Determination of Sample Size for Validation of Allergen-Screening Methods

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For various levels of confidence (i.e., 80 and 90%) and ratios \(K = \frac{\sigma_P^2}{\sigma_N^2}\), where \(\sigma_P^2\) and \(\sigma_N^2\) are the analyte variances for the positive and negative distributions, respectively, sample sizes sufficient to test the requirements that a given method detects \(\geq 90\%\) of the positives (\(\geq 5\) ppm of a given analyte) while misclassifying \(\leq 10\%\) of the negatives (implying a specificity rate, true negatives that will be correctly classified, of 90%) were estimated by using a rationale that minimizes the cost of sampling.

In designing a validation scheme for screening tests used for allergen detection, we had to determine the amount of analytical work that would be necessary to validate a method as having predefined performance specifications. We decided that the sample size should provide \(\geq 80\%\) confidence, when a screening method that generates quantitative data is tested, to verify that the method has a sensitivity rate of 90% for positive samples (samples containing \(\geq 5\) ppm analyte) and a false positive rate of 10% for negative samples (samples free of analyte).

The problem as stated defined the required sensitivity rate \((P_+ = \text{true positives}, \text{samples at levels of } \geq 5 \text{ ppm of a given analyte})\) while misclassifying \(\leq 10\%\) of the negatives (implying a specificity rate, true negatives that will be correctly classified, of 90%) were estimated by using the rationale described by Greenhouse and Mantel (Greenhouse, S.W., & Mantel, N. (1950) Biometrics 6, 399–412.)

To demonstrate the method, it is assumed that the distributions of the method values for the positives and negatives are both normal or can be transformed to achieve normality (Figure 1). In addition, assume that the mean and standard deviation for the positives are defined as \(\mu_P\) and \(\sigma_P\), and for the negatives, \(\mu_N\) and \(\sigma_N\), respectively, as in Figure 1.
Sample Size Estimates

The null hypothesis for the test was specified as \( \Delta \geq 0 \). Assume that if the method is capable of detecting only 80% of the positives (i.e., an 80% sensitivity rate), while having a false positive rate of 10% (analogous to a specificity rate of 90%), we want to be ≥80% certain of rejecting the hypothesis. As before, define \( P_{90} \) as the value corresponding to the 20th percentile of the positive distribution or the value that is exceeded by 80% of the positives (i.e., an 80% sensitivity rate), while having a false positive rate of 10% (analogous to a specificity rate of 90%). To be 80% confident that the hypothesis \( P_{90} = N_{10} \) will be rejected, when in fact the alternative hypothesis \( P_{80} = N_{10} \) is true, it is necessary for \(-0.44\) to be significantly negative or \( \Delta \leq -0.84\sigma_\Delta \). This inequality may be expressed as follows:

\[
\sigma_\Delta \leq \frac{0.44}{1.68} \sigma_P
\]

To determine sample size, we solve the equality:

\[
\sigma_\Delta = \frac{0.44}{1.68} \sigma_P = \left( \sigma_P^2 \left( \frac{1}{n_P} + \frac{(1.282)^2}{2} \right) \right)^{1/2} = \left( \sigma_N^2 \left( \frac{1}{n_N} + \frac{(1.282)^2}{2} \right) \right)^{1/2}
\]

Letting

\[
K = \frac{\sigma_P^2}{\sigma_N^2}
\]

we have:

\[
\frac{\sigma_\Delta}{\sigma_P} = \frac{0.44}{1.68} = \left( \frac{1}{n_P} \left( \frac{1}{n_P} + \frac{(1.282)^2}{2} \right) + \frac{1}{n_N} \left( \frac{1}{n_N} + \frac{(1.282)^2}{2} \right) \right)^{-1}
\]

For a given value of \( K \), values of \( n_p \) and \( n_N \) that satisfy the above equation will reject the hypothesis \( \Delta \geq 0 \), 80% of the time when \( \Delta = -0.44\sigma_P \). To minimize the sampling cost in validating the method, we minimize the total sample size \((n_p + n_N)\), while keeping

\[
\frac{1}{n_P} \left( 1 + \frac{(1.282)^2}{2} \right) + \frac{1}{K n_N} \left( 1 + \frac{(1.282)^2}{2} \right) = \frac{1.822}{n_P} + \frac{1.822}{K n_N}
\]

fixed (see Appendix). The resulting derivation is expressed as follows:

\[
\frac{n_p}{n_N} = \sqrt{K}
\]

Substituting 1 at a time

\[
n_p = n_N \sqrt{K} \quad \text{and} \quad n_N = \frac{n_p}{\sqrt{K}}
\]

derived from

\[
\frac{n_p}{n_N} = \sqrt{K}
\]

in Equation 1, gives the following equations for sample size determinations:

\[
n_p = 26.6 + \frac{26.6}{\sqrt{K}}
\]

and

\[
n_N = \frac{26.6}{K} + \frac{26.6}{\sqrt{K}}
\]

To obtain the proper sample size, as shown in Table 1, one needs a pilot or preliminary estimate of the variation (\( \sigma_P^2 \) and \( \sigma_N^2 \)) of the test samples for the materials (i.e., negatives and positives) under study.

