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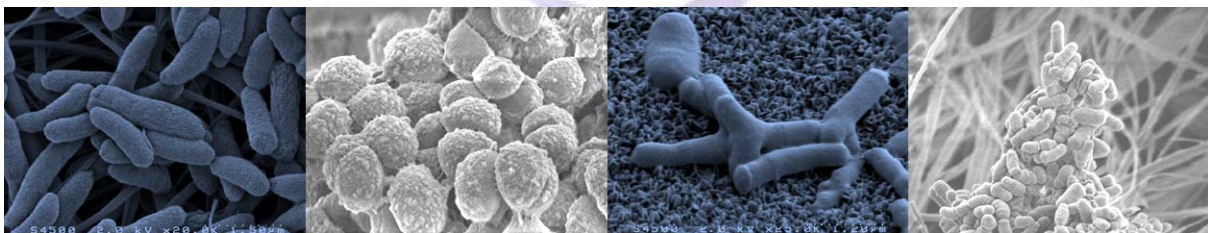
IHMS – QUALITY PROCEDURE
International Human Microbiome Standards
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IHMS SOP 04 V1: STANDARD OPERATING PROCEDURE FOR FECAL SAMPLES

FROZEN PRESERVED SELF-COLLECTION

LABORATORY ANALYSIS HANDLED WITHIN 24 HOURS TO 7 DAYS

(24 HOURS < x ≤ 7 DAYS)



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Introduction

IHMS seeks to coordinate development of standard operating procedures (SOPs) and protocols that will optimize data comparisons in the human microbiome field. The IHMS project concentrates on following objectives:

- Coordinate standardization of procedures and protocols within the existing Human Microbiome research programs and those yet to come,
- Gather and compare the protocols used to collect, identify and process human samples and aid to develop the standard operating procedures for sample collection, identification and processing,
- Compare sequences of genes and genomes of human-associated microorganisms generated by various methodologies and approaches, and to develop standards to define sequence quality and recommend procedures to reach the standards,
- Assess the approaches and procedures used to analyze the sequence data and the associated metadata and recommend standards for data analysis.

Beside these objectives, IHMS project aims at ensuring the optimal public access and use of the data generated by various microbiome projects. The project is supported by the European Commission under the 7th Framework Programme. The consortium gathers 8 partners and 15 contributors across 12 different countries. Its total cost has been evaluated at 2,3 million €, the funding from the European Commission has been set with an upper limit of almost 2 million € and a duration of 4 years, beginning in February 1st, 2011.

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1. OBJECTIVE:

Optimize data comparisons in the human microbiome field by the standardization of the procedure for fecal samples self-collection.

This SOP is of first interest for good fecal samples collection and conservation practice.

2. PRINCIPLE:

This SOP aims to standardize the fecal samples self-collection in case of the samples to be handled by the "laboratory" within 24 hours to 7 days, samples are frozen then shipped on dry-ice to the "laboratory".

3. RELATED DOCUMENTS:

Titles	Codes	Localization
IHMS quality procedure template	IHMS_INS 01 V1	INRA MGP

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4. Persons entitled to use the procedure:

This SOP applies to any person involved in collecting, for good fecal sample collection processing and transportation to the “laboratory” where it will be handled within 24 hours to 7 days.

This person can be the person collecting herself, a nurse, or any health care professional in charge of stool collection.

5. Preliminary steps, specificities:

Protocol should be approved by an ethics committee according to national regulations. Protocol should be declared on international database (e.g. <https://clinicaltrials.gov>). Volunteers and patients should have signed an informed consent according to approved protocol.

Accreditation should have been obtained for sample biobanking, and if necessary for shipping.

Anybody wanting to apply this SOP to the fecal samples collection must be in possession of the kit described in this SOP **§Kit**.

A hand washing is mandatory and essential to the preservation of samples quality.

Please note that this SOP applies only to fecal samples collection which will be handled **within 24 hours to 7 days and for samples preserved at -20°C (frozen) before being shipped on dry-ice** to the “laboratory” in charge of the analysis.

