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# Defining the smallest analyte concentration an immunoassay can measure

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**An immunoassay's minimal detectable concentration (MDC), the smallest analyte concentration the assay can reliably measure, is one of its most important properties. Bayes' theorem is used to unify the five current mathematical MDC definitions. The unified definition has significant implications for defining positive results for screening and diagnostic tests, setting criteria for immunoassay quality control and optimal design, reliably measuring biological substances at low concentrations, and, in general, measuring small analyte concentrations with calibrated analytic methods. As an illustration, we apply the unified definition to the microparticle capture enzyme immunoassay for prostate-specific antigen (PSA) developed for the Abbott IMx® automated immunoassay system. The MDC of this assay as estimated by our unifying approach is shown to be 4.1–7.1 times greater than currently reported. As a consequence, the ability of the assay to measure reliably small concentrations of PSA to detect early recurrences of prostate cancer is probably overstated.**

INDEXING TERMS: Bayesian statistics • detection limit • enzyme immunoassay • minimal detectable dose • prostate disease • sensitivity

One of the most important properties of any immunoassay is its minimal detectable concentration (MDC), the smallest analyte concentration that the assay can reliably measure.[3] The MDC is essential for defining disease states relating to low concentrations of a biological substance, screening for disease with biochemical and molecular markers, characterizing low-dose exposure to a carcinogen or pollutant, and defining lower limits of reliable reporting in screening tests for toxic substances, contaminants, and illicit drugs *[1–7]*. It is the criterion optimized in developing a new assay and the property most often reported when comparing the performance of different assays for the same analyte *[8]*.

The reported MDC is determined by the physical and chemical properties of the immunoassay's constituents and by the mathematical formula used to compute it *[8]*. Although the MDC is defined as the smallest analyte concentration the immunoassay can reliably measure, different interpretations of this statement have led to different mathematical formulations of its definition. Indeed, as we discuss below, the definitions used for immunoassay design are different from those used in assay calibration studies and routine laboratory analyses. Furthermore, research on statistical methods for assay data analysis has not kept pace with the advances in immunoassay technology. Statistical methods currently used were developed 20–25 years ago, when simplifying assumptions and approximations were needed to make analyses computationally feasible. The methods based on these approximations have not been reevaluated, and their impact on immunoassay properties, such as the MDC, has not been reassessed. We summarize the salient concepts underlying the five current mathematical MDC definitions and present a unified definition based on Bayes' theorem. The new definition is compared with the current ones in a study of a microparticle capture enzyme immunoassay (MEIA) for prostate-specific antigen (PSA) developed for the Abbott IMx® automated immunoassay (Abbott Labs., Abbott Park, IL).

## Theory

### FOUR-PARAMETER LOGISTIC EQUATION

Let $X$ denote a concentration of analyte and let $Y$ be the response measured from assaying the analyte sample. We let $[Y|X,\Theta]$ denote the error probability density of the response $Y$ given analyte concentration $X$, where $\Theta$ is the vector of parameters that defines the specific form of the probability density. The error probability density summarizes all uncertainty in the response for a given analyte concentration; its mean function, $E(Y|X,\Theta)$, is the assay dose–response curve; and its variance function, $V(Y|X\Theta)$, describes the variance of the response for a given analyte concentration and level of experimental error *[9]*.

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[3] Nonstandard abbreviations: MDC, minimal detectable concentration; MEIA, microparticle capture enzyme immunoassay; PSA, prostate-specific antigen; and 4PL, four-parameter logistic.

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**Table 1. Current definitions of the MDC.**

| Author | MDC definition |
|---|---|
| Yalow–Berson | $\left\{\frac{dE(Y\|X=0,\theta)}{dX}\right\}$ |
| Ekins–Newman | $\left\{\frac{[V(Y\|X=0,\theta)]^{1/2}}{\frac{dE(Y\|X=0,\theta)}{dX}}\right\}$ |
| Currie (critical limit)[a] | $\{X_c{:}E(Y\|X=0,\theta)+t_{1-\alpha}[V(Y\|X=0,\theta)+V(Y\|X=X_c,\theta)/k]^{1/2}=E(Y\|X=X_c,\theta)\}$ |
| Currie (critical limit, empirical)[a] | $\{X_{c'}{:}\bar{Y}_0+2s_{y_0}=E(Y\|X_{c'},\theta)\}$ |
| Currie (detection limit)[a] | $\{X_d{:}E(Y\|X=X_c,\theta)+t_{1-\beta}[V(Y\|X=X_c,\theta)+V(Y\|X=X_d)/k]^{1/2}=E(Y\|X=X_d,\theta)\}$ |
| Currie (determination limit) | $\left\{X_q{:}\frac{[V(Y\|X=X_q,\theta)/k]^{1/2}}{\frac{E(Y\|X=X_q)}{dX}X_q}=\mathrm{CV}\right\}$ |

[a] The formulae for $X_c$, $X_{c'}$, and $X_d$ assume $E(Y|X,\theta)$ is a monotone increasing function of $X$, as is the case for the Abbott MEIA for PSA.

We make the typical assumption for immunoassay analyses that $[Y|X,\Theta]$ is a gaussian probability density whose mean function is the four-parameter logistic (4PL) or Rodbard equation defined as

$$E(Y|X,\Theta) = \frac{\Theta_1 - \Theta_4}{1 + \left(\frac{X}{\Theta_3}\right)^{\Theta_2}} + \Theta_4 \qquad (1)$$

and whose variance function is $V(Y|X,\Theta) = E(Y|X,\Theta)^{\Theta_5}$, where $\Theta = (\Theta_1, \Theta_2, \Theta_3, \Theta_4, \Theta_5)^T$. For immunoassays in which $Y$ is a counting process, such as radioactivity, fluorescence, or chemiluminescence, $[Y|X,\Theta]$ approximates a Poisson probability mass function with a large mean, and the experimental error is measured by the extra-Poisson error, i.e., the extent to which $\Theta_5 > 1$ *[9]*.

## CURRENT DEFINITIONS OF MDC

Current definitions of the MDC can be expressed as functions of $E(Y|X,\Theta)$ and $V(Y|X,\Theta)$ (Table 1). Yalow and Berson defined the MDC as the slope of the dose–response curve at the zero dose *[10]*. Ekins and Newman defined it as the standard error of the dose–response curve at the zero dose divided by the slope at the zero dose *[11]*. The Yalow–Berson and Ekins–Newman definitions of the MDC have led to the two most widely used theoretical prescriptions for optimal immunoassay design based on mass-action law approximations to the dose–response curve *[12–14]*. Each gives a different prescription for the optimal reagent combination and prediction of the MDC that combination should yield *[12–14]*. In the analysis of immunoassay data, the more common practice is to represent the dose–response curve with the empirically derived 4PL equation (Eq. 1) instead of a mass-action law approximation. With respect to the 4PL equation, the Yalow–Berson and Ekins–Newman definitions are respectively: 0 and undefined if $\Theta_2 = 0$ or $|\Theta_2| > 1$; both finite in the special case of $|\Theta_2| = 1$; and negative infinity and undefined if $|\Theta_2| < 1$. Thus, MDC estimates based on these definitions cannot be reported as part of routine laboratory analyses.

Currie proposed three definitions of the MDC (Table 1) for calibrated analytic methods: the critical limits ($X_c$ or $X_{c'}$), the detection limit ($X_d$), and the determination limit ($X_q$) *[15]*. The critical limit $X_c$ estimates an upper $1-\alpha$ confidence bound of the blank calibrator or zero dose computed from $V(Y|X,\Theta)$. The empirical critical limit $X_{c'}$ estimates an upper 0.975 confidence bound, i.e., upper bound of the 0.95 confidence interval of the zero dose, computed from $s_{y_0}$, the sample standard deviation of the blank responses. The critical limit sets a cutoff at which it can be concluded that an analyte has been detected. $X_d$ is the analyte concentration that has $X_c$ as its $1-\beta$ lower confidence bound. $X_d$ defines a concentration at which the analyte may be reported as reliably detected, where reliably detected means with probability $1-\beta$. $X_q$ is the smallest analyte estimate that can be measured quantitatively with a specified level of precision, where the acceptable level of precision is defined as a coefficient of variation (CV). Currie's definitions separate the neighborhood near the zero dose into three analytic regions: the regions of (*a*) unreliable detection, (*b*) qualitative analysis, and (*c*) quantitative analysis. $X_d$ defines the border between regions *a* and *b*, and $X_q$, the border between regions *b* and *c*. These MDC definitions have been adapted to immunoassays and are used regularly in laboratory analyses *[16–21]*.

