(D) Standard Operating Procedure (SOP) for

Isolation, Transportation, and Storage of stool sample from Human Subjects

1.0 Purpose

This document outlines the procedure to obtain stool sample from human whole blood in Cell Preparation Tubes (CPT) with sodium citrate for transcriptome analysis.

2.0 Scope

This SOP applies to the collection of stools from subjects participating in CCRAS funded project "SYSTEMS BIOLOGY APPROACH TO DELINEATE MOLECULAR SIGNATURES OF PRAKRITI IN HEALTHY HUMANS".

3.0 Safety

3.1. All stool samples should be handled and processed according to institutional Biosafety Guidelines. This procedure should be performed in accordance with all applicable safety procedures.

3.2. It is imperative that stool sample are collected and processed using strict aseptic technique.

3.3. Laboratory personnel are required to be trained on this procedure prior to processing stool samples collected from the study center. Laboratory managers are responsible for documenting the training in accordance with institutional requirements.

4.0 Reagents

4.1 Ethanol (EMSURE®/ MERCK, Cat. 1009831011 or equivalent)
4.2 Phosphate buffer saline (MERCK, Cat,524650-1EA or equivalent)
4.3 buffered Glycerol saline (Thermo Fischer, Cat. R21650 or equivalent)

5.0 Equipment and Materials (Note: equivalent may be used)

5.1 MicroCollect™ Sterile Fecal Collection Container
5.2 Kimberly-Clark Professional™ Purple Nitrile™ Exam Gloves
5.3 Biological Safety Cabinet (Certified)
5.4 RD Plastics Color Coded Ziplock Biohazard Specimen Bags
5.5 10 mL Pipettes
5.6 Filtered Sterile Pipette 20ul-1000ul Universal Tips
5.7 Sonoco™ Thermo Safe Polar Pack™ Foam Bricks
5.8 Kartell™ Sterile Faeces Container
5.9 Fisherbrand™ Commode Specimen Collection System
5.10 Refrigerator (2 to 8°C)

5.11 Freezer (-60°C to -90°C) Shipping/Storage

6.0 Procedure

6.1. Stool Collection

6.1.1 The fecal samples will be self-collected by the volunteer using a disposable paper inverted toilet hat and wearing nitrile gloves during stool collection.
6.1.2 From the disposable paper the stool is transfer to the stool collection container using spoon provided within the lid of MicroCollect™ Sterile Fecal Collection Container (2 container). The container will then place in the zip lock.
6.1.3 After collection of the stool the volunteer must wash their hand with soap. The biological specimen must be collected from the volunteer no more than 24 hours before their clinic visit.

6.2 Precaution

6.2.1 Occupational Safety and Health Act regulations (including standard precautions) should be used for handling all specimens.

6.2.2 Interfering substances (oil-based laxatives, barium, antibiotics) should be avoided when stool specimens are collected.

6.2.3 Contamination with urine or water should be avoided.

6.2.4 For optimal preservation, stool samples should be kept at room temperature and brought at the laboratory within 24 h after collection.

6.3 Shipment

6.3.1 The sample should be kept in cold storage (0°C to 4°C) during the transportation to prevent bacterial growth before arriving the lab for further processing.

6.3.2 In the condition of 0°C to 4°C preservation, the allowable shipment time from participant to laboratory can be 24 to 48 h without significant microbial composition alteration.
6.3.3 Styrofoam box will be used along with 8-10 polar pack for transport of sample (Polar pack must kept below 0°C for 12 hours before use).

6.4 STORAGE

6.4.1 In the laboratory, each fecal specimen will be mixed manually using a spatula, and aliquot in 4 Sarstedt Inc faeces container as subsample.
6.4.2 For each participant, approximately 1 to 2 grams of faeces, representing a full scoop of faeces, will be placed in a Sarstedt faeces tube containing no solution and 2.5 mL of 95% ethanol (Sigma-Aldrich) and Glycerol buffer saline. All theses 3 aliquot will be defreezed

at -80^0 C which will be utilized for DNA extraction and metabolomics study. The fourth subsample will be analysed by use for faecal culture enrichment approach.

6.4.3 In separate sterile test tube approximately, 3 g fecal samples will be diluted with 15 ml sterile saline and homogenized in a standard blender. The slurry is then filtered three times through gauze, strainer, or 0.25 mm stainless steel sieves to eliminate the undigested and small particulate matter in the fecal suspension or fecal suspension could be centrifuged at 6,000 × *g* for 15 minutes

6.4.4 The precipitate, without the supernatant, is re-suspended in fresh sterile saline. All fecal material preparation processes should be carried out at a room temperature of 20–30°C; preferably in an anaerobic incubator.

