Strand Displacement Amplification and Fluorescence Polarization

To the Editor:
The editorial by Diamandis [1] in January discussed a report by Walker et al. [2], who used strand displacement amplification and fluorescence polarization (FP) to detect Mycobacterium tuberculosis. The editorial placed particular emphasis on the homogeneous format of the assay. However, this aspect deserves to be expanded by an outline of the significance and evolution of combining DNA amplification/hybridization with FP. Walker et al. did not attain the reported results with FP in its traditional format but rather used a radically new fluorometric technology.

The use of FP in binding assays is not a recent event. This technology has been known for >20 years [3], and its use in DNA amplification/hybridization procedures has been reported earlier [4]. Traditional FP with fluorescein and steady-state fluorometric instrumentation has been used successfully as a format for assays not requiring high sensitivity, e.g., monitoring the circulating concentrations of therapeutic drugs, for which it has practically displaced most other technologies. Despite its homogeneous format, however, traditional FP has not been able to compete successfully with enzyme-linked immunodiagnostic procedures for the great majority of other assays, and it has not established itself as a viable option for DNA hybridization/amplification procedures either. The reason for this inability is that, in its traditional format, fluorometry has been plagued by substantial background interference, resulting in low sensitivity.

Walker et al. report the use of the new fluorescent dye, La Jolla Blue, and the new detection technique, transient-state fluorometry, both engineered to dramatically reduce background interference and improve signal-to-noise ratio. La Jolla Blue fluoresces at 705 nm, near the infrared region, where intrinsic fluorescence from other serum components is substantially less. Compounding this reduction of autofluorescence is the transient-state detection technique, which performs the excitation by short pulses of a laser diode and measures the decaying fluorescence in the intervals between the pulses. The background noise caused by light-scattering is thus eliminated from the excitation beam. In comparison with traditional fluorometric methods that use fluorescein and steady-state detection, either of the two components of the new technique can improve the signal-to-noise ratio by ~10-fold, and the two together improve the sensitivity by ~100-fold. Earlier, Devlin et al. [5] already reported the use of La Jolla Blue and transient-state fluorometry in connection with another DNA amplification procedure known as 3SR. It is this recent quantum leap in the sensitivity of fluorometric detection and quantification—provided by the combination of La Jolla Blue with transient-state measurement—that has made FP a viable, and in fact superior, format for DNA amplification and hybridization procedures.

References

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Effect of Alum on Methamphetamine Screening Tests

To the Editor:
Recently, alum has been reported to interfere with the detection of methamphetamine by portable test kits [1]. To determine whether other methamphetamine screening tests could also be compromised, we analyzed certified negative urine containing various concentrations of d-methamphetamine and d-amphetamine hydrochlorides (Sigma Chemical Co., St. Louis, MO) and alum. To simulate more realistic tampering conditions, we used alum that is commonly used for pickling (McCormick, Hunt Valley, MD; lot 1056B). Testing methods included RIA (Roche Abscreen®, Roche Diagnostic Systems, Branchburg, NJ; serum reagent lot no. 0017, second-antibody lot no. 0044, 12I-reagent lot no. 0082), fluorescence polarization immunoassay (Abbot TDX® Amphetamine/Methamphetamine II; Abbot Labs., Abbott Park, IL; lot no. 10025Q101) with an Olympus AU800 (Olympus America, Lake Success, NY), and kinetic interaction of microparticles in solution (KIMS; Roche Online®, Roche, Somerville, NJ; lot no. 30195).

RIA was performed on urine supplemented with d-methamphetamine at concentrations of 0, 400, 500, 750, and 1000 µg/L and with alum at 0, 25, 50, 75, and 100 g/L. Solutions containing alum displayed count rates within 10% of the corresponding untreated solutions. No false-positive or false-negative results were observed. Screening with the Abbott TDXs also produced results that were consistent and unaffected by the presence of alum.

When testing was performed with the Online reagents, however, and with d-amphetamine as a calibrator (at a cutoff concentration of 500 µg/L) and a onepoint calibration, increasing the concentrations of alum produced significantly higher readings than those produced by untreated samples. Methamphetamine, which by itself is only ~98% as reactive as d-amphetamine at the 500 µg/L cutoff [2], produces marginally negative readings. However, at alum concentrations of 100 g/L, false-positive results were observed for methamphetamine, phencyclidine, and cocaine in the absence of these compounds (summarized in Table 1). Similarly increased concentration readings were obtained for mixtures containing alum and amphetamine.

