USDA Center for Veterinary Biologics

Statistics Section

Work Instructions

Document:	STATWI0002.04
Title:	Technical Notes for Estimating Diagnostic Sensitivity and Specificity
Author:	Monica Reising
Approved by:	David Siev on 2018-03-23

This document approved for the indicated purposes

Internal use	Yes
External distribution	Yes
CVB public web site	Yes

Technical Notes Regarding Estimating Diagnostic Sensitivity and Specificity

Statistics Section
Center for Veterinary Biologics
United States Department of Agriculture
1920 Dayton Avenue
Ames, Iowa, USA

March 23, 2018

1 Introduction

Veterinary Services Memorandum 800.73 provides guidance on studies required for the licensure of diagnostic test kits. This document provides technical details related to estimating diagnostic sensitivity (Sn) and diagnostic specificity (Sp). A simulation method is described that makes use of the performance characteristics of one or more reference tests, as well as the prevalence of each population sampled. A software package is available to implement the procedure. This document assumes that the reader has a substantial background in mathematical statistics.

2 Notation and Properties

This section provides notation that will be used throughout. Further, properties of probabilities are provided to allow derivations of expected probabilities and hence, expected counts.

Let

- $P(\bullet)$ represent a marginal probability.
- $P(\bullet|\bullet)$ represent a conditional probability
- D+ indicates disease positive

- D− indicates disease negative
- T+ indicates test positive
- T− indicates test negative
- T? indicates test suspect
- $i = 1, 2, \ldots, I$ where I is the number of tests ($i = 1$ refers to the experimental test and $i = 2, \ldots, I$ refers to the $(i-1)$ reference tests)
- $l = 1, 2, \ldots, L$ where L is the number of populations sampled
- $k = 1, 2, \ldots, K$ represents the k^{th} unique combination of test outcomes where K is the total number of unique combinations of test results
- $S_i = \begin{cases} 2, & \text{for a 2-state test} \\ 3, & \text{for a 3-state test} \end{cases}$
- $K = \prod_{i=1}^{I} S_i$, which represents the number of unique test combinations
- $\pi_i = \mathrm{Sn}_i = \mathrm{P}(\mathrm{T}_i+ \mid \mathrm{D}+)$ for the i^{th} test
- $\theta_i = \mathrm{Sp}_i = \mathrm{P}(\mathrm{T}_i- \mid \mathrm{D}-)$ for the i^{th} test
- $\psi_i = \mathrm{P}(\mathrm{T}_i? \mid \mathrm{D}+)$ for the i^{th} test
- $\phi_i = \mathrm{P}(\mathrm{T}_i? \mid \mathrm{D}-)$ for the i^{th} test
- $\delta_i = \begin{cases} 0, & \text{if the } i^{th} \text{ test has 2 states} \\ \frac{\psi_i}{1-\pi_i}, & \text{if the } i^{th} \text{ test has 3 states} \end{cases}$
- $\gamma_i = \begin{cases} 0, & \text{if the } i^{th} \text{ test has 2 states} \\ \frac{\phi_i}{1-\theta_i}, & \text{if the } i^{th} \text{ test has 3 states} \end{cases}$
- $p_l = P_l(\mathrm{D}+)$ for the l^{th} population (l^{th} population prevalence)
- $\mathrm{x}_{k,i} = \begin{cases} \mathrm{T}_i - & \text{if the } i^{th} \text{ test is negative in the } k^{th} \text{ unique combination of test outcomes} \\ \mathrm{T}_i + & \text{if the } i^{th} \text{ test is positive in the } k^{th} \text{ unique combination of test outcomes} \\ \mathrm{T}_i ? & \text{if the } i^{th} \text{ test is suspect in the } k^{th} \text{ unique combination of test outcomes} \end{cases}$
- $\mathbf{M}_k$ is a vector of length I with elements ($\mathrm{x}_{k,1}$, $\mathrm{x}_{k,2}$, …, $\mathrm{x}_{k,I}$) representing the k^{th} unique combination of test outcomes; $\mathbf{M}_k \neq \mathbf{M}_{k'}$ for all $k \neq k'$
- $\mathrm{P}_l(\mathbf{M}_k)$ denotes the probability of observing the k^{th} unique combination of test outcomes within the l^{th} population.
- $\mathrm{n}_{k,l}$ denotes the number of samples (observed count) exhibiting the k^{th} unique combination of test outcomes within the l^{th} population.
- $\mathrm{N}_l = \sum_{k=1}^{K} \mathrm{n}_{k,l}$ is the total number of samples tested from the l^{th} population.

