FOOD SAFETY LABORATORY
CORNELL UNIVERSITY

Title: Receiving External Isolates

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to provide a procedure for uniform handling and receipt of isolates received by the Food Safety Laboratory from external sources (e.g. government agencies, companies, academic institutions).

1.2 Scope

This SOP applies to the Food Safety Lab, including the Laboratory for Food Microbiology and Pathogenesis of Foodborne Diseases and any experimental procedures conducted by laboratory members at other locations.

1.3 Definitions

External isolate: A bacterial strain that has been created or isolated from a sample at a location outside of the Food Safety Lab.

External source: The location outside of the Food Safety Lab that had an isolate in its possession or the location where isolation of the bacterial strain(s) occurred.

SECTION 2 MATERIALS

70% ethanol or freshly prepared 20% Chlorine bleach
Bunsen burner
Disposable gloves
Sterile disposable loops
Sterile media / BHI plates (1 per isolate)*
Sharpie marker and tape
Incubator - temperature is organism dependent

*Refer to troubleshooting section for alternative media choices.

SECTION 3 PROCEDURE

I. Receipt of box & confirmation of contents:

1. Examine exterior of box for damage or leakage.
2. Wearing disposable gloves, apply either 70% ethanol or 20% bleach to bench surface to disinfect working area.
3. Open box and carefully remove its contents over bench.
4. Confirm that contents appear to be in good condition.
5. Cross reference isolates contained within box with packing slip or accompanying paperwork. All numbers should match perfectly. Make a notation of any discrepancy on the paperwork.

II. Assignment of FSL specific identification numbers:

1. Arrange tubes or plates in exact order as listed on paperwork and assign a FSL ID # to each individual isolate. Isolates received in duplicate should only receive one FSL #, but be distinguished by a sub designation of 'a' or 'b'.
   *See protocol "FSL Isolate Designation, ID Assignment & Maintenance" located on the Cornell Food Safety Wiki for additional details on FSL ID # assignment.
2. FSL ID #'s are to be written onto paperwork, original slant or plate and onto the plate that you will sub-streak the external isolate.

III. Aseptically remove growth from slant or plate:

1. Take tube or plate containing external isolate and verify the number on the tube matches the labeled plate you will sub-streak it onto.
2. Remove cap from tube and flame exterior opening of external isolate' tube. Using a sterile disposable loop enter tube and drag loop across surface to capture a bacterial sample within loop. Re-flame exterior of tube and immediately recap tube.
3. Lift cover of labeled Petri plate and apply bacterial sample to media, going back and forth over an area to create a lawn. Cover plate and discard loop.
4. Remove a new sterile disposable loop and drag twice from the lawn to a new section of the plate. Drag loop back and forth, in a tight zig-zag pattern, proceeding in one direction only. Flip loop over and drag from this area twice to a new section of the plate. Drag loop back and forth in a wide zig-zag pattern without touching the other areas you previously streaked.
5. Repeat this procedure with all other external isolates being received.
6. When all of the external isolates have been plated, invert them, tape them together and incubate them at an appropriate temperature overnight.

IV. Visual inspection of plated isolates:

1. After overnight incubation at appropriate temperature, remove plated isolates from incubator and place onto disinfected bench.
2. Visually inspect each plate for contamination. Each plate should display colonies of a single color and morphology, which matches the organism you are expecting to see.

*See troubleshooting section for contamination issues.

3. When you have visually confirmed that you have a pure culture, a single colony from each plate is to be chosen and sub-streaked onto a new plate. This single colony growth will be used in various laboratory procedures and once frozen will become the representative frozen stock for the isolate at the Food Safety Laboratory.

   *Refer to "Protocol for Freezing Bacterial Isolates" on the Cornell Food Safety Wiki page.

SECTION 4 REPORTING and LABELING

- All paperwork is to be retained for external samples. The original is placed into the applicable binder with the date of receipt and the initials of the person that handled their receipt noted at the bottom of the original. A copy should also be made and taped or glued into that person's lab notebook under a dated entry.

- Each individual plate sub-streaked from an external sample is to be clearly labeled with the previous ID, the newly assigned FSL ID and dated and initialed.

- Each isolate is to be frozen in duplicate under an FSL ID. The location of the isolate is to be logged into the freezer binder and entered into the Pathogen Tracker database.

- Any isolate retained at the FSL is to be entered into Pathogen Tracker with pertinent information. Confidential information will be handled as such within the isolate entry.

- Retain all original slants in coldroom for 1 year.

SECTION 5 TROUBLESHOOTING

Contaminated cultures received from external sources require special handling. It is important to know the organism that the external source intended to send to the FSL in order to troubleshoot properly. Once you have ascertained the organism you were to receive, go back to the original culture sent to the FSL and plate it onto media that is selective for the organism.

Listeria → LMPM
Salmonella → MacConkey
Streptococcus → Blood agar
Vibrio → TSA with 2% salt

If you receive cultures and paperwork with conflicting information, contact the external source that provided the external isolates for clarification. Note corrections of information clearly on the paperwork.

SECTION 6 REFERENCES