**APTECA**

***Aflatoxin Proficiency Testing and Control in Africa***

****

Handbook

June 2020

Version 7.1

**Texas A&M AgriLife Research**

**College Station, TX**

**University of Nairobi Chiromo Campus**

**Nairobi, Kenya**

**BACKGROUND**

To manage the economic and food safety risk of aflatoxin contamination, individuals need real-time information about the level of aflatoxin in cereals, oilseeds and their products. That’s why the APTECA program implements a process approach to measure and manage aflatoxin risk. Accurate measurement of aflatoxin is complicated by the nature of the toxin and the challenges associated with sampling and testing maize. To standardize these processes and reduce the variability of aflatoxin test results, APTECA promotes the use of uniform sampling, testing equipment and methods; proficiency testing; use of laboratory control samples; and third party verification.

**ELIGIBILITY & PARTICIPATION:**

Since 2014, the Aflatoxin Proficiency Testing and Control in Africa, Asia, Americas and Europe (APTECA) program focuses on process verification and aflatoxin risk management. Firms agree to:

* Develop a written APTECA Food Safety plan (Appendix A);
* Collect representative samples of incoming maize and finished product (i.e., maize flour or meal) using the methodology described in the APTECA Food Safety plan and handbook**;**
* Prepare samples using methodology that retains the representative property of the sample;
* Test ground maize and finished product using field validated testing methods;
* Maintain records that enable third party audit and verification;
* Perform the above activities with trained personnel; and
* Analyze proficiency samples twice a year and submit results to APTECA.

Firms, government agencies, and researchers in Africa that want to participate in the APTECA proficiency testing program can enroll free of charge in 2017 at <http://apteca.tamu.edu>.

**EMPLOYEE PROFICIENCY**

APTECA participants must have a minimum of two employees capable to perform aflatoxin sample analysis. The employee(s):

* Must review the APTECA handbook and submit an approved written APTECA Food Safety Plan;
* Must be trained prior to performing the APTECA procedures described in the handbook and plan; and
* Must successfully complete aflatoxin proficiency testing administered by APTECA.

**PREVENTIVE CONTROLS**

APTECA participants monitor preventative controls and take corrective actions to maintain equipment and analytical performance at six points within the risk management process (Table 1).

**Table 1.** Preventative control points, objectives, and parameters.

|  |  |  |
| --- | --- | --- |
| ***POINT*** | ***OBJECTIVE*** | ***CONTROL PARAMETER*** |
| **Test kit selection**  Table 2  **Sample collection**  Figure 1, Table 3 | Ensure results are accurate within test kit specified range  Ensure that the sample represents the entire truckload of maize | Field validation by Texas A&M ISO accredited lab  Grind a 2 kg composite sample from individual truckloads. |
| **Grinder check** | Ensure that the sample is finely ground and homogeneous | ≥60% +10% of the ground particles (% fines) pass through a 20 mesh sieve |
| **Lab scale calibration** | Ensure that the scale is calibrated | 50 gram calibration weight measures between 49.8 - 50.2 grams |
| **Control sample analysis** | Maintain analytical performance to accurately measure aflatoxin concentrations | Control sample test results duplicate within acceptable range (Table 2) |
| **Retained File Samples:**  **Corrective Action** | Retain a representative file sample for verification analysis by AgriLife Research  Document changes when a deviation occurs | Verification results duplicate within an acceptable range  A deviation is a failure to meet a control parameter |

**TEST KIT SELECTION**

In order to address the continual introduction of new aflatoxin rapid test kits on the market and expand the scope of the APTECA program, a field validation process for field-based test kits has been adopted as described below.

Process for Field Validation

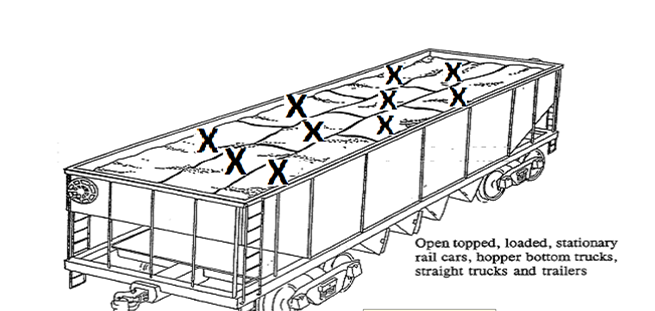
* 1. Mill or grain storage operator selects a Performance Verified Test Kit by a competent authority (e.g. Institute for Reference Materials and Measurements EU-RL, USDA Grain Inspection Packers Stockyard Administration)
  2. Mill or grain storage operator submits APTECA Sampling & Testing Plan;
  3. Mill or grain storage operator analyst passes qualification exercise using selected test kit;
  4. Grain storage operator analyst runs two OTSC control samples (low and high concentrations) once a week and submit results to Texas A&M AgriLife at the University of Nairobi;
  5. Grain or grain storage operator analyst retains a file sample for each sample analyzed and submits to AgriLife at the University of Nairobi;
  6. AgriLife completes statistical data analysis using performance criteria in Table 2.
  7. Aflatoxin test kits that fail to meet performance criteria within the specified testing test range (Table 2) will no longer be used by APTECA program participants.

