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Laboratory Guidebook
Notice of Change

Chapter new, **revised**, or archived: MLG 32.01

Title: Detection and Identification of Extraneous Material in Meat and Poultry Products.

Effective Date: 12/30/04

Description and purpose of change(s):

This Microbiology Laboratory Guidebook method chapter was revised to update the formatting and to meet the requirements of the laboratory's document control system and ISO 17025. Additional content includes sections on quality control and safety precautions. The general content has been re-written to clarify procedures and update the method.

The methods described in this guidebook are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products and acknowledges that equivalent products may be available for laboratory use.

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

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Title: Detection and Identification of Extraneous Material in Meat and Poultry Products.		
Revision: 01	Replaces: Original Chapter 32	Effective: 12/30/04

Procedure Outline

32.1 Introduction

32.1.1 General Introduction

Food Safety and Inspection Service (FSIS) is responsible for insuring that the meat and poultry products offered to the consumer are safe, wholesome, unadulterated and truthfully labeled. In fulfilling this responsibility, the Agency's laboratories perform sanitation analyses of the meat and poultry products including investigations for extraneous or foreign materials. According to law, a meat or poultry product is adulterated if it consists in whole or in part of any filthy substance, is for any reason unsound or unwholesome, or if the product was prepared or packed under unsanitary conditions where it may have been contaminated [21 United States Code 601(m)(3)(4), 21 United States Code 453(g)(3)(4)].

Extraneous material is defined as any foreign material found in a food product and associated with objectionable conditions or practices in production, storage, or distribution. Examples of extraneous materials are: filth, metal, glass, sand, wood, paper or plastic. Filth is defined as any objectionable matter contributed by animal contamination of a product such as: rodent, insect or bird matter; or objectionable material contributed by unsanitary conditions. The presence of extraneous material in a food product is not only unappealing but represents a breakdown in good manufacturing practices and could pose a serious health hazard to the consumer. The isolation and identification of extraneous materials sometimes yields evidence that a product was stored or processed under unsanitary conditions and is unfit as human food.

The study of extraneous materials found in food is called Microanalytical Entomology. The U.S. Food and Drug Administration (FDA) and the Association of Official Analytical Chemists (AOAC) have published reference articles, books and methods on this subject. These publications discuss methods of analyses, contaminant identification, and contaminant significance.

This chapter contains the methods developed and used by FSIS Entomology and Extraneous Materials Laboratories (EEML) to isolate and to identify extraneous materials from meat and poultry products. These methods are intended for the stated product and contaminant. Before using one of these methods on a different product or for a different contaminant, the method must be thoroughly evaluated for that purpose. Aside from the methods developed in our laboratories, FSIS EEMLs use many AOAC methods.

32.2 General Quality Control and Good Laboratory Practices for the Entomology and Extraneous Materials Laboratory

For extraneous materials analyses it is of the utmost importance to maintain a clean and contaminant-free laboratory. All possible action must be taken to prevent the contamination of the sample with insects or extraneous materials. Below are listed general practices and techniques which must be observed in the Entomology and Extraneous Materials Laboratories to insure a quality analysis.

32.2.1 Equipment and Reagents:

a. Sieves

i. Each analyst should be assigned a sieve. The analyst is responsible for maintaining his/her sieve. The sieve should be cleaned immediately after using it to prevent debris from drying on the sieve.

ii. As specified by the AOAC the #230 sieve should be a plain weave, not a twill weave.

iii. Before beginning an analysis, the sieve should be examined for rips and tears. Small tears can be mended with a drop of solder and will not affect the usefulness of the sieve. Sieves with tears and holes should not be used.

iv. The sieve should be cleaned with a soft brush and liquid detergent and backwashed by spraying water through the bottom of the sieve to remove any debris in the sieve after use.

v. Annual Cleaning - The sieves should be cleaned as needed) by the following procedures:

(1) Soaked in a 5% aqueous pancreatin suspension, at pH 8.2-8.5 for 4-5 h at 37-40^{o}C.

(2) Soaked in a 10% EDTA (tetrasodium ethylenediaminetetraacetic acid) for 2-3 h at 40-50°C.

(3) Soaked in a 10% NaOH (w/v) for 2-3 h at 80-90°C.

b. Magnetic Stirrers

i. Magnetic stirrers should be stored in a clean plastic container with lid. This should protect the stirrer from picking up metal fragments while not in use. The interior of this container should be kept clean.

ii. Magnetic stirring bars can be cleaned by removing the large particles with forceps and small filings by soaking in an "aqua regia"† solution (a 1:3 mixture of nitric acid and hydrochloric acids).

c. Filter Paper

Filter paper should be stored in a container that will protect it from extraneous materials contamination. A petri dish or a small plastic sandwich container with a tight fitting lid would be ideal. Of course, this precaution is worthless if the analyst does not replace the lid and leaves the filter paper container on the lab bench uncovered for extended periods of time. As with the container for the magnetic stirrers, the container for the filter paper must be kept clean.

d. Laboratory

The entomologists, technicians and aides will routinely:

i. Wipe the lab bench and the work area with a damp sponge before beginning an analysis.

ii. Clean the lab and the microscope room thoroughly at least once a month. This should include wiping down all benches, table tops and tops of any refrigerators or ovens, and cleaning

or vacuuming all window sills. DO NOT clean up the laboratory while analyzing samples.

e. Glassware

i. Avoid use of plastic beakers, funnels, graduated cylinders, etc. because insect fragments and hairs adhere to plastics.

ii. After cleaning glassware allow it to dry in an inverted position. Store glassware inverted or cover the opening with aluminum foil. When it is not possible to store the glassware inverted or to cover it, the analyst should rinse the glassware with water prior to use.

