



A Screening Test for Rapid Detection of DON in Grain Samples.

ABOUT DON

Deoxynivalenol is also known as vomitoxin or DON. This mycotoxin is produced by fungi living on grain such as wheat, corn and barley. It causes reduced animal feeding and weight gain (especially swine) at levels as low as 1-3 parts per million (ppm). Vomiting and total feed refusal does not occur until DON concentrations are much higher (>10 ppm).¹

The FDA has recommended the following advisory levels for DON:²

- 1 ppm for human food
- 10 ppm for ruminating beef, feedlot cattle and chickens, not to exceed 50% of the diet;
- 5 ppm for swine, not to exceed 20% of the diet; and
- 5 ppm for all other animals, not to exceed 40% of the diet.

INTENDED USE

The ABI DON Test is designed solely for use in preliminary screening of grain samples such as wheat and barley. DON may be detected (as a lighter test line) with quantities of .25 ppm or higher. The cut-off is 1 ppm. The ABI DON Test provides only a preliminary qualitative analytical test result. Other methods must be used to obtain a more confirmed analytical result. Professional judgment should be applied to any test results, particularly when preliminary positive results are used. HPLC or GCMS are recommended as methods of choice for confirmation of positive results obtained with the ABI DON Test.

INTRODUCTION

The ABI DON Test is a qualitative one-step immunoassay for the detection of DON. It detects the presence of DON by utilizing highly specific reactions between anti-DON antibodies and DON in grain samples.

PRINCIPLE

The toxin conjugate competes for antibody binding sites with toxins that may be present in the grain sample. The test device consists of a membrane strip to which a conjugate of the toxin of interest is attached. A colloidal gold labeled antibody is located at one end of the membrane. A control line, produced by a different immunological reaction, is also present on the membrane strip. The control line is not influenced by the presence or absence of mycotoxins in the grain sample, and therefore, it should be present in all reactions. In the absence of toxin in the grain sample, the colloidal gold labeled antibody complex moves with the grain sample by capillary action to contact the immobilized DON conjugate. An antibody-antigen reaction occurs forming a visible line in the 'test' area. When DON is present in the grain sample, it competes with the immobilized toxin conjugate in the test area for the antibody binding sites on the colloidal gold labeled antibody complex. If a sufficient amount of toxin analyte is present, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the toxin conjugate.

MATERIALS PROVIDED

1. DON Test Strip packaged in recloseable desiccant vial.
2. Package insert sheet

MATERIALS REQUIRED BUT NOT SUPPLIED:

1. 100 ml disposable screw cap vials for extracting specimens
2. Pipette to deliver 80 ul +/- 10ul
3. Timer

WARNINGS AND PRECAUTIONS

1. This test is for *screening* use by professionals only.
2. Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
3. The desiccant vial containing the test strips must remain completely closed except for opening to remove test strips. When re-closing, snap lid firmly.
4. Avoid cross-contamination of grain samples by using a new container for each specimen.

STORAGE

The ABI Rapid Test should be stored at room temperature (15° to 30°C) or refrigerated (2° to 8°C). The test strips and grain extract should be at room temperature before using.

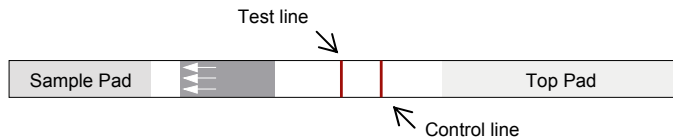
SAMPLE COLLECTION AND EXTRACTION

1. Collect the sample according to accepted GIPSA procedure.
2. Prepare extraction buffer as instructed on buffer packet. (We recommend Sigma Phosphate Buffered Saline packets Item no. P-3813 for convenience. Add 90 ml extraction buffer to a suitable screw cap container. Add 30 gm grain sample to container, tighten cap and shake vigorously for 3 minutes to extract DON from grain sample.
3. Allow sediment to settle for 5 minutes. It is important not to transfer sediment to the test strip since it can interfere with the flow of liquid and this may affect test results. The sample may also be filtered through Whatman no. 1 filter paper.
4. Test line intensity may be lighter when extracts are not fresh. Same day testing is recommended.
5. It is important to allow the recommended settling time. Test line intensity may increase when testing very fine ground samples if sufficient settling has not occurred.

ASSAY PROCEDURE

1. Be certain the test strip and grain extract have equilibrated to room temperature before conducting any testing. Temperature variation can affect test results.
2. Pipet method:
 - Transfer 80 ul of grain extract to sample pad on test strip.
 - Allow test to develop and read results after 5 minutes. After 5 minutes, the test lines may increase in intensity.
3. Dip method:
 - Transfer 500 ul of grain extract to a 12 x 75 mm test tube, 1.5 ml micro centrifuge tube or other suitable tube.
 - Insert test strip into liquid and allow test to develop.
 - Read results after 5 minutes. After 5 minutes, the test lines may increase in intensity.

TEST INTERPRETATION



<u>Control Line</u>	<u>Test Line</u>	<u>Interpretation</u>
No control line present	No test line present	Invalid result
Control line present	Distinct test line present	0 ppm
Control line present	No test line present	1 ppm and higher
Control line present	Light intensity test line present	Between 0 and 1 ppm

QUALITY CONTROL

It is good laboratory practice to use negative controls to ensure proper test performance. It is recommended that each shipment of product be tested with the included controls upon receipt.

LIMITATIONS OF PROCEDURE

This test is intended for screening grain extracts only. Whenever more quantitative results are required, samples should be tested with a quantitative procedure such as HPLC. Qualified testing laboratories can perform such testing. Apply professional judgment to any mycotoxin result, particularly when preliminary positive results are used for determining outcomes. The ABI DON Test provides only a preliminary qualitative test result. Results obtained with the ABI DON Test cannot be considered conclusive evidence that DON is present in quantities greater than the stated threshold. Specimens exhibiting positive results must be submitted to a qualified laboratory for analysis by GCMS or HPLC for definitive detection of DON.

SENSITIVITY

The detection limit for DON was established as 1 PPM as follows: Grain samples previously tested by HPLC and ELISA were confirmed to contain 1 PPM DON and 0 PPM DON respectively. These samples were tested with the ABI Rapid DON Test. 180 extracts were prepared as instructed above (see SAMPLE PREPARATION AND EXTRACTION). Detection limit is defined as the concentration that produced positive responses (no test line visible).

REPRODUCIBILITY

Reproducibility studies were performed using extracts of grain samples that were previously assayed by HPLC and ELISA METHODS.

<u>PPM DON</u>	<u>No. samples</u>	<u>Results</u>	<u>Precision</u>
0	30	Negative	100%
≥1.0	30	Positive	100%

BIBLIOGRAPHY

1. Woloshuk, Charles P.; Corn Diseases; Mycotoxins and Mycotoxin Test Kits BP-47 Department of Botany and Plant Pathology, Purdue University; West Lafayette, IN 47907
2. GIPSA, Grain Inspection, Packers and Stockyards Administration