**Table 2.** Aflatoxin duplication limits.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **If the Aflatoxin control is:** | ≤ 25 ppb | > 25 to ≤ 50 ppb | > 50 to ≤ 100 ppb | > 100 ppb |
| **Acceptable Duplication limit is:** | ± 40 % | ± 34 % | ± 25 % | ± 20 % |

**SAMPLING & TESTING**

Uniform sampling and testing procedures reduce the variability of test results. APTECA procedures are based on the Codex Standard (CX/CF 14/8/9) or other Code by competent regulatory authority. Each participating location submits a customized APTECA Food Safety Plan to describe how standard operating procedures will be implemented.



**Figure 1.** For bulk loads of grain in railcars or trucks, use a 10-probe pattern and collect 2 kg of maize .

Table 3. Number or incremental samples to be takend depending on the weight of the lot.

|  |  |
| --- | --- |
| Lot weight (ton) | Number of incremental sample |
| ≥ 0.05 | 3 |
| > 0.05 - ≤ 0.5 | 5 |
| > 0.5 - ≤ 1 | 10 |
| > 1 - ≤ 3 | 20 |
| > 3 - ≤ 10 | 40 |
| > 10 - ≤ 20 | 60 |
| > 20 - ≤ 50 | 100 |

Source: Codex Alimentarius. CX/CF 14/8/9. 2014. Sampling plan design considerations.

**RECORDKEEPING & REPORTING**

All participating locations agree to keep records for a period of one year and make records available for review by APTECA program representatives. Records include: particle size log; lab scale log; laboratory reference material log; aflatoxin test results log for incoming maize and finished maize products; corrective action record.

**MONITORING & CORRECTIVE ACTIONS**

APTECA monitors personnel, equipment, and performance records to ensure compliance with the program standards.

* Observe sampling procedures;
* Review records; and
* Recommend corrective actions.

Retained file samples sent by the participating mill are analyzed for aflatoxin (without further grinding) by Texas A&M AgriLife Research personnel at the University of Nairobi using Romer FluoroQuant® Afla. APTECA compares the firm’s aflatoxin result with the Texas A&M AgriLife Research laboratory result and reports the level of aflatoxin to the company’s management. When aflatoxin verification results do not agree, mill operators will perform a review to find the cause of the deviation, and initiate and document corrective actions. Corrective actions may include:

* Adjustment or repair of equipment;
* Retraining of personnel;
* Correction of records;
* Assistance from the firm’s management; and/or
* Suspension or removal of an employee or firm from the program.

**CONTACT US**

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Contacts for Aflatoxin Test Kits and price lists (Table 4) are presented below:

Charm – Meikel Brewster, 781-640-2489 US, email meikelb@charm.com

Neogen – F&S Scientific, Rachel Lilies [Rachel.wangui@fnscientific.com](mailto:Rachel.wangui@fnscientific.com) tel +254 727 800 800

or Jitesh Shah [jitesh.shah@fnscientific.com](mailto:jitesh.shah@fnscientific.com) tel: 00254 20 3594777

Vicam – Willen Joubert +27(11)553-2300 [willem.joubert@microsep.co.za](mailto:willem.joubert@microsep.co.za) Microsep