The Ekins–Newman and Currie's first two definitions of the MDC may be interpreted in terms of type I and type II errors. For immunoassay measurements near the zero dose, the type I error of level $\alpha$ (probability of a zero dose being declared as nonzero) is defined by the $1-\alpha$ quantile of the probability density of the zero dose. We show below that the Ekins–Newman and the critical limit definitions have different probabilities of type I error because they estimate different quantiles of the zero dose probability density by using different approximation methods. The type II error of level $\beta$ (probability of a nonzero dose being declared as zero) is defined in terms of the probability density of an analyte concentration near the zero dose whose $\beta$ quantile is the critical limit. The mean of this probability density is the detection limit. The detection limit

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definition takes account of type I error of level $\alpha$ and type II error of level $\beta$.

The Yalow–Berson definition has units of response/analyte instead of analyte and is not a measure of the assay's MDC but rather of its sensitivity at the zero dose. *Sensitivity is often used incorrectly as a synonym of the MDC [22, 23].* Hence, this definition will not be used in formulating our definition of the MDC or in our data analyses.

There are six important shortcomings in the formulation and use of Currie's definitions as applied to immunoassays. First, the critical limit definition considers only the type I error, i.e., the error in the zero dose or blank calibrators. Second, neither the critical limit nor the detection limit provides a measure of precision for its MDC estimate, whereas the determination limit does not provide a measure of either type I or type II error *[24]*. Third, each definition assumes implicitly that the uncertainty in low analyte concentrations can be approximated by symmetric probability densities such as the gaussian or *t*. Because negative analyte values are not possible, the probability density of an analyte concentration near zero must be skewed to the right (see Fig. 1A below). Fourth, the critical limit is the most commonly used MDC definition primarily because the empirical critical limit is the simplest to compute. It appears to be forgotten that Currie placed the critical limit well within his region of unreliable detection and that he considered the detection limit to be the smallest analyte concentration that may be reported as detected reliably. As stated above, the detection limit is the border between the regions of unreliable detection and detection acceptable for qualitative analysis. Fifth, backfitting, inverting the dose–response curve formula in Eq. 1 to compute $X$ for a given $Y$, is the method used in these three definitions to estimate the analyte for a given response. Although backfitting gives reasonable analyte estimates, the associated error estimates derived from this technique can be less reliable *[25]*.

A final shortcoming of the current definitions of the MDC arises from the difference between the experimental paradigm under which the MDC is computed and the one used in routine laboratory analyses. The MDC is usually computed in a calibration analysis. This is an assay run in which the dose–response curve is estimated with 8 to 20 blank calibrators and 2 or more replicates of each nonblank calibrator. A routine assay run dose–response curve, however, is estimated with at most 2 replicates of each calibrator, and an MDC is not determined. Hence, the reported MDC is determined under conditions distinct from those used in routine assay runs and is not a run-specific estimate of the smallest detectable analyte concentration. Assay laboratories often address this ambiguity by setting a conservative lower reporting limit well above the MDC stated by the assay's manufacturer.

### DEFINITION OF MDC BASED ON BAYES' THEOREM

We consider the essential concepts embodied in the current definitions of the MDC to be: (*a*) consideration of type I error; (*b*) consideration of type II error; (*c*) a statement of the measurement precision; and (*d*) a logical framework for making a statement regarding the analyte concentration given the measured response in the assay and for comparing analyte concentrations in terms of their probability densities.

To formulate our definition of the MDC, we state the problem of inferring an analyte concentration from the response measured in the assay in terms of Bayes' theorem. Bayes' theorem is a basic law of elementary probability theory used to compute the probability of one event, given that another has occurred. This law is especially appropriate for immunoassay analyses in which, given a specimen's response, we wish to infer what is the most probable analyte concentration the specimen contains. Bayes' theorem can be used to give a mathematical formulation of this statement in terms of a posterior probability density *[26]*. If $[X|Y,\Theta]$ denotes the posterior probability density of $X$ given $Y$, then by Bayes' theorem

$$[X|Y,\Theta] = \frac{[X][Y|X,\Theta]}{[Y|\Theta]} \qquad (2)$$

where $[X]$ is the prior probability density of the analyte concentration, $[Y|X,\Theta]$ is the error probability density defined in Eq. 1, and $[Y|\Theta]$ is the normalizing constant. The prior probability density represents the range of analyte concentrations likely to be observed in the assay and is defined primarily by the assay's working range *[16]*. An immunoassay is by design a statistically informative experiment in that, for any specimen containing an unknown concentration of analyte, information from the assay analysis dominates that known before performing the assay. Therefore, $[X]$ can be modeled as a uniform probability density *[27]*. We have determined from empirical study of numerous immunoassay systems that $[X]$ is well described by a uniform probability density on the interval from 0 to 1.5 times the largest nonblank calibrator *[28]*. Defining explicit functional forms for the prior and error probability densities is essential to apply Bayes' theorem in immunoassay data analyses.

Given $Y$, the response of a specimen containing an unknown concentration of analyte, Bayes' theorem combines information about the immunoassay's properties summarized in the prior probability density with information from the assay experiment defined by $[Y|X,\Theta]$ to compute the most probable set of analyte concentrations for that response. The set of analyte concentrations over which the posterior probability density $[X|Y,\Theta]$ takes on nonzero values defines the uncertainty in the analyte for that response. In other words, the posterior probability density summarizes all the uncertainty concerning the concentration of analyte in a specimen once the response of that specimen has been measured. To report immunoassay findings, it is necessary to choose a single representative concentration from the set defined by $[X|Y,\Theta]$ to be the analyte estimate for the response. We use the median instead of the mean as the analyte estimate for each posterior probability density because the median provides a more representative single number summary for a skewed probability density. The two are equal for a symmetric probability density *[29]*. Thus, we interpret the median of $[X|Y,\Theta]$ as the analyte estimate for the response $Y$ and the range of $[X|Y,\Theta]$ as defining the uncertainty in this estimate. Application of Eq. 2 to the analysis of immunoassay data requires an estimate of $\Theta$. This parameter may be estimated by fitting Eq. 1 to the immunoassay's calibrators and their responses by using

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either standard least-squares or maximum likelihood methods *[9, 28]*.

We interpret the statement that the MDC is the smallest analyte concentration an immunoassay can reliably measure to mean the smallest analyte concentration that may be reported to be greater than the assay's zero dose with a high probability, say 0.95. We take the zero dose to be the set of analyte concentrations that may be inferred from the response of the blank calibrators and represent it explicitly in terms of a posterior probability density with Eq 2. With this interpretation we can use Bayes' theorem to formulate a definition of the MDC in terms of the probability density of the zero dose and the probability densities of concentrations near the zero dose. In this way, we extend the concept of type I error to the consideration of all the uncertainty in the zero dose and we extend the concept of type II error to consideration of all the uncertainty in any analyte concentration near the zero dose. We can also define the precision with which the MDC is determined both as the probability that it exceeds the zero dose and as the CV of its probability density. Therefore, we arrive at one definition that considers all the concepts in the four MDC definitions. The formal statement of our definition is as follows.