- $\hat{n}_{k,l}$ denotes the expected count for the k^{th} unique combination of test outcomes within the l^{th} population.
- $\hat{n}_{k,l} = \left[[\prod_{i=1}^{I} \mathrm{P}(\omega_{k,i} \mid \mathrm{D}+)]\ \mathrm{P}_l(\mathrm{D}+) + [\prod_{i=1}^{I} \mathrm{P}(\omega_{k,i} \mid \mathrm{D}-)]\ \mathrm{P}_l(\mathrm{D}-)\right] \mathrm{N}_l$

Law of Total Probability: Let $B_1, B_2, \ldots, B_k$ be a collection of mutually exclusive and exhaustive events, then for any event A,

$$P(A) = \sum_{i=1}^{k} P(A \cap B_i)$$

which implies

$$P(\mathrm{T}-|\mathrm{D}+) = 1 - \pi$$

$$P(\mathrm{T}+|\mathrm{D}-) = 1 - \theta$$

in an instance where the test method has 2-states (positive and negative) and

$$P(\mathrm{T}-|\mathrm{D}+) = 1 - \pi -$$

$$P(\mathrm{T}+|\mathrm{D}-) = 1 - \theta - \phi$$

in an instance where the test method has 3-states (positive, negative and suspect).

Conditional Independence:

Two test methods are conditionally independent given disease status if and only if

$$P(\mathrm{T}_1,\ \mathrm{T}_2|\mathrm{D}) = P(\mathrm{T}_1|\mathrm{D})P(\mathrm{T}_2|\mathrm{D})$$

3 Estimating Sn and Sp

3.1 Simulation Method

Identify one or more reference tests. Provide information on the performance characteristics (sensitivity and specificity) of each reference test. Provide information on the prevalence of each of the sampled populations. Estimate the Sn and Sp of the experimental kit by the simulation method. If an infallible reference test is available, follow the method in Section 3.2.

3.1.1 Sn and Sp

- The firm provides reasonable assumed values for Sn (π) and Sp (θ) for each reference test used based on the available information.
- The firm provides reasonable assumed values for δ and γ for each 3-state reference test used based on available information. These express the probability of a suspect result as a fraction of the non-correct test results. See Section 2 Notation.
- When information exists for the distribution of Sn (π) and Sp (θ) it is typically crude or limited. Unless specific data are available about the form and nature of the distribution, rely on the software package default approach of simulating from a beta distribution with fixed variance.

3.1.2 Prevalence

- Provide a known or reasonable assumed value of the prevalence for each population sampled. Please refer to VSM 800.73 for further details regarding prevalence estimates.
- When information exists for the distribution of prevalence it is typically crude or limited. Unless specific data are available about the form and nature of the distribution, rely on the software package default approach of simulating from a beta distribution with fixed variance.

