Fig. 1. Buoyant density gradient separation of benign and malignant prostate cells from peripheral blood.

4. [3H]Thymidine incorporation assay for determination of buoyant density of freshly resected and disaggregated (filled boxes) prostate cancer cells and cells from a benign prostate hyperplasia (open circles). Cells were laid on top of the two-layer gradient after incubation with [3H]thymidine for 12 h and centrifuged for 20 min at 450g. x axis: sequence of 500-μL fractions taken from the top to the bottom of the tube. y axis: β radioactivity in decompositions per minute (dpm). C) Density gradient tube after centrifugation. The different cell fractions are indicated. C) Streptavidin–alkaline phosphatase–NewFuchsin-stained epithelial-derived cells enriched as a cell cluster from peripheral blood of a prostate cancer patient. The anticytokeratin 8/18 antibody (clone K5 5 + 8.22/C 22, IgG2; DAKO, Heidelberg, Germany) was visualized with streptavidin-conjugated alkaline phosphatase (Jackson ImmunoResearch, West Grove, PA: 1.0 g/L, 1:1000 in blocking solution). The reaction with alkaline phosphatase was developed with NewFuchsin (NewFuchsin kit; Dako, Carpinteria, CA).

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The "Correct" PSA Concentration

To the Editor:
The article by van Duijnhoven et al. [1] was a fascinating account of a patient who developed metastatic carcinoma of the prostate (CAP) and subsequently died of this disease. Serum samples from this patient assayed with several commercially available PSA assays had markedly different prostate-specific antigen (PSA) values [both total (T-PSA) and free PSA (F-PSA)]. Similar findings have been reported previously [2–8], showing that PSA results by different assays cannot be used interchangeably.

While comparing various PSA assays, the authors found a patient's serum specimen that they regarded as an "outlier." When this specimen (sample 070993), along with a second specimen (sample 041093) selected from the nine samples available over the 20-month period preceding the patient's death, were assayed
for T-PSA concentration, values were obtained on sample 070993 ranging from 6 \mu L/L (Immulite, 2nd generation; Diagnostic Products Corp., Los Angeles, CA) to 139 \mu L/L (ACS:180; Ciba Corning Diagnostics Corp., East Walpole, MA) and on sample 041093 ranging from 13 \mu L/L (Immulite, 2nd generation) to 276 \mu L/L (ACS:180) (Table 1 in ref. 1).

Van Duijnhoven et al. state that in sample 041093, in contrast to the Coat-A-Count IRMA (Diagnostic Products Corp.), which measured the proportion of \alpha_1-antichymotrypsin (ACT)-bound PSA [or complexed (C-PSA)] as 23\%, "with the IMx, which measured the PSA concentration in the serum of this patient correctly, this fraction was 47\%. The Coat-A-Count IRMA is nearly equimolar for bound and free PSA and the IMx assay results are corrected for the fact that this assay is not equimolar for free PSA and ACT-PSA" [1].

I suggest that these statements are confusing and potentially misleading for two reasons. First, if the basis for signifying the PSA values obtained on this patient's serum samples with the IMx method/instrument (Abbott Labs., Abbott Park, IL) as the "correct" PSA concentration is that the markedly increased IMx PSA values are expected in serum samples collected 2–3 months before the death of a patient from disseminated CAP, then the PSA values obtained with the ACS:180 method/instrument are equally "correct," notwithstanding the fact that higher PSA values are obtained with the PSA_T assay in the ACS:180 instrument, compared with most other commercially available PSA assays. It should be noted that the recently introduced Ciba Corning PSA_T assay has been "restandardized" and reported to provide PSA values similar to those obtained with the Tandem R (Hybritech, San Diego, CA) assay [9].

Second, the PSA values reported on samples 070993 and 041093, obtained with the IMx assay, apparently were not corrected for the fact that the IMx assay is not an equimolar response assay. However, the authors' statement quoted above implies that the IMx instrument "automatically" corrects for this fact. I am not aware of such an automatic correction being performed by this instrument.

