concentration with the automated IDS EIA kit

Etienne Cavalier (1), Véronique Huberty (2), Catherine Cormier (3), Jean-Claude Souberbielle (4)

(1) Department of Clinical Chemistry, University Hospital of Liege, University of Liege, Belgium
(2) Laboratoire de Biologie Clinique, Centre Hospitalier Peltzer-La Tourelle, Verviers, Belgium
(3) Service de Rhumatologie, Hôpital Cochin, APHP, Paris, France
(4) Service dôExplorations Fonctionnelles, Hôpital Necker-Enfants Malades and Faculté de Médecine, Centre de Recherche Croissance et Signalisation (INSERM U845), Université Paris Descartes, Paris, France

Corresponding author :

Dr Etienne Cavalier
Department of Clinical Chemistry
University Hospital of Liege
Domaine du Sart-Tilman
B-4000 Liege, Belgium
Tel: +32 4 3667692
Fax: +32 4 3667691

Running title: Overestimation of 25(OH)D with automated IDS elisa.

Etienne.cavalier@chu.ulg.ac.be, Veronique.huberty@chplt.be, catherine.cormier@cch.aphp.fr, jean-claude.souberbielle@nck.aphp.fr,

l EC) have frequently been contacted by different physicians or clinical biologists who wanted to have an expert point of view on patients presenting very high 25-hydroxy vitamin D (25OHD) results (between 150 and >300 ng/mL). All of these patients had indeed received the equivalent of 400,000 IU of vitamin D3 over a 2-month period (according to a supplementation protocol that we proposed in late 2008 (1)) and these colleagues were thinking that they had induced vitamin D intoxication. As the patients presented strictly normal serum and urine calcium levels, we were able to reassure them. However, after investigation, we noticed that in all cases, 25OHD had been measured with the IDS EIA kit (Boldon, UK) adapted on various ñopenò automated platforms (*i.e.*Evolis, Triturus, Etimax, DSX,é). When we had the opportunity to control these ñhigh-levelledò samples with the DiaSorin RIA kit (Stillwater, MN), we observed concentrations ranging between 50 and 80 ng/mL. We thus suspected that the correct values were given by the DiaSorin RIA, as we have shown that this technique matched well ï even in high 25OHD values ï with a tandem mass spectrometry assay performed in a British reference laboratory (2). To investigate these apparent discrepancies, we initiated a comparison study between DiaSorin RIA and IDS EIA adapted on two different ñopenò automated analyzers, focusing on samples in which high 25OHD levels had been found with DiaSorin RIA in our laboratories. In the first part of the study, 78 samples presenting 25OHD levels of 30-84 ng/mL (mean ±SD: 55.5±12.6 ng/mL; 56 of them with a concentration of 50-84 ng/mL) were assayed in Liège with the IDS EIA adapted on a DSX platform (Dynex Technologies, Chantilly, VA, USA). In the second part, 72 samples presenting 25OHD concentration between 50 and 99 ng/mL (mean ±SD: 62.4±13.9 ng/mL) were assayed in Paris with the IDS EIA adapted on the Evolis platform (BioRad, Marne la Coquette, France). Both groups of samples were also assayed the same days with the IDS EIA performed ñmanuallyò by an experienced technician. All the measurements were performed according to the analytical

ers and when a concentration >100 ng/mL was found with the IDS EIA (either « automated » or « manual »), the serum was diluted 1 : 2 and re-assayed.

Combined together, our results showed that the ñautomatedò IDS EIA produced 25OHD concentrations (mean ± SD: 87.3±65.6 ng/mL) that were significantly higher ($p<0.0001$) than those obtained with the DiaSorin RIA (58.9±12.6 ng/mL) and with the IDS EIA run manually (59.9±38.6 ng/mL). Even if the manual IDS EIA gave results that were only slightly but significantly ($p=0.03$) higher than those of the DiaSorin RIA, two samples presented however a concentration that was still out of range (>154 ng/mL), even after a 1:2 dilution. The Bland-Altman plot showed an obvious systematic bias that started at a mean level close to 50 ng/mL (Figure 1A). This bias was much less obvious when comparing the DiaSorin RIA and the manual IDS EIA (Figure 1B).

