	FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM *Standard Operating Procedure*	

Title: Receiving External Isolates			
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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 and MQIP Lab

1.3 Definitions

Externally 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 the DNA in its possession or the location where isolation & purification of the bacterial DNA occurred.

Selective Media: Inhibits the growth of certain species of bacteria in a mixed culture while allowing others to grow

1.4 Safety

As a preventative measure, all isolates received from an external source should be treated as BSL2 organisms.

SECTION 2 MATERIALS

- 70% ethanol or freshly prepared 20% Chlorine bleach
- Bunsen burner
- PPE - Disposable gloves, lab coat and safety glasses
- Sterile inoculating 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 PROCEDURES

3.1 Receipt of box & confirmation of contents

3.1.1 Wearing disposable gloves, apply either 70% ethanol or 20% bleach to bench surface to disinfect working area

3.1.2 Wearing lab required PPE, apply either 70% ethanol or 20% bleach to bench surface to disinfect working area.

3.1.3 Open box and carefully remove its contents over bench.

3.1.4 Confirm that contents appear to be in good condition.

3.1.5 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.

3.2 Assignment of FSL specific identification numbers

3.2.1 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 the SOP for FSL Isolate Assignment on the Cornell Food Safety WIKI)

3.2.2 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.

3.2.3 Slants are to be retained in storage for one year from date of receipt.

3.3 Aseptically remove growth from slant or plate

3.3.1 Take tube or plate containing external isolate and verify the number on the tube matches the labeled plate you will sub-streak it onto.

3.3.2 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.

3.3.3 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.

3.3.4 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.

3.3.5 Repeat this procedure with all other external isolates being received.
3.3.6 When all of the external isolates have been plated, invert them, tape them together and incubate them at an appropriate temperature overnight.

3.4 Visual inspection of plated isolates

3.4.1 After overnight incubation at appropriate temperature, remove plated isolates from incubator and place onto disinfected bench.

3.4.2 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.

3.4.3 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.

3.5 Reporting and Labeling

3.5.1 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 dated entry should also be made in that person's lab notebook.
3.5.2 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.

3.5.3 Each isolate is to be frozen under an FSL ID. The location of the isolate is to be logged into Food Microbe Tracker database.

3.5.4 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.
3.5.5 Retain all original slants in cold room for 1 year.

SECTION 4 TROUBLESHOOTING

(1) Any discrepancies between the packing slip and the contents, should be noted on the packing slip and communicated to the sender.

(2) Any broken, or leaking tubes should be reported to the sender. Segregate compromised tubes from the remainder of the contents of the package. The uncompromised tubes should be wiped with a Kim Wipe treated with a 20% bleach solution and allowed to sit for 20 minutes. Following this treatment, the tubes should be wiped with a Kim Wipe wetted with water and followed by a 70% ETOH wipe.

(3) 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 → XLD
Streptococcus → Blood agar
Vibrio → TSA with 2% salt

(4) 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 5 REFERENCES

SECTION 6 METHOD VERSION & CHANGES

VERSION	DATE	EDITOR	COMMENTS
Version 1	03/07/2007	E.D.F.	Original SOP
Version 2	04/22/2020	Ser15	Reformatted SOP to new template.