*Definition 1.* Let $X_y$ and $X_{y_0}$ be the random variables whose probability densities are respectively $[X|Y,\Theta]$ and $[X|Y_0,\Theta]$, where $Y_0$ is the immunoassay response of the blank calibrators. Find $X_y$ that satisfies the condition

$$\int_{\{x>0\}} [X^*(x)|Y,\Theta]dx = \rho \qquad (3)$$

where $[X^*|Y,\Theta]$ is the probability density of the random variable $X^* = X_y - X_{y_0}$, $x$ represents a specific value of the random variable $X^*$, and $0.5 < \rho \leq 1$. Then, the MDC of the immunoassay is $X_b$, the median of $[X|Y,\Theta]$, defined as

$$\left\{X_b: \int_0^{X_b} [X(x)|Y,\Theta]dx = \int_{X_b}^{x_{max}} [X(x)|Y,\Theta]dx = 0.5\right\} \qquad (4)$$

where $x_{max}$ is the upper endpoint of the range of the prior density. As stated above, $x_{max}$ is taken to be 1.5 times the largest nonblank calibrator. The lower limit of 0.5 for $\rho$ follows from Lemma *1* in the *Appendix*.

To understand this definition of the MDC, we describe its logic with the aid of a graphical representation in Fig. 1. First, we set $\rho$ equal to an acceptable level of certainty such as 0.95. Once $Y_0$, the responses of the blank calibrators, have been measured and the assay dose–response relation defined by Eq. 1 has been estimated by using the calibrators and their responses, we may use Eq. 2 to compute the posterior probability density $[X|Y_0,\Theta]$ (broken curve in Fig. 1A). This probability density defines the error in the zero dose, i.e., the set of concentrations that is most likely, given the responses of the blank calibrators. This probability density illustrates the extent to which the uncertainty in the blank calibrators extends into nonzero concentrations. As mentioned above, it is necessarily skewed to the right because negative analyte concentrations are not physically possible. Similarly, for any $Y$, we may use Eq. 2 to compute the posterior probability density $[X|Y,\Theta]$ (solid curve in Fig. 1A) and take the median of $[X|Y,\Theta]$ (solid vertical line in Fig. 1A) as the associated analyte estimate for that response. To compute our definition of the MDC, we must compute the probability that an analyte estimate is greater than the zero dose. This probability is the same as the probability that the difference between the analyte estimate and zero dose is greater than zero. The probability density $[X^*|Y,\Theta]$ (Fig. 1B) is the probability density of this difference and is computed from $[X|Y,\Theta]$ and $[X|Y_0,\Theta]$ by convolution *[30]*. The black region under $[X^*|Y,\Theta]$ in Fig. 1B is the area to the right of zero and thus defines the probability that the difference between the analyte estimate and the zero dose is greater than zero. This region is defined mathematically by the left side of Eq. 3. If the area of this region is equal to 0.95, then the analyte estimate in Fig. 1A—median of $[X|Y,\Theta]$—is the MDC.

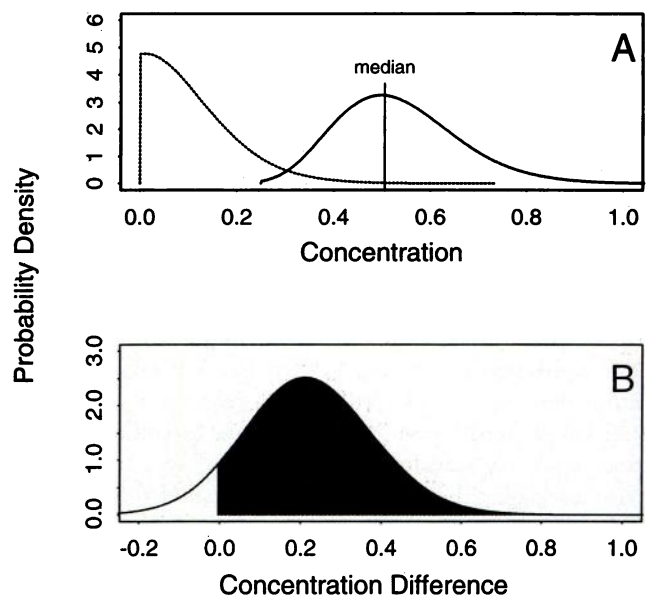


Fig. 1. Graphical representation of the $X_b$ definition of the MDC.

(*A*) The probability density $[X|Y_0,\Theta]$ (*broken curve*) summarizes the uncertainty in the zero dose given the response $Y_0$. The probability density $[X|Y,\Theta]$ (*solid curve*) summarizes the most probable set of analyte concentrations given the response $Y$. Its median (*solid vertical line*) is the analyte estimate given for this response. (*B*) The probability that an analyte estimate is greater than the zero dose equals the probability that the difference between the two is greater than zero. $[X^*|Y,\Theta]$ is the probability density of this difference. The *black area* under $[X^*|Y,\Theta]$ is the left side of Eq. 3 and defines the probability that the difference is greater than zero. If the *black area* is $\rho$, then the median in (*A*) is $X_b$.

The MDC is computed numerically by searching among the responses in the neighborhood of $Y_0$ to locate the analyte concentration in the neighborhood of the zero dose that satisfies Eq. 3. If the number of specimen replicates is >1, i.e., if $Y$ is a vector, there may be a range of analyte concentrations whose probability densities satisfy Eq. 3. In this case, $X_b$ is taken to be the largest analyte concentration in this range.

### RELATION AMONG DEFINITIONS OF MDC

Our definition unifies the current MDC definitions because each of the latter either approximates some component of $X_b$ or

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agrees with $X_b$ under a set of special conditions. These relations are established in *Propositions 1–5* in the *Appendix*. $X_{c'}$ ($X_c$) is a large-sample approximation to the 0.975 ($1-\alpha$) quantile of $[X|Y_0,\Theta]$, the probability density of the zero dose, computed by backfitting and gaussian ($t$-) density approximations (*Propositions 1* and *2*). The Ekins–Newman definition is a large-sample gaussian approximation to the 0.84 quantile of $[X|Y_0,\Theta]$ for a dose–response curve with a nonzero derivative at the zero dose (*Proposition 3*). The same gaussian approximation is used to compute $X_q$ in Table 1. The detection limit, $X_d$, is a large-sample approximation to $X_b$ since it finds the analyte concentration that is greater than the zero dose with probability $1-\beta$. The zero dose is estimated by $X_c$, an approximation of the $1-\alpha$ quantile of the zero dose probability density. The formula for the detection limit in Table 1 uses backfitting and a $t$-density approximation to estimate both $X_c$ and the mean of the probability density that has $X_c$ as its $\beta$ quantile. $X_b$, in contrast, is computed by comparing the probability density of the zero dose with the probability density of every analyte concentration near the zero dose. Moreover, $X_b$ can be computed for any choice of $[X]$ and $[Y|X,\Theta]$. In *Proposition 4* we show that $X_d$ may be derived as a special case of $X_b$.

The concept of precision used in the definition of $X_q$ is an explicit property of $X_b$. This is because each posterior probability density defined by Eq. 2 for $[X]$, a uniform probability density, and $[Y|X,\Theta]$, a gaussian probability density, has a well-defined CV. More generally, because $X_b$ has an associated probability density, its precision may be stated in terms of the probability that it is greater than the zero dose, its CV, or any other property of its probability density that may be analytically important. In *Proposition 5* we show that if $\gamma$ is given and Eq. 2 is used in lieu of the gaussian approximation of Table 1 to compute $X_q$, then it is possible to find $\rho$ in *Definition 1* such that $X_q = X_b$. Conversely we show that, given $\rho$, it is possible to find $\gamma$ such that $X_b = X_q$. Therefore, if a certain level of measurement precision is given in terms of an immunoassay CV, then the probability that the analyte concentration associated with that CV is greater than the zero dose can be determined. On the other hand, if we know how sure we must be to conclude that a concentration is greater than the zero dose, then for the analyte concentration associated with that level of certainty, we can find its precision in terms of its CV.

In *Proposition 6* we show that both the probability of a measurement from $[X|Y_0,\Theta]$ being misclassified as greater than the MDC and the probability of a measurement from the probability density of the MDC being misclassified as indistinguishable from the zero dose equal $1-\rho$.

