Comparison of a Commercial ELISA Kit and TLC for Detection of Deoxynivalenol in Wheat

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Fusarium graminearum Schwabe, the causal agent of wheat scab (head blight), may produce the mycotoxin deoxynivalenol (DON), also known as vomitoxin, in wheat heads under certain environmental conditions. DON is the most commonly produced mycotoxin in Indiana and is a concern where grain is to be used for feeding swine and certain other monogastric animals. Hogs generally reduce feed consumption when 3 ppm DON is present in the ration and refuse the feed at 5 ppm or greater.

Testing grain for the presence of mycotoxins has traditionally been performed by the Animal Disease Diagnostic Laboratory at Purdue. However, the Plant and Pest Diagnostic Laboratory also has occasion to test grain. Traditional analytical methods of assaying for DON (e.g., gas chromatography, mass spectrometry) are relatively rapid but require elaborate chemical equipment not usually found in a plant disease diagnostic laboratory. More recently, thin-layer chromatography (TLC) has become a recommended procedure, but TLC is still complex enough to be impractical in a plant diagnostic laboratory where speed of sample processing is important. Neogen (620 Lesher Place, Lansing, MI 48912-1509) has developed a commercially available rapid diagnostic test for DON based on ELISA technology. Neogen's Agri-Screen lab screening DON kit is simple to use and requires no special equipment or reagents.

During the course of a survey for DON in wheat in Indiana during June 1991, we compared the results of the Agri-Screen DON kit and the TLC procedure in 112 wheat samples collected from randomly selected fields. First, 100 heads of wheat per sample were dried to 4–7% moisture content and hand-threshed, then the grain was ground in a coffee mill to pass easily through a No. 10 sieve (2-mm-diameter openings). One portion of each sample was used for the Agri-Screen DON test and one for TLC (1).

The Agri-Screen kits were purchased from Neogen. All solutions were prepared the day of use, and six to 24 samples were assayed at one time. For each sample, 5 g of ground wheat was added to 50 cc of deionized water and shaken for 10 min on a mechanical rotary shaker at 200 rpm (kit instructions specify shaking vigorously by hand for 3 min). The extract was filtered through Whatman No. 1 filter paper, and a portion of the eluent was used for the Agri-Screen assay.

The Agri-Screen DON kit uses a colorimetric reaction to distinguish between positive and negative samples, and qualitative observations were made according to kit directions. In each assay, test wells were compared with a negative control (conjugate and substrate only) and the positive control provided with each kit. The positive control was labeled by Neogen as containing 2 ppm DON and gave a purple reaction. Sample wells "as blue as or bluer" than the positive control indicate the sample contains less DON than the control; "if sample wells show less blue color (more red color)" than the control, the samples contain more DON than the positive control. All test wells were visually evaluated against a white background by the same person.

A colorimetric test of this nature requires good color perception, and we found it difficult at times to distinguish between a positive and a negative result. Six samples were an unambiguous blue, indicating <2 ppm DON; these results were confirmed by TLC. Results were ambiguous with 39 of the samples, i.e., it was difficult to determine whether the test wells were "bluer than the positive" or "less blue." We decided that these samples probably contained <2 ppm DON and considered them to be "negative." Analysis by TLC found that eight of the 39 samples contained ≥2 ppm DON (positive) and 31 contained between 0.5 and 1.5 ppm DON (negative). The remaining 67 samples were rated positive (≥2 ppm) with the test kit, whereas analysis by TLC found 63 to be positive and four to contain <2 ppm; these four could be considered false positives.

Overall, results obtained with the two test systems did not show a statistically significant difference (χ^2 goodness-of-fit test, $P > 0.25$, df = 1). Under our conditions, we considered the Agri-Screen DON test kit results to be comparable to those obtained by TLC for purposes of identifying samples with ≥2 ppm DON. Four false positives (red sample wells) and eight false negatives (purple sample wells) were obtained with the Agri-Screen kit. Samples that give an ambiguous result (purple wells) should be tested by another method.

Quick test kits based on technology such as ELISA have become more prevalent in the market within the past 10 yr. These kits allow plant disease diagnostic labs to perform in-house immunologic based tests to detect compounds that previously required expensive analytic equipment. The Neogen Agri-Screen lab screening kit for detection of the mycotoxin DON was simple to use, required no special equipment, and was fast (results within 1 hr). Results did not differ significantly from those obtained by TLC in detecting samples with ≥2 ppm DON, but both false negatives and false positives were obtained. Since this particular kit is not designed to be quantitative, there was no way to distinguish grain samples with only 2 ppm DON from those with as much as 40 ppm. (Quantitative test kits for DON are available from Neogen but were not evaluated for this comparison.) In years when the incidence of head scab is low, the Agri-Screen kits would be useful as a preliminary screening tool in which positive or questionable samples could be identified, then quantified with TLC.

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