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Letters

Determination of Oestrogen Receptors by Enzyme Immunoassay

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THE ARTICLE by S. Romain *et al.* (pp. 740–746) states in the discussion section that the Abbott oestrogen receptor enzyme immunoassay (ER-EIA) Monoclonal Kit standardisation is being recalibrated and will be in kits by early 1993. Based on studies from Professor Martin's laboratory, from Professor Goussard's publication [1] and data from other EORTC Receptor Group laboratories, Abbott performed studies to determine if antibodies or calibration changed, two possibilities proposed by Goussard. Although we could find no significant difference between beads and conjugates made with file samples of antibodies produced in the mid-1980s versus current lots of antibodies, we did discover a slow but cumulative change in standardisation from 1988 to 1992.

File samples of the original primary standard (1984 Reference Standard), that had been lyophilised and stored at 2–8°C, were used to make standard curves, and were compared to ER-EIA standards from 1992 kits. All other assay reagents were identical for the comparison. A total of 129 frozen breast cancer specimen cytosols were prepared as described in the package insert, and assayed by both versions of ER-EIA. We found that the 1992 standard (y) versus the 1984 reference standard (x) yielded a linear regression equation for specimen values of $y = 1.35x - 5.9$ with $r = 0.988$. Specimens were well distributed from 0 to 350 fmol/mg cytosol protein for the reference standard with no individual outliers biasing the slope. It appeared that standardisation changed by 35% over the 8-year period. When the area near the cutoff was analysed, 56 specimens from 0 to 24 fmol/mg gave a linear regression equation of $y = 1.04x + 0.2$ with $r = 0.983$. Again, there were no outliers biasing the slope. When standardisation data from individual lots were analysed, we found that standardisation began to change slowly in 1988 when a secondary standard was first used, and continued to change slowly with subsequent lots of standards, giving a cumulative change of 35%. Individual changes from reference lot to reference lot were 0 to 6% for five lots and 11% for another lot, with each lot having a slightly lower (or equal) potency (results in higher specimen values) than the previous lot. We confirmed in three EORTC Receptor Group laboratories that the ratio of ER-EIA to oestrogen receptor steroid binding assay (ER-SBA) values increased from their original 1984–1987 studies to a higher ratio in a 1992 confirmatory study. The assay has now been calibrated with a new reference standard to match the 1984 reference standard. Our internal quality documentation reflects this calibration, and will minimise the chance of recurrence of a standardisation drift.

The recalibrated standards (y) when tested on 215 frozen breast cancer cytosols and compared to the 1984 reference standard (x) gave a linear regression equation of $y = 0.95x + 0.3$ with $r = 0.990$. The samples were again well distributed over the range of 0 to 400 fmol/mg with no significant outliers. Thus, ER-EIA was recalibrated as closely as possible to the 1984 reference standard. Concordance using a 15-fmol/mg cutoff was 98% for those 215 specimens, with one discordant specimen reading 0.5 fmol/mg higher by the recalibrated assay and three specimens reading 1.5 to 3.6 fmol/mg lower by the recalibrated assay as compared to the 1984 reference standard. Therefore, the cutoff of 15 fmol/mg should be used on the recalibrated assay just as it has been since 1984. ER-EIA kits containing the recalibrated standards started shipping on 1 March 1993 and are clearly identified as such.

To allay any concerns about whether there were specimens near the cutoff reading higher and thus giving more positive samples, it is important to note that the correlation data shows that the slope near the cutoff was only 4% higher, an insignificant amount. For the 23 specimens in the 10–20 fmol/mg range, the 1992 standards gave values an average of 3.5 fmol/mg (range 0.2 to 9.6) higher than 1984 standards.

With respect to the dilution studies described in the paper, the reconstitution and dilution buffers, diluents and procedures are specified in the Abbott ER-EIA package insert. Reconstitution of the kit controls with anything other than the Abbott reconstitution buffer, and dilution of the kit controls with anything other than the 0 fmol/ml receptor standard are not recommended. Preparation and dilution of cytosols with anything other than the Tris homogenisation buffer described in the package insert is also not recommended. As also stated in the package insert, cytosols with protein concentrations of 0.2 to 4 mg/ml have been demonstrated to yield valid ER results. Protein concentrations outside this range may result in ER values which are suspect.

It is important to note that the Abbott progesterone receptor enzyme immunoassay (PgR-EIA) monoclonal has not changed in calibration over time. The original PgR-EIA reference standard is still in use today. No lot-to-lot drift of reference material has occurred.

We concur with Romain *et al.* that standardisation between laboratories of homogenisation parameters is an important step toward reducing inter-laboratory variation.

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