## Materials and Methods

### THE ABBOTT MEIA FOR PSA

To investigate the implications of our definition, we studied the Abbott MEIA for PSA *[31]*. Serum PSA concentrations are measured to screen for prostate cancer and to monitor its recurrence after medical or surgical therapy *[32, 33]*. The American Cancer Society recently recommended annual screening with a serum PSA concentration and digital rectal examination in all men over age 50 years, and of all African American men and those men with a family history of the disease at a younger age *[34]*. The Abbott MEIA is a widely used PSA assay. Based on $X_{c'}$ computed in calibration runs with 20 blank calibrator replicates and five nonblank calibrators each with two replicates, its MDC is 0.03–0.04 μg/L *[32, 33]*. In this assay's routine runs, each unknown is assayed once and the dose–response curve is estimated with two replicates of the blank and each of the five nonblank calibrators. In our laboratory we set the lower limit of reliable reporting for routine runs at 0.5 μg/L.

**Table 2. Summary of analysis protocol for the study of Abbott MEIA for PSA.**

| Analysis | No. of blank calibrator replicates | No. of specimen replicates ($k$) |
|---|---|---|
| F (routine) | 2 | 1 |
| G | 12 | 2 |
| H (calibration) | 20 | 2 |
| I (excess) | 20 | 4 |

For each analysis denoted F through I, the second column defines the number of blank calibrator replicates used to compute $[X|Y_0,\theta]$ and the third column defines $k$, the number of specimen replicates used to compute each $[X|Y,\theta]$. For example, in each H analysis there were 20 blank calibrator replicates and the number of specimen replicates was 2. Therefore, for this analysis the vectors $Y_0$ and $Y$ had respectively 20 and 2 elements. The F and H analyses correspond respectively to routine and calibration runs of this immunoassay.

### EXPERIMENTAL DESIGN AND DATA ANALYSIS PARADIGM

The protocol was approved by the Committee on Human Studies of the Massachusetts General Hospital. The PSA assay experiment was repeated five times according to Abbott's specifications on its IMx analyzer *[31]*. In each repetition of the experiment 20 replicates of the blank calibrators and four replicates of each of the five nonblank calibrators at concentrations of 2.0, 10.0, 30.0, 60.0, and 100.0 μg/L were assayed. $[Y|X,\Theta]$ was the gaussian density defined in Eq. 1. Since the largest nonblank calibrator was 100 μg/L, we took $[X]$ to be the uniform density on [0, 150] as discussed in *Theory*. The parameter $\Theta$ was estimated by maximum likelihood in each repetition of the experiment from the complete set of 40 observations *[28]*. The blank calibrators were made from sera of normal, healthy women blood donors. Because the IMx analyzer can accommodate only 24 specimens, each run was performed in two parts by using the analyzer's mode one calibration between parts.

Four MDC analyses denoted F through I were performed on the data from each repetition of the experiment (Table 2). In each analysis $X_c$, $X_{c'}$, $X_d$, and $X_q$ were computed from the formulae in Table 1. For $X_c$ and $X_d$ we set $\alpha$ and $\beta = 0.05$. For $X_q$, we set $\gamma = 0.20$, since CVs ranging from 0.183 to 0.276 for PSA concentrations near the MDC have been reported *[32]*. The Ekins–Newman definition is not included in the analyses because, as stated in *Theory*, its formula computed in terms of the 4PL equation gives undefined estimates except for the

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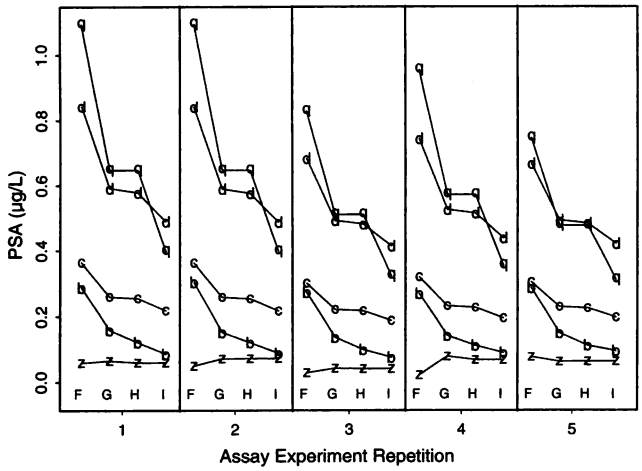


Fig. 2. Estimates of the MDC for the five repetitions of the assay experiment: *F, G, H,* and *I* denote the analyses within each experiment as described in Table 2.

The symbols for the definitions of the MDC are: $z = X_{c'}$, $b = X_b$, $c = X_c$, $d = X_d$, and $q = X_q$.

special case of $|\Theta_2| = 1$.[4] Under the assumption that the random variables $X_y$ and $X_{y_0}$ are independent, given the maximum likelihood estimate of $\Theta$, we computed each $[X^*|Y,\Theta]$ by numerical convolution, using a rectangular integration algorithm based on the fast Fourier transform *[35]*. $X_b$ was then computed numerically from the definition in Eq. 3 with $\rho = 0.95$.

## Results

The same relations among the MDC estimates held in each repetition of the experiment (Fig. 2). In all analyses $X_b$ was less than the conservative estimates of $X_c$, $X_d$, and $X_q$, yet greater than the optimistic estimates of $X_{c'}$. $X_{c'}$ agreed most closely with reported MDC values, ranging from 0.021 to 0.076 µg/L (median 0.059 µg/L). It did not approach the 0.95 quantile of $[X|Y_0,\Theta]$ until the number of blank replicates was 20, mainly because the latter probability densities were all right-skewed (Fig. 3).[5] $X_b$ is 1.6–2.3 times greater than the 0.95 quantile of $[X|Y_0,\Theta]$ in the third repetition of the assay experiment, indicating that, even when well characterized, the latter appreciably underestimates the MDC (Fig. 3 and Table 3). The percent CVs for the zero doses in Fig. 3 suggest that the errors in this calibrator are larger than presently appreciated. Identical percent CVs (74–82%) were found when blank calibrators made

[4] Because the Ekins–Newman definition is an alternative form of Currie's critical limit definition, which estimates the 0.84 quantile of the probability density of the zero dose, the formula for $X_c$ in Table 1 could be used to compute the Ekins–Newman definition by taking $\alpha = 0.16$. We did not do this because by definition, each value of the Ekins–Newman definition computed in this manner will be systematically smaller than $X_c$.

[5] $X_{c'}$ should not equal the 0.95 quantile of $[X|Y_0,\Theta]$ or $X_c$ because in our analysis $X_{c'}$ approximates the 0.975 quantile, whereas $X_c$ approximates the 0.95 quantile. We did not choose the same $\alpha$ for both $X_{c'}$ and $X_c$ because the choice of two in the definition of $X_{c'}$ implicitly sets $\alpha = 0.025$, whereas $X_c$ is usually computed with $\alpha = 0.05$ *[18–21]*. In our analyses, relative to the probability density $[X|Y_0,\Theta]$, $X_{c'}$ is an underestimate of its quantile whereas $X_c$ is an overestimate of its quantile.

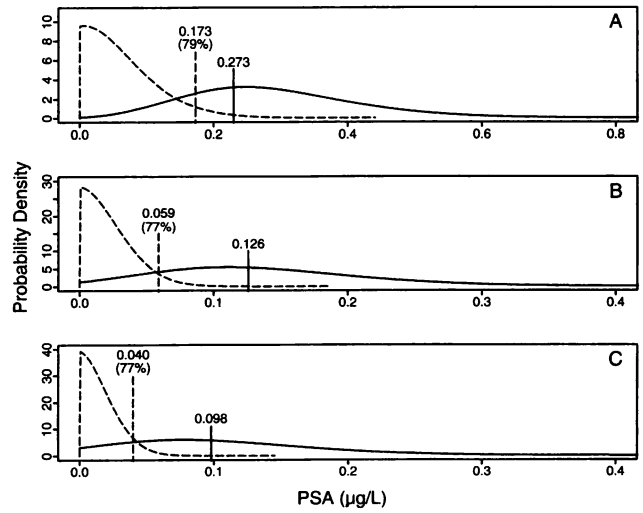


Fig. 3. Posterior probability densities for the third repetition of the assay experiment: $[X|Y_0,\Theta]$ (*broken curve*) and the $X_b$ posterior density (*solid curve*).

