Standard Operating Procedure (SOP)

1. Purpose and Scope:
   Provide a brief description of why you’re performing this procedure.

   1.1 The purpose of this study is to determine the prevalence of *E. coli* O157:H7 in animal and environmental samples obtained from petting zoos in NY State.
   1.2 Bacteria in the samples will be lysed and PCR will be used to amplify *E. coli*-specific genes.

2. Hazard Identification, Exposures, Control and Containment:
   Describe the biohazardous materials (e.g., infectious agent), routes of infection (e.g., mucous membranes, respiratory), and symptoms of disease or exposure. Indicate engineering controls (e.g., biosafety cabinet, sealed secondary containers for transport) and personal protective equipment (e.g., gloves, lab coat) that must be used to contain hazards and minimize exposures.

   2.1 The primary hazards are *E. coli* O157:H7 and other gram negative bacteria.
   2.2 The primary route of transmission with these gastrointestinal bacteria is ingestion and contact with the mucous membranes of the mouth, nose and eyes. Symptoms of exposure include abdominal cramping and diarrhea, perhaps bloody at times. If you experience any of these symptoms, contact your supervisor immediately.
   2.3 All work with live bacteria will take place within the biosafety cabinet.
   2.4 All lab workers must wear a lab coat and gloves. Include disposable sleeves when working within the biosafety cabinet. Change gloves and sleeves frequently to avoid potential contamination of equipment and surfaces within the lab. Remove PPE before leaving the lab.
   2.5 Wash hands frequently especially after removing gloves, handling samples, leaving lab, etc.

3. Experimental Procedures:
   Describe specific experimental procedures and equipment that must be used. Also, identify any procedures that may be high risk (e.g., centrifugation steps, handling sharps).

   3.1 Transport samples in sealable secondary containers. Include absorbent material in the secondary containers to soak up any spilled material.
   3.2 Don appropriate PPE (see section 2.4). Open secondary containers only in the biosafety cabinet. Refer to section 5 for spilled material.
   3.3 Place tubes in rack. Transfer a 15 ul aliquot from each sample tube to the lysis multiwell strip. Add lysis buffer to each well and cover strip. Use aerosol-barrier tips for all operations.
   3.4 Transfer strips to heat blocks. Heat in 37°C block for 10 min and then transfer strips to the 95°C block for 5 min.

4. Inactivation, Decontamination, Waste Treatment and Disposal:
   Indicate if and how the organisms will be inactivated prior to further manipulations. Specify the type of disinfectant, frequency and procedures. Describe the types of waste that will be generated and how they will be treated or disposed (e.g., intact plasticware in red biohazard bags, needles and razor blades in sharps disposal container, treat liquid wastes with bleach).

   4.1 Decontaminate work surfaces before commencing work, at the end of the day, and after any spills.
   4.2 Change gloves often to prevent contamination of strips, pipettors, refrigerator handles, etc. Decontaminate outer surfaces of strips with disinfectant-saturated towel before transferring to heat blocks.
   4.3 Discard excess sample tubes in red biohazard bag located within the biosafety cabinet. Red biohazard bags are treated as regulated medical waste.
   4.4 Place red biohazard bags in covered transport bin. Transport waste to waste management facility.
   4.5 Decontaminate pipettors and other pieces of equipment when work is complete.

   Alternatively, this section could be a separate SOP.
5. Spills and Emergency Exposure Procedures:

Specify procedures used to clean up spills or releases, what to do if personnel become exposed.

5.1 Spills
5.2 Cover any spills of live bacteria with paper towels.
5.3 Saturate towels with 1:10 dilution of household bleach (made fresh daily) and allow 5-10 min contact time. Wipe up area and discard towels in red biohazard bag. Repeat disinfection procedure
5.4 Exposures
5.5 Wash exposed area with soap and water. Perform first aid if necessary.
5.6 Notify supervisor and seek medical evaluation at Gannett Health Services. If the exposure should occur after normal working hours or on the weekend, seek evaluation at Cayuga Medical Center (notify Gannett Occupational Medicine at 255-6960 the next business day).
5.7 Along with supervisor, complete accident report form.

Alternatively, this section could be a separate SOP.

6. Miscellaneous Information, References:

Optional. For example, information regarding immunizations, medical surveillance, procedural references, etc.

Emergency Phone Numbers
Police, Fire, and Medical Emergencies 911
Environmental Health & Safety 255-8200
Gannett Health Center 255-5155
Principal Investigator xxx-xxxx
Principal Laboratory Contact xxx-xxxx
Secondary Laboratory Contact xxx-xxxx

Written and Prepared by: ____________________________________________________________________________

Approved by: ____________________________________________________________________________________

Signatures and Acknowledgement:

All personnel associated with this project must read and be trained to this Standard Operating Procedure. Additionally, all personnel must understand the hazards and safe work practices described herein, and receive appropriate training before handling biohazardous materials and engaging in high risk procedures.

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