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# Protocol for ESBL-EC recovery from stool samples

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

Understanding transmission pathways of important opportunistic, drug resistant pathogens, such as extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli, i*s essential to implementing targeted prevention strategies to interrupt transmission and reduce the number of infections. To link transmission of ESBL-producing *E. coli* (ESBL-EC) between two sources, single nucleotide resolution of *E. coli* strains as well as *E. coli* diversity within and between samples is required. However, the microbiological methods to best track these pathogens are unclear. Here we compared different steps in the microbiological workflow to determine the impact different pre-enrichment broths, pre-enrichment incubation times, selection in pre-enrichment, selective plating, and DNA extraction methods had on recovering ESBL-EC from human stool samples, with the aim to acquire high quality DNA for sequencing and genomic epidemiology. We demonstrate that using a 4-hour pre-enrichment in Buffered Peptone Water, plating on cefotaxime supplemented MacConkey agar and extracting DNA using Lucigen MasterPure DNA Purification kit improves the recovery of ESBL-EC from human stool and produced high-quality DNA for whole genome sequencing. We conclude that our optimised workflow can be applied for single nucleotide variant analysis of an ESBL-EC from stool.

## Guidelines

All the samples should be treated as potentially infectious. Therefore, samples must only be opened in a class II Microbiological Safety Cabinet (MSC) until considered inactivated. The use of lidded, aerosol-tight centrifuge buckets is compulsory.

## Materials

- 15 mL falcon tubes
- Cryovials
- Cryoboxes
- 2ml microcentrifuge tubes
- 1000 uL pipette tips
- 200 uL pipette tips
- 20 uL pipette tips
- 10 uL pipette tips
- Sterile filter units
- 5 uL serological pipette
- 10 uL serological pipette
- 25 uL serological pipette
- 50 uL serological pipette
- Stirrer bar
- 1 uL loops
- Petri dishes

## Before start

Before starting, thoroughly clean the Class II MSC with Chemgene (5% solution) and 70% ethanol. Switch the Class II MSC on and leave it running until airflow is safe. All other solid waste is considered contaminated and must be disposed of by autoclaving followed by incineration.

## Reagent preparation

1 **Preparation of Sterile Buffered Peptone Water (BPW) pre-enrichment broth**

1.1 Add 20 g of BPW powder to 1 L of distilled water.

1.2 Mix well and sterilise by autoclaving at 121 °C .

1.3 Store at room temperature for up to 1 month.

1.4 Aliquot 5 mL into 15ml falcon tubes in preparation for samples.

2 **Preparation of cefotaxime stock solution**

2.1 Dissolve cefotaxime powder in water to [M] 200 mg/mL .

2.2 Dilute this to [M] 10 mg/mL in water to make working stock solution.

2.3 Store at -20 °C .

3 **Preparation of MacConkey agar**

3.1 Suspend 50 g in 1 L distilled water.

3.2 Mix well and sterilise by autoclaving at 121 °C .

3.3 Cool to 50 °C in a water bath and add cefotaxime to a final concentration of 1 µg/mL.

3.4 Pour into sterile petri dishes and allow to dry, then store at 4 °C for up to 1 month.

4 **Preparation of Stool Solution (adapted from maltodextrin:trehelose method in Burz et al. 2019)**

4.1 Dissolve 9.4 g of maltodextrin and 3.1 g of trehalose in 450 mL of PBS.

4.2 Put into a waterbath set to 40 °C until dissolved.

4.3 Add 50 mL glycerol (to give a final concentration of [M] 10 % volume glycerol).

4.4 Filter sterilise.

4.5 Aliquot 500 µL into cryovials. Cryovials can be stored at Room temperature for up to 6 months. 2 cryovials will be needed for each sample.

## Sample processing

5 **Stool and rectal swab processing**

5.1 **A) Swabs:** Transfer the rectal swab into a 15mL falcon tube containing 5 mL of BPW enrichment broth. Cut the end of the swab and replace the cap of the falcon tube.

**B) Stool samples**: weigh stool sample pot (use an empty pot to zero the balance) and resuspend in sample in stool solution. Use 5x the volume of stool solution per g of stool (e.g. 0.5g stool = 2.5ml of stool solution). Add a small stirrer bar to the pot and place on a magnetic stirrer to agitate the sample. Vortex sample to resuspend if necessary. Add 100 µL of resuspended stool into a 15mL falcon tube containing 5 mL of BPW.

5.2 Incubate the tubes shaking at 220 rpm, 37°C for 4 hours.

5.3 After 4 hours, transfer samples to the Class II microbiological safety cabinet (MSC) and streak each sample out onto MacConkey agar supplemented with 1 µg/mL cefotaxime using a 1 µL loop. Incubate the plates overnight at 37 °C .

5.4 Pick several positive morphology colonies (colonies that appear red/pink on MacConkey agar) and streak each colony onto a separate section of a new MacConkey agar plate supplemented with 1 µg/mL cefotaxime. Incubate the plates overnight at 37 °C .

6 **DNA extraction**

6.1 Transfer a 1 µL loopful of each colony restreak to a 1.5 mL tube.

6.2 Extract DNA according to the Lucigen MasterPure Complete DNA and RNA Purification kit manual, available at: https://biosearchtech.a.bigcontent.io/v1/static/manual_NAEXPU-001_MasterPure-Complete

6.3 Measure the concentration of all DNA extracts using a Qubit according to the manufacturer's recommendations (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/Qubit_dsDNA_BR_Assay_UG.pdf).

## Protocol references

Burz, S. D. *et al.* A Guide for Ex Vivo Handling and Storage of Stool Samples Intended for Fecal Microbiota Transplantation. *Sci Rep* **9**, (2019).