The *broken vertical line* is the 0.95 quantile of $[X|Y_0,\Theta]$, the parentheses contain the CVs of $[X|Y_0,\Theta]$, and the *solid vertical line* is $X_b$. (*A*) Analysis F, (*B*) analysis G, (*C*) analysis H.

from the diluent in the Abbott PSA kit were examined with our paradigm.

Unlike the other MDC estimates, $X_{c'}$ did not always decrease with increases in the number of blank replicates, because of sampling variation in the estimation of $\bar{Y}_0$ and $s_{y_0}$. Because the CVs for the $X_b$ posterior densities were much greater than 20% (44–64% in the third repetition of the experiment and 42–64% in all five repetitions), the choice of 0.20 in the formula for $X_q$ led necessarily to conservative MDC estimates. In no analysis was $X_c$ close to $X_{c'}$, nor was $X_d$ close to $X_b$.

These latter observations are not surprising, since *Proposition 1* shows that, even when the same values of $\alpha$ are used in both definitions of the critical limit, $X_c$ will tend to be larger because of the prediction error variance term in its formula. Similarly, *Proposition 4* shows that $X_d$ is typically larger than $X_b$.

In each repetition of the experiment, $X_b$ decreased the most between analyses F and G (Figs. 2 and 3). With 20 blank replicates, increasing the number of specimen replicates from two to four produced only a marginal decrease in $X_b$. As the number of calibrators increased, the $X_b$ probability densities became more right-skewed (Fig. 3). The median values of $X_b$ in analyses F, H, and I were respectively 0.287 (range 0.270–0.304 µg/L), 0.111 (range 0.098–0.121 µg/L), and 0.087 µg/L (range 0.074–0.094 µg/L). The ratio of $X_b$ in an F analysis to $X_{c'}$ in the

**Table 3. Estimates of the MDC for analyses F through I in the third repetition of the assay experiment.**

| Analysis | $X_{c'}$ | $X_c$ | $X_d$ | $X_q$ | $X_b$ (CV[a] × 100%) |
|---|---|---|---|---|---|
| F | 0.028 | 0.320 | 0.684 | 0.830 | 0.273 (44.39) |
| G | 0.040 | 0.220 | 0.490 | 0.511 | 0.126 (55.63) |
| H | 0.039 | 0.216 | 0.482 | 0.511 | 0.098 (63.74) |
| I | 0.039 | 0.187 | 0.413 | 0.323 | 0.074 (59.50) |

[a] The percent CVs are those of the $X_b$ posterior probability densities.

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corresponding H analysis ranged from 4.09 to 7.05 (median 4.71). This latter comparison is important because $X_b$ in the F analyses would be the MDC estimates for routine runs under our paradigm, whereas $X_{c'}$ in the H analyses would be this assay's reported MDC.

## Discussion

On the basis of our findings, a representative value for this PSA assay's lowest limit of reliable reporting is 0.29 μg/L instead of 0.5 μg/L in routine runs, and 0.11 μg/L instead of 0.03–0.04 μg/L in calibration runs. To use this PSA assay for prostate cancer diagnostic and therapeutic decision-making, we recommend that $X_b$ be computed for each run and only those measurements that exceed it be reported as significantly greater than zero. We further recommend that changes be made in this assay's present design, to measure reliably PSA concentrations <0.29 μg/L in the routine assay.[6]

The Abbott IMx PSA assay has been shown to have correlations of 0.9909 and 0.99 with Hybritech's Tandem-R® and Tandem-E® PSA assays (Hybritech, San Diego, CA), respectively *[32, 33]*. Dnistrian et al. also showed that the MDCs based on $X_{c'}$ for the IMx and the Tandem E PSA assays were in very good agreement *[33]*. They were both 0.04 μg/L in one comparison and 0.08 μg/L for the IMx and 0.12 μg/L for the Tandem E in a second. Yu and Diamandis reported an ultrasensitive PSA assay with $X_{c'}$ = 0.002 μg/L and a correlation of 0.96 with the Tandem PSA assay *[39]*. The greater-than-fourfold median ratio we found between $X_b$ in the routine assays and $X_{c'}$ in the calibration assays together with the high interassay correlations found in these studies raises the possibility that a ratio of similar magnitude may exist in these three PSA assays.

Two other specially designed PSA assays have reported MDCs based on $X_{c'}$ of 0.009 and 0.03 μg/L, computed with respectively 20 and 12 replicates of the blank calibrator *[40, 41]*. Our analysis suggests that these MDC estimates may be understated because of the shortcomings in the definition of $X_{c'}$ coupled with the fact that in routine use of the assay it is unlikely that as many blank calibrators would be used. Fig. 2 further suggests that if the detection limit or the determination limit, the definitions Currie considered to be reliable measures of minimal detection, were computed instead of the empirical critical limit, then the reported MDC of the Abbott IMx PSA assay would be much higher. The same is most likely true for these new ultrasensitive immunoassays as well.

Our approach makes two improvements in the current MDC computational formulae: Bayes' theorem is used to compute $[X|Y,\Theta]$ from $[Y|X,\Theta]$, and exact numerical convolution methods are used to compute $[X^*|Y,\Theta]$. The first obviates the use of backfitting and large-sample approximations to compute the most likely analyte concentration for a given response. It also formulates the MDC definition explicitly in terms of the probability that an analyte is greater than the blank calibrators. The second obviates the need to combine backfitting and *t*-density or gaussian approximations to right-skewed probability densities to treat simultaneously the error in the blank calibrators and in analyte concentrations to the right of zero. These improvements are primarily responsible for the differences we found between the current MDC definitions and $X_b$, and why the latter is a more reliable MDC measure determined with either a small or large number of specimen replicates.

$X_b$ may be reported for any immunoassay run that includes blank calibrators in the estimation of its dose–response curve. We have computed $X_b$ for immunoassays for several different analytes (antithyroid globulin, *cis*-platinum-DNA adducts, cortisol, melatonin, parathyroid hormone, thyroid-stimulating hormone, and thyroxine), with different labels (chemiluminescent, enzyme, fluorescent, and radioactive) and different formats (competitive binding and sandwich) *[28* and unpublished data*]*. $X_b$ eliminates the current ambiguity in MDC interpretation caused by differences among the MDC definitions. This is because we used Bayes' theorem to construct our inference paradigm. As a consequence, for any analyte concentration, including the MDC, we can make a quantitative statement regarding its precision in terms of the probability that it (the analyte) is greater than the zero dose, its CV, or any other property of its probability density relevant to an analysis (Table 3). Therefore, we remove the need for four different MDC definitions and for Currie's distinction between regions of unreliable, qualitative, and quantitative detection for analyte concentrations near the zero dose.

$X_b$ also eliminates the ambiguity in MDC interpretation caused by the difference between an assay's calibration and routine runs. Reporting $X_b$ for each immunoassay provides an assay-specific estimate of the smallest reportable analyte concentration and provides a reliable means of monitoring assay quality control at low concentrations. $X_b$ can be made more or less conservative by choosing a smaller or larger probability for $\rho$ in Eq. 3. Our definition also suggests a new, readily interpretable paradigm for optimal immunoassay design: Use the concentration of reagents and experimental conditions that minimize $X_b$ for a given number of calibrators and replicates. This would allow the same MDC definition to be used for immunoassay development and laboratory analysis. Several new technologies are being used to enhance immunoassay performance at low analyte concentrations *[42]*. For example, the new immuno-PCR assay reports its MDC as <600 molecules *[43]*. Our paradigm offers a rigorous means of quantifying improvements in assay MDCs.

The high specificity of monoclonal antibodies and sandwich assays suggests that for certain immunoassays the accuracy of our methods can be enhanced by deriving the dose–response curve from the mass-action laws. Improvements may also be made by developing a more physically based probability model of the assay experimental error and by taking account of interassay variation. To account for interassay variation in the definition of $X_b$, we can define the probability densities

[6] Improving assay reliability at ever smaller PSA concentrations is one current strategy for improving early detection of prostate cancer and its recurrence *[36]*. The approach is complicated because PSA exists in at least two forms in serum and conditions other than cancer are associated with measurable amounts of PSA in the serum *[37]*. The best choice of analyte for a PSA assay and the assay's most efficient use for early detection are topics of current debate *[38]*.

