DEQAS

D · E · Q · A · S

VITAMIN D EXTERNAL QUALITY ASSESSMENT SCHEME

REVIEW 2014

Endocrine Laboratory · Charing Cross Hospital · Fulham Palace Road · London W6 8RF · UK
Telephone +44 (0)20 331 33645 · Facsimile +44 (0)20 331 17007
E-mail administrator@deqas.org · Website www.deqas.org

Introduction

DEQAS is now well established as the largest specialist external quality assessment (proficiency testing) scheme for the vitamin D metabolites 25-hydroxyvitamin D (25-OHD) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$).

In 2012, DEQAS was accepted by the College of American Pathologists (CAP) as an alternative proficiency testing scheme for 25-OHD; CAP accredited laboratories in the US and Canada can now use DEQAS as their primary proficiency testing scheme for 25-OHD.

In April 2013 DEQAS became an accuracy-based scheme (for 25-OHD) with values assigned to all samples by the Reference Measurement Procedure of the US National Institute for Standards and Technology (NIST) (1). Participants can now assess the accuracy of their results by comparing them to an internationally recognized reference method.

An important role of DEQAS is to investigate particular aspects of 25-OHD and $1,25(OH)_2D$ methods; these have included linearity, specificity and the effect of anticoagulants. Occasionally, samples with abnormal levels of other constituents (eg. high lipid content, haemoglobin) are distributed to assess methods' resistance to matrix effects. Because DEQAS has over 1100 participants in 53 countries using 26 25-OHD methods or variants of methods (October 2014), the statistics are very robust and much more representative than studies done in a single laboratory or among small groups of collaborators.

Another important service is the provision of advice and/or additional samples to participants and manufacturers wishing to introduce or develop new methods and troubleshoot existing methods. Participants are not normally charged for this.

DEQAS has a panel of Advisors, which includes acknowledged experts in the field of vitamin D, proficiency testing schemes and biostatistics. All are available to provide participants with help and advice should they require it. Initial contact should be made by e-mailing <administrator@ deqas.org>.

DEQAS can justly claim to offer its participants an enhanced service beyond that of basic performance assessment.

Accreditation

DEQAS operates from within the clinical biochemistry department at Imperial College Healthcare NHS Trust which is accredited by Clinical Pathology Accreditation (CPA). DEQAS is not separately accredited.

CPA is now a subsidiary of the UK Accreditation Service (UKAS) which is applying the internationally recognized ISO 17043 standard to proficiency testing schemes. DEQAS is actively adopting procedures set out in the ISO 17043 standard and intends to seek separate accreditation in due course.

Scheme Design

Each quarterly distribution consists of five serum samples which, with some exceptions, are dispatched by the UK Royal Mail - first class post (UK) and airmail (Europe) or express delivery (non-European laboratories).
The exceptions are the US, Mexico and Canada, for which most samples are shipped overnight to our agent in Atlanta who forwards them through the US postal service.

Some overseas laboratories that have experienced delays in receiving their samples have opted to pay for delivery by a courier service e.g. FedEx, which we are happy to arrange on the client's account.

Source of Serum

Until recently, serum was obtained from blood donated by haemochromatosis or polycythaemic patients undergoing therapeutic venesection at Charing Cross Hospital where DEQAS is based. In a reorganization of haematology clinics, patients have gradually been transferred to Hammersmith Hospital, another teaching hospital of Imperial College.

Following unsubstantiated claims that plasticisers leached from plastic collection bags were interfering in an automated 25-OHD immunoassay (see below), it was decided to purchase 'plasticiser free' serum from a commercial supplier (Solomon Park) in the US. Serum from this supplier (also used in the CAP and NIST accuracy based surveys) is harvested from blood collected according to the CLSI C37-A guidelines (2), which minimizes the possibility of leached substances appearing in the sera. This source has been used for the 25-OHD and $1,25(OH)_2D$ schemes since April 2014.

Sample stability

Sample stability is an important issue for DEQAS as samples are sent worldwide at ambient temperature. Solutions of vitamin D and its metabolites are known to be light sensitive and relatively unstable. However, in experiments conducted before the regular dispatch of samples, both 25-OHD and $1,25(OH)_2D$ were shown to be very stable in serum, probably as a result of the tight binding to vitamin D binding protein and the relative opacity of aqueous solutions to UV radiation. However the stability studies were performed using a chromatographic method and we cannot guarantee that matrix changes (e.g. a rise in serum pH) that inevitably occur at ambient temperature might not affect the performance of less rigorous methods

A 'Plasticiser Problem' – The Facts!

1. A mysterious peak

In July 2013, NIST reported that all DEQAS samples from blood donated in the Hammersmith clinic contained a substance that apparently co-eluted with 3-epi-25-OHD_3 and produced an M/S peak overlapping that of the 3-epi-25-OHD_3. This had not been seen in serum from Charing Cross donations and the most likely explanation was that something, possibly a plasticiser, was leaching from the collection bags. Information from the manufacturers (Fenwal Europe sprl, Belgium)) revealed that their collection bags contained the plasticiser Di(2-ethylhexyl) phthalate (DEHP). The bags used in the Hammersmith clinic were from the same manufacturer but were sterilised by gamma irradiation whereas the Charing Cross bags were steam sterilized.

It was later confirmed that DEHP was present in the DEQAS samples.

However, DEHP has no structural similarities with 25-OHD and further investigations by NIST effectively eliminated this as the interfering peak. It is likely that DEHP is simply a surrogate for other substances leached from the bags, of which there are many.

2. Immunoassays – the definitive experiment

After the 'plasticiser problem' was reported to DEQAS participants and manufacturers in the October 2013 report it was suggested, with no direct evidence, that DEHP might interfere in immunoassays. To investigate this, it was decided to compare serum 25-OHD results on blood donations collected simultaneously in plastic bags and plain glass tubes. Preliminary investigations have been conducted on 4 automated immunoassays (DiaSorin Liaison, IDS iSYS, Abbott Architect and the Siemens ADVIA Centaur)
In a comparison of serum 25-OHD results, only the Siemens assay showed slightly higher results from blood collected in plastic. Investigations continue.

3. A Change of Policy

Plasticisers are so ubiquitous in the environment that, were they shown to interfere in 25-OHD (or 1,25$(OH)_2$D) methods, this would or should be of major concern to manufacturers.

Whilst we have no direct evidence that there is a problem, DEQAS has decided to continue using commercial serum for both the 25-OHD and 1,25(OH)D schemes. This has two advantages:

1. The serum is known to have minimal plasticiser content and its use removes any lingering doubts about leached substances in DEQAS samples interfering in methods for 25-OHD or 1,25$(OH)_2$D.

2. We will be able to specify what range of values are required. This will enable us to send out samples with higher levels of 25-OHD and more containing 25-OHD_2.

