Lower limits of detection, biological detection limits, functional sensitivity, or residual cancer detection limit? Sensitivity reports on prostate-specific antigen assays mislead clinicians

THOMAS A. STAMEY

It is increasingly important for clinicians to know whether prostate-specific antigen (PSA) is detectable or undetectable in serum of a patient being treated for prostate cancer. The lower the PSA falls after treatment, the more sensitive the cancer is to that therapy and the longer the patient is likely to live.

However, clinicians are faced with a bewildering series of reports from different reference laboratories, often laboratories using the same assay. To take only one example, the "sensitivity" (lower limit of detection?) of the Hybritech Tandem®-R assay (San Diego, CA) is variously reported as <0.05 µg/L, <0.1 µg/L, <0.2 µg/L, and 0.3–0.5 µg/L.

Some of this confusion is caused by the terminology used by laboratorians, which is then misinterpreted by the clinicians. We should all remember G.B. Shaw’s famous comment that the biggest single problem with communication is the illusion that it has been accomplished. A good example is the laboratorian’s use of the term “lower limit of detection” (LLD). Clinicians who see this term rightly believe that any serum PSA value above this number indicates a “positive” result; i.e., there is detectable PSA in the serum of the patient. Unfortunately, commercialism has led assay manufacturers to tout their differences in LLD among assays to the point where laboratory believe that the LLD has clinical significance and subsequently relay these values to clinicians on the laboratory report.

Traditionally, the LLD is determined by running the zero calibrator of the assay many times in the same assay run (intraassay variation) to determine the imprecision of the zero calibrator determination [1, 2]. Many assays for PSA utilize bovine serum albumin (BSA) as their matrix, although other animal sera are also used. However, BSA is a highly purified simple protein that bears no resemblance to the complexities of human serum. Intraassay variation with such simple proteins might be elegantly low, but this would be misleading for the clinician and potentially harmful to the patient. Determination of the LLD for thyrotropin assays in the diagnosis of euthyroidism is quite similar to the problem with PSA assays. As Spencer et al. note, the “American Thyroid Association now considers such intraassay determinations of detection limit to be clinically irrelevant and to give an overly optimistic estimate of assay performance” [1].

Clinicians responsible for management of patients with prostate cancer need to know what value of PSA in male serum indicates the presence of PSA-producing cells. With 100,000 men undergoing radical prostatectomy each year in the US, urologists must identify as early as possible the 40% of men who “fail” radical prostatectomy in order to initiate potentially curative salvage therapy. The confidence limits for determining early postradical prostatectomy failure should approach 99% now that reports of successful salvage therapies are beginning to appear (Gong M, Ferrari M, Stamey TA. Combined androgen deprivation and radiation therapy for treatment of residual prostate cancer after radical prostatectomy; submitted ms.). In addition, urological surgeons need to know with some degree of certainty whether or not their patients have been cured in case modifications of surgical technique are indicated (avoidance of positive surgical margins, etc.).

Vessella et al. [3] and Lange et al. [4] were the first to recognize that the LLD for PSA assays is a misleading number for physicians seeking clinical relevance. They introduced the term “biological detection limit,” which included the LLD as a baseline value, but added to it 2 SD of interassay (run-to-run) precision derived from “determining low level concentrations of human serum PSA close to the LLD”. This important concept recognized that human sera are much more complex than simple matrix solutions, which manufacturers usually design to have the least noise with the lowest CV. Vessella et al. reported a LLD of 0.09 but a biological detection limit of 0.2 µg/L for the Hybritech Tandem-R assay with BSA as the matrix [3]. The problem with determining the “biological detection limit,”

Department of Urology S-287, Stanford University School of Medicine, Stanford, CA 94305. Fax 415-498-6106.

Received October 11, 1995; accepted February 16, 1996.

1 Nonstandard abbreviations: PSA, prostate-specific antigen; LLD, lower limit of detection; BSA, bovine serum albumin; and RCDL, residual cancer detection limit.
however, is that the selection of low-concentration sera to measure assay precision is not easy when the LLD is very low (<0.1 μg/L). Adding seminal fluid PSA to “zero PSA” female sera leads only to free, uncomplexed PSA, rather than the more common form of PSA in serum, i.e., complexed to α1-antichymotrypsin [3]. Because the free form of PSA is one-third the molecular size of the complexed form, antibodies to PSA recognize the free fraction more readily than the complexed form—which can potentially distort assay precisions. One can dilute low-concentration PSA male sera with female serum but, since some female sera contain PSA at concentrations >0.10 μg/L [6], an assay known to be accurate at ultrasensitive values must be used to find female sera with “zero” concentrations of PSA that can serve as a diluent for male sera.