3.1.3 For each simulation cycle, the Sn (π) and Sp (θ) for the experimental test are estimated as the values that minimize the sum of the squared deviations between the observed and expected counts. For a 2-state experimental test,

$$\min_{\pi_1,\,\theta_1} \sum_{k=1}^{K} \sum_{l=1}^{L} (n_{k,l} - \hat{n}_{k,l})^2$$

For a 3-state experimental test,

$$\min_{\pi_1,\,\theta_1,\delta_1,\gamma_1} \sum_{k=1}^{K} \sum_{l=1}^{L} (n_{k,l} - \hat{n}_{k,l})^2$$

3.1.4 Using the above equations, interval estimates are obtained from the set of optimized values for each set of simulations by the highest density method. A software package[1] in the R language may be found online.

3.2 Estimating Sn and Sp with an Infallible Reference test

An infallible reference test is one that can detect either the presence or absence of a disease without error. There are very few instances in which an infallible reference test exists for

[1] https://github.com/ABS-dev/DiagTestKit/blob/master/README.md

determining the positive or negative status of a sample. If an infallible reference test is available for one or the other disease status, then the corresonding parameter estimate (Sn or Sp) is a simple fraction.

For example, for a 2-state experimental kit the results of the experimental kit and the true disease status can be cross-classified as follows:

	Disease	
Test	Positive	Negative
Positive	a	b
Negative	c	d

Then Sn and Sp are determined as follows:

$$\text{Sn} = P(\text{T}+|\text{D}+) = \frac{a}{a+c}$$

$$\text{Sp} = P(\text{T}-|\text{D}-) = \frac{d}{b+d}$$

Use the Clopper-Pearson (Clopper & Pearson (1934)) method for obtaining interval estimates.

A Appendix

A.1 A Single 2-State Reference Test, One population, 2-State Experimental Test

When a single 2-state reference test (T_2) is used to estimate the sensitivity and specificity of a 2-state experimental test (T_1), there are 4 unique test combinations (i.e. $K = 4$). If only one population is sampled, there are a total of 4 expected counts.

Let

- $\mathbf{M}_1 = (\text{T}_1-, \text{T}_2-)$
- $\mathbf{M}_2 = (\text{T}_1-, \text{T}_2+)$
- $\mathbf{M}_3 = (\text{T}_1+, \text{T}_2-)$
- $\mathbf{M}_4 = (\text{T}_1+, \text{T}_2+)$

Therefore,

$$\begin{aligned} \mathrm{P}_1(\mathbf{M}_1) &= \mathrm{P}(\mathrm{T}_1-|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}+)\mathrm{P}_1(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1-|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}-)\mathrm{P}_1(\mathrm{D}-) \\ &= (1-\pi_1)(1-\pi_2)p_1 + \theta_1\theta_2(1-p_1) \end{aligned}$$

$$\begin{aligned} \mathrm{P}_1(\mathbf{M}_2) &= \mathrm{P}(\mathrm{T}_1-|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}+)\mathrm{P}_1(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1-|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}-)\mathrm{P}_1(\mathrm{D}-) \\ &= (1-\pi_1)\pi_2 p_1 + \theta_1(1-\theta_2)(1-p_1) \end{aligned}$$

$$\begin{aligned} \mathrm{P}_1(\mathbf{M}_3) &= \mathrm{P}(\mathrm{T}_1+|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}+)\mathrm{P}_1(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1+|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}-)\mathrm{P}_1(\mathrm{D}-) \\ &= \pi_1(1-\pi_2)p_1 + (1-\theta_1)\theta_2(1-p_1) \end{aligned}$$

$$\begin{aligned} \mathrm{P}_1(\mathbf{M}_4) &= \mathrm{P}(\mathrm{T}_1+|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}+)\mathrm{P}_1(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1+|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}-)\mathrm{P}_1(\mathrm{D}-) \\ &= \pi_1\pi_2 p_1 + (1-\theta_1)(1-\theta_2)(1-p_1) \end{aligned}$$

The expected counts are

- $\hat{n}_{1,1} = \mathrm{P}_1(\mathbf{M}_1)N_1$
- $\hat{n}_{2,1} = \mathrm{P}_1(\mathbf{M}_2)N_1$
- $\hat{n}_{3,1} = \mathrm{P}_1(\mathbf{M}_3)N_1$
- $\hat{n}_{4,1} = \mathrm{P}_1(\mathbf{M}_4)N_1$