f. Trap Rods

i. Clean the trap rod with soap and water after use.

g. Balances

i. All balances should be inspected and serviced by a trained service technician once a year.

ii. Every day the balance is used, the lab analyst should clean the exterior of the balance, level (if possible) and check the accuracy of the balance with a 100 g calibration weight (or other weight as deemed appropriate for that balance).

h. Microscopes

i. All microscopes should be inspected and serviced by a trained service technician on a yearly basis.

ii. Each analyst should be assigned a microscope and will be responsible for the daily maintenance of that instrument. The analyst will clean the exterior surface of the microscope, the eyepieces and the illuminators.

i. Reagents

i. Before mixing reagents, be sure to clean the top of the reagent bottles to prevent contaminants from falling into the solution.

ii. Rinse out carboys before preparing solutions.

iii. Label reagents with the "date prepared" and the "expiration date" (if the latter is applicable).

iv. Request the "Certification of Analysis" for chemicals, such as paraffin oils.

j. Sample Handling Procedures

When opening the sample container maintain control of the closure mechanism. Remove rubber bands from bags. Do not cut or otherwise break rubber bands. Remove the staples from bags and paperwork. Do not pull open bags sealed with staples or rubber bands.

32.2.2. Laboratory Quality Control

a. Air Quality

A petri dish with filter paper wetted with glycerin should be left exposed for 24h in the laboratory to detect any air borne contaminants. Place these petri dishes on the lab bench, in the fume hood, and near a window. Examine microscopically at 30X. Perform once a week. Record the results of this examination in a bound "Quality Control Notebook".

b. Water Quality

Sample the tap water (hot and cold) by running the water through a #230 sieve for 15 minutes. Wash the trappings from the sieve on to filter paper and examine microscopically at 30X. Perform this analysis once a week. Record the results in a bound "Quality Control Notebook".

c. Hairs and Fibers

The analyst should prepare microscope slide mounts of their head, arm, and eyebrow hair and be able to recognize their own hair from other hair. Include a slide mount with facial hair, if applicable. Analyst should prepare microscope slide mounts of fibers from personal clothes which have a loose knit and could fall into a product. All of these slides should be maintained in the lab as a record.

d. Alternative/Analytical Point Quality Control

A blank sample consisting of the same amounts of compounds, solvents and solutions used in an analysis is run alongside actual samples whenever a digestion, light flotation, or other chemical/isolation method is performed. Isolates from the blank sample are enumerated, identified and documented in a Quality Control notebook. The date of the analysis and actual samples the blank accompanied are listed with the blank findings for traceability purposes.

PART A: DETECTION OF LIGHT FILTH IN PREPARED INFANT FOODS CONTAINING MEAT AND POULTRY

32.3. Introduction

The presence of any objectionable animal material in a food product is defined as filth. Oleophilic filth is defined as light filth. Examples of light filth include insects, insect fragments, hairs, and feather barbules. These adulterants can be detected in a food product by separating them from the food in the oil phase of an oil/aqueous mixture.

The methods described here isolate insect fragments and rodent hairs from prepared baby food containing meat or poultry. Bovine hairs and feathers can also be recovered from the pure meat and poultry. The product is digested in a hydrochloric acid solution and the solubilized material is washed through a #230 sieve. In a pure meat/poultry product, the meat tissue is totally digested and can be washed through the sieve. The material remaining on the sieve can be transferred directly to filter paper. In the baby food dinners, meat products combined with cereals or vegetables, the plant material is not completely digested and thus does not pass through the sieve. In this case, a light filth flotation using paraffin oil is necessary to separate the filth material from the plant material. This flotation step will provide cleaner filter paper, thus easier and more accurate enumeration of the light filth.

32.4. Equipment and Reagents

32.4.1. Equipment

a. Laboratory Balance,
b. Beaker, 2 L
c. Beaker, 600 ml
d. Wildman trap flask, 1 L
e. Hot plate, magnetic stirring
f. Sieve, stainless steel, U.S. Standard No. 230
g. Magnetic stirrer bar, Teflon coated (1 X 5 cm)
h. Buchner funnel
i. Vacuum Pump
j. Watch Glass for a 2 L beaker
k. Petri Dish (2), 100 X 10 mm
l. Filter Paper, S&S #8 Ruled
m. Stereoscopic Microscope, 10 - 30X
n. Trap Rod

o. Aerator, local hardware store
p. 1 L graduated cylinder
q. 50 ml graduated cylinder
r. 25 ml graduated cylinder

32.4.2. Reagents

a. Igepal CO-730 (nonionic detergent, active at low pH) available through GAF Corporation, 140 W. 51st St. NY, NY 10020
b. Concentrated Hydrochloric acid (HCl) †
c. Tergitol #4, Sigma Chemical Co.
d. 40% isopropanol in filtered, distilled water.
e. Paraffin oil (Saybolt viscosity 125/135) Sargent - Welch
f. Glycerin/Ethanol mixture (vol:vol 1:1)
g. Sodium Bicarbonate

32.5. Procedures

32.5.1. Procedure for Meat and Poultry

a. Preparation - Wash the exterior of the jar, particularly around the lid to remove any contaminants which may be drawn into the jar upon opening.

b. Quantitatively transfer contents of one jar strained or one jar junior infant food to a two liter beaker with distilled water. Be sure to rinse the inside of the lid into the beaker.

c. Bring volume to around 800 ml with distilled water.

d. Add 5 ml Igepal CO-730 and 45 ml concentrated HCl with stirring. Cover with watch glass.

e. Bring to a boil and boil for 30 minutes.

f. Transfer the hot mixture to a 230 mesh sieve and wash with a forcible stream of hot aerated tap water until washings are clear and acid is removed. Wash the remaining material to one side of sieve. Retain the washings in a pan to neutralize at a later time with sodium

bicarbonate.