Table 4: Price list for companies that have offered discounts to APTECA participants that are GIPSA validated and approved.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **ROSA FAST (Charm Sciences)** | **Veratox**  **(Neogen)** | **Reveal Q+ (Neogen)** | **AflaTest**  **(Vicam)** |
|
| **Range**  (GIPSA guideline) | 5 – 150 ppb | 5 – 100 ppb | 5-100 ppb | 5 – 1000 ppb |
| **Mechanism** | Antigen/Antibody color reading of strip | Antigen/Antibody color reading of solution | Lateral flow | Fluorescence of analyte eluted through a column |
| **Extraction Solvent** | 100 ml  70/30  Methanol/Water | 250 ml  70/30  Methanol/Water | 125 ml  65/35  Ethanol/Water | 100 ml  80/20  Methanol/Water |
| **# of samples/run** | Up to 4 | Up to ~ 40 | 1 | 1 |
| **Est. time/kit** | 3 min. | 15 min. | 12 min | 7 min. |
| **Comments** | Keep cold & bring to room temp. for testing | Keep cold & bring to room temp. for testing | Refrigeration not required; Test kit dev. & extractor react quickly | Refrigeration not required; Test kit dev. & extractor react quickly |
| **Reader Cost** | 2000 samples per year for free reader | No cost if purchasing 20 kits **No longer available** | No cost if purchasing 20 kits **No longer available** | On loan, $1000 order limit for free shipping of kits |
| **Accessory**  **Part # Est. Cost** | Incubator  No cost if purchasing 2000 tests | No cost if purchasing 20 kits | Each kit comes complete with all test & accessory materials | 12 position pump stand w/ 6 pumps  $1062 |
| **Calibration Set**  **Part # & Est. Cost** | Included w/ kit | Included w/ kit | Included w/ kit | 33020  $31 |
| **Kit Part # Est. Cost**  **# Analyses/Kit** | LF-AFQ-FAST-100K  $400 + VAT (100 analyses /kit) | 8030  $180 + VAT  (40 analyses/kit) | 8085  $175 + VAT  (25 analysis/kit) | AflaTest Columns  12022  $378 (50 pack) VAT included |

**APPENDIX A**

**[COMPANY NAME] APTECA Food Safety Plan**

# Company Information

Primary Contact:

Address:

Phone Number:

Fax Number:

Email:

# Scope / Field of Application

This APTECA Food Safety Plan applies to the following products:

# Equipment

Aflatoxin Test Kit and Reader:

# Responsibilities

Quality Assurance manager:

General Manager:

Laboratory manager:

Production manager:

Analyst:

# Definitions

**Aflatoxin**: A toxic fungal metabolite that is a group 1 carcinogen

**Composite Sample:** A sample formed by compositing or accumulating and combining a number of discrete   
 samples; useful in determining the average composition of a large amount, such as a   
 shipload, carload, or truckload.

**Control Sample:** A sample that is carefully subdivided with portions sent to a number of laboratories   
 for analysis and used as a check on laboratory assay procedures.

**Control Sample Log**: A mandatory record of control samples performed weekly

**Corrective Action Record**: A mandatory record of corrective actions

**Flour Aflatoxin Log**: A mandatory record of finished product (i.e., maize flour or meal) aflatoxin analysis

**Lab Scale Log**: A mandatory record of scale calibration performed daily

**Maize Aflatoxin Log**: A mandatory record for every load of maize received

**Particle Size**: Measured as percent passing through a US 20 mesh sieve (841 microns openings)

**Particle size Log**: A mandatory record of particle size analysis performed weekly

**Retained File Sample:** A duplicate portion of a lot retained in case an analysis is needed following use or   
 distribution of the lot.