Using PSA calibrators kindly provided by Hybritech, we recently studied the response of the IMx and Tandem E (Hybritech) PSA assays to samples containing different ratios (0:100, 25:75, 50:50, and 100:0) of F-PSA to C-PSA (bound) and an expected T-PSA concentration (T-[PSA]_exp) of 2.5, 5, 10, or 20 \mu g/L (Fig. 1). At a ratio of F:C = 50:50, the IMx assay overestimated the T-[PSA]_exp by 52\% (30.4 \mu g/L vs 20 \mu g/L) to 60\% (80 \mu g/L vs 5 \mu g/L; 16.0 \mu g/L vs 10 \mu g/L), while the Tandem E assay yielded values in closer agreement with the T-[PSA]_exp at all ratios of F:C and all T-PSA concentrations (Fig. 1). At a ratio of F:C = 50:50, the range of variability between the observed T-PSA concentration, quantified with the Tandem E assay, vs T-[PSA]_exp ranged from +12\% (T-[PSA]_exp = 2.5 \mu g/L and 5 \mu g/L) to +22\% (T-[PSA]_exp = 20 \mu g/L).

Presumably, this variability represents, predominantly, analytical imprecision, and not a skewed response of the Tandem E assay to the samples containing different proportions of F:C PSA, because all of the curves obtained with these samples have a slope nearly equal to zero (Fig. 1B). When we used least-squares linear regression analysis to determine the equation for the line of best fit for each curve, values for the slope of these lines (bottom curve to top curve) were 0.34, −0.04, −0.10, and 0.10.

The slope of the line of best fit for each of the curves for the IMx assay, on the other hand, is significantly different from zero (i.e., the lines are "skewed" and not horizontal with a slope nearly equal to zero) as the proportion of F-PSA in each of the calibrators increases (Fig. 1A). Values for the slope of these lines (bottom curve to top curve) were 0.66, 1.39, 2.64, and 5.44. In addition, the observed PSA concentration in each calibrator containing 100\% F-PSA is increased approximately twofold over the observed PSA concentration in these calibrators when they contain 0\% F-PSA (Fig. 1A).

If we make the following assumptions: (a) the PSA concentration of 101 \mu g/L, obtained on sample 070993 by using the IMx assay, was not corrected for the unusually large percentage (53\%; F:C = 53:47) of F-PSA present in this sample; (b) a similar ratio (i.e., ~50:50) of F:C occurs in sample 041093; (c) the IMx assay overestimates by 52–60\% the total PSA concentration in serum samples containing ~0.50% F-PSA and C-PSA (Fig. 1); and (d) the Tandem R assay is similar to the Tandem E assay and "overestimates" the T-PSA concentration in sample 070993 similarly (i.e., 12\% to 22\%), then the Tandem R value of 68 \mu g/L on sample 070993 is likely to be closer to the "correct" T-PSA concentration in this sample than the PSA value of 101 \mu g/L obtained by using the IMx assay. On the basis of our determination (Fig. 1) of the percentage by which the IMx assay overestimates the T-PSA concentration, I estimate that the corrected IMx T-PSA concentration ranges from 40 \mu g/L to 48 \mu g/L, values closer to 68 \mu g/L (or 53 \mu g/L to 60 \mu g/L, if we correct for the imprecision of the Tandem E assay) than to 101 \mu g/L. Unfortunately, sample 041093 was not assayed with the Tandem R method, possibly because of the insufficient quantity of serum remaining. On the basis of the above, if this sample had been assayed with this method, I predict that a T-PSA concentration of 95 to 109 \mu g/L would have been obtained.