g. Add 2 ml of "Tergitol #4" to contents of sieve and wash with forcible stream of hot aerated water, until foaming subsides. Wash all of the remaining residue to one side of sieve.

h. Wash the contents of the sieve onto lined filter paper in a buchner funnel with isopropyl alcohol. Wash down the sides of the filter paper. Aspirate the paper to near dryness.

i. Add a small amount of glycerine/ethanol (32.33 f) to a petri dish. Using forceps, remove the filter paper from the buchner funnel and place in the petri dish.

j. Examine microscopically at 30X. (See Section 32.36)

32.5.2. Procedure for Baby Food Dinners

a. As an initial preparation, wash the exterior of the jar, particularly around the lid, to remove any contaminants which may be drawn into the jar upon opening.

b. Quantitatively transfer contents of one jar strained or one jar junior infant food to a two liter beaker with distilled water. Be sure to rinse the inside of the lid into the beaker.

c. Bring volume to around 800 ml with distilled water.

d. Add 5 ml of Igepal CO-730 and 45 ml of concentrated HCl with stirring. Cover with watch glass.

e. Bring to a boil and boil for 30 minutes.

f. Transfer the hot mixture to a 230 mesh sieve and wash with a forcible stream of hot aerated water until washings are clear and acid is removed. Wash the remaining material to one side of sieve. Retain the washings in a pan to neutralize at a later time.

g. Add 2 ml of Tergitol #4 to contents of sieve and wash with a forcible stream of hot water, until foaming subsides. Wash the remaining material to one side of sieve.

h. Transfer contents quantitatively to a 1 L Trap Flask with 40% isopropanol.

i. Bring volume of the liquid in the flask to 500 ml with 40% isopropanol.

j. Bring to a boil and continue simmering boil for 5 minutes with magnetic stirring.

k. Remove from heat and let stand for 1 minute. Insert trap rod into flask. With disc held just below liquid surface slowly add paraffin oil (29.33 e) by pouring it slowly down the trap rod.

l. Stir magnetically for 3 minutes at a speed sufficient to draw a vortex to the stirring bar without splashing and without introducing air into the liquid.

m. Allow the mixture to stand for 1 minute.

n. Fill the flask to the neck with 40% isopropanol by pouring slowly down the trap rod with the disc just below the oil layer.

o. Allow the mixture to stand for 20 minutes. Resuspend the material at the bottom of the flask by turning the flask in a clock-wise or counter clock-wise direction on the bench at 5 and 10 minutes to release any trapped oil, taking care not to disturb the oil layer.

p. Trap off the oil layer into a 600 ml beaker. With 40% isopropanol, rinse the neck of flask and stem of trap rod and pour rinsings into same beaker. Repeat rinsing procedure as necessary.

q. Pour the contents of the above beaker on to lined filter paper in a buchner funnel. Rinse the beaker with 100% isopropanol until all the oil is gone.

r. Wet a petri dish with a small amount of glycerine/ethanol and place the filter paper on this dish.

s. Examine microscopically at 30X. (See Section 32.36)

t. Add 25 ml of paraffin oil to the flask.

u. Slowly push the oil into the aqueous phase with the trap rod. Continue to slowly plunge up and down, about 1/2 the height of the flask, for 1 minute. Be careful not to introduce any air into the liquid.

v. Fill the flask up to the neck with 40% isopropanol and let stand for 15 minutes. Turn the flask at 5 and 10 minute intervals to release any trapped oil drops.

w. Trap off the oil layer into a 600 ml beaker. With 100% isopropanol, rinse the neck of flask and stem of trap rod and pour rinsings into the same beaker. Repeat rinsing procedure as necessary.

x. Continue as in Steps q & r. Examine at 30X. (See Section 32.36)

32.6. Results

The lined filter paper should be examined line by line at 30X magnification. Identify and count any hairs and insect fragments observed. Report the following:

a. whole or equivalent insects (adults, pupae, maggots, larvae, cast skins)

b. insect fragments, identified

c. insect fragments, unidentified

d. aphids, scale insects, mites, spiders, psocids, thrips, etc. and fragments of the above

e. rodent hairs (state the length of the hairs)

32.7. Quality Control

See Section 32.2.2.

32.8. Safety Caution

a. Do not dispose of hazardous waste by pouring down sink drains.

b. Collect in separate containers and dispose of this waste according to standard waste management procedures for your laboratory.

c. Use caution when working with hydrochloric, other acids and strong bases.

d. Wear goggles and gloves to protect eyes and skin when preparing the solution and when moving and wet sieving the sample.

e. Digest and wet sieve samples under a safety hood.

Part B: DETECTION AND IDENTIFICATION OF EXTRANEOUS MATERIALS IN NON-MEAT FOOD INGREDIENTS - MACROSCOPIC EXAMINATION

32.9. Introduction

A food is considered adulterated if "it consists in whole or in part" of any filth or decomposed substance or if the food is "otherwise unfit for human food." Extraneous materials detected in the ingredients indicate the product was prepared under unsanitary condition where it may have become contaminated. The presence of extraneous materials in the product ingredients would render the final product adulterated. The purpose of this procedure is to presumptively determine the presence of rodent excreta, insects, insect webbing, mold and other extraneous materials in the dry non-meat food ingredient. This method is intended as a screening procedure. A vast majority of samples analyzed by this procedure will be free of extraneous materials.

This method will allow for prompt examination of samples by all FSIS laboratories and insure that compliant samples are reported promptly to the operating inspectors. This procedure will also reserve the analytical time the analyst has for the smaller number of non-compliant samples that will require more time consuming analyses. This method is recommended only for screening; all positives or apparently violation samples are to be confirmed by AOAC or other accepted microscopic methods.

