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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 0003.01

Title: Alkaline Phosphatase Test for Mammalian Fecal Material

Effective Date: 02/24/20

Description and purpose of change(s):

Removal of alternate boiling step in 3.3.1 Blank Preparation.

Safety Precautions Section 3.2 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

3.1 Introduction

This procedure is for the detection of mammalian fecal material in samples submitted to the Eastern Laboratory with an analytical request for rodent excreta or food animal fecal material. The alkaline phosphatase isoenzyme found in the intestinal tract of most mammals (including mice and rats) splits the phosphate radical from phenolphthalein diphosphate in the Work Test Media (WTM) to produce a free phenolphthalein displaying a reddish ("hot pink") color reaction.

3.2 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.

3.3 Quality Control Procedures

Rat or mouse excreta shall be obtained from a documented source. Store the rat or mouse excreta and prepared pellets in a closed container in a freezer. The rat/mouse excreta and prepared pellets should be kept no longer than five years.

3.3.1 Blank Preparation

Autoclave rat or mouse excreta for 15+ minutes at 15+ psi on a liquid cycle (to avoid excessive dehydration).

3.3.2 Positive Control

Use known rat or mouse excreta pellets from the same documented source as those used for the blank.

3.3.3 Control Media

Work Test Media (WTM) shall be used for the blank and positive control in the alkaline phosphatase test.

3.4 Equipment, Supplies, and Reagents

3.4.1 Equipment

a. Balance, analytical, 10-150 g capacity
b. Stirrer/Hot plate
c. Water bath, circulating
d. Digital camera

3.4.2 Supplies

a. Petri dishes- sterile, disposable polystyrene, 85-95 mm diameter, or equivalent
b. Qualitative filter papers- No. 1, 70 mm diameter, or equivalent
c. Fine forceps- Dumont No. 3, 120 mm long (2 pair), or equivalent
d. Volumetric flask, glass, 1 liter, with glass stopper
e. Pipet, calibrated to deliver (TD), 1 mL
f. Beakers, glass, 100 mL and 800-1000 mL
g. Graduated cylinders, glass, 25-50 mL and 500-1000 mL

h. Stirring bars
i. Tissue culture plate with lid, non-treated, 24 well, clear polystyrene, sterile, 2.0 cm^2 well area (per well), 3.5 mL well volume (per well)
j. Thermometer
k. Utility tray, stainless steel, (adequate volume to support tissue culture plate in water bath)

3.4.3 Reagents

a. Deionized water
b. Magnesium chloride hexahydrate, crystal ($MgCl_2$•$6H_20$), ACS reagent-grade
c. Sodium borate decahydrate ($Na_2B_4O_7$•$10H_2O$), crystal, ACS reagent-grade
d. Sodium carbonate, anhydrous (Na_2CO_3), powder, ACS reagent-grade
e. Phenolphthalein bisphosphate tetrasodium salt ($C_{20}H_{12}Na_4O_{10}P_2$), ~95%
f. Agar, granulated

3.5 Procedure

3.5.1 Reagent Preparation

a. Magnesium Chloride Solution – Dissolve 0.406 g magnesium chloride hexahydrate ($MgCl_2$•$6H_2O$) in deionized water and dilute to 1 liter in a volumetric flask.

b. Stock Test Reagent – Dissolve 9.5 g sodium borate decahydrate ($Na_2B_4O_7$•$10H_2O$) and 3.14 g sodium carbonate, anhydrous (Na_2CO_3) in 500 ml deionized water with stirring. Add 0.47 g phenolphthalein bisphosphate (may be used in the form of tetrasodium salt, approximately 95%) and stir while adding 1 ml magnesium chloride solution. Preparation is stable for one year when stored in the refrigerator.

c. Work Test Media (WTM) – 1:1 mixture of stock test reagent and 2% agar dispersion. Generally, for one 24-well culture test plate, 50 ml of WTM is adequate. To make the agar dispersion for this amount, heat 0.5 g agar (granulated) in 25 ml deionized water, stirring with a magnetic stirring bar. Once boiling/foaming starts, add 25 ml stock test reagent to the beaker, move to an unheated stirrer and mix for 1+ minute. The WTM must be no hotter than 41°C when adding material to be tested (40-41°C is optimal).

d. Prepare Blank and Positive control as described in section 3.3 (Quality Control Procedures).

3.5.2 Sample Test

a. Using forceps, collect at least one suspect excreta pellet from each sample and place on a filter paper in a separate (labeled) petri dish.

b. Measure the width and length of the excreta pellet as described in PLG 2 (if a distinct pellet is discernible) and record.

c. Label a plastic multiwell tissue culture test plate to denote which wells the different test materials will be placed in.

d. For each test sample, place a small piece of excreta in one of the wells of the culture test plate and immerse in 1-2 ml of working test media (no hotter than 41^{o}C).

e. Once the blank, the positive control, and the test materials are deposited into separate wells with WTM, cover the test plate and place in a tray floating in a filled water bath stabilized at 40-41^{o}C. Record the water bath temperature and start time. If multiple plates are used, a blank and positive control must be incorporated in each plate.

f. At four hours, observe the test reactions, photograph, and record. Also, record the water bath temperature and end time. No color change of the working test media means a negative reaction (a yellowish to brownish tinge to the WTM around test material is also considered a negative reaction). A reddish (bright or hot pink) color is a positive reaction.

3.6 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.

Whitlock, L. L. (ed.). 2016. Extraneous materials: isolation, Chapter 16 [Method 16.16.08 (AOAC Official Method 981.22)]. In Official Methods of Analysis of the Association of Official Analytical Chemists International, 20th Edition. AOAC International, Rockville, MD 20850-3250.