Sensitivity and specificity of the experimental kit are estimated as the values that minimize the sum of the squared residuals. Specificially,

$$\min_{\pi_1,\,\theta_1}\left[(n_{1,1}-\hat{n}_{1,1})^2 + (n_{2,1}-\hat{n}_{2,1})^2 + (n_{3,1}-\hat{n}_{3,1})^2 + (n_{4,1}-\hat{n}_{4,1})^2\right]$$

This can easily be extended to multiple populations.

A.2 Two, 2-State Reference Tests, One population, 3-State Experimental Test

When two, 2-state reference tests are used to estimate the performance characteristics of a 3-state experimental test, there are 12 unique test combinations (i.e. K=12). If only one population is sampled, there are a total of 12 expected counts.

Let

- $\mathbf{M}_1 = (\mathrm{T}_1-,\, \mathrm{T}_2-,\, T_3-)$
- $\mathbf{M}_2 = (\mathrm{T}_1-,\, \mathrm{T}_2-,\, T_3+)$

- $\mathbf{M}_3 = (\mathrm{T}_1-,\ \mathrm{T}_2+,\ T_3-)$
- $\mathbf{M}_4 = (\mathrm{T}_1-,\ \mathrm{T}_2+,\ T_3+)$
- $\mathbf{M}_5 = (\mathrm{T}_1+,\ \mathrm{T}_2-,\ T_3-)$
- $\mathbf{M}_6 = (\mathrm{T}_1+,\ \mathrm{T}_2-,\ T_3+)$
- $\mathbf{M}_7 = (\mathrm{T}_1+,\ \mathrm{T}_2+,\ T_3-)$
- $\mathbf{M}_8 = (\mathrm{T}_1+,\ \mathrm{T}_2+,\ T_3+)$
- $\mathbf{M}_9 = (\mathrm{T}_1?,\ \mathrm{T}_2-,\ T_3-)$
- $\mathbf{M}_{10} = (\mathrm{T}_1?,\ \mathrm{T}_2-,\ T_3+)$
- $\mathbf{M}_{11} = (\mathrm{T}_1?,\ \mathrm{T}_2+,\ T_3-)$
- $\mathbf{M}_{12} = (\mathrm{T}_1?,\ \mathrm{T}_2+,\ T_3+)$

Therefore,

$$\begin{aligned}
\mathrm{P}_1(\mathbf{M}_1) &= \mathrm{P}(\mathrm{T}_1-|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}+)\mathrm{P}(\mathrm{T}_3-|\mathrm{D}+)\mathrm{P}(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1-|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}-)\mathrm{P}(\mathrm{T}_3-|\mathrm{D}-)\mathrm{P}(\mathrm{D}-) \\
&= (1-\pi_1)(1-\delta_1)(1-\pi_2)(1-\pi_3)p_1 + \theta_1\theta_2\theta_3(1-p_1)
\end{aligned}$$

$$\begin{aligned}
\mathrm{P}_1(\mathbf{M}_2) &= \mathrm{P}(\mathrm{T}_1-|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}+)\mathrm{P}(\mathrm{T}_3+|\mathrm{D}+)\mathrm{P}(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1-|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}-)\mathrm{P}(\mathrm{T}_3+|\mathrm{D}-)\mathrm{P}(\mathrm{D}-) \\
&= (1-\pi_1)(1-\delta_1)(1-\pi_2)\pi_3 p_1 + \theta_1\theta_2(1-\theta_3)(1-p_1)
\end{aligned}$$