32.10. Terms and Concepts

The following terms are used in the macroscopic examination and reporting results:

a. Thrus: Any material going through the sieve.

b. Overs: Any material remaining on the sieve after sieving.

c. Animal Contaminated: Any material showing animal excreta or evidence of rodent or other animal chewing or gnawing.

d. Insect Infested: Any non-meat ingredient that contains live or dead insects, webbing, excreta, or definite evidence of insect feeding.

e. Miscellaneous Extraneous Material: Includes stones, dirt, wire, string, non-toxic foreign seeds, etc.

f. Moldy: A product bearing any evidence of mold.

g. Rodent Excreta: Excretory pellets of the black rat (Rattus rattus), Norway rat (Rattus norvegicus) or the house mouse (Mus musculus), or pieces/fragments thereof, as determined by the presence of murine rodent hairs in the matrix of the fecal material.

h. Other Animal Excreta: Any excretory product, other than rodent, as identified by microscopic examination.

i. Whole Insects: Includes an adult insect, a pupa, a larva, or a major portion thereof.

32.11. Equipment and Reagents

32.11.1. Equipment:

a. Jones Riffle Sampler (8261-C10 Arthur H. Thomas)

b. Balance, Top-loading, 1 kg capacity

c. Balance, analytical, 500 g capacity

d. Sieves, U.S. Standard Series (4-881 Fisher Scientific Co.) 3 1/2 through 20

e. Magnifier-Lamp (L6039-2 Scientific Products) (LUXO-LFM2FE)

f. Trays, Cutting, (62686-363, VWR Scientific)

32.11.2. Reagents:

i. Isopropyl Alcohol-Propylene Glycol Solution

1. Isopropyl alcohol, 100 ml

2. Propylene glycol, 300 ml

3. Picric acid, 160 mg (optional; follow safety precautions)

Preparation: Mix isopropyl alcohol and propylene glycol. If yellow staining is desired in preparation of specimens, add picric acid and stir until dissolved. Heat hair specimens in the solution to deaerate for microscopic examination. The solution can be stored at room temperature.

ii. Hydrogen Peroxide-Sodium Lauryl Sulfate Solution

1. 30% Hydrogen peroxide, 1-5 ml

2. 5% sodium lauryl sulfate solution, 1-2 drops

Preparation: Mix two reagents in a small crucible, or in a glass or porcelain serological plate well just before use. Heat dark hair specimens in the solution to remove enough pigment to allow microscopic examination. Reagents should be stored in separate containers in the refrigerator.

iii. Glycerin Jelly

1. Gelatin, 10 g

2. Glycerin, 70 ml

3. Distilled water, 60 ml

4. Phenol, 1 g

Preparation: Soak gelatin for 1-2 hours in water; add glycerin and phenol. Warm for 10-15 minutes (not above 75^{o}C) and stir until homogeneous. Pour into petri dishes and store in refrigerator. Or microwave gelatin and water in bottle until it starts to boil. Cap bottle and shake until homogenous. Add phenol and glycerin and shake bottle to mix. Remove small portions as needed. Melt small lump on a microscope slide for mounting hair, feather, and insect/arthropod specimens. Coverslip, let set (horizontally) for several hours, and ring with nail polish (or similar substance) for semi-permanent slides.

iv. Hoyer's Chloral Hydrate (Berlese's Fluid)

1. Water, 50 ml
2. Gum arabic (ground-up crystals or powder), 30 g
3. Chloral hydrate, 200 g
4. Glycerine, 20 ml

Preparation: Stir until homogenous. Filter through glass wool; store at room temperature. Dehydrate insect/arthropod specimens in increasing concentrations of alcohol (70, 95, and 100%) and then in xylol before mounting in medium on microscope slides. Coverslip and let harden (horizontally) for several hours for permanent slides.

v. Nesbitt's Solution

1. Chloral hydrate, 40 g
2. Concentrated HCl acid, 2.5 ml
3. Distilled water, 25-50 ml (more for lightly sclerotized specimens)

Preparation: Stir. Use cold to clear insect/arthropod specimens (takes several hours to a day or more).

vi. Potassium Hydroxide Solution (10-15% aqueous KOH solution)

Preparation: Stir until homogenous. Use cold, warm, or boiling to clear insect/arthropod specimens (cold solution takes several hours to a day or more; boiling takes a few minutes but may distort the specimen). Rinse specimen in water (preferably with small amount of acetic acid added) after clearing to remove excess KOH.

32.12. Procedure

32.12.1. Examination of Ground Spices

a. Mix sample received by passing through a riffle sampler 4 times, recombining separations before each pass.

b. Separate approximately 200 g of sample and weigh. NOTE: Retain excess sample for use in confirmatory analysis, if needed.

c. Sift sample portion-wise through a #20 sieve, retain "thrus".

d. Transfer "overs" to a cutting tray and spread evenly so that all material can be observed.

e. If sufficient material is present to preclude spreading, place material on one side of tray and move portion-wise to middle.

f. Examine the middle portion at 3 to 5 magnifications with strong, even light. (A bench-top magnifier-lamp is suitable for this purpose).

g. Note and identify (if possible) all categories of extraneous material observed.

h. If confirmatory analysis is needed, place "thrus" and "overs" into a plastic bag along with the excess sample.

i. Send all portions for confirmatory analysis to an advanced reference laboratory, if available and necessary.

j. A written report of the extraneous material observed should accompany samples submitted for confirmation.