Klee et al. [7], also recognizing the clinical limitations of reporting sensitivities of PSA assays in terms of the LLD, used the concept “functional sensitivity,” which assesses the precision of human serum assays by determining the lowest concentration from a series of serum pools that achieves an interassay CV of <20% [1, 8]. Creation of these pooled sera, however, has the same problems in selection as discussed above for the biological detection limit.

Because of these difficulties, we chose a different term and a different technique to indicate the presence of low concentrations of real PSA in human male serum [9, 10]. We believe the clinical term “residual cancer detection limit” (RCDL) carries the clear implication that the PSA measured in sera from patients treated for prostate cancer is real PSA and not noise from the assay. The RCDL would be appropriate for several or even all therapeutic efforts to treat prostate cancer, including the reappearance of detectable PSA after radical prostatectomy, after hormonal therapy, and after chemotherapy. If radiation therapy or cryosurgical therapy ever achieve the ideal goal of obliterating all prostate epithelial cells, RCDL would be appropriate for these therapies too.

The RCDL can be more simply and directly determined than the “biological detection limit” or “functional sensitivity”; it is especially appropriate when comparing one assay with another because the same male sera can be used to set the RCDL for each assay [10]. To determine the RCDL, clinicians need sera collected 5 years or more after radical prostatectomy from patients who had very small organ-confined cancers without any high Gleason grade 4 or 5 cancer in the prostatectomy specimen and who, on 3-mm step-sections of their prostate, had very small volumes of prostate cancer. In fact, ~10–20% of all radical prostatectomy specimens have cancer volumes of <0.5 mL, a volume so small that one can argue [11] they should never have had the prostatectomy in the first place. Prestigiacomo and I chose 118 sera from 10 men with 5.2–8.8 years of follow-up (mean ± SD 6.5 ± 1.1 year, median 6.3 years) and defined the RCDL as the mean of these sera + 3 SD [10]. For the automated Tosoh AIA-PACK PA® (Foster City, CA) and the Corning-Nichols (San Juan Capistrano, CA) reference chemiluminescence assay, the mean of these sera + 3 SD was 0.02 ± 0.05 (0.07 μg/L) and 0.02 ± 0.03 (0.05 μg/L), respectively [10]. Thus, a serum is 0.07 μg/L by the Tosoh assay (provided one follows the extra precautions for performing the assay as an ultrasensitive one, as described in our report [10]) or 0.05 μg/L by the Corning-Nichols assay, that patient has only 1 chance in 100 of his serum representing a male cured of his cancer by radical prostatectomy. The minimal follow-up of 5 years is important; in a current unpublished analysis of our Stanford series of 366 men treated only by radical prostatectomy and followed for at least 4 years with an ultrasensitive assay, only 4 of 142 failures occurred after 5 years, despite the fact that 161 of the 366 men were followed for >5 years.

Examination of a recent publication [12] emphasizes how much more complicated, and potentially misleading, calculation of the biological detection limit can be in comparison with the RCDL. On the basis of replicate intraassay determinations of a BSA zero calibrator, the reported assay had a LLD of 0.002 μg/L. The biological detection limit, however, was reported as 0.01 μg/L, far lower than any previously published. The methodology for determining the biological detection limit differed from that of Vessella et al. [3]. Instead of determining interassay (run-to-run) variations of several low-concentration human sera close to the LLD of 0.002 μg/L, the authors chose to run only one human serum (0.016 μg/L) 12 times in a single assay (intraassay rather than interassay). Had they followed the recommendations of Vessella et al. [3], their biological limit of detection would have been much higher. When I sent Yu and Diamandis 30 male sera from the 118 used in our earlier report [10] to determine the RCDL of their new ultrasensitive PSA assay, they found it to be 0.09 μg/L. The difference between biological detection limits of 0.01 μg/L and 0.09 μg/L is clearly substantial and leads to very different clinical decisions. Moreover, when they assayed sera from Stanford from whose PSA concentrations initially fell to undetectable amounts, but who ultimately failed radical prostatectomy [10], their assay detected recurrences no earlier than the automated Tosoh assay, for which the RCDL is 0.07 μg/L. Although one might argue that no firm guidelines exist for establishing Vessella’s and Lange’s biological detection limit, I think it clear that to use one assay instead of several near the LLD and to use intraassay variation rather than interassay variation to establish assay precision yields a lower and clinically unrealistic biological detection limit. This example argues strongly for the simpler use of male sera from men known to be cured of their prostate cancer to determine the RCDL. Clinicians will understand the concept of RCDL more easily if the mean + 3 SD of male sera from men known to be cured by radical prostatectomy constitutes the true assay control for their patients because any value greater than the control has a 99% chance of not representing a cured patient—i.e., there is real PSA (not assay noise) in their patient’s serum.