$$\begin{aligned}
\mathrm{P}_1(\mathbf{M}_3) &= \mathrm{P}(\mathrm{T}_1-|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}+)\mathrm{P}(\mathrm{T}_3-|\mathrm{D}+)\mathrm{P}(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1-|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}-)\mathrm{P}(\mathrm{T}_3-|\mathrm{D}-)\mathrm{P}(\mathrm{D}-) \\
&= (1-\pi_1)(1-\delta_1)\pi_2(1-\pi_3)p_1 + \theta_1(1-\theta_2)\theta_3(1-p_1)
\end{aligned}$$

$$\begin{aligned}
\mathrm{P}_1(\mathbf{M}_4) &= \mathrm{P}(\mathrm{T}_1-|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}+)\mathrm{P}(\mathrm{T}_3+|\mathrm{D}+)\mathrm{P}(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1-|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}-)\mathrm{P}(\mathrm{T}_3+|\mathrm{D}-)\mathrm{P}(\mathrm{D}-) \\
&= (1-\pi_1)(1-\delta_1)\pi_2\pi_3 p_1 + \theta_1(1-\theta_2)(1-\theta_3)(1-p_1)
\end{aligned}$$

$$\begin{aligned}
\mathrm{P}_1(\mathbf{M}_5) &= \mathrm{P}(\mathrm{T}_1+|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}+)\mathrm{P}(\mathrm{T}_3-|\mathrm{D}+)\mathrm{P}(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1+|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}-)\mathrm{P}(\mathrm{T}_3-|\mathrm{D}-)\mathrm{P}(\mathrm{D}-) \\
&= \pi_1(1-\pi_2)(1-\pi_3)p_1 + (1-\theta_1)(1-\gamma_1)\theta_2\theta_3(1-p_1)
\end{aligned}$$

$$\begin{aligned}
\mathrm{P}_1(\mathbf{M}_6) &= \mathrm{P}(\mathrm{T}_1+|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}+)\mathrm{P}(\mathrm{T}_3+|\mathrm{D}+)\mathrm{P}(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1+|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}-)\mathrm{P}(\mathrm{T}_3+|\mathrm{D}-)\mathrm{P}(\mathrm{D}-) \\
&= \pi_1(1-\pi_2)\pi_3 p_1 + (1-\theta_1)(1-\gamma_1)\theta_2(1-\theta_3)(1-p_1)
\end{aligned}$$

$$\begin{aligned}
\mathrm{P}_1(\mathbf{M}_7) &= \mathrm{P}(\mathrm{T}_1+|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}+)\mathrm{P}(\mathrm{T}_3-|\mathrm{D}+)\mathrm{P}(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1+|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}-)\mathrm{P}(\mathrm{T}_3-|\mathrm{D}-)\mathrm{P}(\mathrm{D}-) \\
&= \pi_1\pi_2(1-\pi_3)p_1 + (1-\theta_1)(1-\gamma_1)(1-\theta_2)\theta_3(1-p_1)
\end{aligned}$$

$$\begin{aligned}
\mathrm{P}_1(\mathbf{M}_8) &= \mathrm{P}(\mathrm{T}_1+|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}+)\mathrm{P}(\mathrm{T}_3+|\mathrm{D}+)\mathrm{P}(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1+|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}-)\mathrm{P}(\mathrm{T}_3+|\mathrm{D}-)\mathrm{P}(\mathrm{D}-) \\
&= \pi_1\pi_2\pi_3 p_1 + (1-\theta_1)(1-\gamma_1)(1-\theta_2)(1-\theta_3)(1-p_1)
\end{aligned}$$

$$\begin{aligned}
\mathrm{P}_1(\mathbf{M}_9) &= \mathrm{P}(\mathrm{T}_1?|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}+)\mathrm{P}(\mathrm{T}_3-|\mathrm{D}+)\mathrm{P}(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1?|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}-)\mathrm{P}(\mathrm{T}_3-|\mathrm{D}-)\mathrm{P}(\mathrm{D}-) \\
&= \delta_1(1-\pi_1)(1-\pi_2)(1-\pi_3)p_1 + \gamma_1(1-\theta_1)\theta_2\theta_3(1-p_1)
\end{aligned}$$