32.12.2. Examination of Whole Spices, Seeds, and Large Flake Leafy Spices

a. Mix sample received by passing through a riffle sampler 4 times, recombining separations before each pass.

b. Separate approximately (200 g for whole spices and seeds; 50 g for leafy spices and herbs).

c. Sift sample portion wise through a sieve of such size that more of the whole spices are retained as "overs". NOTE: The sieve should never be of a smaller opening size than "Tyler Standard #8" or USA Standard 2.36 mm".

d. Transfer both "thrus" and "overs" separately on to a cutting tray and spread evenly so that all material can be observed.

e. Examine both portions at 3 to 5X magnification under strong, even light. (A bench-top magnifier lamp is suitable for this purpose). NOTE: If sufficient material is present to preclude obtaining a single layer, place all material on one side of the tray and move portion-wise to middle and examine.

f. Note and identify (if possible) insects, insect damage, other filth and extraneous material observed.

g. If confirmatory analysis is needed place "thrus" and "overs" into a plastic bag along with the excess sample.

h. Send all sample portions for confirmatory analysis to an advanced reference laboratory, if available and necessary.

i. A written report of the extraneous material observed should accompany samples submitted for confirmation.

32.12.3. Preparation of Hair and Feather Specimens

a. Remove with fine forceps hairs and feathers observed in the sample isolates and place in a small porcelain crucible or in a well of a glass or porcelain serological plate for deaeration. Add sufficient amount of isopropyl alcohol-propylene glycol (1:3) solution (picric acid is optional; use 40 mg per 100 ml solution for staining, if desired) to cover the specimen(s), and heat to sub-boiling for ten minutes to two hours. Mount the specimen in glycerine jelly on a microscope slide, coverslip, and seal with clear nail polish.

b. Alternately, place the specimen in one or more drops of glycerine on a microscope slide, coverslip, and heat the slide on a slide warmer set at 55-65^{o}C for one to 18 hours or until the specimen is deaerated. For storage, remount the specimen in glycerine jelly as described in step (a).

c. For hair specimens too dark for microscopic examination, substitute for or follow the deaeration process in step (a) with bleaching of the hair. Bleaching is accomplished by heating the hair to sub-boiling in 1-5 ml of 30% hydrogen peroxide with 1-2 drops of 5% sodium lauryl sulfate solution added (in crucible or serological plate). Add more hydrogen peroxide as needed during heating – do not let the hair dry and burn. The specimen must be observed during the heating process and removed when a light red to colorless state is reached (one to 30 minutes). Heating for too long will result in a dissolved specimen.

32.12.4. Preparation of Small Insect/Arthropod and Fragment Specimens

a. Remove with fine forceps specimens observed in the sample isolates and place on a microscope slide for mounting. Temporary mounts for microscopic examination can be made by adding glycerine and coverslip.

b. Semi-permanent aqueous mounts can be made by transferring the specimen directly to glycerine jelly on a slide, coverslipping, warming the slide, then allowing the medium to firm up (at room temperature), and ringing the coverslip with nail polish or other suitable sealant.

c. Permanent resin mounts can be made with Hoyer's chloral hydrate (Berlese's fluid). Specimens must first be dehydrated with solvents before mounting and coverslipping. The slide mounts need to set/dry.

d. Clearing of dark or thick-bodied specimens can be done in potassium hydroxide (KOH) or Nesbitt's solution before mounting on a slide.

32.13. Reporting Results

a. Identify all categories of extraneous materials observed and record the quantity in each category.

b. To report the results as "percent extraneous material by weight", transfer the extraneous material to a tared dish and weigh.
Use the following formula to calculate the percentage:

$$\text{Percent (\%)} = \frac{\text{extraneous material (gm) in category}}{\text{Sample Weight}} \times 100$$

32.14. Criteria for Confirmatory Analysis

These criteria are presented as internal guidelines to assist the analyst trained for macroscopic analysis in determining whether or not a sample should be subjected to a more extensive examination. Each type of contamination observed should be considered both on its own sanitation significance and in conjunction with other observations reported by the field or seen by the analyst.

Any sample exhibiting the following characteristics must be confirmed by analysts trained in more sensitive microscopic or chemical analytical techniques:

a. Any sample showing evidence of active or current infestation with insects and/or other animal contamination.

b. Any sample of whole seeds, herbs or other spice material that exhibits evidence of mold and/or insect damage.

c. Any sample that appears to contain in excess of 0.5% by weight of any non-hazardous extraneous material (stones, soil and non-toxic seeds).

d. Any sample appearing to contain animal excreta, including insect excreta identified during macroscopic examination.

32.15. Quality Control and Quality Assurance

See Section 32.2.2.

Part C: DETECTION OF GLASS AND NON-ALUMINUM METALS IN MEAT AND POULTRY PRODUCTS

32.16. Introduction

Meat and poultry products are exposed to a wide variety of materials during processing and packaging. Due to faulty processing, breakage in machinery or improper handling, pieces of the processing equipment or packaging material can be introduced into the finished product. The presence of extraneous materials in a finished product may pose a serious health risk to all consumers.

This method provides a fast, simple, and reliable means for isolating glass or metal contaminants from meat and poultry products. The sample is digested in an alkaline solution. The glass and non-aluminum metals are unaffected by the digestion. These contaminants are separated from other undigested material in a brine solution.

The laboratory equipment used in the analysis will depend on the type of contamination. When the suspected contaminant is glass, use of laboratory glassware in analysis must be avoided. Similarly, when the contaminant is suspected to be a metal, use of metal utensils and containers should be avoided. This will serve to protect the integrity of the sample during analysis.