Our results, obtained in two laboratories which used two different automated analyzers, showed that the automated IDS EIA gave frankly higher concentrations than the DiaSorin RIA when values measured with the RIA are higher than 50-60 ng/mL. This may have important clinical consequences as a physician who finds a 25OHD concentration >80-100 ng/mL in one of his vitamin D-treated patient will probably stop the vitamin D supplementation. This situation may become quite frequent as many experts consider that a serum 25OHD level above 30 ng/mL is required for an optimal vitamin D status (1;3-7), and that the dietary recommended intakes (DRI) for vitamin D are insufficient to reach this optimal range. Thus, different protocols for vitamin D supplementation that use dosages that are much higher than the DRI, bringing the 25OHD level of vitamin D insufficient patients to a mean level close to 40-50 ng/mL, have been published recently (1;3;6-9). Interestingly, our finding was not detectable through the vitamin D External Quality Assessment Scheme (DEQAS) as, for all the DEQAS samples tested in 2009 (n=20), the IDS ñautomatedò EIA

re very close to those produced by the DiaSorin RIA, and to the IDS Laboratory Trimmed Meanò (ALTM). This may be explained by the fact that the ALTM of the 2009 DEQAS sample with the highest concentration was around 37 ng/mL, which is in agreement with our finding that the systematic overestimation with the IDS EIA seems only present for serums with a DiaSorin RIA 25OHD level of 50 ng/mL or more. It may be thus very useful that the DEQAS proposes, in a near future, at least one serum sample containing a 25OHD concentration of approximately 80-100 ng/mL. Similarly, other studies that compared the automated IDS EIA kit with the DiaSorin RIA (10-12) did not report such a bias but used samples with concentrations mostly in the range of 10-60 ng/mL. One study (10) even reported a modest positive bias (the DiaSorin RIA giving higher levels than the automated IDS EIA) for concentrations mostly between 5-40 ng/mL.

We donôt have clear explanation for this bias; Older RIA's, both DiaSorin and IDS, are performed using sample destruction with acetonitrile. That means that samples are deprotinized and delipidated prior to assay. This technique removes all vitamin D binding protein (DBP) and other matrix components that have the capacity to interfere with the assay. The newer 25OHD assays try to remove the 25OHD from its DBP-binding pocket with various displacement techniques such as pH. This type of assay leaves a somewhat functional DBP and all other serum components that may contribute to unwanted matrix effects. In the case of the manual IDS EIA the effects are not so noticeable although it still seems to occur at 25OHD levels > 70 ng/ml. For some reason this matrix problem is much worse in the IDS automated procedure. The exact cause for this will be difficult to determine and whether the problem is similar with other automated analyzers will deserve other studies.

In conclusion, we suggest to the users of the IDS EIA to use this kit in its ñmanualò procedure rather than to adapt it on an automated platform until clarification and correction of the problem is achieved. We also suggest to interpret very cautiously a 25OHD level >100 ng/mL

try to know what kind of vitamin D supplement the patient has taken), to re-measure the sample after dilution, and to use another assay-method if the high value persists. Finally, we think that proficiency testing providers, like UK-DEQAS, should integrate high-levelled samples in their schemes.

Acknowledgements: We would like to thank Pr Bruce Hollis for the discussion aiming to help in understanding the discrepancies observed. We also thank IDS for the donation of the EIA kits

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of the comparison of the 25OHD levels measured with
lò IDS EIA (Figure 1A), or the ñmanualò IDS EIA
(Figure 1B) in 78 serum samples assayed in the Clinical Chemistry Laboratory of the Liège University Hospital (open circles) and 72 serum samples assayed in the Hormonology Laboratory of the Necker Hospital in Paris (crosses). A systematic negative bias is obvious in Figure 1A (lower values with the DiaSorin RIA).

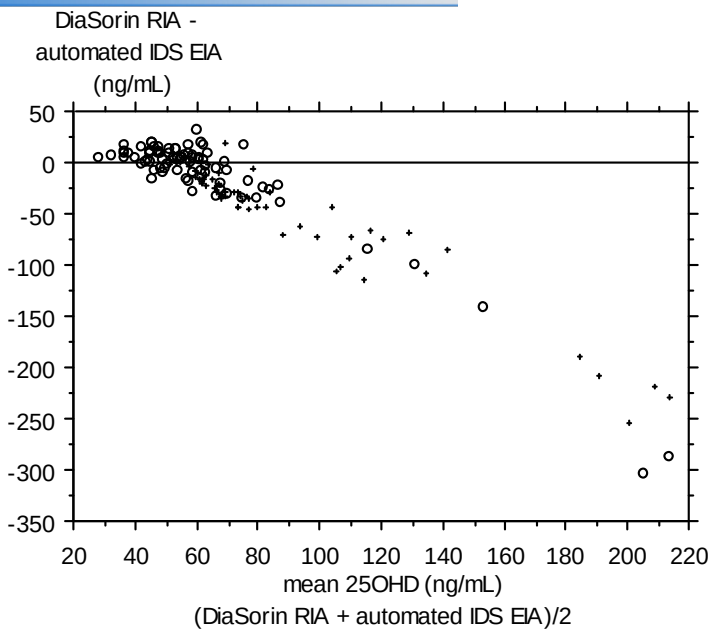

Figure 1B