United States Department of Agriculture
Agricultural Marketing Service
Federal Grain Inspection Service

Criteria & Specifications

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Biotechnology Test Kit Criteria Supporting Document

Contents

1. BACKGROUND

To meet the grain industry's demand for accurate and reliable qualitative testing for genetically engineered (GE) traits in grains and oilseeds, test kit manufacturers have asked the United States Department of Agriculture's Federal Grain Inspection Service (FGIS) to maintain a program to verify test kit performance claims and subsequently issue Certificates of Performance (COP) for test kits meeting these claims. In response to this request, FGIS has established a program for verifying the performance of these qualitative rapid test kits.

2. ISSUE

Test kit manufacturers have approached FGIS with their concern that sourcing 100% conventional grain to serve as control material for sample analysis is becoming increasingly difficult. FGIS understands these concerns given the increasing prevalence of adventitious presence of GE content in grain. Therefore, FGIS has applied a statistical approach to modify the manufacturer's data submission requirements.

A component of the test kit verification process requires the manufacturer to submit test data to FGIS. Relative to previous requirements, FGIS has increased the number of independent tests to 156 and now allows up to 3 failures for both control and fortified samples. At this failure rate, the test kit will be verified to have a 95% sensitivity and specificity at 95% confidence level.

- Control Samples

  One hundred fifty-six (156) independent analyses performed using thirteen (13) different samples tested across three (3) manufacturing lots, at four (4) replicates per sample. Up to three (3) false positive results for the genetically engineered material of interest are allowed.

- Fortified Samples

  One hundred fifty-six (156) independent analyses performed using thirteen (13) different samples fortified at the claimed detection threshold. Samples will be tested across three (3) manufacturing lots, at four (4) replicates per sample. Up to three (3) false negative results for the genetically engineered material of interest are allowed.

3. CONCLUSIONS

a. GE-Free Base Grain Sample

FGIS asserts that the kit manufacturer should make every effort to provide GE-free grain samples for the purpose of test kit verification. If GE-free base material cannot be sourced, the concentration of GE- grain should be one order of magnitude smaller than the LOD of the protein-based kit, or two orders of magnitude smaller than the LOD of the PCR-based kit, under the critical assumption of sampling m grains and the LOD of PCR is 1/m.

(1) For protein-based kits, which are generally one order of magnitude less sensitive than PCR-based kits, if the kit can detect 1 GE grain out 1,000 grains, the base grain samples must contain 1 or fewer GE-grains out of 10,000 grains. Assuming the sample is 10,000 grains, this level is not expected to adversely affect the verification of the 156 control and fortified samples. Table 1 shows that if the concentration of GE-grain in the base sample is one order of magnitude smaller than the LOD of the kit, the probability of detecting a positive sample due to adventitious presence is negligible (Table 1).

Table 1. Probabilities of detecting a positive sample by the test kit when the concentration of GE- grain is 1／m and the LOD of the kit is 10／m , assuming m grains sampled for test

Grains in Sample (m)	**100**	**500**	**1,000**	**5,000**	**10,000**	**50,000**
Probability of "+" Sample	7.6×10^{-8}	1.0×10^{-7}	1.1×10^{-7}	1.1×10^{-7}	1.1×10^{-7}	1.1×10^{-7}
*Based on a binomial distribution. Similar results can also be obtained by Poisson distribution.						

(2) For PCR-based kits, if and sample size is m and the LOD of the kit is 1/m, no matter how big is m, the contamination level should be two orders of magnitudes smaller than the LOD, that is, < 1 / (100×m), to obtain reasonably small number of false positives from the 156 controls (Table 2).

Table 2. Probabilities of detecting a positive sample by the test kit when the concentration of GE- grain is one or more orders of magnitude smaller than the LOD of the kit (1／m), assuming m grains sampled for test

Grains in Sample (m)		**100**	**500**	**1,000**	**5,000**	**10,000**	**50,000**
cont.	1／10*m*	0.0952	0.0952	0.0952	0.0952	0.0952	0.0952
	1／100*m*	0.00995	0.00995	0.00995	0.00995	0.00995	0.00995
	1／1000m	9.9×10^{-4}	9.9×10^{-4}	9.9×10^{-4}	9.9×10^{-4}	9.9×10^{-4}	9.9×10^{-4}
	1／10000*m*	9.9×10^{-5}	9.9×10^{-5}	9.9×10^{-5}	9.9×10^{-5}	9.9×10^{-5}	9.9×10^{-5}
*Based on a binomial distribution. Similar results can also be obtained by Poisson distribution.							

Compare Table 1 and Table 2, it is not difficult to see that the size of grain sample (m), not the working mechanism of the kit—whether it is based on protein or DNA—is the critical factor determining the probability of false positives.

- If the LOD of the kit is 1⁄x or 1 out of x grains, the number of grains sampled for test should be m=10×x. With this way to determine m, the adventitious presence of GE grains in the base sample at the level less than1⁄m will contribute negligibly the occurrence of false positive result.

- If the LOD of the kit is 1⁄x, and the number of grains sampled for test is m=x, the probability of adventitious presence of GE grains in the base sample increases dramatically. This probability reaches about 1⁄k if the level of GE contamination is 1⁄((k×m)). The probability of false positive is no longer negligible if the contamination level is about one order of magnitude smaller than the LOD.

In either strategy to determine m, there is no way to prepare negative control samples if the concentration of GE-grain in the base sample is close to LOD of the test kit. The manufacturer is advised to find an alternative source of material.

b. Sensitivity and Specificity of Test

The following calculation provides an estimate to determine the sample sizes required for calculating sensitivity and specificity. At a 95% confidence level, the minimal sensitivity varies along the number of negative samples among the n (=156) fortified samples tested by the kit. If we want a minimal sensitivity of 0.95, then the number of false negative among 156 fortified samples must be no more than 3 (Table 2).

Table 3. Relationship between observed false negatives and sensitivity

# Negative samples	Sensitivity
0	**0.981**
1	**0.970**
2	**0.960**
3	**0.951**
4	**0.942**
5	**0.934**

The number of false positive samples allowed for a given level of specificity can be found from the table below. At a 95% confidence level, the minimal specificity varies along the number of positive samples among the n (=156) base control samples tested by the kit. If we want a minimal specificity of 0.95, then the number of false positives among 156 base control samples must be no more than 3 (**Table 4**).

Table 4. Relationship between observed false positives and specificity

# Negative samples	Sensitivity
0	0.981
1	0.970
2	0.960
3	0.951
4	0.942
5	0.934