32.17. Reagents and Equipment

32.17.1. Reagents

a. 7% Alcoholic Potassium Hydroxide (KOH) †
Dissolve 7 g of KOH in 100 ml of 95% Ethyl Alcohol
NOTE: KOH pellets can be used.

b. Sodium Chloride (NaCl) Solution
Prepare 2 L of NaCl solution at room temperature by adding 300 g of NaCl/L of distilled water.

c. Tergitol #4

d. Glycerol/Ethanol Mixture (vol:vol 1:1)

32.17.2. Supplies

32.17.2.1. For Metallic Contaminants

a. Heavyweight Plastic Picnic knives and forks or 40 lb. test Monofilament

b. Magnetic Stirring Hot Plate and Magnetic Bar (AOAC XIV 44.002n)

c. #230 Sieve (AOAC XIV 44.002 r)

d. Filter Paper (AOAC XIV 44.002 i)

e. Hirsch Funnel with Screen (AOAC XIV 44.002 k)

f. 2 L Beaker, glass

g. 600 ml "tall" beaker, i.e. Pyrex #1060

h. 2 L Graduated cylinder

i. Watch Glass for a 2 L beaker

32.17.2.2. For Glass Contaminants

The equipment is the same as above except do not use glassware in analysis and substitute with the following for glass beakers:

a. Stainless Steel Beaker with 2 L capacity

b. Reusable Plastic Beaker with 600 ml capacity (Nalgene Polypropylene #1201)

c. Polypropylene Graduated Cylinder

d. Plastic Basin to cover 2 L beaker, Nalgene #69010040 or equivalent

32.18. X-ray Screening

a. Follow safety procedures pertinent to the use of the cabinet X-ray system. In this case, a 'Radioactive Material Use Permit' must be provided by the Radiation Safety Staff under the United States Department of Agriculture. The steps outlined here are generally those followed for use of a cabinet X-ray system which emits ionizing radiation at a controllable range of 10-110 kVp.

b. Turn the X-ray unit on with a key and allow warming up for a minimum of 15 minutes. Set to an emission of 50 kVp for five minutes (turn on by pressing the "START" button). Once the emission has stopped, turn the kVp setting back to zero before opening the door.

c. Place the product sample (in its retail container, plastic bag, or spread out on an open plastic tray) on a film negative or on a film holder loaded with a film negative. Center the film on the marked surface of the shelf inside the X-ray unit.

d. Close the door to the X-ray unit. Set the desired emission and exposure time, and press the "START" button.

e. After exposure, remove the sample. Develop the film following directions for the type of film used. Examine the radiograph for dark, sharply outlined areas. Isolate these portions of the product sample for further analysis by other methods. The X-ray unit is most applicable in screening for metal, glass, and bone particles.

f. Turn off the cabinet X-ray unit with the key after use.

32.19. Procedure

a. Cut sample to be digested into 1" x 1" pieces to facilitate the digestion process (Use plastic utensils or monofilament if examining for suspected metal contamination).

b. Weigh 225 g sample into a 2 L beaker.

c. Add 1.5 L 7% Alcoholic KOH. (see Section 32.56, Safety Caution)

d. Cover with a watch glass. Heat to a boil while stirring on a stirring hot plate until the sample is completely digested (approximately 1 h). Initially, it will be difficult to stir the sample magnetically but after 10 min at medium to low heat the sample will be

sufficiently digested to permit magnetic stirring.

e. Transfer sample to a No. 230 sieve. Apply a moderately forceful stream of hot water to push digested residues through sieve. Retain washings in a pan for hazardous chemical disposal.

f. If there is only a little residue present, transfer this directly to filter paper and examine microscopically.

g. If large amounts of undigested material remain, add 2 ml of Tergitol to help solubilize remaining residues. Repeat washing until suds subside. Transfer sample to a "tall" 600 ml beaker with distilled water.

h. Add 400 ml NaCl solution.

i. Wait 30 seconds, and then pour off suspended material. Be careful not to disturb or pour off material on bottom.

j. Repeat steps h and i.

k. Wash the material remaining on bottom of beaker onto ruled filter paper with distilled water and examine microscopically.

NOTE: Check the magnetic stirring bar for metal contamination.

32.19.1. Procedure for Index Sample

a. If an index sample of the contaminant is available, put a portion of the index sample in the 7% Alcoholic KOH Solution. (see Section 32.56 Safety Caution)

b. Bring the solution to a boil and examine the index sample noting any chemical reaction it may have undergone.

c. Repeat the boiling and examine again. If the sample reacts with the solution, do not use an alkaline digestion. Use an acid or enzymatic digestion instead.

32.20. Result

The lined filter paper should be examined line by line at 30X magnification. Report the following:

a. Metal

1. Count the number of pieces of metal recovered.

2. Record the size or the number of contaminants within a size range.

3. Provide a general description of the contaminants recovered. Note the shape, thickness, color or discoloration, magnetism, and surface markings.

b. Glass

1. Count the number of fragments recovered.

2. Record the size or the number of contaminants within a size range.

3. Provide a general description of the contaminants. Note the presence or absence of the following characteristics: very thin, cube shaped, mold markings, rounded edges, smooth curved surfaces, color, conchoidal fractures.

4. Examine suspected fragments (immersed in mineral oil or other immersion oil) under polarized light to determine if they are isotropic (glass is isotropic or non-birefringent; i.e., fragments will disappear when polarizing filter is positioned under the microscope stage to create a dark field).

5. If refractive index is to be measured, take the following steps:

i. Compare the refractive index of any suspect glass fragments with refractive index oils by immersing in oil on a microscope slide, coverslipping, and examining microscopically (immersion method). A bright white “halo” (the Becke line) appearing along the edges of the fragment moves in the direction of higher refractive index as the focal distance is increased (and toward lower refractive index when focal distance is decreased).

ii. Rinse the fragment with benzene (or other suitable solvent), let dry, immerse in oil with a lower or higher refractive index than the oil just used, and reexamine.

iii. Repeat the procedure until the edges of the fragment are indistinguishable (i.e. no Becke line is visible) from the oil it is immersed in; yellow and blue may be observed as a refractive index match is approached. These observations indicate that the refractive index of the fragment is similar to that of the oil used. [More complex measurements can be made at different temperatures or different wavelengths of light using more elaborate instrumentation, but the method just described is simple and sufficient for general purposes.]

