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RAPID COMMUNICATION

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Overestimation of serum levels of rheumatoid factor caused by the presence of an incorrect calibrator in the Dade Behring kit

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The level of rheumatoid factor (RF) in sera is a widely used criterion that has been adopted by the American College of Rheumatology (ACR) for the diagnosis of rheumatoid arthritis (RA).[1] Measurement of RF was done initially using the Waaler–Rose test.[2] This was succeeded by the latex agglutination test, in which denatured human IgG is absorbed by latex granules. At present, moreover, automated analyzers are being used worldwide with quantitative methods such as turbidimetry or nephelometry to measure RF.[3,4] For instance, the RF measurement kit developed by Dade Behring (DB) (Marburg, Germany) uses a latex agglutination method quantified by nephelometry; its market share is 22% in Japan and 17 % worldwide.[5]

In June 2006, DB notified us that they discovered a discrepancy between their reference material (RM) and the international RM for RF, and that the company would replace its RF standard with new lots whose consistency with the international RM had been confirmed. According to the notification, DB had re-evaluated the traceability of RF on the basis of ISO/EN17511 (quantification of biological samples – traceability for the indicated values of standard preparations and control substances)[6] and found that there was a 50% discrepancy between the company RM and the international RM for RF (NIBSC 64/002)[7], i.e., levels determined based on the company RM were approximately twofold higher than those determined based on the international RM. Our laboratory had been using the DB kit since March 2000. Upon receiving this notification, we realized that it would be necessary to warn our clinicians of the possibility that they may have misdiagnosed patients as having RA on the basis of falsely positive RF tests.

Accordingly, we immediately investigated the time frame and the extent to which this problem had impacted our clinical laboratory. For this analysis, we first assayed a set of samples for RF, comparing the results obtained using commercial standard materials (SMs) from old and new lots. We then reassayed stored specimens (collected from 2000 to date) with the new lot. Finally, we analyzed the changes in monthly RF positivity rates using past data imported from the laboratory information system. RF was measured using a combination of N-latex RF II (DB) and a Behring Nephelometer II (BNII, DB).

In the first step, the correlations relating RF readings made with old (No. 183836) and new (No. 183837) lots of N-rheumatology standard SL were analyzed using 60 clinical samples sent to our laboratory for RF measurement. The correlation coefficient for the RF levels obtained based on calibration curves prepared using the old and new lots was high ($r = 0.983$), but the regression line was defined by the formula $Y = 2.03X + 1.96$ ($n = 44$; Y, old; X, new), as shown in Fig. 1. Thus, the RF levels measured with the old lot appeared to be almost twice as high as those measured with the new lot, which was consistent with the information supplied by DB.

Next, 141 samples collected from five patients during the period extending from 2000 to 2006 (7 RF negative samples from one patient, 35 weakly positive samples from one patient, and 99 positive samples from three patients) and stored at –40°C were assayed for RF using commercial SM from the new lot for calibration. When we compared the new RF levels with those determined from past measurements, we found that RF levels measured after May 2003 tended to be higher than those measured with the new lot (Fig. 2A). All of the past samples from one RF-negative RA patient were also judged as RF-negative based on the

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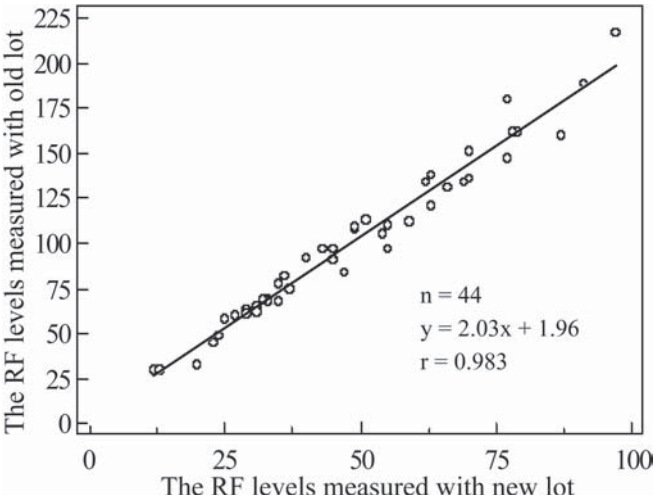

Fig. 1. The correlation between the rheumatoid factor (*RF*) levels measured with the old and new calibrators