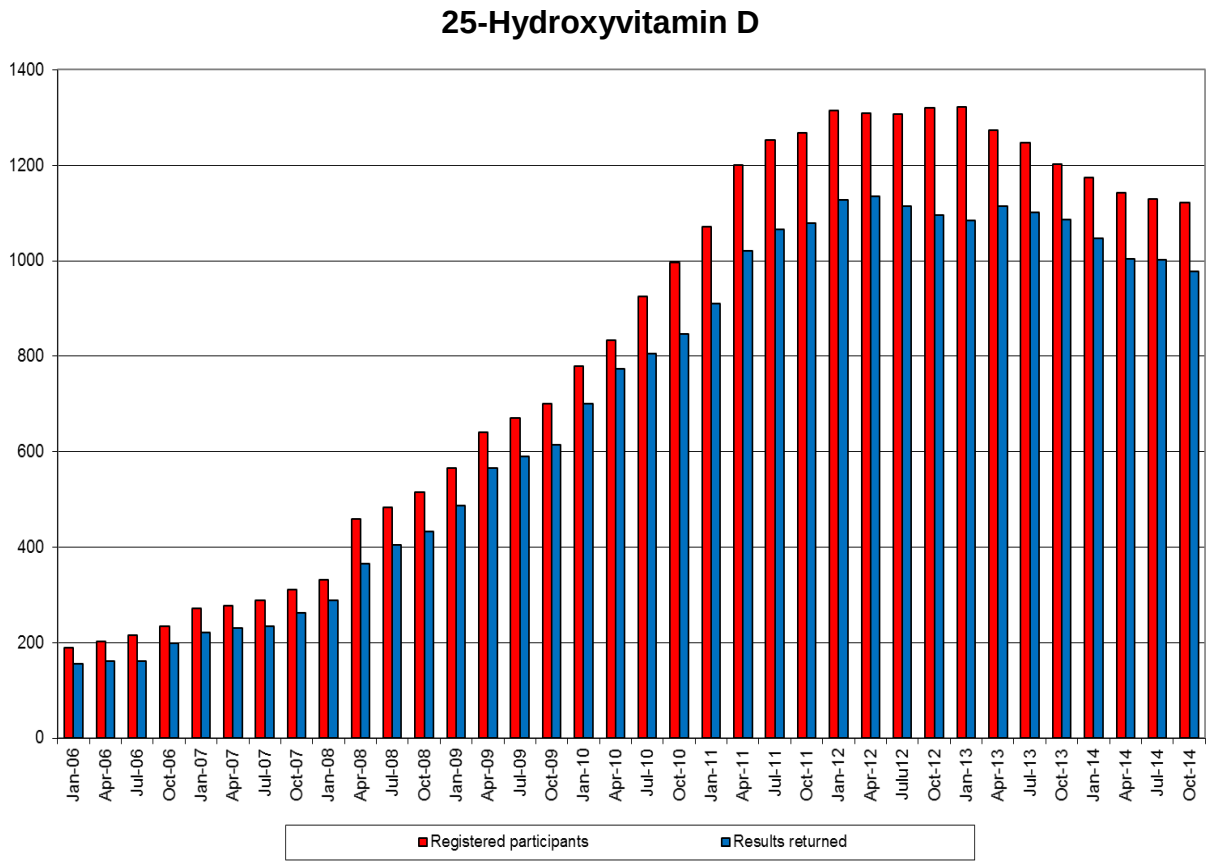

Fig. 1. Number of participants and results submitted in the distributions from January 2006 to October 2014 inclusive.

Comment

The number of registered participants has shown a slight decline recently. The reasons for this probably include worries about the commutability of DEQAS samples (see below) and the rationalization of laboratory services which resulted in some laboratories no longer running 25-OHD assays.

DEQAS Collaboration with NIST (US National Institute of Standards and Technology)

From **April 2013**, every DEQAS sample will have had values assigned by the NIST Reference Measurement Procedure (RMP) for 25-OHD. In addition to 'Total 25-OHD' (25-OHD_3 + 25-OHD_2) participants are given the NIST values for the individual metabolites **25-OHD_3, 25-OHD_2 and 3-epi – 25-OHD_3**. This will be of particular interest to those laboratories using HPLC/UV and LC-MS/MS methods.

The NIST assigned value has replaced the All-Laboratory Trimmed Mean (ALTM) previously used as the target value for performance assessment although the ALTM will continue to be reported.

During the period of uncertainty about which methods, if any, were affected by the presence of leached substances in DEQAS samples, participant performance was assessed against the Method Mean (October 2013 and January 2014).

From April 2014, DEQAS samples used for performance assessment were prepared from commercial serum and we reverted to using the NIST assigned values as the performance target.

College of American Pathologists (CAP): Acceptance of the DEQAS 25-OHD Scheme

DEQAS is now accepted by CAP as an alternate PT provider for 25-hydroxyvitamin D assays and has been returning the performance scores for over 60 laboratories in the USA and 4 laboratories in Canada since April 2013. Performance scores are submitted to CAP after the results deadline for each distribution (April, July, October and January).

Any laboratory in the USA or Canada that is participating in the CAP Laboratory Accreditation Program and is interested in using DEQAS as their PT provider for 25-OHD should contact DEQAS at administrator@deqas.org and provide their CAP identification code (LAP number).

Exclusion of Sample 5 from Performance Assessment (25-OHD only)

Occasionally, DEQAS may include an 'experimental sample' as part of a special investigation. Only the fifth sample from each distribution is used for investigative purposes and results will be excluded from performance assessment, *whether used for this purpose or not*. Despite not using this sample for performance assessment, all the usual statistics will be published.

.

Vitamin D Standardisation Program (VDSP)

DEQAS is an active participant in the VDSP which was inaugurated in 2010 by the US Office of Dietary Supplements (ODS).

The objectives of the VDSP are:

- Standardize the laboratory measurement of 25(OH)D to the NIST Reference Measurement Procedure (RMP) in national health surveys worldwide.
- Promote standardized 25(OH)D measurement in commercially developed laboratory procedures, clinical and research laboratory procedures.
- Study differences in 25(OH)D data found among standardized national health surveys worldwide.
- Conduct an international research program devoted to improving the laboratory measurement of 25(OH)D.
- Conduct commutability study of Proficiency Testing samples, including DEQAS.

Commutability of DEQAS samples

Introduction

Proficiency Testing samples are said to be 'commutable' in an assay when they behave identically to 'normal' patient samples.

A commutability study was organized by the VDSP in 2012. The principles of commutability are explained in Fig. 2

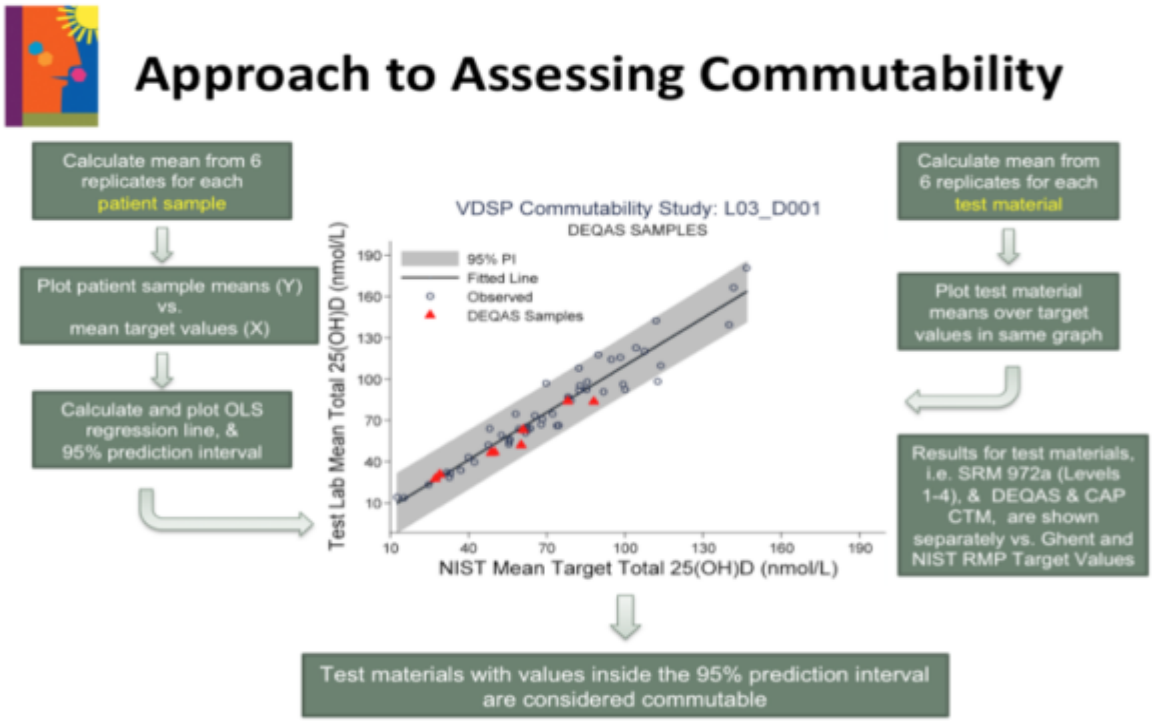

Fig. 2. Assessment of Commutability (courtesy of Dr. Christopher Sempos). The chart shows real data obtained from a widely used automated immunoassay.

Method

Briefly, participating laboratories were sent a panel of 50 single donor samples prepared from blood collected according to the C37-A guidelines, together with samples from DEQAS (collected in plastic bags), CAP and NIST accuracy based surveys. All samples were assigned 25-OHD concentrations by the NIST and Gent Reference Measurement Procedures. Results from the 50 samples obtained from each participating laboratory were plotted against the RMP values and a linear regression line constructed.
PT samples were said to be commutable if the results fell within the 95% confidence limits of the regression line.

Results

Results of all DEQAS samples fell within the 95% prediction interval of the regression line and were deemed to be commutable on the analytical platforms used.

Conclusions

This study was done on samples prepared from blood collected in plastic bags from our clinic patients. This is further evidence that, in the methods studied so far, leached materials from plastic bags do not cause problems in immunoassays.