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$$[X|Y] = \int [X|Y,\Theta(\Theta)][\Theta(\Theta)]d\Theta \quad (5)$$

$$[X|Y_0] = \int [X|Y_0,\Theta(\Theta)][\Theta(\Theta)]d\Theta \quad (6)$$

where $[\Theta]$ characterizes the uncertainty in $\Theta$ among assays with acceptable quality control. $X_b$ is then computed by substituting $[X|Y]$ and $[X|Y_0]$ for $[X|Y,\Theta]$ and $[X|Y_0,\Theta]$, respectively, in its definition. The biologic detection limit was proposed by Vessella et al. *[32]* to correct for optimistic values of $X_{c'}$ as an approximation to the 0.975 quantile of $[X|Y_0]$. These considerations are under investigation.

Commonly used immunoassay-based tests predicated on detecting small concentrations of a biologic substance depend critically on the MDC for defining a positive result. Among these are tests for viral infections (HIV, hepatitis B and C), thyroid dysfunction, food contamination, exposure to polyaromatic hydrocarbons, presence of forensic evidence, and illegal use of controlled substances *[1–7]*. Our paradigm suggests a reliable means of defining a positive result for these procedures. Because the measurement of small analyte concentrations with calibrated methods (spectroscopy, chromatography, and quantitative PCR) is an important problem in many disciplines, our paradigm for determining the MDC should be applicable to other analytic procedures.

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## Appendix

In the following propositions we assume that $[Y|X,\Theta]$ is defined by Eq. 1, $[X]$ is a uniform probability density on the interval 0 to $x_{max}$, where $x_{max}$ is 1.5 times the largest nonblank calibrator, $[X|Y,\Theta]$ is defined by Eq. 2, $X_b$ is given in *Definition 1*, and $X_c$, $X_{c'}$, $X_d$, and $X_q$ are defined by the formulae in Table 1.

**PROPOSITION 1**

For $\alpha = 0.025$, the difference between $X_c$ and $X_{c'}$ tends to 0 as the number of replicates at each calibrator becomes large.

Proof: To compute $X_c$ and $X_{c'}$ from experimental data, we replace $\Theta$ by its maximum likelihood estimate $\hat{\Theta}$ in their formulae. $\bar{Y}_0$ and $E(Y|X=0,\hat{\Theta})$ are respectively the method of moments and maximum likelihood estimates of $E(Y|X=0,\Theta)$. Similarly, $s_{y_0}$ and $V(Y|X,\hat{\Theta})$ are respectively the method of moments and maximum likelihood estimates of $V(Y|X=0,\Theta)$. If the 4PL equation is a good approximation to the true dose–response curve, then as $k$, the number of replicates at each calibrator, tends to infinity, both $\bar{Y}_0$ and $E(Y|X=0,\hat{\Theta})$ converge to $E(Y|X=0,\Theta)$ and both $s_{y_0}$ and $V(Y|X=0,\hat{\Theta})$ converge to $V(Y|X=0,\Theta)$. That is,

$$\bar{Y}_0 \xrightarrow{p} E(Y|X = 0,\Theta) \quad (A.1)$$

$$E(Y|X = 0,\hat{\Theta}) \xrightarrow{p} E(Y|X = 0,\Theta)$$

$$s_{y_0} \xrightarrow{p} V(Y|X = 0,\Theta)$$

$$V(Y|X = 0,\hat{\Theta}) \xrightarrow{p} V(Y|X = 0,\Theta),$$

where $\xrightarrow{p}$ indicates convergence in probability *[44]*. Similarly,

$$V(Y|X = X_c, \hat{\Theta}) \xrightarrow{p} V(Y|X = X_c, \Theta)$$

as $k \to \infty$ and therefore,

$$V(Y|X = X_c, \hat{\Theta})/k \xrightarrow{p} 0.$$

Also as $k \to \infty$, the $t$-density converges to a standard gaussian probability density and in particular, with $t_{1-\alpha} = t_{0.975}$, the 0.975th quantile of the $t$-density converges to $1.96 \approx 2$, the 0.975th quantile of a standard gaussian density *[45]*. Combining these results with the definitions of $X_c$ and $X_{c'}$ in Table 1 shows that when $k$ is large, both $X_c$ and $X_{c'}$ are defined by

$$E(Y|X = 0,\Theta) + 1.96V(Y|X = 0,\Theta) = E(Y|X = X_c,\Theta) \quad (A.2)$$

The numerical differences between $X_c$ and $X_{c'}$ arise because they are different approximations used to estimate the same quantity from finite samples. $X_c$ will tend to be larger than $X_{c'}$ because the definition of $X_c$ has the extra term of $V(Y|X = X_c,\Theta)/k$. This term is the prediction error variance for the 4PL equation at the point $X = X_c$. It must appear because the point $X_c$ was not used in the estimation of the 4PL equation. A discussion of prediction error variances for simple regression models may be found in Draper and Smith *[46]*, for nonlinear regression models in Seber and Wild *[47]*, and for immunoassay dose–response curves in Munson *[20]* and in Davidian et al. *[21]*.

**PROPOSITION 2**

If $\alpha = 0.025$, then $X_c$ and $X_{c'}$ are large-sample gaussian approximations to the 0.975 quantile (upper limit of a 95% confidence interval) of $[X|Y_0,\Theta]$, the zero dose probability density.

Proof: For a gaussian probability density with mean $\mu$ and variance $\sigma^2$, the lower and upper limits of the standard 95% confidence interval are respectively the 0.025 and 0.975 quantiles, defined as

$$z_{0.025} = \mu - 1.96\sigma \quad (A.3)$$

$$z_{0.975} = \mu + 1.96\sigma$$

If the parameters of the dose–response curve are known, then for a given dose $X_0$, the 95% confidence interval for the mean response is

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$$E(Y|X = X_0,\Theta) - 1.96V(Y|X = X_0,\Theta) = z_{Y|X_0,0.025} \quad (A.4)$$

$$E(Y|X = X_0,\Theta) + 1.96V(Y|X = X_0,\Theta) = z_{Y|X_0,0.975}$$

For a monotone increasing dose–response curve, $z_{Y|X_0,0.975} = E(Y|X,\Theta)$ for some $X,Y$ pair. For any monotone dose–response curve, Eq. A.4 also gives an implicit 95% confidence interval for $X$ *[48]*. This confidence interval is simply the set of doses corresponding to the responses in the range defined by Eq. A.4. If we take $X_0 = 0$, then for a monotone increasing dose–response curve the upper limit of the $X$ 95% confidence interval is defined by

$$E(Y|X = 0,\Theta) + 1.96V(Y|X = 0,\Theta) = E(Y|X = X_{0.975},\Theta) \quad (A.5)$$

where $X_{0.975}$ is the approximate 0.975 quantile of $[X|Y_0,\Theta]$. When the number of replicates of each calibrator is sufficiently large, Eq. A.2 and Eq. A.5 together imply $E(Y|X = X_c,\Theta) = E(Y|X = X_{0.975},\Theta)$ or $X_c = X_{0.975}$.

PROPOSITION 3

If the dose–response curve is a monotone, differentiable function of $X$, then the Ekins–Newman MDC definition is a gaussian approximation to the 0.84 quantile of $[X|Y_0,\Theta]$.

