comparison for serum cholesterol between an accuracy-based reference method (applying isotope-dilution gas chromatography/mass spectrometry) and a routine method. However, the simulation truly mimics a “real world” comparison that has been done before (7). The y axis represents the differences of the routine method from the reference method, expressed in percentage of the values of the reference method. This approach is recommended for data that span a “medium” range, where a more or less constant CV can be expected (8). Moreover, the 1.96 SD_{diff} deviations can directly be related to the CV of the routine method. The bias of the routine method was assumed to be 2.3% and the CV to be 3%. We used as acceptance limits for the routine method SE = 3% (9) and TE = 10% (10). For an overview about strategies for setting quality specifications, the reader is referred to a recent conference report (11).

Visual interpretation of Fig. 1A (n = 80) easily allows one to conclude that the routine method satisfies the limits for SE as well as TE (UCL_{d} ≤ SE and UCL_{1.96 SD_{diff}} ≤ TE). From Fig. 1B (n = 40), on the other hand, the conclusion would be that the routine method does not satisfy the SE limit (UCL_{d} > SE), but does satisfy the TE limit (UCL_{1.96 SD_{diff}} ≤ TE). From Fig. 1C (n = 20), one would conclude that the routine method satisfies neither the SE nor the TE limit (UCL_{d} > SE and UCL_{1.96 SD_{diff}} > TE).

In summary, the example demonstrates that the incorporation of confidence limits and predefined error limits in a Bland–Altman plot allows easy visual interpretation of a method-comparison study. Moreover, the confidence limits directly show the importance of the sample size for decisions about method acceptance, a fact that is usually not considered.

Finally, we want to remark that the confidence intervals (and indeed, limits of agreement) are by convention set at 95% but that other values might be used. Most obviously, one might in some situations require 99% limits of agreement to meet a predefined specification.

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Dietmar Stöckl
Diego Rodríguez Cabaleiro
Kathleen Van Uytfanghe
Linda M. Thienpont

1 STT Consulting Horebeke, Belgium
2 Laboratorium voor Analytische Chemie Faculteit Farmaceutische Wetenschappen Universiteit Gent Gent, Belgium

* Address correspondence to this author at: Laboratorium voor Analytische Chemie, Faculteit Farmaceutische Wetenschappen, Universiteit Gent, Harelbekestraat 72, B-9000 Gent, Belgium. Fax 32-9-264-81-98; e-mail linda.thienpont@ugent.be.

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No Interference by Diclofenac with the New Vitros FT3II Assay Reagent

To the Editor:

Measurements of serum concentrations of free triiodothyronine (FT₃), thyroid-stimulating hormone (TSH), and free thyroxine (FT₄) are useful in the diagnosis and treatment of thyroid diseases. Several nonsteroidal antiinflammatory drugs (NSAIDs) are known to interfere with thyroid function tests (1, 2). Cross-reaction with the assay antibody is one of the mechanisms of NSAID interference (3). The FT₃ antibody included in the Vitros FT₃ assay (Ortho-Clinical Diagnostics Inc.) cross-reacts with diclofenac, a phenylacetic acid derivative with structural resemblance to T₃ (4). To avoid interference by diclofenac, a new reagent has been developed and is included in the Vitros FT3II assay. In this study, we examined the interference by diclofenac with the Vitros FT3II.

We analyzed the correlation between the old assay reagents and the new reagents with 133 random samples obtained from hospitalized patients and sent to the laboratory for FT₃ analysis. Five of the patients were taking diclofenac. Precision was evaluated according to NCCLS protocol EP5-T2 (5). In addition, two replicates of each of three freeze-dried control serum samples were assayed on at least one occasion per day for at least 13 days over a 28-day period.

The within-day CVs at representative concentrations of 1.65, 4.71, and 13.0 ng/L were 3.9%, 1.8%, and 2.4%, respectively; the within-calibration CVs were 7.4%, 4.2%, and 4.7%, respectively.