It is intended to repeat the commutability study in 2015. We are hoping to test the commutability of DEQAS samples on all the major platforms used by DEQAS participants. Any participant or manufacturer who wishes to participate in the commutability study or who would like further information about the VDSP should contact the VDSP Organiser: Dr. Chris Sempos <Sempos@mail.nih.gov>

25-HYDROXYVITAMIN D METHOD TIMELINE

From	Method	Returns	
		April 2014	April 2013
Oct 1989	Chromatographic competitive protein binding assay		1
April 1991	HPLC	31	32
April 1993	IncStar RIA (until January 1999)		
July 1999	DiaSorin RIA (formerly IncStar)	10	15
	IDS RIA	5	7
July 2001	Nichols Advantage (discontinued in April 2006)		
Oct 2002	IDS EIA (OCTEIA)	58	78
April 2004	DiaSorin Liaison		
Oct 2005	LC-MS/MS	151	143
Jan 2006	IDS Automated EIA	25	53
April 2007	DiaSorin Liaison Total	284	356
Oct 2007	Roche 25-OHD3		
July 2008	DIASource 25-OHD3 RIA (formerly BioSource)	1	
Jan 2009	IDS iSYS automated chemiluminescence immunoassay	125	149
Jan 2011	Abbott Architect	75	77
April 2011	Siemens ADVIA Centaur	58	65
	Roche Total 25-OHD	138	122
Jan 2012	Diazyme 25-OHD EIA	1	2
	DiaSource Total 25-OHD RIA	3	2
	DiaSource Total 25-OHD ELISA	1	1
July 2012	Euroimmun ELISA	1	1
Oct 2012	DRG ELISA	1	1
	Tosoh AIA	6	2
Jan 2013	Ortho Total 25-OHD	5	4
	Immunodiagnostik ELISA	1	1
April 2013	Quidel Microvue 25-OH Vit D		1
July 2013	SNIBE Maglumi 25-OH Vit D	3	
Jan 2014	Beckman Access 2 Total 25-OHD	2	
	Beckman Unicel Dxi Total 25-OHD	10	
April 2014	Diazyme 25-OH VitD Chemistry Analysers	1	
	Fujirebio Lumipulse G 25-OH Vit D	1	

Table 1. 25-OHD Methods used by DEQAS participants – first appearance in DEQAS and number of results submitted for each method in April 2014 compared with April 2013

Comment

The number of laboratories using a radioimmunoassay has declined to less than 2% of participants whereas the non-isotopic manual/partially automated and automated assays now comprise approximately 9% and 69% of returned results respectively. The latter include the fully automated methods; Abbott Architect, DiaSorin Liaison, IDS iSYS, Roche Total 25-OHD and Siemens ADVIA Centaur. LC-MS/MS methods account for 15% of the results returned in April 2014.

Performance

Method Accuracy

The following charts (Figs. 3 to 9) illustrate the long-term changes in % bias from the NIST assigned values for each of the major 25-OHD methods. The 40 samples were distributed between October 2012 and July 2014. Commercial serum collected according to C37-A guidelines was used to prepare samples 451 to 454 (April 2014 – round 7) and 456 to 459 (July 2014 – round 8). All other samples were prepared from donations made in the Hammersmith Hospital haematology clinic and will contain varying amounts of substances leached from the plastic bags. Further details of individual samples are highlighted in table 2.

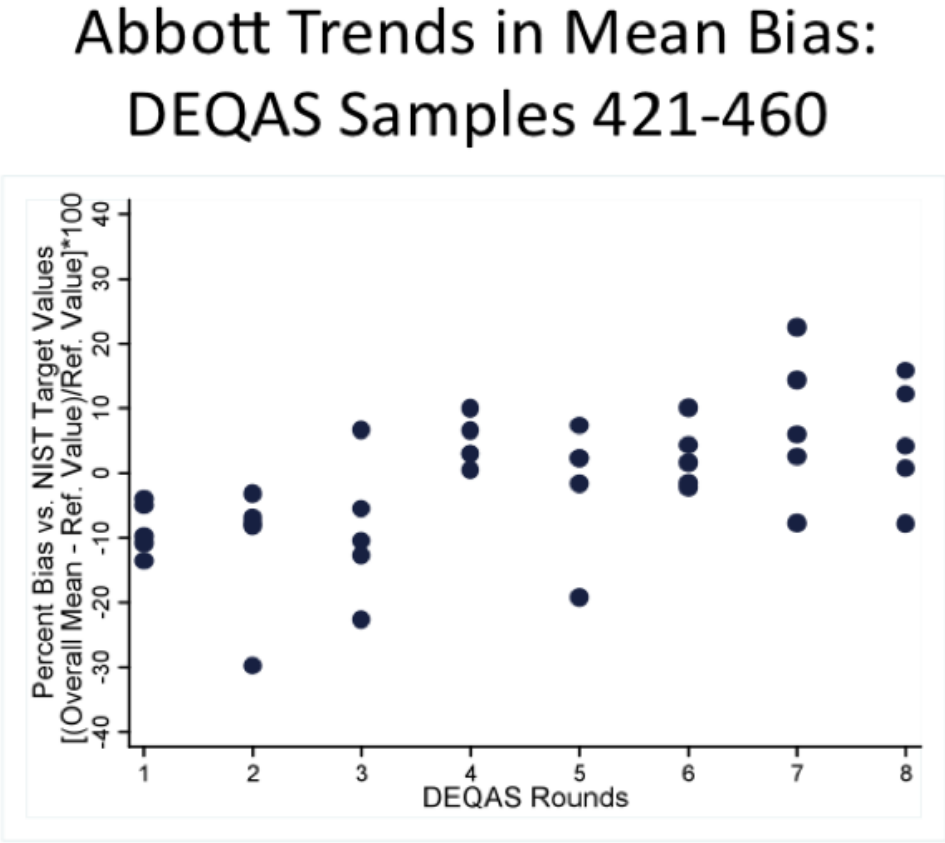

Fig. 3. Abbott Architect; Mean % bias (from the NIST assigned target values) for the 8 distributions (rounds) Oct 2012 to July 2014 inclusive.

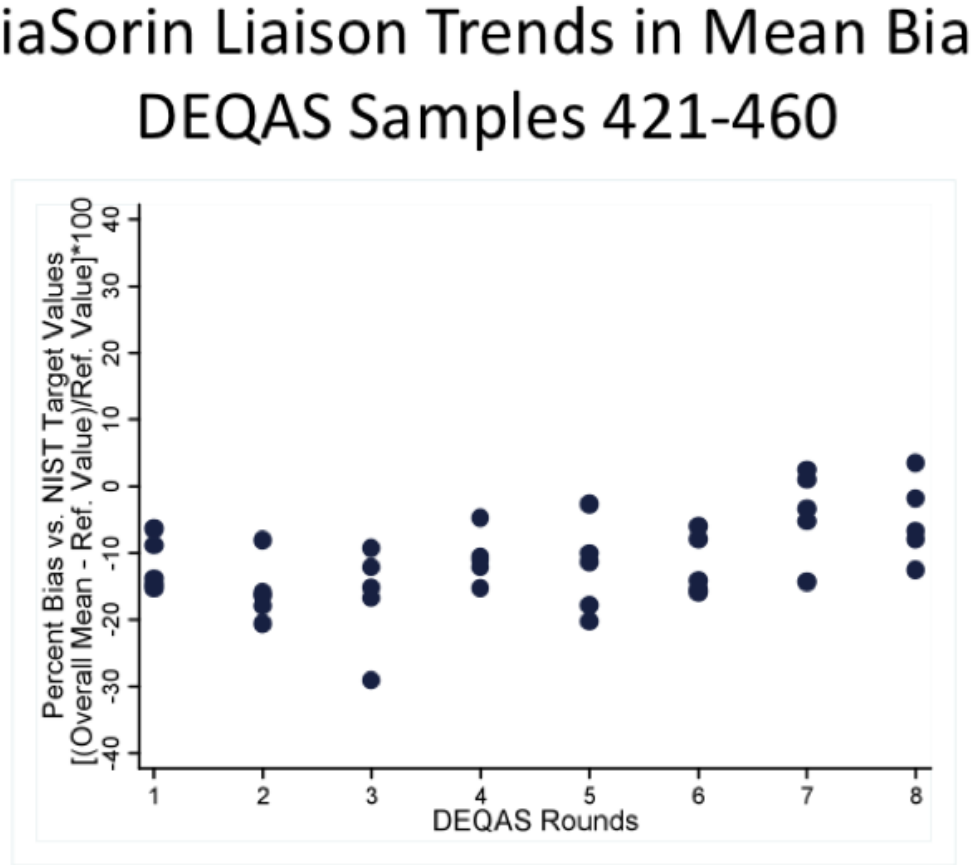

Fig. 4. DiaSorin Liaison; Mean % bias (from the NIST assigned target values) for the 8 distributions (rounds) Oct 2012 to July 2014 inclusive.