Proof: For a gaussian density with mean $\mu$ and variance $\sigma^2$, the 0.84 quantile is $z_{0.84} = \mu+\sigma$, and if $\mu = 0$, then $z_{0.84} = \sigma$. The probability density $[Y|X,\Theta]$ defined by Eq. 1 is assumed to be gaussian. Because the dose–response curve is a monotone, differentiable function of $X$, then by Taylor series expansion, applications of Slutsky's theorems and the implicit function theorem, the analyte concentration for a given response is a gaussian random variable whose variance may be approximated as *[49, 50]*:

$$V(X|Y,\Theta) \approx \left(\frac{dE(Y|X,\Theta)}{dX}\right)^{-2} V(Y|X,\Theta). \quad (A.6)$$

If $Y = Y_0$, the response of the zero dose, and the slope of the dose–response curve at the zero dose is nonzero and finite, then the variance of the zero dose is approximately

$$V(X|Y = Y_0,\Theta) \approx \left(\frac{dE(Y|X = 0,\Theta)}{dX}\right)^{-2} V(Y|X = 0,\Theta). \quad (A.7)$$

If we apply Eq. A.7 under the assumption that the true expected dose for $Y_0$ is zero, then the 0.84 quantile of $[X|Y_0,\Theta]$ is approximately

$$z_{X|Y_0,0.84} = \frac{[V(Y|X = 0,\Theta)]^{1/2}}{\dfrac{dE(Y|X = 0,\Theta)}{dX}}$$

which is the Ekins–Newman definition of the MDC.

The approximation in Eq. A.6 is also used in the determination limit definition in Table 1.

PROPOSITION 4

If (*a*) the critical and detection limits are computed by using Eq. 2 in lieu of their backfit approximations, (*b*) the probability density of $X_b$ is approximately symmetric, and (*c*) the value of the random variable $X_{y_0}$ in *Definition 1* is fixed at its $1-\alpha$ quantile, then $X_d \approx X_b$.

Proof: Expressed in terms of posterior probability densities, $X_c$ is

$$\left\{X_c: \int_{\{x>X_c\}} [X(x)|Y_0,\Theta]dx = \alpha\right\} \quad (A.8)$$

and $X_d$ is the mean of the probability density that satisfies the condition

$$\int_{\{x>X_c\}} [X(x)|Y,\Theta]dx = 1 - \beta. \quad (A.9)$$

From *Definition 1*, $X_b$ is the median of the probability density $[X|Y,\Theta]$, which satisfies the condition

$$\begin{aligned}\Pr(X_y>X_{y_0}) &= \Pr(X_y - X_{y_0}>0) \quad (A.10)\\ &= \Pr(X^*>0)\\ &= \int_{\{x>0\}} [X^*(x)|Y,\Theta]dx\\ &= \rho,\end{aligned}$$

where

$$[X^*(x)|Y,\Theta] = \int_{x^*_{min}}^{x} [X(x)|Y,\Theta][X(x+u)|Y_0,\Theta]du, \quad (A.11)$$

and $x^*_{min}$ is the minimum value of the random variable $X^*$. If we fix $X_{y_0}$ at $x_{1-\alpha}$, the $1-\alpha$ quantile of $[X|Y_0,\Theta]$, then the condition for $X_b$ in Eq. A.10 becomes

$$\begin{aligned}\Pr(X_y>X_{y_0}) &= \Pr(X_y>X_{y_0}|X_{y_0} = x_{1-\alpha}) \quad (A.12)\\ &= \Pr(X_y>x_{1-\alpha})\\ &= \int_{\{x>x_{1-\alpha}\}} [X(x)|Y,\Theta]dx\\ &= \rho.\end{aligned}$$

By Eq. A.8, $X_c = x_{1-\alpha}$. The probability density of $X_b$ is approximately symmetric, so that its median and its mean are approximately equal. It follows that Eq. A.9 and Eq. A.12 agree and that $X_d \approx X_b$ with $\rho = 1-\beta$.

Because $X_b$ considers the entire probability density of the zero dose, whereas $X_d$ estimates the zero dose by its $1-\alpha$ quantile, $X_d$ will tend to be larger than $X_b$.

*Lemma 1.* If $X_y$ is the random variable associated with $[X|Y,\Theta]$ and $X_{y_0}$ is as given in *Definition 1*, then $0.5 \le \Pr(X_y > X_{y_0}) \le 1$, where the lower bound holds approximately.

Proof: The upper bound is obvious. Since the zero dose is the smallest analyte concentration possible, we establish the lower bound by letting $X_y$ have the same probability density as $X_{y_0}$.

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Then $\Pr(X_y > X_{y_0}) = \Pr(X_{y_0} \geq X_y) = 0.5$. If $Y$ is any response vector, and $E(Y|X,\Theta)$ is monotone increasing (decreasing), then the components of $Y_0$ need not be uniformly smaller (larger) than the components of $Y$. However, because the zero dose is the smallest analyte concentration and because the vector of responses for any concentration near the zero dose must be near $Y_0$, the lower bound holds approximately.

PROPOSITION 5

Suppose $\gamma$ in the definition of $X_q$ is given. If (*a*) Eq. 2 is used in lieu of the asymptotic approximation in Eq. A.6 to compute $X_q$ and (*b*) $X_b$ is taken to be the mean, rather than the median, of the probability density that satisfies Eq. 3, then there exists $\rho \in [0.5,1]$, such that $X_q \approx X_b$, where $\rho$ is the probability that $X_b$ is greater than the zero dose. Conversely, suppose $\rho$ in *Definition 1* is given and (*a*) and (*b*) hold. Then there exists a $\gamma$, such that $X_q$ and $X_b$ agree.

Proof: If we use Eq. 2 to compute $X_q$, then we find a probability density $[X|Y_q,\Theta]$ such that

$$\sigma_x/\mu_x = \gamma$$

where

$$\mu_x = \int x[X(x)|Y_q,\Theta]dx$$

$$\sigma_x^2 = \int (x - \mu_x)^2[X(x)|Y_q,\Theta]dx,$$

and $X_q = \mu_x$. Hence, if $X_{y_q}$ is the random variable associated with $[X|Y_q,\Theta]$, then by Eq. 3 the probability that $X_q$ is greater than the zero dose is $\Pr(X_{y_q} > X_{y_0}) = \rho$, where $\rho \in [0.5,1]$ by *Lemma 1*. Hence, by *Definition 1* and (*b*), $X_b = \mu_x$ and $X_q = X_b$. Conversely, for a given $\rho$, we can use *Definition 1* to compute $X_b$ and its associated probability density. If we denote the mean and standard deviation of this probability density as respectively $\mu_{X_b}$ and $\sigma_{X_b}$, then its CV is $\mathrm{CV}_{X_b} = \mu_{X_b}/\sigma_{X_b}$ and by (*b*) $X_b = \mu_{X_b}$. If we now use Eq. 2 to compute $X_q$ with $\gamma = \mathrm{CV}_{X_b}$, then by the definition of $X_q$, $X_q = \mu_{X_b}$ and $X_b = X_q$.

PROPOSITION 6

The probability of a measurement from $[X|Y_0,\Theta]$ being misclassified as greater than the zero dose is $1-\rho$. Similarly, the probability of a measurement from the probability density of the MDC being misclassified as indistinguishable from the zero dose is $1-\rho$.

Proof. A measurement from $[X|Y_0,\Theta]$ is misclassified as greater than the zero dose if $X_{y_0} > X_y$, where $X_y$ is the random variable associated with the probability density of the MDC. By *Definition 1*, the probability of this event is $\Pr(X_{y_0} > X_y) = 1-\Pr(X_{y_0} \leq X_y) = 1-\Pr(X_{y_0} < X_y) = 1-\rho$, since $X^*$ is a continuous random variable. A measurement from the probability density of the MDC is misclassified as indistinguishable from the zero dose if $X_y \leq X_{y_0}$. The probability of this event is $\Pr(X_y \leq X_{y_0}) = 1-\Pr(X_y > X_{y_0}) = 1-\rho$.