$$\begin{aligned}
\mathrm{P}_1(\mathbf{M}_{10}) &= \mathrm{P}(\mathrm{T}_1?|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}+)\mathrm{P}(\mathrm{T}_3+|\mathrm{D}+)\mathrm{P}(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1?|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2-|\mathrm{D}-)\mathrm{P}(\mathrm{T}_3+|\mathrm{D}-)\mathrm{P}(\mathrm{D}-) \\
&= \delta_1(1-\pi_1)(1-\pi_2)\pi_3 p_1 + \gamma_1(1-\theta_1)\theta_2(1-\theta_3)(1-p_1)
\end{aligned}$$

$$\begin{aligned}
\mathrm{P}_1(\mathbf{M}_{11}) &= \mathrm{P}(\mathrm{T}_1?|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}+)\mathrm{P}(\mathrm{T}_3-|\mathrm{D}+)\mathrm{P}(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1?|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}-)\mathrm{P}(\mathrm{T}_3-|\mathrm{D}-)\mathrm{P}(\mathrm{D}-) \\
&= \delta_1(1-\pi_1)\pi_2(1-\pi_3)p_1 + \gamma_1(1-\theta_1)(1-\theta_2)\theta_3(1-p_1)
\end{aligned}$$

$$\begin{aligned}
\mathrm{P}_1(\mathbf{M}_{12}) &= \mathrm{P}(\mathrm{T}_1?|\mathrm{D}+)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}+)\mathrm{P}(\mathrm{T}_3+|\mathrm{D}+)\mathrm{P}(\mathrm{D}+) + \mathrm{P}(\mathrm{T}_1?|\mathrm{D}-)\mathrm{P}(\mathrm{T}_2+|\mathrm{D}-)\mathrm{P}(\mathrm{T}_3+|\mathrm{D}-)\mathrm{P}(\mathrm{D}-) \\
&= \delta_1(1-\pi_1)\pi_2\pi_3 p_1 + \gamma_1(1-\theta_1)(1-\theta_2)(1-\theta_3)(1-p_1)
\end{aligned}$$

The expected counts are

- $\hat{n}_{1,1} = \mathrm{P}_1(\mathbf{M}_1)N_1$
- $\hat{n}_{2,1} = \mathrm{P}_1(\mathbf{M}_2)N_1$
- $\hat{n}_{3,1} = \mathrm{P}_1(\mathbf{M}_3)N_1$
- $\hat{n}_{4,1} = \mathrm{P}_1(\mathbf{M}_4)N_1$
- $\hat{n}_{5,1} = \mathrm{P}_1(\mathbf{M}_5)N_1$
- $\hat{n}_{6,1} = \mathrm{P}_1(\mathbf{M}_6)N_1$
- $\hat{n}_{7,1} = \mathrm{P}_1(\mathbf{M}_7)N_1$
- $\hat{n}_{8,1} = \mathrm{P}_1(\mathbf{M}_8)N_1$
- $\hat{n}_{9,1} = \mathrm{P}_1(\mathbf{M}_9)N_1$
- $\hat{n}_{10,1} = \mathrm{P}_1(\mathbf{M}_{10})N_1$
- $\hat{n}_{11,1} = \mathrm{P}_1(\mathbf{M}_{11})N_1$
- $\hat{n}_{12,1} = \mathrm{P}_1(\mathbf{M}_{12})N_1$

Sensitivity and specificity of the experimental kit are estimated as the values that minimize the sum of the squared residuals. Specificially,

$$\min_{\pi_1,\theta_1,\delta_1,\gamma_1} \sum_{k=1}^{K} (n_{k,1} - \hat{n}_{k,1})^2.$$

References

Clopper, D., & Pearson, E. (1934). The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika*, *26*, 404–413.