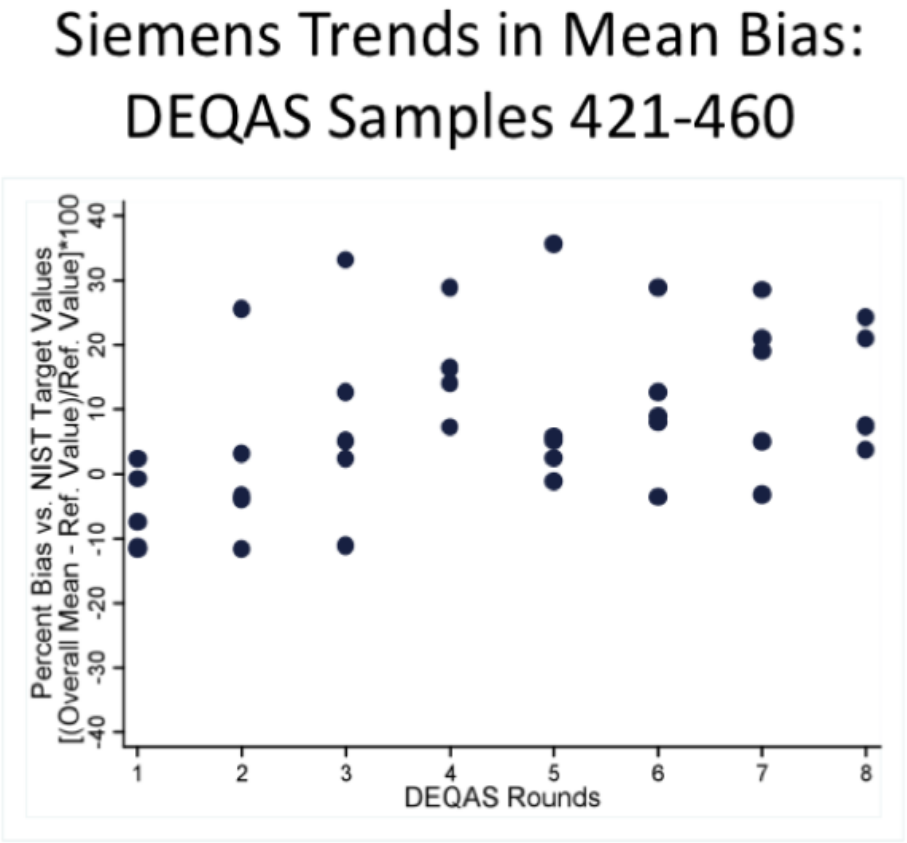

Fig. 5. Siemens ADVIA Centaur; Mean % bias (from the NIST assigned target values) for the 8 distributions (rounds) Oct 2012 to July 2014 inclusive.

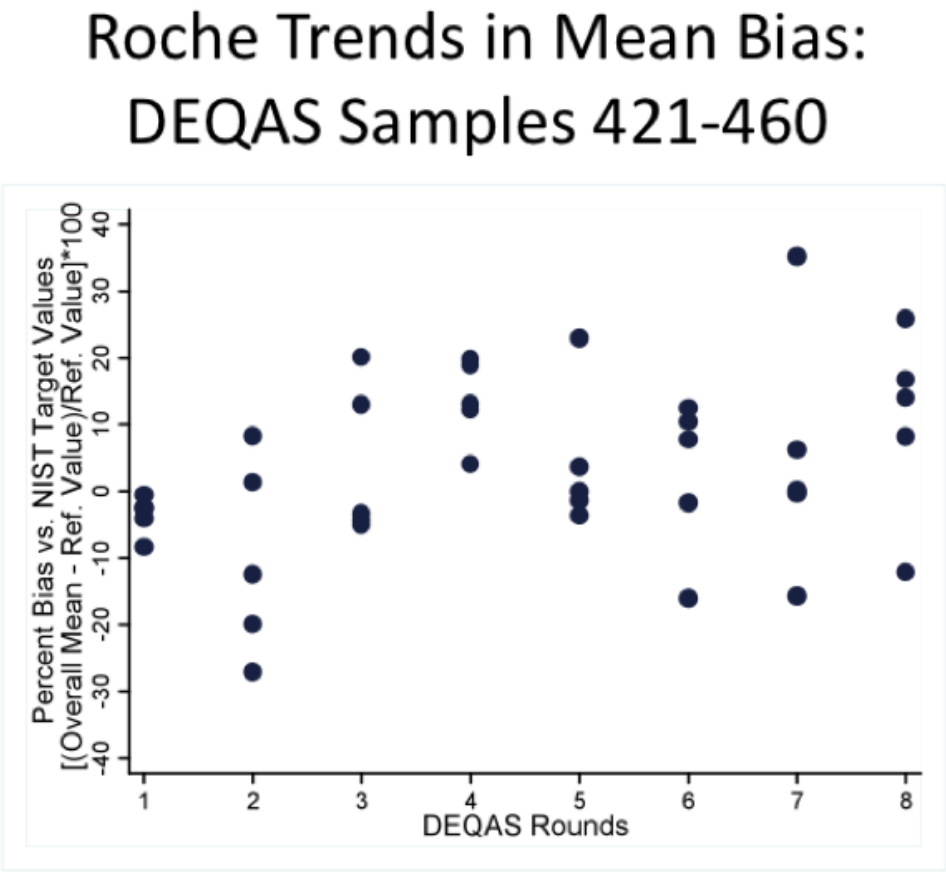

Fig. 6. Roche Total 25-OHD: Mean % bias (from the NIST assigned target values) for the 8 distributions (rounds) Oct 2012 to July 2014 inclusive.

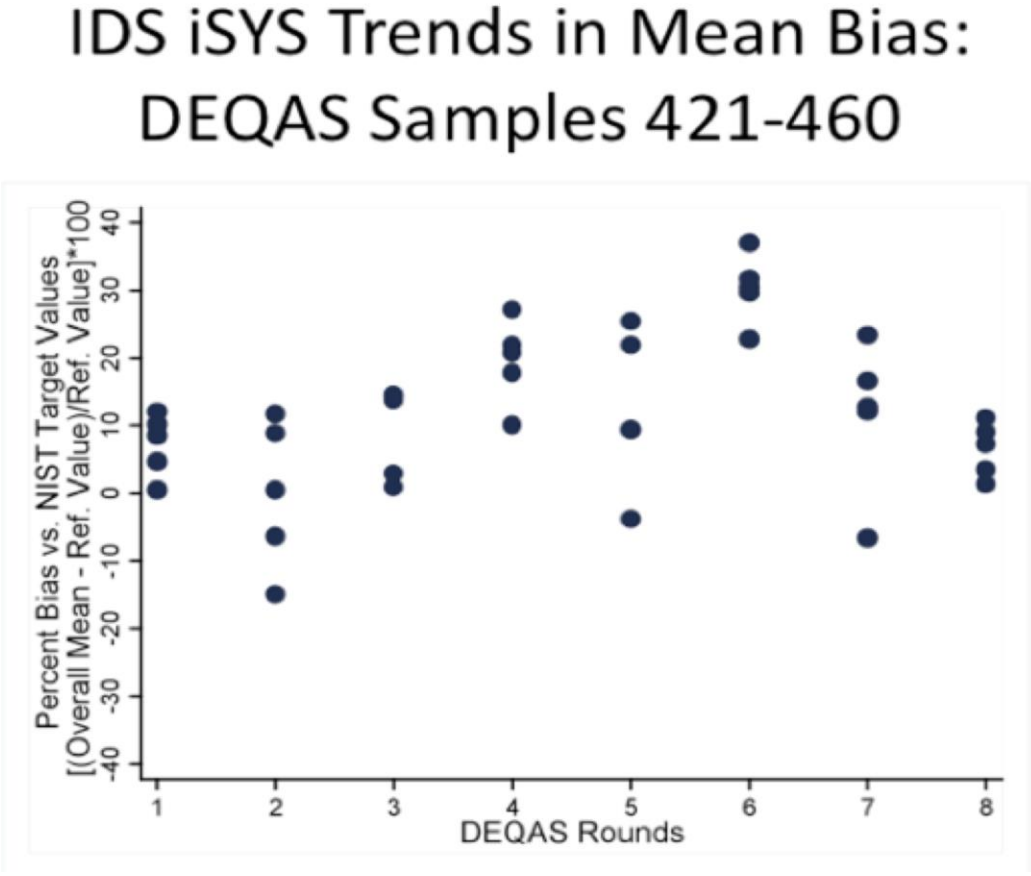

Fig. 7. IDS iSYS: Mean % bias (from the NIST assigned target values) for the 8 distributions (rounds) Oct 2012 to July 2014 inclusive.

