Zearalenone Test Strip
1 ppm

A Screening Test for Rapid Detection of Zearalenone
(Currently validated for corn)

ABOUT ZEARLEANONE
Zearalenone, a secondary metabolite with estrogenic properties, is produced by several Fusarium species that colonize cereal grains in the field and in storage. Zearalenone has been shown to cause estrogenic disturbances in farm animals. Recently, there have been reports of zearalenone contamination in corn, oats, barley, wheat, and grain sorghum.[1]

INTENDED USE
The Zearalenone Test is designed solely for use in preliminary screening of grain samples. It is a competitive inhibition immunoassay for the qualitative detection of zearalenone in samples such as corn and corn meal. The test produces a positive result in grain samples containing zearalenone in quantities of 1 ppm or higher. This 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 this test.

INTRODUCTION
The Zearalenone Test is a qualitative one-step immunoassay for the detection of zearalenone. It detects the presence of zearalenone by utilizing highly specific reactions between anti- zearalenone antibodies and Zearalenone 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 test strip membrane. The control line is not influenced by the presence or absence of zearalenone 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 toxin conjugate. An antibody-antigen reaction occurs forming a visible line in the 'test' area. When zearalenone 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.

The formation of one visible line (Figure 1) represents a positive result, indicating presence of the toxin at or above 1 ppm.

The formation of two visible lines (Figure 2) represents a negative test result. This means the test did not detect the toxin above the cut-off point of 1 ppm. The intensity of the test line increases with lower toxin levels.

MATERIALS PROVIDED
1. Zearalenone Test Strips in desiccant vials
2. Package insert sheet

MATERIALS REQUIRED BUT NOT SUPPLIED:
1. 50 ml screw cap vial for extracting specimen
2. Graduated cylinder for preparing Extraction Solution (70% Methanol/DI Water)
3. Methanol
4. Deionized Water
5. 1.7 ml microcentrifuge tubes
6. Pipets to deliver 250 ul
7. Timer

WARNINGS AND PRECAUTIONS:
This test is for screening use only. Prior to use, verify that the date of use is prior to the expiration date on the foil pouch.
The foil pouch containing the test strip must remain completely sealed before use. Do not use if foil pouch seal is not intact. Avoid cross-contamination of grain samples by using a new container and pipette for each specimen.

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

PREPARATION OF EXTRACTION SOLUTION
Prepare 200 ml 70 % Methanol/DI Water as follows:
Mix 60 ml DI Water with 140 ml Methanol. The mixture may become somewhat warm. Allow to cool to room temperature before using for extracting grain samples.

SAMPLE COLLECTION AND EXTRACTION AND DILUTION
Collect the sample according to accepted GIPSA procedure.
Add 5 gm grain sample to a 50 ml screw cap vial. Then add 10 ml 70% Methanol, tighten cap, shake for 3 minutes and allow sediment to settle for 5 minutes.
In a microcentrifuge tube, dilute .25 ml extract with .25 ml DI Water and mix well.

ASSAY PROCEDURE
Be certain the test strip and grain extract have equilibrated to room temperature before conducting any testing. Temperature variation can affect test results.
Remove test strip from the foil pouch. Do not use if foil pouch seal is not intact. Label strip in the designated area.
Dip test strip into microcentrifuge tube containing diluted extract.
Remove test strip after 5 minutes, and read results as explained below under Interpretation of Results. Negative results may be visible within 1 minute.

INTERPRETATION OF RESULTS

Read results after five minutes.

Figure 1: Positive Result – Zearalenone level greater than 1 ppm. Lower Zearalenone levels produce test line of increasing intensity.

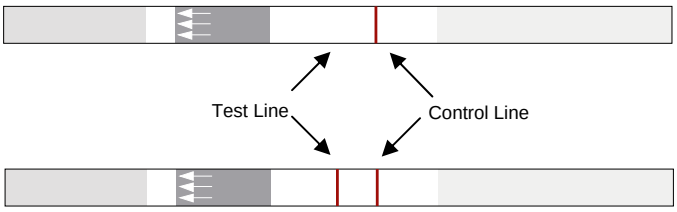

Figure 2: Negative Result – Zearalenone level less than 1 ppm

Control Line	Test Line	Interpretation
Control line present	Test line present	Negative
Control line present	Faint or no test line present	Positive

Test is invalid if no Control Line is visible

Test line ***is not visible*** in specimens with Zearalenone content greater of 1 ppm or greater. Test line ***is visible*** with zearalenone content less than 1 ppm and very intense in negative samples.

QUALITY CONTROL

It is good laboratory practice to use positive and negative controls to ensure proper test performance. It is recommended that each shipment of product be tested with controls upon receipt and each day tests are performed. Suitable control material is available from Trilogy Analytical Lab (Washington, MO) and other sources.

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 Zearalenone Test provides only a preliminary qualitative test result. Results obtained with this test cannot be considered conclusive evidence that Zearalenone is present in quantities greater than the stated threshold. Specimens exhibiting positive results should be submitted to a qualified laboratory for analysis by GCMS or HPLC for definitive detection of Zearalenone.

SENSITIVITY

The detection limit for Zearalenone was established as 1 ppm as follows: Extracts prepared from corn samples were previously tested for Zearalenone by HPLC . 25 extracts were prepared as instructed above (see Sample Preparation and Extraction). Sensitivity is defined as that concentration which produced positive responses in all 25 replicate samples.

REPRODUCIBILITY

Reproducibility studies were performed using extracts of grain samples that were previously assayed by HPLC and certified for ZON content.

PPM ZON	No. samples	Results	Precision
0	30	Negative	100%
1.0	30	Positive	100%

[1] Bennett GA, Shotwell OA. Zearalenone in Cereal Grains, J. AOCS, Symposium: Mycotoxins I and II; 56,9,September 1979