To be 90% confident that the hypothesis \( P_{90} = N_{10} \) will be rejected, when in fact the alternative hypothesis \( P_{80} = N_{10} \) is true, it is necessary for \(-0.44\sigma_P + 1.282\sigma_\Delta\) to be significantly negative or \( \Delta \leq -1.282\sigma_\Delta \). In which case

\[
\frac{\sigma_\Delta}{\sigma_P} \leq \frac{0.44}{2.564}
\]

and

\[
\frac{\sigma_\Delta}{\sigma_P} = \frac{0.44}{2.564} = \left( \frac{1}{n_P} \left( \frac{1}{n_P} + \frac{(1.282)^2}{2} \right) + \frac{1}{K n_N} \left( \frac{1}{n_N} + \frac{(1.282)^2}{2} \right) \right)
\]

The technique described above was used to arrive at the appropriate sample size for a specified

\[
K = \frac{\sigma_P^2}{\sigma_N^2}
\]

where \( \sigma_P^2 \) and \( \sigma_N^2 \) are the analyte variances for the positive and negative distributions. Substituting 1 at a time and \( n_p \) and \( n_N \) derived from
In Equation 2, gives the following equations for sample size determinations:

\[
\frac{n_p}{n_N} = \sqrt{K}
\]

(see Appendix), in Equation 2, gives the following equations for sample size determinations:

\[
n_p = 62 + \frac{62}{\sqrt{K}}
\]

and

\[
n_N = \frac{62}{K} + \frac{62}{\sqrt{K}}
\]

To obtain the proper sample size, as shown in Table 2, one needs a pilot or preliminary estimate of the variation \(\sigma^2_p\) and \(\sigma^2_N\) of the test samples for the materials (i.e., negatives and positives) under study.

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**Appendix**

For validating a method with a sensitivity of 90% and false positive rate of 10%, we want to minimize \(T = n_p + n_N\) while keeping

\[
\frac{1}{n_p} \left( 1 + \frac{(1.282)^2}{2} \right) + \frac{1}{n_N} \left( 1 + \frac{(1.282)^2}{2} \right) = \frac{1.822}{n_p} + \frac{1.822}{n_N}
\]

fixed. Letting \(f\) represent the fixed constant, we have

\[
f = \frac{1.822}{n_p} + \frac{1.822}{n_N} \quad (A1)
\]

Using Equation A1 to solve for \(n_p\), we have

\[
\frac{1.822}{n_p} = f - \frac{1.822}{n_N} \Rightarrow n_p = \frac{1.822n_N}{fKn_N - 1.822}
\]

In minimizing \(T\), we first substitute \(n_p\) into \(T\) as follows:

\[
T = n_N + \frac{1.822n_N}{fKn_N - 1822}
\]

Setting

\[
\frac{\partial T}{\partial n_N} = 0 \Rightarrow 1 + \frac{1.822K}{fKn_N - 1.822} + n_p \left[ \frac{-1.822fK}{(fKn_N - 1.822)^2} \right] = 0 \quad (A2)
\]

Simplifying \(fKn_N - 1.822\), we get

\[
fKn_N - 1.822 = \left[ \frac{1.822}{n_p} + \frac{1.822}{Kn_N} \right]n_N - 1.822 = \frac{1.822Kn_N}{n_p}
\]

Substituting this result in Equation A2, we get

\[
\frac{1.822}{n_p} + \frac{1.822}{Kn_N}
\]
\[
\frac{\partial T}{\partial n} = 1 + \frac{n_p}{n_N} - \frac{n_p^2 f}{1.822n_N} = 0
\]

Substituting \( f \) from Equation A1 above, we get

\[
\frac{\partial T}{\partial n} = 1 + \frac{n_p}{n_N} - \frac{n_p^2 \left( \frac{1.822}{n_p} + \frac{1.822}{Kn_N} \right)} = 0 \Rightarrow
\]

\[
\frac{\partial T}{\partial n} = 1 + \frac{n_p}{n_N} - \frac{n_p^2 \left( Kn_N + n_N \right)}{Kn_N n_N} = 0 \Rightarrow
\]

\[
- \frac{n_p^2}{n_N} = K \Rightarrow - \frac{n_p}{n_N} = \sqrt{K}
\]