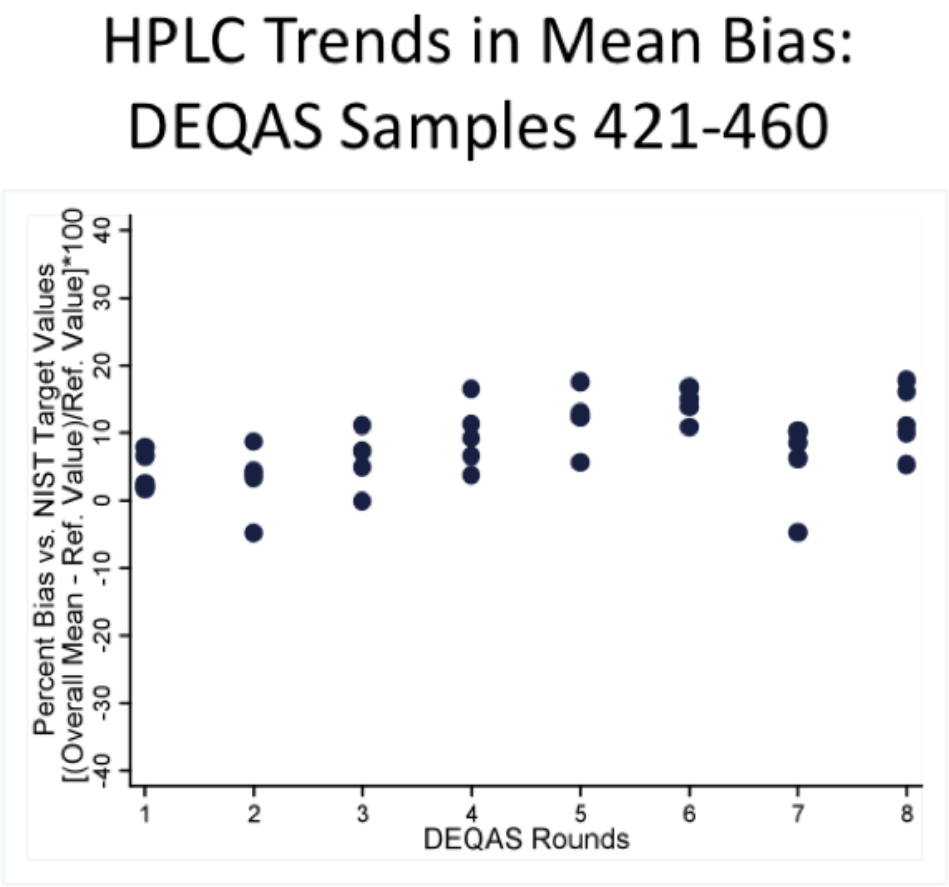

Fig. 8. HPLC/UV: Mean % bias (from the NIST assigned target values) for the distributions Oct 2012 to July 2014 inclusive.

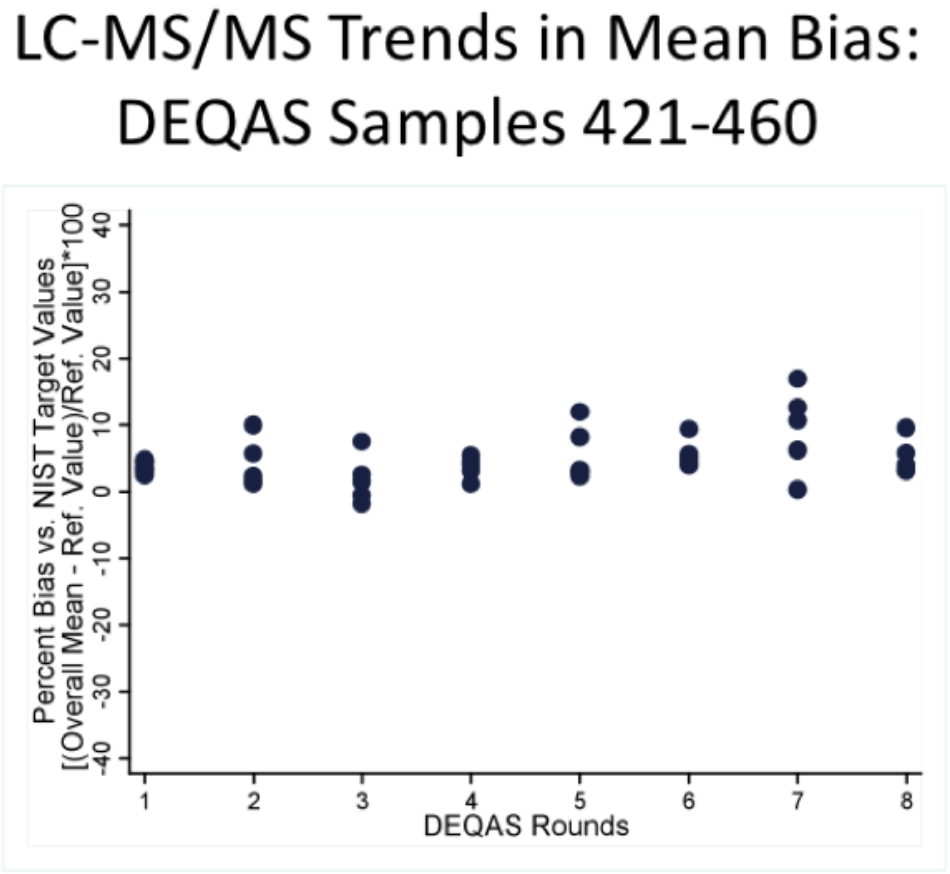

Fig. 9. LC-MS/MS: Mean % bias (from the NIST assigned target values) for the distributions Oct 2012 to July 2014 inclusive.

Distribution	Sample No.	NIST 3-epi-25-OHD3	NIST 25-OHD2	NIST 25-OHD3	NIST 'Total' 25-OHD (25-OHD3 + 25-OHD2)	DEQAS ALTM	% Difference *
		nmo/L	nmol/L	nmol/L	nmol/L	nmol/L	
October 2012	**421**	2.3	1.0	57.5	58.5	55.4	-5.3
	422	1.7	1.7	36.6	38.2	36.2	-5.2
	423	5.7	1.0	84.6	85.6	81.2	-5.1
	424	2.6	1.0	46.2	47.2	47.0	-0.4
	425	2.5	1.0	46.2	47.1	46.9	-0.4
January 2013	**426**	0.4	1.1	33.4	34.5	32.0	-7.2
	427	4.5	0.9	75.2	76.1	72.5	-4.7
	428	2.8	2.5	52.3	54.8	51.8	-5.5
	429	3.0	0.6	58.9	59.5	58.5	-1.7
	430 ***	0.8	22.3	17.6	39.9	35.0	-12.3
April 2013	**431**	1.2	1.3	22.6	23.9	24.9	4.2
	432	2.9	2.7	48.6	51.3	50.0	-2.5
	433	11.7	1.2	90.4	91.6	88.2	-3.7
	434 !	2.4	4.4	74.2	78.6	67.2	-14.5
	435	2.4	0.5	46.1	46.6	45.8	-1.7
July 2013	**436**	4.0	1.3	76.7	78.0	83.2	6.7
	437	1.4	1.4	33.3	34.7	35.2	1.4
	438	2.7	1.8	54.8	56.6	57.3	1.2
	439	2.3	1.2	39.7	40.9	42.6	4.2
	440	2.3	1.3	47.1	48.4	51.3	6.0
October 2013	**441**	7.1	1.6	89.0	90.6	93.6	3.3
	442	4.6	1.6	73.8	75.4	82.3	9.2
	443	1.3	1.9	29.5	31.4	31.3	-0.3
	444	1.9	1.6	41.8	43.4	42.9	-1.2
	445 ***	1.4	14.8	39.3	54.1	49.3	-8.9
January 2014	**446**	5.7	0.9	91.8	92.6	96.7	4.4
	447	2.2	1.1	47.0	48.1	51.3	6.7
	448	1.3	1.8	30.7	32.5	32.7	0.6
	449	4.1	1.5	70.8	72.3	69.5	-3.9
	450	2.5	1.0	63.5	64.4	65.2	1.2
April 2014	**451**	1.4	1.9	27.3	29.2	32.4	11.0
	452	7.8	1.1	99.0	100.1	114.9	14.8
	453	3.2	2.3	64.1	66.4	60.7	-8.6
	454	2.5	2.3	45.2	47.5	50.3	5.9
	455	na	1.4	50.9	52.3	55.5	6.1
July 2014	**456**	3.9	1.5	72.6	74.1	81.8	10.4
	457	1.4	2.0	39.0	41.0	42.0	2.4
	458	5.8	1.3	69.9	71.2	74.5	4.6
	459	4.8	0.9	92.5	93.4	99.4	6.4
	460	na	1.3	50.9	52.7	55.2	4.7
October 2014	**461**	2.7	2.1	55.0	57.1	56.6	-0.9
	462	4.5	1.2	80.0	81.2	80.1	-1.3
	463	5.6	0.7	85.7	86.4	91.9	6.4
	464	3.1	2.0	57.7	59.7	56.5	-5.4
	465 **	3.1	2.0	57.9	59.9	88.4	47.6
	*****	% Difference of the ALTM from the NIST RMP assigned value					
	******	Sample spiked with 24,25-dihydroxyvitamin D					
	*******	Sample contained a mixture of endogenous 25-OHD2 and 25-OHD3					
	!	Lipaemic sample					
	na	Result not available					