Finally, there is another and important reason to determine and report serum PSA concentrations in relation to RCDL in men treated for prostate cancer. If the manufacturer has set the zero calibrator too high, the PSA assay will yield artifically low LLD values, which are misleading. The reports by Vessella et al. [3] and Klee et al. [7] are both sophisticated analyses of the Abbott IMx® assay, but neither detected the fact that the IMx zero calibrator had been set too high by the manufacturer; consequently, this assay failed to detect low concentrations of
real PSA that other assays were able to detect [10]. The only way this error can be detected is to compare one ultrasensitive assay with another; both Vessella et al. [3] and Klee et al. [7] compared the IMx assay with the Tandem-R, which, at a biological detection limit of 0.2 \( \mu \text{g/L} \), is not very sensitive. If an assay has an extremely low LLD and yet does not exceed the calculated RCDL for that assay until long after the comparison assay has indicated failure of radical prostatectomy, the zero calibrator has been set too high in that assay. This is precisely what happened with the Abbott IMx assay when it was compared with three other ultrasensitive assays [10]. The RCDL for the IMx assay, determined with sera from prostatectomy-cured males, was an incredible 0.01 \( \mu \text{g/L} \), much lower than the other three assays, but the IMx assay could not detect the recurrence of PSA in postradical prostatectomy sera until long after the other three assays had indicated recurrence of the cancer. That the Abbott IMx zero calibrator had been set too high was confirmed by the observation that female sera were generating less signal than the zero calibrator [10]. Thus, one of the great advantages of using the RCDL is that the same control sera from cured radical prostatectomy patients can be run in different assays to establish comparative RCDL values without being captive to how different investigators calculate the biological detection limits, e.g., Vessella et al. [3] vs Yu and Diamandis [12].

It is true that establishing the RCDL requires close examination of the radical prostatectomy specimen by a knowledgeable and careful pathologist to select small, organ-confined cancers of low volume and grade, but numerous institutions have this capability. Moreover, patients who at 5 to 10 years of follow-up after radical prostatectomy have very low nonincreasing PSA concentrations can be easily found today in most medical centers, whereas 5 years ago this was more difficult. In our Department, we are currently collecting large volumes of serum (500 mL) from 10 men known to be cured by the criteria described here. We believe that assaying 10 such sera on each of 10 different days to determine interassay variation will establish a solid RCDL value for any assay. Moreover, the same sera should serve to assign correct signals to the zero calibrator.

Some will object to the introduction of a new term, the RCDL, but PSA is a unique tumor marker for the most common of all cancers. With the single exception of thyrotropin, no other serum protein can so clearly indicate the recurrence of cancer after removal or treatment of the parent organ. Moreover, the term “residual cancer detection limit” precisely describes the clinical situation [9]. Indeed, the use of male serum from men known to be cured of their prostate cancer by the criteria reviewed here [10] is actually no different in principle from the statistical method described by Arbümstruer et al. [13] and Lawson [14], who use a “certified negative urine blank with subsequent calculation of the mean and standard deviation of the blank response” to qualitatively indicate the presence or absence of abused drugs.

One additional laboratory approach can add validity to the absence of detectable PSA in the serum of men apparently cured of their prostate cancer 5 years after radical prostatectomy. Huland et al. (unpublished) have recently shown that a fourfold concentration of 1 mL of serum by lyophilization can increase the concentration of PSA from undetectable amounts into the detectable range of the RCDL or above [15]. We have confirmed their observations in our laboratory, using both lyophilization and Millipore-filtered centrifuged sera. This technique can further facilitate valid selection of sera from men 5 years after radical prostatectomy who have no detectable PSA.

The question of whether ultrasensitive assays can detect periurethral gland leakage of PSA into the serum has been decided only recently by a collaborative study between the Department of Urology at Stanford and the Mayo Clinic [16]. We compared PSA concentrations of 46 men whose bladder, prostate, and urethra had been removed surgically for bladder cancer with those of 92 men who had only their bladder and prostate removed for the same disease. By three different ultrasensitive assays, the greatest difference in median PSA concentrations between the two groups was 0.01 \( \mu \text{g/L} \), strongly suggesting that periurethral gland secretion of PSA into urine [17] does not contribute to serum PSA.

References


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