

Letters to the Editor

Comments regarding publication on 'Performance assessment of rapid immunological test'

Dear Editor,

It has come to our attention that a study conducted and authored by Fagan *et al.* (2000) has been published in the April issue of the *International Journal of Food Science and Technology*. The authors conclude that the use of lateral flow test strips to analyse unknown samples of whole soybeans for the presence of biotech soybeans resulted in a high incidence of false negative and positive responses. The authors conclude that, '...operator performance, not the inherent characteristics of the kit material, were found to be the primary factor influencing the field performance of the test'. It is our opinion that the study organizers did not provide the participants with sufficient information to allow them to generate results consistent with the manner in which the authors intended to analyse the data. The design of the study and the authors' interpretation of the findings are inconsistent with the underlying methodology and seem to have resulted in the formation of some erroneous conclusions.

According to the authors, operators at 21 grain handling facilities and two state grain analytical laboratories were given duplicate blind samples of 10,000 soybeans prepared at concentrations of 0, 0.01, 0.1, 0.5, 1.0 and 10% 'GM content'. The authors did not tell the labs what concentrations to screen for, but instead instructed the labs to use their 'normal sampling procedure'. It is our understanding that, in practice, each user of the test screens at different concentrations determined by their unique business considerations. It is therefore an absolute requirement of the methodology that screening and confidence levels be specified prior to running the test in order to generate meaningful data for a particular concentration. The authors conclude that the methodology resulted in a large number of 'false negative' results when in actuality the majority of the sub-samples in the study clearly did not contain biotech soybeans and therefore were correctly identified as being negative.

Because the protocol did not specify what levels the samples were to be screened at, participants used

sub-sample sizes as low as 50 beans. Using the Poisson probability distribution employed by the test procedure a sample containing 50 beans has 0.5, 4.9, 22, 39 and 99% probability of containing a biotech soybean when the actual concentration of biotech beans is 0.01, 0.1, 0.5, 1 and 10% respectively (the study concentrations). Clearly, from the above considerations, a large percentage of 50 bean sub-samples would not contain a biotech soybean given the low concentrations provided by the authors and therefore would correctly result in a negative result. It is important to emphasize that the analytical test result on these sub-samples would be negative without regard to the actual method of analysis (i.e., PCR, microtitre Plate ELISA or strip test).

Although a 50 bean sub-sample may be too small for many applications, the fact is that the appropriate number of beans in the sub-sample is dictated by the specific circumstances. A sample size of 50 beans may be appropriate for certain applications. For example, a soybean producer has a 92% probability of detecting a load containing 5% biotech soybeans (current Japanese threshold for labelling) when testing a single sample of 50 beans using the strip test. If the objective of the method was to have a 99% probability of detecting 0.1% biotech beans then the participants could have used a sample size appropriate for that screening level (five sub-samples of 1000 beans). As it was, the participants seem to have used sample sizes that were appropriate for their own specific applications – not for detection of the concentrations provided by the authors in this study.

The samples were provided to the labs in duplicate and the authors cite the variability between replicate analyses as evidence of failure of the methodology stating that 'since sampling procedures were identical for replicates, the inconsistencies in results obtained for replicate analyses is not likely to be related to sampling limitations, but is more likely to be because of operator-related variability'. We do not agree with this conclusion. If a sample of 10,000 soybeans contains 1% biotech soybeans, simple probability distributions dictate that there is a 63% probability

that a sample of 100 beans will contain a biotech bean and therefore a 37% probability that it will not. Therefore, if 10 sub-samples of 100 were taken from the same sample of 10,000 soybeans it is expected that three or four of the samples would *not* contain a single biotech bean and would result in a negative test, while six or seven of the samples would indeed contain biotech soybeans and would result in a positive response. The fact that both positive and negative responses are observed in the same sample is not an 'inconsistency' of the analytical method but the expected outcome and is certainly not because of 'operator-related variability'.

The authors further conclude that the test sensitivity is limited to concentrations above 1%. The test performed best at high concentrations in this study because most users have designed their individual sampling strategies for detection at these levels. Most of the users of these tests are testing soybeans at concentrations around the regulatory thresholds specified by their customers selling into Europe (1%) and Japan (5%) and therefore their sample sizes are designed around these screening levels – not 0.01 and 0.1%. Failure to use appropriate procedures to detect these low concentrations cannot be viewed as operator error because the labs were instructed by the organizers to use their 'normal sampling procedures'.

The sensitivity of the method is determined partly by the number of beans in the sample and the number of samples analysed from the load and can be adjusted to various levels of sensitivity with very high reliability. For example, if a person wanted to determine if a load contained 0.01% biotech soybeans, the analyst could test 10 samples of 1000 beans ground together, and providing the method is always positive when there is a single biotech bean in 1000, then 10 negative tests indicates that there was not a single biotech soybean in 10,000. This strategy can be employed to achieve any detection limit as long as the maximum number of beans in the sample is limited to a size where one biotech bean will always be detected. Ultimately, sensitivity of a method is usually limited by practical considerations like cost, time of analysis, etc. and not by the detection level of the analytical test.

The authors do point out that 'one facility included in the study used much larger sample sizes than the other facilities (2400 beans)'. This facility achieved a perfect accuracy score.

If the laboratories with the information that the authors intended to evaluate the test's capacity to detect at the level of 0.01, 0.1 or even 0.5% then the laboratories could have selected the correct sampling strategies to detect these levels. The authors explicit

instructions to the laboratories and lack of information regarding the threshold screening concentrations, confidence levels and intended purpose of the study prevented the laboratories from using the test in a way that they could detect biotech beans in the blind samples.

In summary, it is our opinion that the experimental design of this study is flawed and as a result the experimental data generated does not support many of the conclusions as stated by the authors. This study does not add any substantial scientific information to the literature on biotech testing methods. It simply reinforces the necessity to choose an appropriate sampling strategy and testing method based on the particular testing application. No single sampling strategy or testing method can be used effectively for all applications but we do believe that immunoassay strip test method as specified by the USDA–GIPSA test method protocol is appropriate for the designated application.

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Reference

- Fagan, J., Schoel, B., haegert, A., Moore, J. & Beeby, J. (2000). Performance assessment under field conditions of a rapid immunological test for transgenic soybeans. *International Journal of Food Science and Technology*, **36**, 357–367.

Reply

Dear Editor,

I am writing in response to the comments made by Dr Grothaus of AEIC Biotechnology Consortium and Dr Porter of the American Crop Protection Association, on our article entitled, 'Performance assessment under field conditions of a rapid immunological test for transgenic soybeans', which was published in the April issue of this journal.

The respondents raised three major objections, which we discuss below:

Objection 1

The authors did not understand the methodology for the strip tests, and therefore the design of their study was not valid.

Quite to the contrary, it would appear that the respondents must have misunderstood the purpose and rationale of our study. It was not designed to evaluate the manufacturer's recommended protocol.