Table 2. The mean % Bias from values given by the NIST RMP from October 2012. Sample 434 was lipaemic with a triglyceride concentration of 4.0 mmol/L (twice the upper limit of the local reference range). Samples 430 and 445 had significant amounts of 25-OHD_2; 22.3 nmol/L (55.9% of the total 25-OHD) and 14.8 nmol/L (27% of the total 25-OHD) respectively.

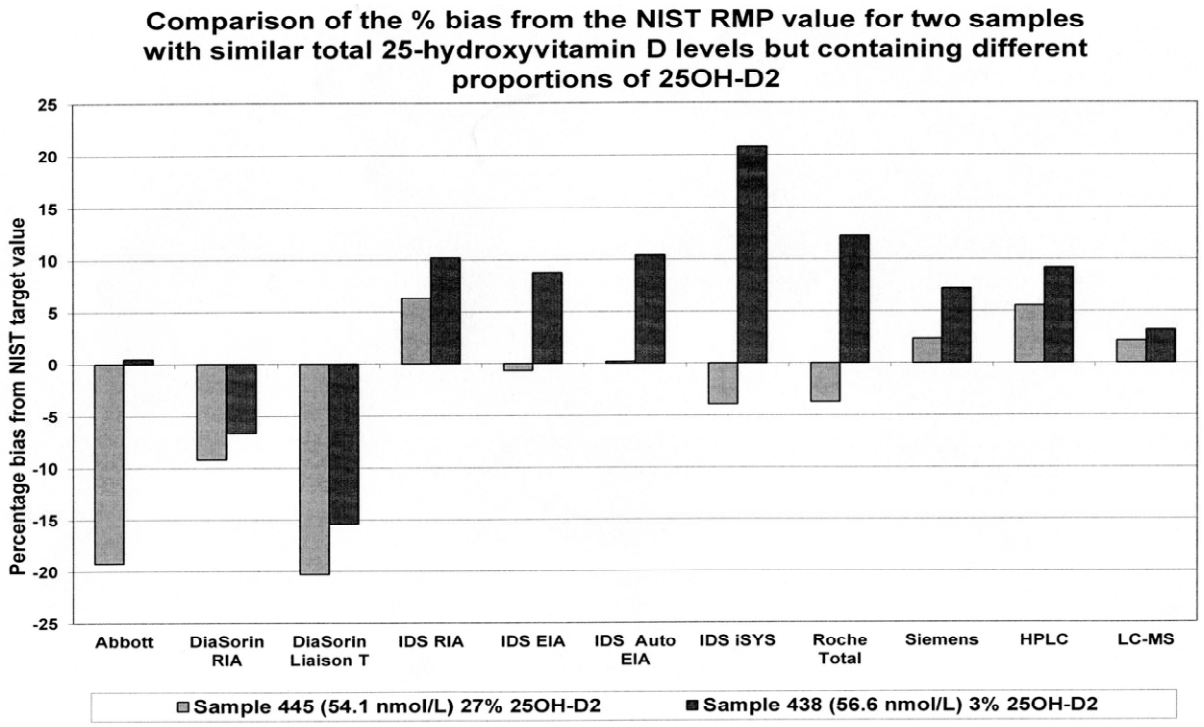

Fig. 10. The effect on assay bias of 25-OHD_2

Comment

All the major ligand binding assays and HPLC apparently under-recovered 25-OHD_2. The exception was the Siemens assay that showed a large positive bias in sample 430 suggesting an over-recovery of 25-OHD_2, although this was not apparent in sample 445 (see Fig. 10). Previous experience has suggested that recovery of 25-OHD_2 is not consistent from sample to sample and may be affected by other constituents of the sample matrix.

Matrix Effects

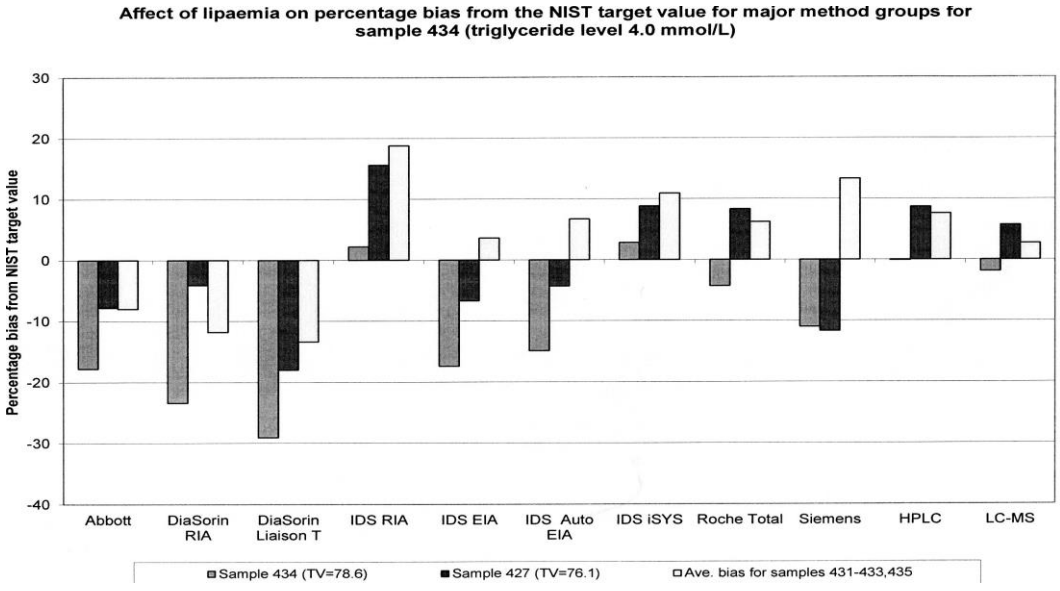

Fig. 11. Comparison of %Bias of 2 samples with similar total 25-OHD concentration; one sample (434) has a modestly raised triglyceride concentration (4.0 mmol/L)

Comment

Sample 434 was lipaemic in appearance and was found to have a triglyceride concentration of 4.0 nmol/L, a level about twice as high as the upper limit of the local reference range but lower than the concentration that many manufacturers state that interference might occur. When judged against a previously distributed non-lipaemic sample (427) of similar total 25-OHD concentration or the 4 other (non-lipaemic) samples in the same distribution, 25-OHD results were apparently reduced in all assays. Thus, the % bias in already negatively biased assays was increased and the bias of positively biased assays was reduced.