## References


1. Tamashiro H, Maskill W, Emmanuel J, Fauquex A, Sato P, Heymann D. Reducing the cost of HIV antibody testing. Lancet 1993;342:87–90.
2. Donahue JG, Munõz A, Ness PM, Brown DE, Yawn DH, McAllister HA, et al. The declining risk of post-transfusion hepatitis C virus infection. N Engl J Med 1992;327:369–73.
3. Krusell L, Skovgaard N. Evaluation of a new semi-automated screening method for the detection of salmonella in foods within 24 h. Int J Food Microbiol 1993;20:123–30.
4. Spencer CA, Schwarzbein D, Guttler RB, LoPresti JS, Nicoloff JT. Thyrotropin (TSH) releasing hormone stimulation test responses employing third and fourth generation TSH assays. J Clin Endocrinol Metab 1993;76:494–8.
5. Weston A, Caporaso NE, Taghizadeh K, Hoover RN, Tannenbaum SR, Skipper PL, et al. Measurement of 4-aminobiphenyl-hemoglobin adducts in lung cancer cases and controls. Cancer Res 1991;51:5219–23.
6. Cox KJ, Thomas AS. The application of immunoblotting to the phenotyping of haptoglobin. J Forensic Sci 1992;37:1652–5.
7. Cone EJ, Dickerson S, Paul BD, Mitchell JM. Forensic drug testing for opiates. V. Urine testing for heroin, morphine, and codeine with commercial opiate immunoassays. J Anal Toxicol 1993;17:156–64.
8. Kricka LJ. Use of different labels in immunoassays. In: Nakamura RM, Kasahara Y, Richnitz GA, eds. Immunochemical assays and biosensor technology for the 1990's. Washington, DC: American Society of Microbiology, 1992:44–5.
9. Finney DJ. Radioligand assays. Biometrics 1976;32:721–40.
10. Yalow RS, Berson SA. Special problems in the radioimmunoassay of small polypeptides. In: Margoulies M, ed. Protein and polypeptide hormones. Amsterdam: Excerpta Medica, 1969:71–6.
11. Ekins R, Newman B. Theoretical aspects of saturation analysis. Acta Endocrinol Suppl (Copenh) 1970;147:11–30.
12. Schuurman HJ, deLigny CL. Physical models of radioimmunoassay applied to the calculation of the detection limit. Anal Chem 1979;51:2–7.
13. Chan DW. General principle of immunoassay. In: Chan DW, Perlstein MT, eds. Immunoassay: a practical guide. San Diego: Academic Press, 1987:10–1.
14. Nakamura RM. General principles of immunoassays. In: Nakamura RM, Kasahara Y, Richnitz GA, eds. Immunochemical assays and biosensor technology for the 1990's. Washington, DC: American Society of Microbiology, 1992:14–6.
15. Currie LA. Limits for qualitative detection and quantitative determination. Anal Chem 1968;40:586–93.
16. Borth R. Discussion of theoretical aspects of saturation analysis. Acta Endocrinol Suppl (Copenh) 1970;147:32–6.
17. Rodbard D. Statistical estimation of the minimal detectable concentration ("sensitivity") for radioligand assays. Anal Biochem 1978;90:1–12.
18. Oppenheimer L, Capizzi TP, Weppleman RM, Mehta H. Determining the lowest limit of reliable assay measurement. Anal Chem 1983;55:638–43.
19. Kalman SM, Clark DR, Moses LE. Limits of detection and quantification, as applied to an assay for digoxin. Clin Chem 1984;30:515–7.
20. Munson PJ. Radioimmunoassay curve shape and assay detection limits. Proceedings of the 1984 biopharmaceutical section of the American Statistical Association. Alexandria, VA: American Statistical Association, 1984:46–52.
21. Davidian M, Carroll RJ, Smith W. Variance functions and the minimum detectable concentration in assays. Biometrika 1988;75:549–56.


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**22.** Rudy JL. Differentiating between sensitivity and limit of detection [Letter]. Clin Chem 1989;35:509.

**23.** Anderson DJ. Determination of the lower limit of detection [Letter]. Clin Chem 1989;35:2152–3.

**24.** Feldkamp CS. Evaluation and clinical validation of immunoassays. In: Nakamura RM, Kasahara Y, Richnitz GA, eds. Immunochemical assays and biosensor technology for the 1990's. Washington, DC: American Society of Microbiology, 1992:96.

**25.** Seber GAF, Wild CJ. Non-linear regression. New York: Wiley, 1989;246–50.

**26.** Press SJ. Bayesian statistics: principles, models, and applications. New York: Wiley, 1988;39–40.

**27.** Box GEP, Tiao GC. Bayesian inference in statistical analysis. New York: Wiley, 1992;20–5.

**28.** Brown EN. New statistical methods for immunoassay data analysis [Abstract]. Life Science and Space Medicine Conference '95. Washington, DC: American Institute of Aeronautics and Astronautics, 1995:202.

**29.** Rosenberger JL, Gasko M. Comparing location estimators: trimmed means, median and trimeans. In: Hoaglin DC, Mosteller F, Tukey JW, eds. Understanding robust and exploratory data analysis. New York: Wiley, 1983:297–302.

**30.** Hoel PG, Port SC, Stone CJ. Introduction to probability theory. Boston: Houghton Mifflin, 1971;145–50.

**31.** Fiore M, Mitchell J, Doan T, Nelson R, Winter G, Grandone C, et al. The Abbott IMx automated benchtop immunochemistry analyzer system. Clin Chem 1988;34:1726–32.

**32.** Vessella RL, Noteboom J, Lange PH. Evaluation of the Abbott IMx automated immunoassay of prostate-specific antigen. Clin Chem 1992;38:2044–54.

**33.** Dnistrian AM, Schwartz MK, Smith CA, Nisselbaum JS, Fair WR. Abbott IMx evaluated for assay of prostate-specific antigen in serum. Clin Chem 1992;38:2140–2.

**34.** Mettlin C, Jones G, Averette H, Gusberg SB, Murphy GP. Defining and updating the American Cancer Society guidelines for the cancer-related checkup: prostate and endometrial cancers. CA Cancer J Clin 1993;43:42–6.

**35.** Press WH, Teukolsky SA, Vetterling WT, Flannery BP. Numerical recipes: the art of scientific programming (FORTRAN version). Cambridge: Cambridge Univ Press, 1986:407–13.

**36.** Yu H, Diamandis EP, Prestigiacomo AF, Stamey TA. Ultrasensitive assay of prostate-specific antigen used for early detection of prostate cancer relapse and estimation of tumor-doubling time after radical prostatectomy. Clin Chem 1995;41:430–4.

**37.** Graves HCB. The dilemma of prostate cancer. Cancer 1993;72: 3141–4.

**38.** Garnick MB. Standardization of immunoassays for prostate-specific antigen. Sci Am 1994;270:72–81.

**39.** Yu H, Diamandis EP. Ultrasensitive time-resolved immunofluorometric assay of prostate-specific antigen in serum and preliminary clinical studies. Clin Chem 1993;39:2108–14.

**40.** Liedtke RJ, Kroon G, Batjer JD. Modified assay of prostate-specific antigen with a detection limit <0.01 μg/L. Clin Chem 1993;39: 2150–4.

**41.** Leinonen J, Lövgren T, Vornanen T, Stenman U-H. Double-label time-resolved immunofluorometric assay of prostate-specific antigen and of its complex with $\alpha_1$-antichymotrypsin. Clin Chem 1993;39:2098–103.

**42.** Kricka LJ. Selected strategies for improving sensitivity and reliability of immunoassays [Review]. Clin Chem 1994;40:347–57.

**43.** Sano T, Smith CL, Cantor CR. Immuno-PCR: very sensitive antigen detection by means of specific antibody–DNA conjugates. Science 1992;258:120–2.

**44.** Serfling RJ. Approximation theorems of mathematical statistics. New York: Wiley, 1980;122–5:143–50.

**45.** Johnson NL, Kotz S. Continuous univariate distributions, Vol. 2. New York: Wiley, 1970:95.

**46.** Draper NR, Smith H. Applied regression analysis. New York: Wiley, 1966:21–4.

**47.** Seber GAF, Wild CJ. Non-linear regression. New York: Wiley, 1989:191–4.

**48.** Carroll RJ, Ruppert D. Transformation and weighting in regression. New York: Chapman and Hall, 1988:54.

**49.** Serfling RJ. Approximation theorems of mathematical statistics. New York: Wiley, 1980:18–9.

**50.** Johnson GF. Data reduction techniques for immunoassay. In: Chan DW, Perlstein MT, eds. Immunoassay: a practical guide. San Diego: Academic Press, 1987:138–9.


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