32.21. Quality Control

See Section 32.2.2.

a. In step 32.19.e, be sure to wash the heavy contaminants from the bottom of the beaker to the sieve. Heavy contaminants settle quickly to the bottom of the beaker and an ample stream of water is needed to wash them from the beaker. The beaker should be inverted over the sieve and the material in the beaker should be washed into the sieve with a gentle stream of water.

b. Check the magnetic stirring bar for small magnetic filings before beginning analysis.

32.22. Safety Caution

† Do not dispose of hazardous waste by pouring down sink drains. Collect in separate containers and dispose of as hazardous waste as per standard waste management procedures for your laboratory.

Use caution when working with potassium hydroxide. Wear goggles and gloves to protect eyes and skin when preparing the solution and when moving and wet sieving the sample. Digest and wet sieve samples under a safety hood.

Part D: METHOD FOR THE ISOLATION OF GLASS FROM PREPARED MEAT AND POULTRY BABY FOODS

32.23. Introduction

The recommended procedure for isolating glass from a food product is the heavy sediment procedure for that product. There is no heavy sediment procedure for meat/poultry baby food products. The method outlined below was developed in response to the need for a standard procedure for isolating glass from meat/poultry baby food products.

Bottled food can become contaminated with glass in a number of ways. The container may already be contaminated when it arrives at the food processors. The finished food product may become contaminated by glass breakage during processing. Containers can break during storage, shipping, retail, and consumer handling and fragments from broken containers can contaminate the exterior of other containers. If these exterior contaminants are in or around the jar opening, they could contaminate the product when the jar is opened.

This method is quick and easy. The sample is washed in a #60 sieve. The bulk of the sample is washed through the sieve. The remaining material is transferred to a beaker and mixed with a brine solution. In a brine solution the heavy contaminants, such as glass, settle to the bottom of the beaker. The brine solution and the suspended food material are poured off and discarded. These two steps, adding and pouring off the brine solution, are repeated three times. The heavy contaminants remaining on the bottom of the beaker are washed on to a filter paper which is examined microscopically. This isolation procedure takes less than 15 minutes. Suspect particles must be tested to confirm that they are in fact glass.

To protect the integrity of the sample, no glass should be used in any part of this method. After adding the brine solution to the plastic beaker containing the sample, any glass fragments will settle to the bottom within seconds. The longer the settling step, the more food material settles to the bottom which creates dirty plates. Ten seconds is plenty of time for glass fragments to settle to the bottom.

32.24 Regents and Equipment

32.24.1. Reagents

a. Sodium Chloride (300 gm/L)

Add 2 L of distilled water to 2 L plastic beaker. Add 600 g of NaCl while magnetically stirring. The above recommended plastic beaker will accommodate 2 L of salt solution. Cover beaker with plastic basin and continue stirring until NaCl is completely dissolved.

32.24.2. Equipment

NOTE: DO NOT USE ANY GLASS APPARATUS DURING THE ANALYSIS.

a. Plastic Beaker w/ 600 ml capacity, ie. Nalgene #1201

b. Plastic Beaker w/ 2 L capacity, ie. Nalgene #1201

c. Plastic Basin to cover 2 L beaker, ie. Nalgene #69010040

d. Plastic Graduated Cylinder

e. #60 Sieve (AOAC 16.1.01(B)(r) 16th Ed.)

f. #230 Sieve (AOAC 16.1.01(B)(r) 16th Ed.)

g. Magnetic Stirrer and Bar (AOAC 16.1.01(B)(n) 16th Ed.)

h. Ruled Filter Paper (AOAC 16.1.01(B) (i) 16th Ed.)

i. Disposable Petri Dish (100 X 10 mm)

j. Lab Spatula

k. Hirsch Funnel

l. Side arm trap flask connected to vacuum pump

m. Laboratory Balance, 1 kg capacity

n. Compound Microscope with polarizer

o. Aerator, Water (AOAC 16.1.01(B)(a) 16th Ed.)

32.25. Procedure

32.25.1. Cleaning of Exterior of Sample Container

a. Thoroughly rinse exterior of jar and around lid on to a #230 sieve using hot water. Use the spatula to clean food residues from lid and jar threads. This step is included to be sure no glass is on exterior of the jar.

b. Wet filter paper with water and center it in Hirsch funnel. Turn on vacuum and fit filter paper to sides of funnel.

c. Transfer material on the sieve to filter paper with distilled water. Do not aspirate the paper to dryness otherwise the glass fragments will "pop" off the paper. If needed, wet the paper with a drop or two of water. The paper should be moist enough so that it adheres to the petri dish but it should not be soaked. If the paper is too wet, the water will hide small, flat pieces of glass.

d. Transfer filter paper to petri dish and examine paper microscopically for glass fragments.

e. Confirm any suspect particles using a compound microscope with polarized light.

f. Count, measure and describe all glass fragments found on the exterior of container. Report any particles of glass as contaminants found on the exterior of the sample container. Report number of fragments found within a size range. Fragments less than 1 mm can be reported as "Less than 1 mm." An excessive number of fragments can be reported as "Too Numerous To Count."