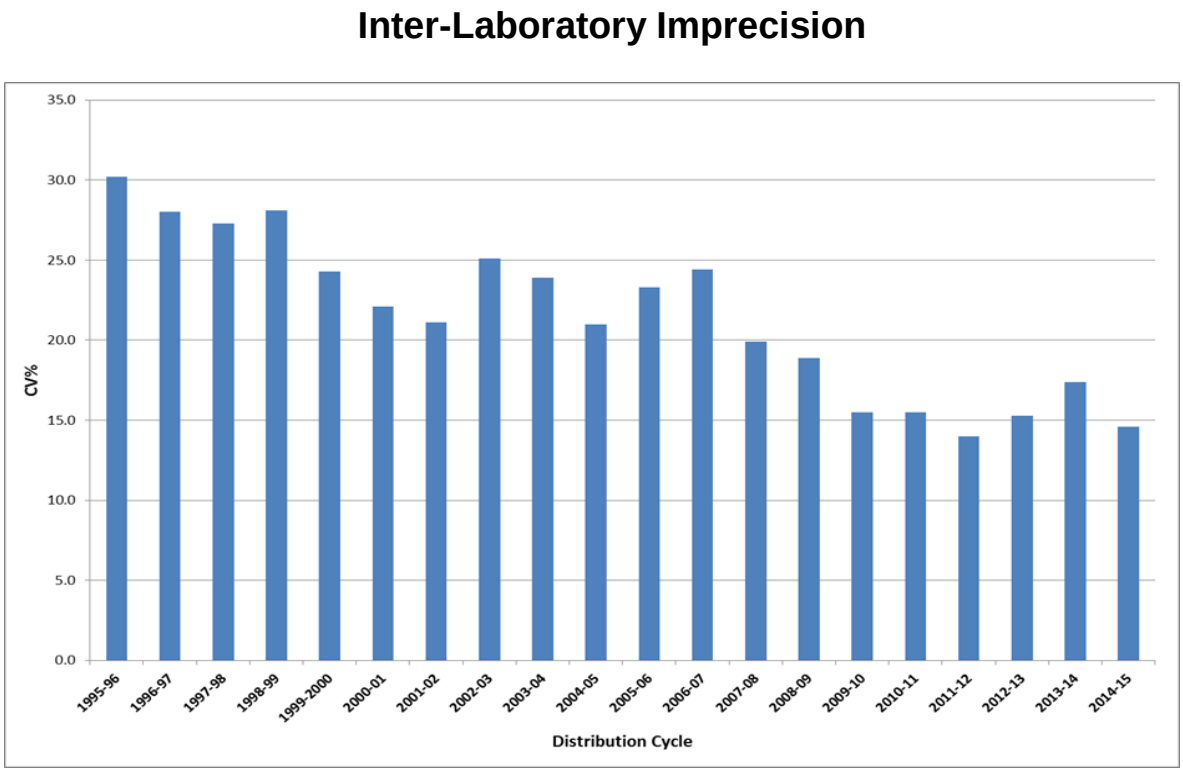

Fig. 12. Mean inter-laboratory imprecision (CV%) of 25-OHD results for distribution cycles since 1995

Comment

There has been an improvement in overall inter-laboratory agreement over the last 18 years (Figure 12). The increase in imprecision (CV%) between 2002 and 2006 probably reflected the introduction of new methods and standardisation problems. The long-term downward trend was restored in 2007 after the Nichols advantage was withdrawn from the market and the IDS EIA assay had been recalibrated (in 2006).

1,25-DIHYDROXYVITAMIN D

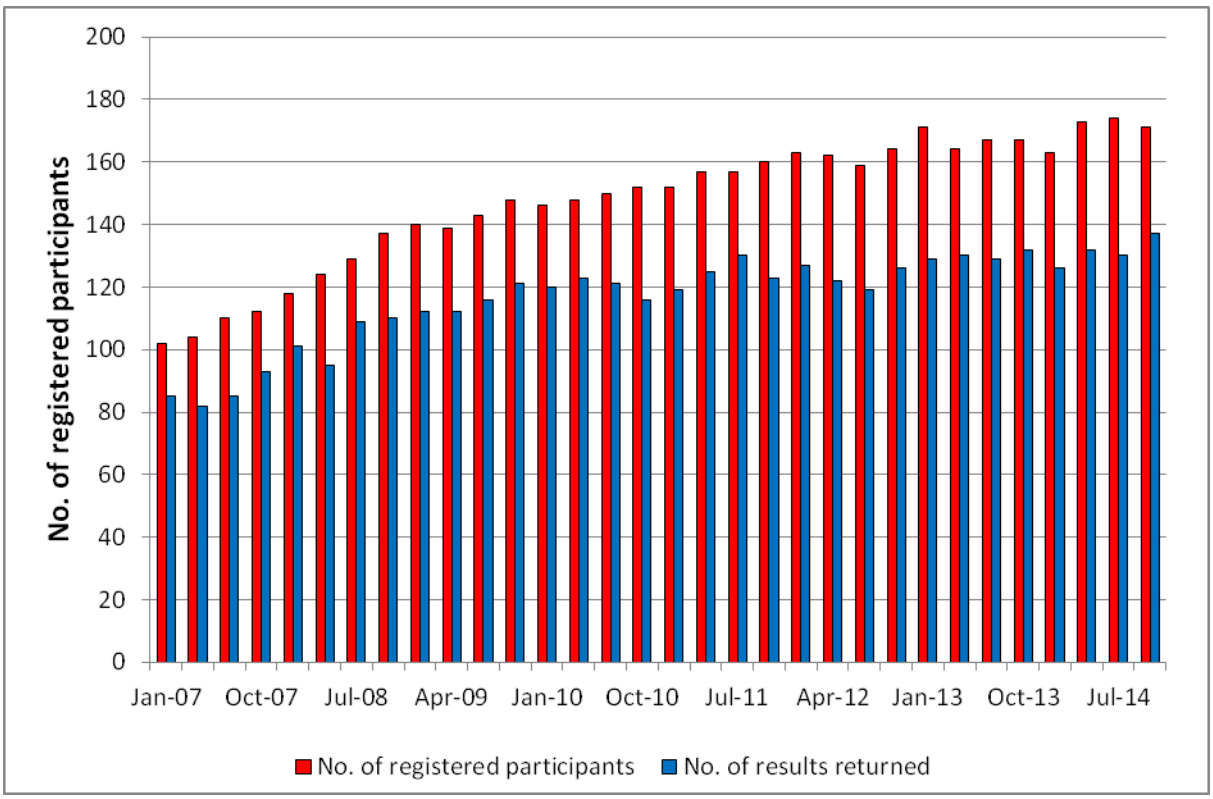

Fig. 13. Number of DEQAS participants registered for 1,25-dihydroxyvitamin D for distributions between January 2007 and October 2014 and results submitted.

1,25-DIHYDROXYVITAMIN D METHOD TIMELINE

From	Method	Returns		
		April 2013	April 2014	Oct 2014
April 1997-1998	HPLC + RIA	1	1	1
	IDS RIA	62	47	37
	In-house Receptor assay	0	0	0
	In-house RIA	0	0	0
	Incstar Receptor assay	0	0	0
	DiaSorin RIA (formerly Incstar)	21	20	16
	Nicholls Receptor assay	0	0	0
October 2004	DIAsource CT assay (formerly BioSource CT)	4	1	3
April 2005	IDS EIA	19	17	16
October 2007	LC-MS/MS	8	11	13
July 2009	AMP RIA	0	0	0
April 2010	Immunodiagnostik ELISA	1	1	1
April 2012	IDS iSYS	14	31	32
January 2014	Cusabio ELISA	0	1	1
April 2014	DiaSorin Liaison XL	0	2	17

Table 3. Methods used for measuring 1,25(OH)2D and results returned for each over the last 18 months

Comment

The number of participants has remained fairly stable in recent years. There was a slight rise in April 2014 coinciding with the introduction of the DiaSorin Liaison XL method for 1,25(OH)$_2$D. Now that this metabolite is available on a fully automated platform, it will be interesting to see how many laboratories start offering it on a routine basis.

Method Bias

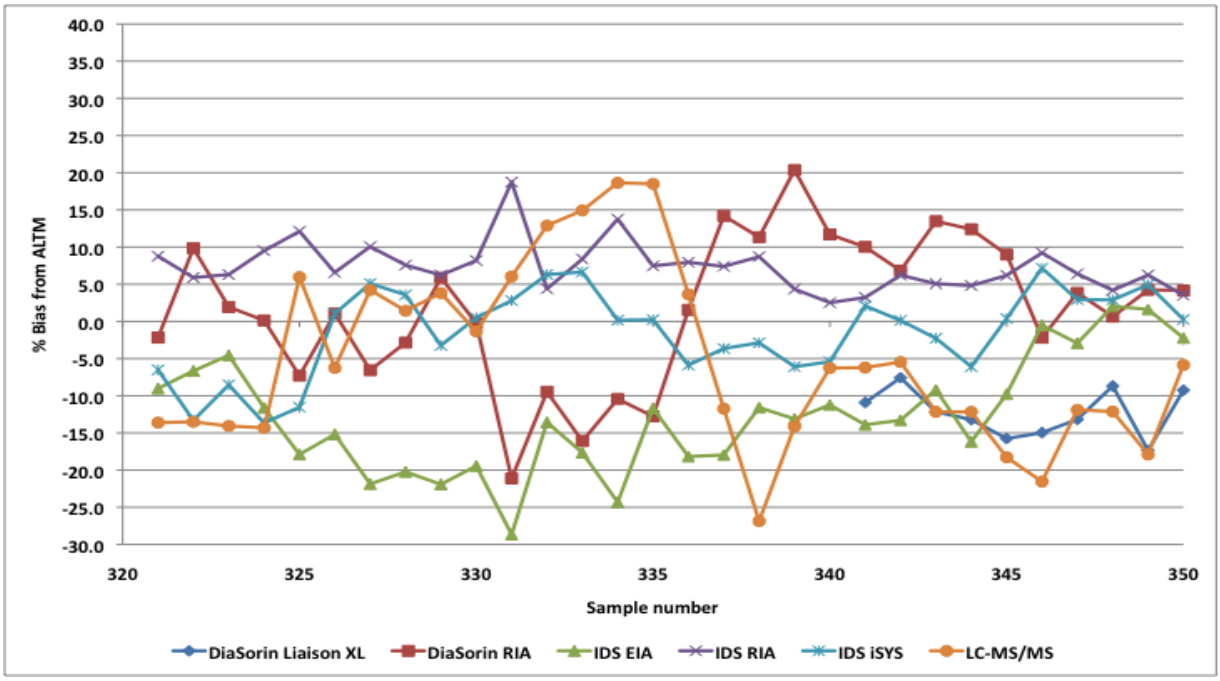

Fig. 14. The % bias from the ALTM of each of the major 1,25 (OH)$_2$D immunoassay methods for the 35 samples distributed between April 2013 and July 2014