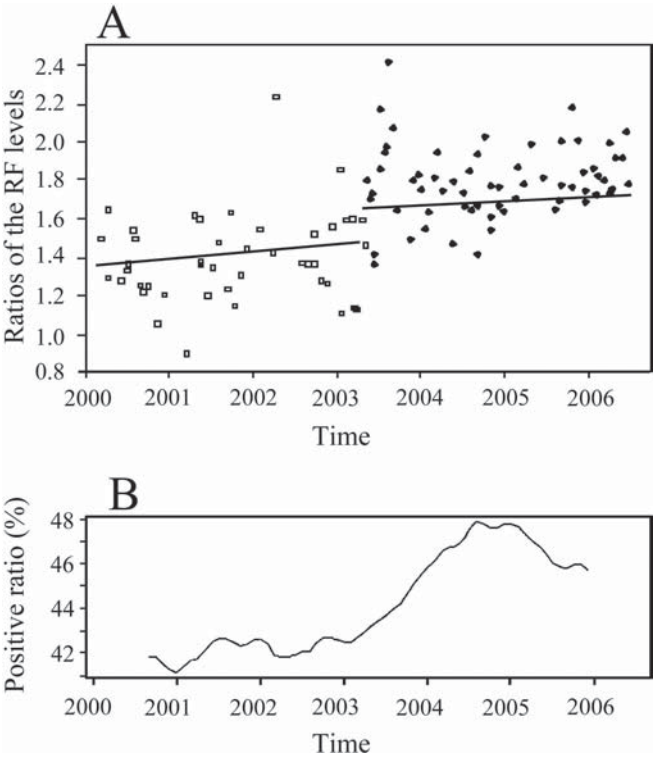

Fig. 2A,B. Time course of the ratios of the rheumatoid factor (*RF*) levels and the RF-positivity ratio. Ratios of the RF levels measured using reference material from the old lot to the level measured using material from the new lot for the samples collected from 2000 to date. *Open symbols* indicate data collected between 2000 and May 2003, *closed symbols* data collected between May 2003 and July 2006. The regression lines for each period are shown in **A**. Time series analysis of the monthly RF-positivity rate ratio during the period from 2000 to 2006 is represented in **B**

measurements made with the new lot. However, a discrepancy was noted in 5 of the 35 samples from the one weakly RF-positive RA patient: 5 samples had been rated as RF-positive (16–23 IU/ml) in the past, but the new measurements indicated them to be RF-negative (<15 IU/ml).

Because our findings suggested that RF levels had become abnormally high at around May of 2003, we then calculated the monthly positivity ratios for RF by collecting all the RF values measured at our clinical laboratory for the period extending from 2000 to the present (about 390 samples/month, 28 897 samples in all). After we analyzed the monthly RF-positivity ratios, time series analysis revealed that the ratios tended to rise during the period extending from early 2003 to mid-2004 (Fig. 2B).

The RF kits produced by DB are being used by many facilities around the world. Because of DB's large market share in Japan, there was the potential for confusion among a large number of Japanese clinicians and laboratories. Unfortunately, DB could not determine when this problem initially appeared, so we began investigating the RF values with the aim of estimating the period during which the incorrect calibrator was in use and then informing our hospital and the broader medical community. The analysis of monthly RF-positivity ratios suggested that RF levels determined at our hospital shifted upward during the period between early 2003 and mid-2004, which was confirmed by comparison of RF measurements of the same samples made using new and old lots of the commercial SM. However, because we could not identify the specific lot involved during that period, other hospitals and laboratories should not simply apply the same time frame to the problem.

We also measured 8281 RF samples collected during the 18 months between January 2005 and June 2006, and obtained RF values between 15 and 30 IU/ml in 592 samples (7.1%). This means that, on average, 33 cases a month were falsely identified as positive for RF during that period; their true values are thought to be about half those reported (<15 IU/ml). In our hospital, we discussed the information from DB, as well as a report from the Department of Clinical Laboratory at the Committee of Medical Safety. We then made a thorough disclosure to our clinicians with the cooperation of the Department of Medical Information and issued a warning of the potential for misdiagnosis.

The quantitative methods used in clinical laboratories require the use of reliable RM. To establish such RM, kit manufacturers first perform a value assignment of the company RM using World Health Organization (WHO) SM as the international RM, and then perform a value assignment of the commercial SM using the company RM in place of international RM. Notably, in that regard, RF is unstable and cannot be kept for prolonged periods in a serum base. Nonetheless, DB has been checking the traceability of new company RM not by using the international RM, but using the older company RM. This procedure would be expected to gradually cause a discrepancy to exist between RF levels determined using the international RM and those determined using the company RM. In an average clinical laboratory, the validity of the cutoff value is usually checked only once, at the time of introduction of each reagent kit. Consequently, a discrepancy between the user's RF levels and the international RM can be easily overlooked when the wrong commercial SM is provided. In the present case, because the cutoff level had been set at a level

lower than the true value, false-positive samples were readily generated, but false-negative samples probably were not. Since RA is usually diagnosed based on ACR criteria, the weakly-positive RF levels would not, by themselves, lead clinicians to a misdiagnosis of RA. However, it is possible that unnecessary clinical investigations may have been performed as a result.

Dade Behring has agreed to implement two quality controls: (1) to check the traceability of WHO SM and the company RM twice a year, and (2) to use paneled samples with long-term stability for product certification during manufacture. Nonetheless, we recommend that users proceed carefully with respect to quality control. For example, users are advised to refer not only to the control material attached to the kit, but also to other controls supplied by a third party. Finally, reagent users should keep in mind that this type of error could happen with any labile RM.

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