32.25.2. Sample Analysis

a. Quantitatively transfer contents of jar to #60 sieve. A spatula can be used to remove the bulk of the sample. Use water from a squirt bottle to thoroughly rinse interior of jar. Retain jar for further examination at step one.

b. Thoroughly wash sample in sieve with hot aerated water. When no more material passes thru sieve, wash remaining material to one side of sieve.

c. Quantitatively transfer contents of sieve to a plastic beaker w/ distilled water. Use no more than 200 ml of water.

d. Dilute to 400 ml with NaCl solution.

e. Let material settle 10 seconds, then pour off suspended material. More than 10 seconds is not needed. Glass will settle to bottom in 10 seconds. Waiting longer than 10 seconds allows more food material to settle to the bottom. Be careful not to disturb or pour off residues on bottom.

f. Repeat Steps d & e to remove excess plant material, usually twice more.

g. Wet filter paper with water and center it in Hirsch funnel. Turn on vacuum and fit filter paper to sides of funnel.

h. Wash residues remaining on bottom of beaker to ruled filter paper. Do not aspirate the paper to dryness otherwise the glass fragment will "pop" off the paper. If needed, wet the paper with a drop or two of water. The paper should be moist enough so that the paper adheres to the petri dish but the paper should not be soaked. If the paper is too wet, the water will hide small, flat pieces of glass.

i. Transfer paper to petri dish and examine microscopically.

j. Count, measure, and describe all glass fragments found in the food product. Report number of fragments found within a size range. Fragments less than 1 mm can be reported as " Less than 1 mm." An excessive number of fragments can be reported as "Too Numerous To Count."

k. Confirm any suspected particles using a compound microscope and polarized light. Glass is an isotropic compound and will not transmit crossed polar light. Sand or quartz is birefringent, thus will transmit crossed polar light.

l. Examine all retail sample jars for chips, fractures, or other defects if any glass fragments are found within product.

m. Maintain in reserve all glass fragments and all jars from which glass fragments were removed.

32.25.3. Characterization of Contaminants

If classification or comparison of glass contaminants is needed to identify a possible source, determine the refractive index of the glass contaminants.

32.26. Quality Control

See Section 32.2.2.

a. Do not use any glassware in this analysis.

b. Before beginning an analysis, wipe down or wash the entire work area.

c. Rinse the beakers and graduated cylinder before using them.

d. Backwash the sieve by spraying water through the bottom to remove any debris in the sieve before using it.

Part E: Analysis of Fecal Material

32.27. Introduction

Diagnostic characteristics of excreta pellets and the alkaline phosphatase test provide a method to aid in the identification of the excreta pellets of animals and insects. The alkaline phosphatase isoenzyme is in the intestinal tract of most mammals, including mice and rats. In this procedure, the alkaline phosphatase will split the phosphate radical from phenolphthalein diphosphate in the Working Test Media to produce a reddish free phenolphthalein.

32.28. Reagents and Equipment

32.28.1. Reagents

a. Magnesium Chloride Solution--Dissolve 0.203 g $MgCl_2 \cdot 6H_2O$ in H_20 and dilute to 500 ml.

b. Stock Test Reagent--Dissolve 4.75 g borax ($Na_2B_4O_7 \cdot 10H_2O$) and 1.57 g anhydrous Na_2CO_3 in 250 ml H_2O with stirring. Add 0.235 g phenolphthalein diphosphate and stir while adding 0.5 ml $MgCl_2$ solution. Preparation is stable.

c. Work Test Media (WTM)--Measure equal volumes stock test reagent and H_2O. Place cold H_2O into a media bottle or beaker on a stirring hot plate, add stirring bar, and, with rapid stirring, add sufficient agar to yield a 2% agar dispersion. Heat solution to boil on stirring hot plate or in microwave. Pour equal amount of stock test reagent into container of boiled agar solution. Stir rapidly or cap bottle and shake for one minute. Add ca 1 ml portions of WTM to wells before cooling to 40-41^oC. WTM must be cooled to 40-41^oC before contacting samples.

Short Term Storage: WTM may be held ca 48 h at 40-41^oC if covered snugly with foil or plastic.

Long Term Storage: WTM may be stored up to 12 months if refrigerated and protected from direct sunlight. Discard if pink color and/or volume loss is observed.

32.28.2. Equipment

a. Pasteur pipets--1 ml capacity

b. Water bath--set at 40-41^oC

c. Tissue culture multi-well plate with cover-capacity

d. Laboratory Balance, 1 Kg capacity

e. Beakers, 100 ml and 800 ml or 1 L capacity

32.29. Procedure

a. Measure suspect pellet.

b. Moisten suspect pellet with H_2O and examine for hair and other diagnostic characteristics.

c. Place small part of particle into WTM well.

d. Cover particle with ca 1 ml portion of cooled (40-41^oC) WTM.

e. Place plate in 40-41^oC H_2O bath.

f. Check for development of red color near particles.

32.29.1. Test Response

Time varies according to species from 2-3 min for most mouse samples, up to 4 hours for samples from some grass eaters, such as deer or rabbit.

32.30. Quality Control

32.30.1. Blank Preparation

a. Autoclave pellets 15+ min. at 15 psi.

b. Store in closed container in freezer.

c. Alternatively, place 100 ml beaker in 800 ml or 1 L beaker and add ca 25 mm depth H_2O to each.

d. Place small test tube with 1 ml WTM in smaller beaker.

e. Heat H_2O to boiling ca 2 min.

f. Remove small tube and quickly transfer crushed pellet material to tube.

g. Return tube to rapidly boiling H_2O ca 2 min.

h. Remove tube and with small glass rod work all particles from side wall of tube down into liquid.

i. Replace tube in small beaker, cover large beaker with watch glass and continue to boil ca 5 min.

j. Remove small tube, mix contents quickly and transfer to negative test well.

32.30.2. Positive Control

Store known rat and mouse excreta pellets in closed container in freezer.

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