position that is used in many Delfia kits (d) but not in the Delfia FT4 kit. Contrary to what we say in the article, the Delfia FT4 Assay Buffer does not contain any bovine serum albumin, Tween 40, or diethylaminoethylpentacetic acid. The buffer used in the first incubation in the Delfia FT4 assay is a 0.05 mol/L Tris buffer, pH 7.4, containing 0.07 mol/L NaCl, 0.01 mol/L sodium azide, and an inert red dye. The formulation of the buffer has not been changed over the period that this kit has been commercially available. We regret the confusion this incorrect information may have caused.

References

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Rapid Glycohemoglobin Measurements

To the Editor:

Herold et al. (1) compared the Primus CLC330 automated HPLC system (Primus Corp., Kansas City, MO), using a 4-min run time, with one manual and two custom-programmed robotic systems. All systems used boronate affinity technology. Since this study was done, significant advancements have been realized with the Primus CLC330. These enhancements include modified sample preparation, which provides lysis without vortex-mixing; software upgrades; and a 2-min run time, making the new version more than twice as fast as the earlier system. The rapid 2-min assay achieves even better precision (CV = 0.4–1.2%) than the "remarkably small within-run and total standard deviation" described by Herold et al.

These changes have reduced sample preparation time to 0.38 min per sample (faster than any of the other three systems) and reduced total analysis time to 2.20 min per sample for the first run of the day, including a 15-min warm-up period, and 2.04 min per sample for subsequent runs. In comparison with the manual method, technologist time is decreased by 85% and total analysis time is decreased by 29%.

In summary, the enhanced version of the Primus CLC330 offers significant advantages in accuracy, precision, and speed, requiring less hands-on time than any of the other three methods, less total analysis time than either the manual or the Accu-Flex robot method, and only slightly more total analysis time than the Hamilton robot (2.2 vs 1.9 min per sample).

Reference

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Human Anti-Mouse Antibodies against OC-125 Do Not Interfere In a CA-125 Assay That Uses OC-125 and M11 Antibodies

To the Editor:

Recent papers in this journal have addressed the problem of increased concentrations of apparent serum CA-125 caused by human anti-mouse antibodies (HAMA) against the murine OC-125 antibody (1-3). We now report that these HAMAs seem no longer to interfere in a new assay that uses the monoclonal antibody M11 in addition to the OC-125 antibody. We observed five patients who had been operated on for ovarian cancer during 1987 and who had received one injection of the murine monoclonal antibody OC-125 during late 1987 or early 1988 before their second-look operation (SLO) (4). Soon thereafter we found large increases in the CA-125 values, which could not be explained at that time by the clinical findings during the SLO, because all patients were considered to be without evidence of disease.

However, given that microscopic disease can never reliably be ruled out in ovarian cancer, we offered the patients the opportunity to participate in a study involving a gonadotropin-releasing hormone (GnRH) analog (5). The procedures followed in this study were in accordance with the ethical standards of our institution's ethical committee. During the next 12 months, CA-125 concentrations decreased steadily in all patients and remained at a plateau for the next few years (an example of the pattern is shown for one patient in Figure 1).

Until 1992, the CA-125 serum concentrations were measured with an immunoradiometric solid-phase sandwich assay based on the murine monoclonal antibody OC-125 as capture antibody as well as labeled antibody (ELSA-CA-125 I; ID-CIS, Dreieich, Germany). Since July 1992, the same manufacturer has provided a new CA-125 assay, called ELISA CA-125 II. In that assay, a new murine monoclonal antibody [M11, developed by O'Brien et al. (6)] is bound to the solid phase and the radiolabeled OC-125 is used as tracer. In agreement with van Kamp et al. (7), our first comparisons of the two assays revealed close agreement: I = 1.14 (II) - 16 kIU/L (n = 31, r = 0.992).

With the new assay (II), CA-125 concentrations in the OC-125-treated patients suddenly dropped to <25 kIU/L. Frozen serum samples from these patients were thawed and remeasured with the new assay. The CA-125 results in samples drawn before the injection of the OC-125 were nearly identical in the two assays, but six months after the injection the increased values for CA-125 observed with the old method were replaced with normal values in the new assay. We knew from previous studies that the sudden increase of the serum CA-125 in these patients was caused by the development of human antibodies against the mouse OC-125 (HAMA) (8).

The HAMA concentrations had been determined by a two-step immunoradiometric assay (Biorama, Edmonton, Canada), which we have previously described in detail (8). Briefly, OC-125 F(ab)2 was immobilized on polysyrene wells and incubated with a suitable dilution of either patient's serum or normal serum. Bound HAMA were detected with goat anti-human IgG (H + L) radiolabeled with 125I. The radioactivity in the wells was counted in a multi-well gamma counter to determine the bound 125I, and the data were expressed as the percent of the input that was bound. Patients were considered to
have statistically significant HAMA if their HAMA value was greater than 2 SD above the mean for a healthy control group (in our series, 10.9% ± 6%) (8).

Besides these effects on the CA-125 assay, comparable interferences of the HAMA with the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) assays were suspected. That suspicion was raised by an observation during the treatment with the GnRH analog. Usually, secretion of LH and FSH by the pituitary is blocked immediately, and their serum concentrations drop below the limits of detection of the corresponding assays (<0.5 IU/L). Surprisingly, only in these five patients did the LH and FSH remain measurable—something we had never observed in other patients who received GnRH analogs (Figure 1).

LH and FSH were measured with immunoradiometric assays with a magnetic solid phase (LH MAIAclone, FSH MAIAclone; Serono Diagnostics, Freiburg, Germany), which are based on the binding of murine monoclonal antibodies to either LH (1st IRP 68/40) or FSH (2nd IRP 78/549).

When we analyzed these sera for their LH bioactivity, we found that these sera were not able to induce testosterone production in mouse Leydig cells. That meant that only immunoreactive LH-like material was present or there was a cross-reaction with the HAMA.

We conclude that the HAMA, which were produced after injection of the murine monoclonal antibody OC-125, do not disturb the CA-125 measurements with the new murine monoclonal antibody M11 (at least, not the HAMA concentrations found in our patients). Because the concentration of added mouse immunoglobulin in the reagent of the new assay remained the same as in the old assay (100 mg/L according to the manufacturer), this improvement should not be caused by the binding of the HAMA to these added mouse proteins.

The course of the remeasured concentrations of CA-125 is better correlated to the clinical course of the patients than were the previous measurements, given that these patients are now 4–5 years after surgery without any sign of relapse. The results further indicate that, even several years after injection of a murine antibody, some HAMA still circulate. Although these HAMA were not measurable with conventional assays, the persistently increased concentrations of LH and FSH in our patients made us suspect continuing interference from HAMA.

It has been speculated that the HAMA induced after the injection of the murine monoclonal antibody OC-125 may be anti-idiotypic (2, 3). If that should be the case, it would lead to two interesting conclusions: (a) the HAMA raised against OC-125 were also responsible for the effects in the LH and FSH assays, which means that CA-125 and LH/FSH share a common epitope (however, preliminary studies with purified LH and FSH could not support that concept), and (b) the M11 antibody acts on a different idioype than OC-125 does, which would mean that we did not measure CA-125, but something probably closely related but different ("CA-126")..

References

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CLINICAL CHEMISTRY, Vol. 39, No. 7, 1993 1557