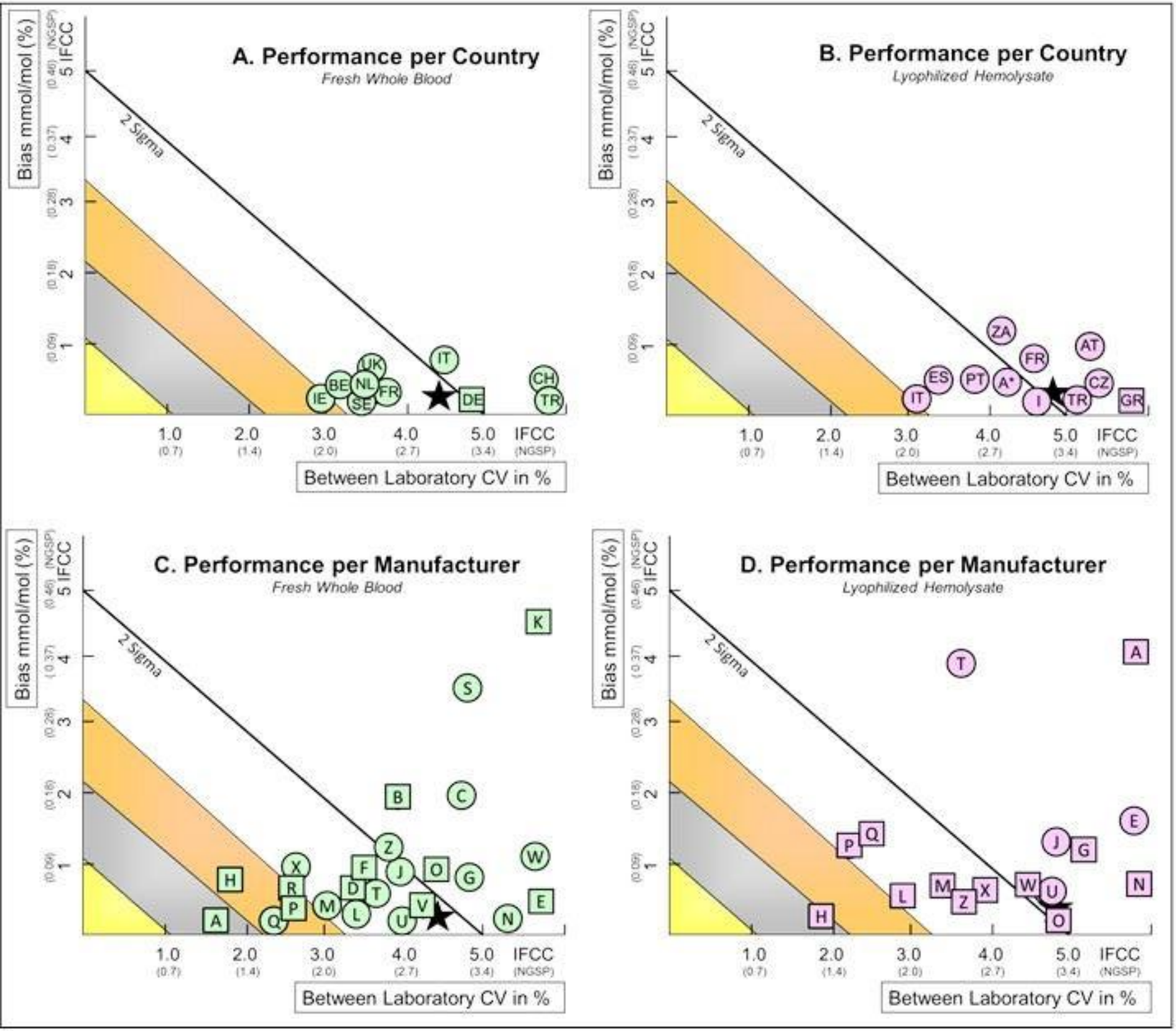
IT

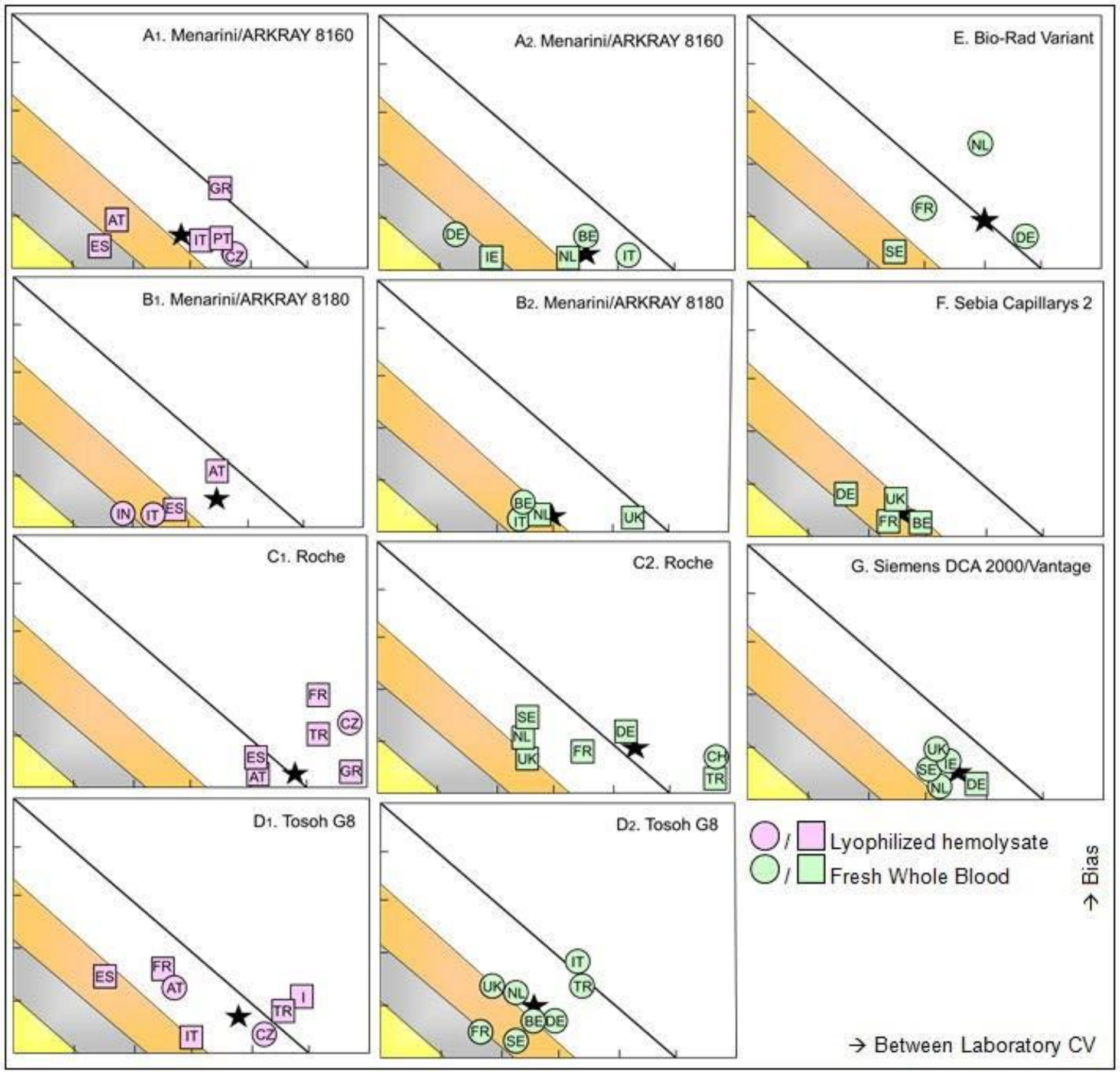
PT

TR

ZA

Performance across Countries and Manufacturers in terms of IFCC Model for Quality Targets


A. Performance per Country
Fresh Whole Blood
B. Performance per Country
Lyophilized Hemolysate
C. Performance per Manufacturer
Fresh Whole Blood
D. Performance per Manufacturer
Lyophilized Hemolysate
Bias mmol/mol (%)
IFCC
(NGSP)
(0.46)
(0.37)
(0.28)
(0.18)
(0.09)
2 Sigma
1.0 (0.7)
2.0 (1.4)
3.0 (2.0)
4.0 (2.7)
5.0 (3.4)
Between Laboratory CV in %


A1. Menarini/ARKRAY 8160
GR
AT
ES
IT
PT
CZ
A2. Menarini/ARKRAY 8160
DE
IE
BE
NL
IT
E. Bio-Rad Variant
NL
FR
DE
SE
B1. Menarini/ARKRAY 8180
AT
IN
IT
ES
B2. Menarini/ARKRAY 8180
BE
IT
NL
UK
F. Sebia Capillarys 2
DE
UK
FR
BE
C1. Roche
FR
CZ
TR
ES
AT
GR
C2. Roche
SE
NL
UK
FR
DE
CH
TR
G. Siemens DCA 2000/Vantage
UK
IE
SE
NL
DE
D1. Tosoh G8
ES
FR
AT
IT
TR
I
CZ
D2. Tosoh G8
IT
TR
UK
NL
BE
DE
FR
SE
Lyophilized hemolysate
Fresh Whole Blood
→ Bias
→ Between Laboratory CV