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Food Safety and Inspection Service
Office of Public Health Science
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Laboratory Guidebook
Notice of Change

Chapter new, **revised**, or archived: PLG 0002.01

Title: Rodent Adulteration and Infestation Sample Analysis

Effective Date: 02/24/20

Description and purpose of change(s):

Elimination of rodent urine testing step in 2.5.2. This assay is no longer fit for purpose.

Safety Precautions Section 2.2 was updated to include safety precautions for the potential risk of hantavirus transmission.

References were updated to current versions of references.

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Revision: .01	Replaces: Original	Effective: 02/24/20

Procedure Outline

2.1 Introduction

This procedure serves as a decision tree for determining rodent adulteration or infestation by systematic analysis of samples. It includes the series of steps to be used in the analysis of samples with an analytical request for rodent droppings/excreta, rodent gnawing, or hair from rodent adulteration of meat and poultry products, or rodent infestation as in a processing plant or cold storage warehouse. Note: Some analytical steps may not be necessary dependent on findings from preceding steps.

2.2 Safety Precautions

Laboratory transmission of hantaviruses from rodents to humans via the aerosol route is well documented. Exposures to rodent excreta and urine are presumed to be associated with risk. A risk assessment should be performed to determine the risk of hantavirus transmission. Primary physical containment devices including Biological Safety Cabinets (BSCs) should be used whenever procedures with potential for generating aerosols (centrifugation, vortex-mixing, etc.) are conducted. Samples from potentially infected rodents should be handled at BSL-2 facilities using BSL-3 practices, containment equipment and procedures.

2.3 Quality Control Procedures

Controls which may be used in this decision tree are detailed in the referenced procedures.

2.4 Equipment, Supplies, and Reagents

2.4.1 Equipment

a. Balance, top loading, 5-10 kg capacity
b. Balance, analytical, 10-150 g capacity
c. Stereomicroscope, 5-50X magnification
d. Compound microscope, 20-1000X magnification
e. Hot plate
f. Stirrer/Hot plate
g. Slide warmer
h. Digital camera

2.4.2 Supplies

a. Petri dishes- sterile, disposable polystyrene, 85-95 mm diameter, or equivalent
b. Filter paper circles- grade 8 ruled, 70 mm diameter, or equivalent
c. Fine forceps- Dumont No. 3, 120 mm long (2 pair), or equivalent
d. Spot test Plate- 9 well, transparent, glass, 85 x 100 mm, or equivalent
e. Microscope slides
f. Microscope cover glass
g. Beakers, glass, 200-600 ml and 2 liter
h. Stirring bars
i. Balsam bottles

2.4.3 Reagents

a. Deionized water
b. 2-Propanol (isopropyl alcohol), ACS reagent-grade
c. 1,2-Propanediol (propylene glycol)
d. Glycerol (Glycerin), ACS reagent-grade
e. Gelatin, laboratory grade
f. Phenol
g. Nylon nail enamel (clear, colorless)

2.5 Procedures

2.5.1 Initial Treatment of Sample

a. Upon receipt of a sample, log in, make initial observations and record. For a product, weigh the sample including retail packaging or make a note if sample weight exceeds the capacity of the top loading balance used. If the sample merely consists of suspect excreta or stained packaging/cardboard/etc., make note of the type of sample and weigh only if deemed necessary.

b. Photograph the sample if deemed necessary as in the case of a very large sample of which only a small portion will be kept for a reserve.

2.5.2 Succession of Analytical Steps

a. If the sample includes retail packaging, examine for rodent gnawing (scalloped cut pattern made with small incisors) and record observations.

b. If suspect excreta pellets are observed, remove at least one pellet and place on a piece of filter paper in a petri dish using forceps. Measure (width and length), moisten with deionized water, and remove striated hairs (generally five to ten) for deaeration. Move the remainder of the pellet to one side of the petri dish (for alkaline phosphatase test).

c. Perform the alkaline phosphatase test as described in PLG 3 on the remainder of the same suspect excreta pellet set aside in step b and photograph the test reaction.

d. Deaerate hairs by gently heating in a spot test plate or beaker (until fumes are observed but not to boiling) in a 1:3 solution of isopropyl alcohol-propylene glycol for at least 30 minutes and mount in glycerin jelly (procedure described below) on a microscope slide, coverslipped, for microscopic examination.

   i. Glycerin jelly components:
   10 g gelatin (laboratory grade); 70 ml glycerin; 60 ml deionized water; and 1 g phenol. Boil water in a beaker, remove from heat and add gelatin (stir with magnetic stirring bar) before adding glycerin and phenol. Pour into petri plates or balsam bottles (good for 10 years).
   ii. If needed for removal of air bubbles from mounting medium, place the slide on a slide warmer set at 55 $\pm$ 5ºC for 12-72 hours. [When preparing

slide for long-term storage, allow to cool (at ambient temperature) for at least 30 minutes, and ring coverslip with nylon nail enamel.]

iii. Alternately, mount the hairs directly into glycerol on a microscope slide, coverslip, and heat on a slide warmer (65 $\pm$ 5ºC) for one to two hours (or at 55 $\pm$ 5ºC for 12-72 hours for overnight or over-the-weekend preparation). Record beginning and ending times and temperatures for all slide preparations employing a slide warmer. Slide mounts can be checked under a compound microscope for quality of preparation (no air in hair; hair not overheated).

e. If no excreta is found, examine packaging and product surfaces macroscopically and microscopically for striated hairs. Remove any striated hairs found, deaerate, and mount as above for microscopic examination.

f. Microscopic examination of the hairs is performed by a properly trained analyst according to FDA Technical Bulletin #1 to determine if the hairs are rat or mouse hairs.

2.6 Selected References

Chosewood, L.C. and Wilson, D. E. (eds.) 2009. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition. HHS Publication No. (CDC) 21-112.

Gorham, J. R. (ed.). 1981. Principles of Food Analysis for Filth, Decomposition, and Foreign Matter, FDA Technical Bulletin #1. U.S. Dept. of Health and Human Services, Public Health Service, Food and Drug Administration, Washington, DC.

Olsen, A. R., T. H. Sidebottom, and S. A. Knight (eds.). 1996. Fundamentals of Microanalytical Entomology: A Practical Guide to Detecting and Identifying Filth in Foods. CRC Press, Inc., Boca Raton.

Whitlock, L. L. (ed.). 2016. Extraneous materials: isolation, Chapter 16. In Official Methods of Analysis of the Association of Official Analytical Chemists International, 20th Edition. AOAC International, Rockville, MD 20850-3250.