We note that adulteration of a collected specimen at an alum concentration of 100 g/L would involve the addition and surreptitious dissolution of a relatively large amount of powder and would be relatively difficult to achieve during an observed urine collection, had false-negative results been observed. Also, any addition of alum markedly changes the urine pH (in these cases from ~5.6 to pH 2-3), as previously reported [1], and would present an indication of tampering. To determine whether alum was affecting the Online test through a simple change of acidity, we assayed urine...
specimens containing 625 μg/L of amphetamine, adjusted to pH values between 2 and 9. Changes in pH had no effect on the instrument readings for amphetamine.

References

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Prostate-Specific Antigen Immunoactivity in Women with Breast Cancer

To the Editor:

Human prostate-specific antigen (PSA), an androgen-dependent monomeric glycoprotein of 30–34-kDa molecular mass structurally and functionally related to the kallikrein family of serine proteases, has long been considered a gender- and tissue-specific protein [1]. Recently, however, several reports described the detection of PSA immunoactivity and mRNA in normal and malignant non-prostatic tissues [2, 3], including breast tumors [4]. This last report led us to measure PSA immunoactivity in several tumor tissue cytosols routinely prepared in our laboratory for analysis for steroid hormone receptors.

Frozen tissue (0.2–0.5 g wet weight) was pulverized in a microdismembrator; homogenized with 5- to 10-fold excess 10 mmol/L Na2MoO4, 10 mmol/L NaH2PO4, 1.5 mmol/L EDTA, 10 mmol/L α-monoothioglycerol, 100 mL/L glycerol, pH 7.4; and centrifuged for 45 min at 105 000 g. The supernatant was analyzed for protein concentration with a Coomassie Brilliant Blue binding assay (BioRad Labs., D-80901 Munich, Germany) and for estrogen (ER) and progesterone receptor (PR) content by Scatchard saturation analysis with the dextran-coated charcoal method and with [3H]-labeled estradiol and ORG 2058 as tracers (Amersham Buchler, D-38110 Braunschweig, Germany). An aliquot of the cytosol was stored in liquid nitrogen for 1–3 months until tested for PSA immunoactivity with the Tandem-R PSA immunoradiometric assay (Hybritech, D-50996 Cologne, Germany). Instructions given by the manufacturer for determination of PSA in human serum were followed, except that the calibration curve (2–100 μg/L) was extended by concentrations of 0.5 and 1 μg/L. Inter-assay CVs as measured with serum-based controls were 17% at 0.21 μg/L and ±8% at 1.1, 3.1, and 25.7 μg/L mean PSA concentrations.

We analyzed 100 successfully prepared female tumor cytosols (87 primary breast cancer, 3 lymph node metastases of breast cancer, and 10 ovary carcinoma). PSA immunoactivity was below the lower limit of quantification (0.1 μg/L) in 69 cytosols, including all the samples from lymph node metastases and ovarian tumors. However, 19 breast tumor cytosols reproducibly exhibited apparent PSA concentrations between 0.1 and 0.6 μg/L (0.03–0.15 ng/mg protein), and 12 cytosols had apparent PSA concentrations between 1.2 and 51.6 μg/L (0.3–11.9 ng/mg protein). When the latter samples were serially diluted and re-tested, we obtained the same results, thus demonstrating parallelity of cytosolic PSA immunoactivity and the dose-response curve measured with standard PSA. For 34 cytosols, including the one with the highest apparent PSA concentration and 10 other PSA-immunoreactive cytosols, corresponding serum samples were available that had been taken on the day of tumor resection; PSA immunoactivity could not be detected in any serum.

Of the 19 cytosols that yielded PSA immunoactivities between 0.1 and 0.6 μg/L, 4 exhibited ER and PR concentrations above the lower limit of detection (5 fmol/mg protein) but below our level of significance for receptor-positive breast cancer cytosol (20 fmol/mg protein). Four more samples showed ER concentrations of 8–15 fmol/mg protein and PR concentrations >20 fmol/mg protein, and 11 cytosols had ER and PR concentrations both >20 fmol/mg protein. All cytosols with PSA immunoactivities ≥1.0 μg/L were ER- and PR-positive. However, no significant linear correlation was observed between the apparent PSA concentration and the concentrations of ER (r = 0.03) or PR (r = 0.25), patient’s age (r = 0.05), or tumor size (r = 0.15).

Our findings demonstrate that PSA immunoactivity is clearly detectable by a frequently used IRMA in ~30% of female breast cancer cytosols (in agreement with Yu et al. [4]) but not in the corresponding sera [5]. Its biological function as well as the factor(s) that control its expression in breast tumor tissue.