6.4.5 Subsequently, the resulting suspension should be added to glycerol to get a final concentration of 10%. Finally, the fecal suspensions must be labelled accurately and then stored at -80°C

7.0 Documentation

The project clinical site implementing this SOP will document the procedure. Any deviation from the SOP should be recorded. This documentation will be stored indefinitely as per CCRAS guidelines.

Work Flow Chart for Collection, storage and processing Faecal specimen

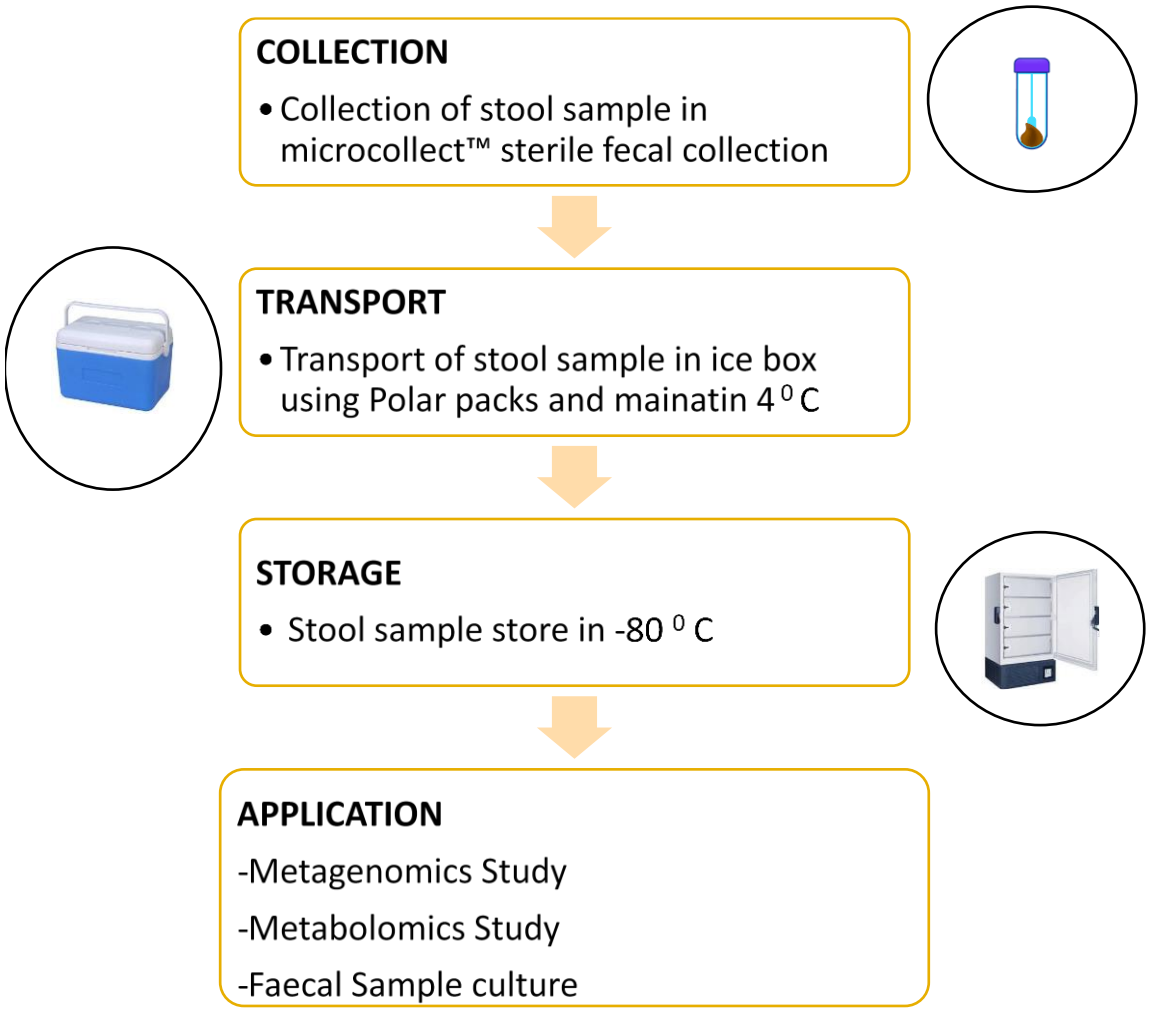

Figure represents the flow chart of faecal sample processing