# Procedure

1. **The [COMPANY NAME] APTECA Food Safety Plan**
2. This plan includes methodology for sample collection of incoming maize and finished product, sample grinding, sample preparation and aflatoxin testing of maize and flour, recordkeeping, analyst training and qualification, participation in proficiency testing, lab scale calibration, use of control samples, and sample retention for third party verification.
3. This plan includes monitoring frequency, critical limits, corrective action, testing verification procedures and record keeping.
4. The APTECA Food Safety Plan supports, but does not eliminate or preempt, the [COMPANY NAME] HACCP plan.
5. **Employee Training and Qualification**
6. Analyst reads the test kit instructions;
7. Analyst observes the experienced analyst perform test;
8. Analyst runs 6 samples tested by the previous analyst and compares results using the Dixon outlier test (<http://www.apteca.tamu.edu>)
9. Analyst runs three sets of control samples and plots results on the control chart; and
10. Analyst runs quarterly APTECA proficiency analyses.
11. **Preventive Controls: Incoming Truckloads of Maize**
    1. Maize Sample Collection and Subsampling: All incoming loads of maize will be representative and follow a prescribed pattern and frequency as follows:
12. Sample Collection:
    1. Bulk Loads: A \_\_-probe sampling pattern performed with a slotted spiral grain probe is used to collect a minimum of \_\_ kg.
    2. Bags: A bag probe is used to collect a minimum of \_\_ kg.
13. Subsampling:
    * 1. The entire \_\_kg maize sample is ground;
      2. A representative 500 g subsample is obtained using a quartering or riffling technique;
      3. 50 g are analyzed for aflatoxin and the remaining 450 g are retained as a file sample.
    1. Maize Particle Size: Particle size of ground maize will be analyzed once per week or when the analyst suspects there is a need to adjust the grinder as follows:
       1. A 50 g representative subsample is weighed and placed on a 20 mesh sieve then covered by pan and lid;
       2. The 50 g sample is shaken for 60 seconds;
       3. The material passing through the 20 mesh sieve is weighed;
       4. The percentage of material passing through the 20 mesh sieve is expressed as a percentage (target particle size ≥70% +10% passing through a 20 mesh sieve);
       5. Adjust the grinder if less than 60% ground maize passed through the 20 mesh sieve; and
       6. Record results in the Particle Size Log.
    2. Lab Scale Calibration: The laboratory scale will be calibrated daily before use and after electrical outage.
       1. Calibrate the scale daily before use with a 50 g calibration weight (target calibration 49.8- 50.2 grams (±0.2 g);
       2. Record results on the Lab Scale Log; and
       3. Adjust scale if the weight deviates by more than 0.2 g.
    3. Control Sample Analysis: Control samples provided by Texas A&M AgriLife Research will be run at the beginning of the week and when new lots of test kits and reagents are used.
       1. Results of the control sample will be plotted on the control chart.
       2. If the laboratory results are outside the aflatoxin control sample limits, corrective action by the laboratory will be implemented to determine the cause of the deviation (inaccurate measurement).
14. Retained Maize File Samples: Twice a month, \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ (Name and Title) will send \_\_, 450 g retained maize samples to the Texas A&M AgriLife Research laboratory at the University of Nairobi for verification.
15. **Finished Product (i.e., maize flour or meal) Preventative Controls**
16. Flour Sample Collection: Each hour, a 200 g sample will be collected from the finished product stream. The point of collection will be at the \_\_\_\_\_\_\_ line spout on the \_\_\_\_ floor. Samples will be collected \_\_\_ (e.g. hourly) and composited every \_ hours (total \_ kg of flour) and tested for aflatoxin following test kit directions.
17. Retained Flour Samples: Every two weeks, \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ (Name and Title) will send \_\_, 500g retained flour samples to the Texas A&M AgriLife Research laboratory at the University of Nairobi for verification.
18. **Maize and Finished Product Sample Identification**
    1. Samples submitted to the Texas A&M AgriLife Research laboratory at the University of Nairobi will have a unique identification number that will provide traceability to the truck or flour production shift and lot as follows:
       1. Maize: All retained maize file samples will be traceable to the time, date, truck ID (if appropriate), and aflatoxin level.
       2. Finished Product: All retained flour or meal samples will be traceable to the product run, date, lot, and aflatoxin level.
19. **Third Party Verification Labeling**
    1. A logo prescribed by the verification provider will be used for only those products contained in this plan and conforming to the [COMPANY NAME] APTECA Food Safety Plan that contain <10 ppb in the finished product.
20. **Records and Reporting Responsibilities**
    1. The following records will be retained for a period of one year: particle size log, lab scale log, control sample log, control chart, maize aflatoxin log, flour aflatoxin log, corrective actions record.
    2. Records will document any deviations and corrective actions.
    3. Retained maize file samples are kept for \_\_ days
    4. Retained finished product file samples are kept for \_\_ days.
21. **Corrective Actions**
    1. [COMPANY NAME] will reject incoming truckloads of maize that exceed 10 ppb.
    2. [COMPANY NAME] will not distribute finished product that contains greater than 10 ppb aflatoxin with the APTECA seal.
    3. Inability to replicate control sample results within the acceptable range will result in a root cause analysis to investigate reagents, kits, reader, scale, and analyst. Once the process is corrected and corrective actions have been documented on the Corrective Action Record, the firm may proceed to analyze samples.
    4. If a sample of finished product is found to contain >10 ppb aflatoxin by Texas A&M AgriLife Research at the University of Nairobi, [COMPANY NAME] will perform a root cause analysis and document corrective actions on the Corrective Action Report.
    5. An investigation will be taken if [COMPANY NAME] quarterly proficiency results have a Z-value score >2 and a corrective action if the Z-value score is >3.

# Revision History