A comparison of the results obtained with the Vitros FT3II with the results obtained with the FT₃ assay is shown in Fig. 1. The regression equation for 128 patients, not including the 5 patients taking diclofenac, was: y = 0.87x + 0.49 ng/L (r = 0.994). The FT₃ values for the five patients taking diclofenac were above the upper limit of the reference interval in the Vitros FT₃ assay but within the reference interval in the FT3II assay.
Three of the five patients were evaluated as euthyroid because their TSH and FT4 concentrations were within the appropriate reference intervals. The other two patients were hypothyroid and were taking levothyroxine sodium. Levothyroxine therapy for hypothyroidism reportedly can cause increases in FT4 with FT3 values remaining within the reference interval (6). Therefore, the five patients taking diclofenac showed correct FT3 values with the Vitros FT3II assay but altered FT3 values with the Vitros FT3 assay.

In conclusion, the new Vitros FT3II assay showed no interference by diclofenac.

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Kunihiro Iwahara
Chizuko Tanabe
Masato Maekawa

Department of Laboratory Medicine
Hamamatsu University School of Medicine
Hamamatsu, Japan

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Extraction/Chromatographic Testosterone RIA Can Be Used as the “Gold Standard” for Determining the Reliability of Direct Testosterone Immunoassay Measurements

To the Editor:
In their reply to my letter to the editor published in a recent issue of Clinical Chemistry (1), Herold and Fitzgerald state that there are no convincing data showing that extraction/chromatography testosterone RIAs work. In addition, they conclude that unless an extraction/chromatographic RIA has been validated against a gas chromatography–mass spectrometry (GS-MS) method, the results generated from such tests are subject to question. I disagree with their point of view.

The extraction/chromatography RIA method has been used for measurement of testosterone and many other steroid hormones ever since the RIA method was first developed by Abraham (2) for measuring estradiol in 1969. Over the years, this RIA method has remained essentially the same, with the exceptions that tritiated ligands used in the RIAs have been replaced with iodinated derivatives to improve assay sensitivity and that more-specific antibodies are used. When properly validated with respect to sensitivity, accuracy, precision, and specificity and an internal standard is added to each sample to monitor procedural losses, the extraction/chromatography testosterone RIA gives highly reliable results.

It is surprising that Herold and Fitzgerald make the point that there are no convincing data showing that extraction/chromatography RIAs work. For more than 30 years now, testosterone measurements obtained with the extraction/chromatographic RIA have been carried out in numerous studies that have enriched the field of reproductive endocrinology with new knowledge. Furthermore, during those years, this RIA method has been used for diagnostic testing in highly respected laboratories such as Quest Diagnostics Nichols Institute (San Juan Capistrano, CA) and Esoterix Inc. (Culver City, CA) and has provided physicians with valuable information for diagnosing and treating countless numbers of patients. Without question, the extraction/chromatography testosterone RIA works.

The argument presented by Herold and Fitzgerald that there are “considerable problems” with extraction/chromatographic RIAs (1) is based on data in two reports that show comparison of serum testosterone concentrations measured by extraction/chromatography RIA and GC-MS. However, as pointed out in my original letter to the editor, neither report describes a study that was carried out thoroughly. One set of data was included only as a figure.

Three of the five patients were evaluated as euthyroid because their TSH and FT4 concentrations were within the appropriate reference intervals. The other two patients were hypothyroid and were taking levothyroxine sodium. Levothyroxine therapy for hypothyroidism reportedly can cause increases in FT4 with FT3 values remaining within the reference interval (6). Therefore, the five patients taking diclofenac showed correct FT3 values with the Vitros FT3II assay but altered FT3 values with the Vitros FT3 assay.

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Kunihiro Iwahara
Chizuko Tanabe
Masato Maekawa

Department of Laboratory Medicine
Hamamatsu University School of Medicine
Hamamatsu, Japan

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