Inter-laboratory imprecision

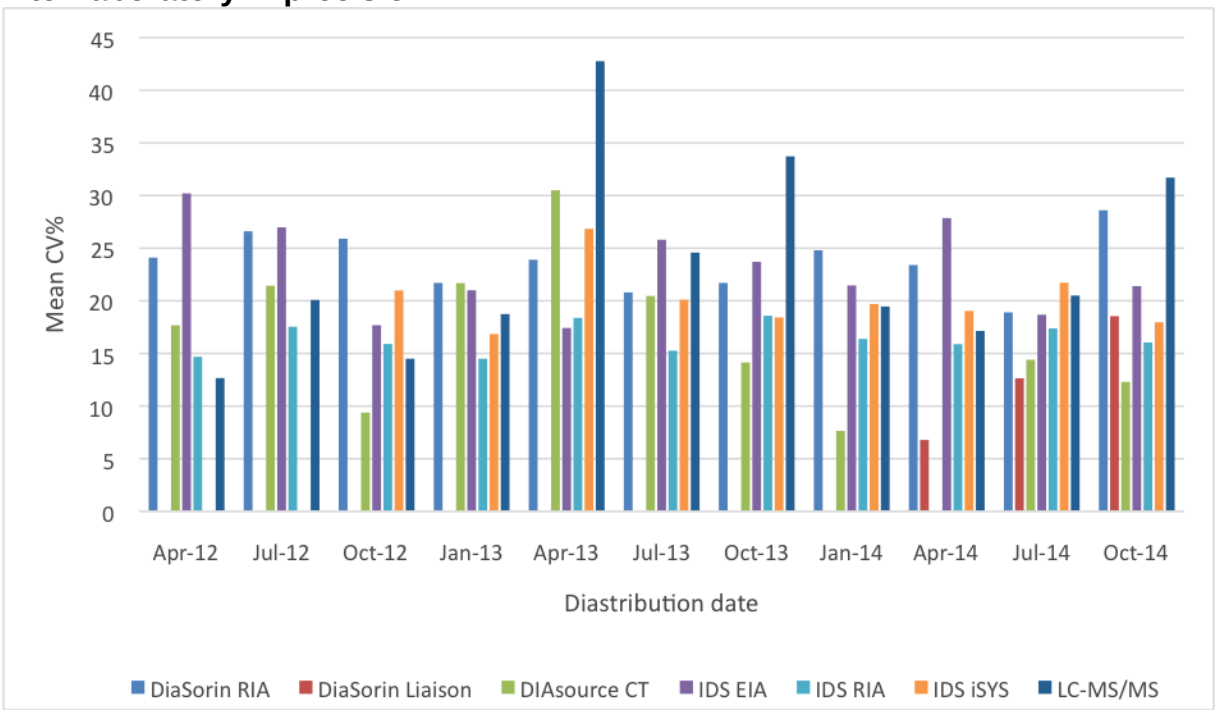

Fig. 15. Mean inter-laboratory imprecision (CV%) of 1,25(OH)$_2$D results for distribution cycles since April 2012

Comment

The most notable changes in 1,25$(OH)_2$D methodology was the introduction of LC-MS/MS in October 2007 and the more recent appearance of the IDS iSYS (July 2012) and the DiaSorin Liaison XL method in April 2014 (13, 32 and 17 users respectively in October 2014). Other popular methods remain the DiaSorin RIA (16), IDS RIA (37) and the IDS EIA (16).
Minor methods include the DIASource coated tube RIA (3 participants), the Cusabio ELISA, Immunodiagnostik ELISA and HPLC/RIA, each with 1 user (October 2014)

Performance Assessment

The bias limits for acceptable performance for 1,25$(OH)_2$D has remained the same since it was introduced for the 2009-2010 distribution cycle. To achieve acceptable performance over the distribution cycle, participants are required to return results for all 4 distributions and to have 80% of their results within 30% of the Target Value (until recently the ALTM). For the 2012-2013 cycle, 80 of the 158 eligible laboratories were awarded a certificate (51%). Fifty- seven (36%) failed to return any results on one or more occasions and were therefore unable to achieve acceptable performance.

Due to the considerable method-related variability the use of the ALTM as a target value for 1,25-dihydroxyvitamin D has been questioned. However at a recent DEQAS Advisory Panel meeting it was decided that there was no reasonable alternative. The development of a RMP for 1,25-dihydroxyvitamin D is unlikely to happen in the foreseeable future.

From October 2013 performance was judged against the Method Mean rather than the ALTM. This was due to the large disparity of results given by the different methods. However, agreement has improved in the latest distributions and this policy will be kept under review.

24,25$(OH)_2$D

There has been renewed interest recently in 24,25$(OH)_2$D and it has been suggested that, in some circumstances, the ratio 24,25$(OH)_2D_3$: 25-OHD_3 might be an additional useful indicator of vitamin D status (3,4,5,6)

We would be pleased to hear from any participant who is measuring this metabolite and who would like to submit results on DEQAS samples. From April 2015 the Results form will contain a section for entering 24,25$(OH)_2D_3$ results

If there is sufficient demand we will do a statistical analysis and publish a table of results in an attachment to the main report.

There will be no additional charge for this service.

Summary

The biggest challenge recently faced by DEQAS was the discovery that samples prepared from blood donated in our haematology clinic contained materials leached from the plastic collection bags. Although there was no direct evidence of interference in 25-OHD assays we decided as a precaution to purchase serum guaranteed to be free of leached materials.

DEQAS has continued to challenge assays with its investigations although results have not always been what was expected! The apparently anomalous behaviour of exogenously added 25-OHD and more recently 24,25$(OH)_2D_3$ has not so far been explained but serves as a warning to manufacturers that cross reactivity data obtained with spiked samples are likely to be unreliable.

Whilst the primary function of DEQAS is to monitor the accuracy of participants' results, we believe that DEQAS, together with colleagues in NIST and the VDSP, has made and will continue to make an important contribution to the improvement of assays for vitamin D metabolites.

Graham Carter Julia Jones Jayne Shannon Emma Walker

February 2015

References

1. Development of a Candidate Reference Measurement Procedure for the Determination of 25-Hydroxyvitamin D3 and 25-Hydroxyvitamin D2 in Human Serum Using Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry. Susan S.-C. Tai, Mary Bedner, and Karen W. Phinney. Anal. Chem. 2010**;** *82*: 1942–1948

2. C37-A. Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Approved Guideline. Clinical and Laboratory Standards Institute, November 1999.

3. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med 2013; 369: 1991–2000.

4. Carter GD and Phinney KW. Assessing Vitamin D Status: Time For a Rethink? Clin Chem 2014; 60, 809-811.

5. Kaufmann M, Gallagher C, Peacock M, Schlingmann KP, Konrad M, Deluca HF, et al. Clinical Utility of Simultaneous Quantitation of 25-hydroxyvitamin D & 24,25-dihydroxyvitamin D by LC-MS/MS involving derivatization with DMEQ-TAD. J Clin Endocrinol Metab. 2014 Mar 26:jc20134388.

6. Significance of serum 24,25-dihydroxyvitamin D in the assessment of vitamin D status: a double-edged sword? Kevin D Cashman, Aoife Hayes, Karen Galvin, Joyce Merkel, Glenville Jones, Martin Kaufmann, Andrew N Hoofnagle, Graham D Carter, Ramon A Durazo-Arvizu, and Christopher T Sempos. Clin Chem 2015, in press.