Even if all the above assumptions are correct, the advantages of so-called equimolar response over skewed-response PSA assays are controversial, and the exact meaning of these terms is confusing to both laboratorians and clinicians. A T-PSA value of 101 \mu g/L is not likely to change diagnostic decision making, treatment, or patient outcome, compared with a value of 68 \mu g/L, especially...
in a patient with well-defined metastatic disease by other clinical criteria. Moreover, these authors suggest (I believe correctly) that the prevalence of this discrepancy [i.e., serum samples from patients with CAP containing such a large percentage (53%) of F-PSA] is unknown, but probably very low" [1]. In a previous study of the percent of F-PSA in 653 serum samples from patients with CAP or benign prostatic hyperplasia, which affects men in the same age group as CAP and can confound the discrimination of malignant prostate disease on the basis of T-PSA measurement alone [10], <5% of these patients had ≥50% F-PSA in their serum [11]. It would have been interesting to determine, using several different PSA assays, the T-PSA concentration in the serum samples from this subgroup of patients.

To my knowledge, no published clinical studies have provided unequivocal evidence to support the diagnostic advantage of one type (i.e., equimolar response vs skewed response) of T-PSA assay over the other. In my view, the controversy over this issue can be resolved only by demonstrating conclusively in a large, well-controlled, and well-designed clinical trial, whether patients' serum samples containing an abnormally large percentage of F-PSA occur with a frequency that clearly affects the diagnostic accuracy, efficiency, and predictive value of T-PSA values obtained by one type of assay over the other—when compared over the full range of medically important total PSA values (e.g., undetectable to 4 µg/L; 4–10 µg/L; >10 µg/L).

Unfortunately, the controversy over this issue is delaying the much-needed effort to standardize PSA values obtained with different assay methods by achieving consensus an internationally recognized PSA reference preparation. Until this happens, we are back at the beginning: Not all PSA assays are created equal, and the "correct" PSA value may be in the eye of the beholder.

References

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Two of the authors of the article referred to above reply:

To the Editor:
We identified this patient by chance and chose to study the discrepancy further. Many laboratories might have discarded the result as being an outlier. In the course of extensive comparisons between PSA tests, we could have found discrepancies the other way around too. We did not want to contribute to the battle that is going on in the literature about the quality of PSA assays but rather intended to focus the attention to a remarkable observation.

We want to clarify the possible confusion that might have arisen. In the Results section we described the immunogram data and compared the IMx and Coat-A-Count results [1]. On the basis of the clinical data it was clear that the Coat-A-Count results of the neat sample were falsely low. The results of IMx were in agreement with the clinical status of the patient. In this experiment, for calculation of the percent of ACT-bound PSA, the IMx results were manually corrected for the skewed response of the IMx assay by using data presented earlier (ref. 17 in [1]). These data are in agreement with the data submitted by Wians (Fig. 1). Apart from the fact that we considered the results of the Immulite, Coat-A-Count, and Immuno-1 assays to be falsely low, we made no specific statement that any of the other assays would provide incorrect results.

As we stated in our paper, clearly standardization of PSA assays is a major issue. We also mentioned that "the higher results reported by ACS:180 were due to differences in standardization and lack of equimolarity of this ACS:180 method" and that "the IMx assay is not equimolar for free PSA and ACT-PSA." Based upon the high fraction of free PSA in these samples, equimolarity will markedly influence the results of the assays. The suggestion that equimolar assays would, by definition, be better than non-equimolar assays is disputed by the difference in results between these assays, i.e., Tosoh, Tandem-R, and Immulite. If these assays are truly equimolar and the only problem with our patient was the percentage of free PSA, we would expect the different equimolar assays to yield the same results. In the absence of a true test result, we agree with Wians's estimation of 95–109 µg/L as being the most likely Tandem-R result for specimen 041093. On the basis of the difference between this value and the measured result of the Tosoh AIA-Pack assay (35 µg/L), which utilizes the same antibodies as the Tandem-R assay, Wians's presumption that "the Tandem R assay is similar to the Tandem E assay" might not be valid.

It is undoubtedly clear in the eye of every beholder that for this particular patient the PSA results of the Immulite, Coat-A-Count, and Immuno-1 assays are incorrect. We have clearly and explicitly stated in the last paragraph of our paper that "the results of this study do not imply that the assays reporting falsely low PSA concentrations are less suitable for routine use." The real issue of course is the fact that the patient is aberrant, not the assays. In this sense, Wians may have