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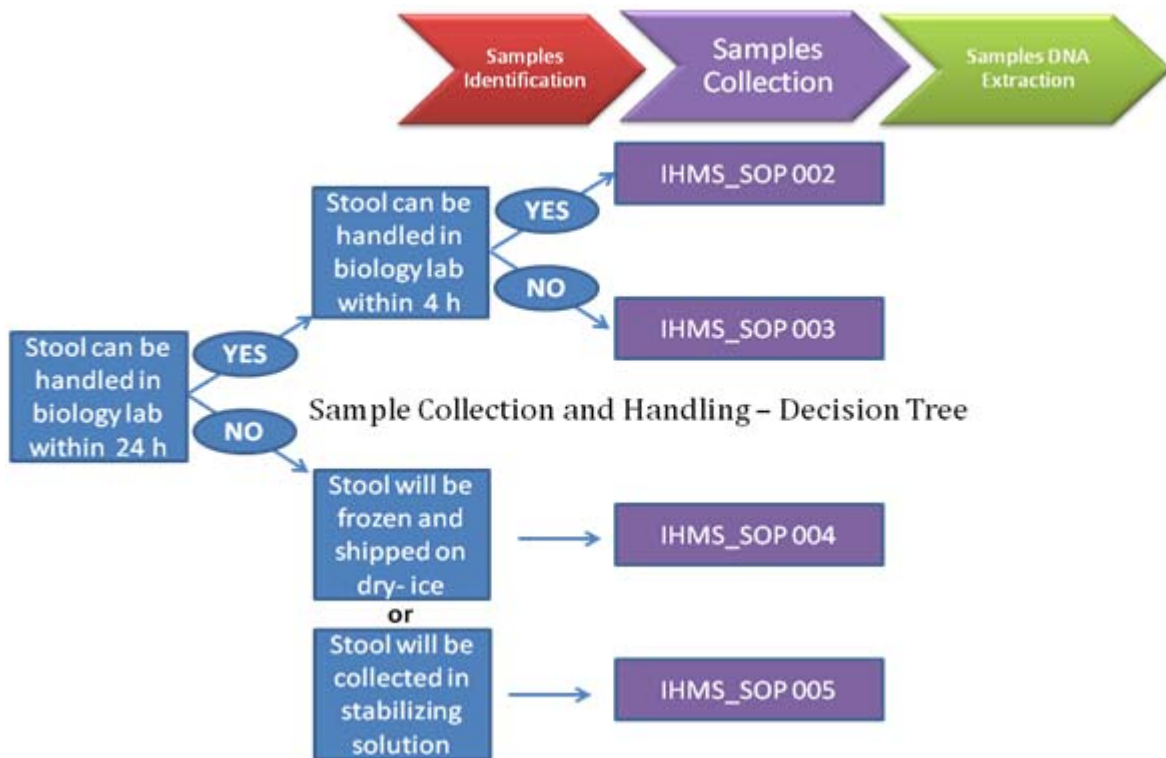
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If it is not your case, please refer to the decision Tree below to select the appropriate SOP for your fecal sample collection:



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6. Kit:

Materials	Picture 1	Picture 2	Suppliers Names and references
A plastic lining to cover the toilet (i.)			Care Bag [®] protège WC by 20 pcs. Supplier in France: Cleanis Ref 9538534
A tissue lining to put inside the plastic lining (ii.)			Care Bag [®] protège WC by 20 pcs. Supplier in France: Cleanis Ref 9538534
Two plastic tubes with a spoon attached to the lid (iii.)			Plastic vessel with spatula by 1000. Supplier in France: Starsted Ref 80 623 022
A small transparent plastic bag (iv.)			Plastic bags by 1000. Supplier in France: VWR Ref 129-0297
A series of labels (v.)			NA*
A large opaque plastic bag (vi.)			Plastic bag opaque gastight by 1000. Supplier in France: Protective Packaging

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A small grey sachet containing a white anaerobic generator paper bag (vii.)			Anaerocult® P anaerobic incubation sachet (AnaeroGen or GenBox)
A clipping system (viii.)			Clips for Gaz tight opaque plastic bag by 200. Supplier in France: VWR Ref 129-9869

NA* Non Available

7. Conditions and usage constraints to follow:

- 1- Before fecal sample collection it is better if you urinate
- 2- Hand washing is mandatory and essential before placing and after removing the plastic lining (i.) over the toilet
- 3- The fecal sample minimal volume must be at least 2/3 of the volume of the spoon
- 4- The fecal sample maximal volume must be at least the volume of the spoon
- 5- The plastic tubes (iii.) must NEVER be closed
- 6- Close the opaque bag (vi.) tightly with the clipping system (viii.)
- 7- Stool samples should be kept at minimum -20°C for a short time before transportation at -80°C
- 8- Stool samples should be send to the 'laboratory' within 24 hours to 7 days maximum after collection for aliquoting and storage at -80°C
- 9- The white anaerobic generator paper bag must NEVER be open
Keep it CLOSED during shipment for appropriate use
- 10- The logo 3373 is mandatory for each biological sample transportation
- 11- Labelling with same label the plastic tubes (iii.), the small transparent plastic bag (iv;) and the opaque bag (vii.) is mandatory

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8. Step by step procedure:

How to collect the fecal sample (self-collection)?

Numbers correspond to numbers on the attached pictures below:

1) Use the complete kit that you received by mail (see kit's picture below).

It contains :

- i. A plastic lining to cover the toilet
- ii. A tissue lining to put inside the plastic lining
- iii. Two plastic tubes with a spoon attached to the lid
- iv. A small transparent plastic bag
- v. A series of labels
- vi. A large opaque plastic bag
- vii. A small sachet of catalyst in a bag (AnaeroGen or GenBox)
- viii. A clipping system to close the large opaque plastic bag



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- 2) Label each tube (iii.), the small transparent plastic bag (iv.), the opaque bag (vi.) with the labels (v.) cf. movie IHMS SOP 04 V1 part 1



Please urinate (if possible) before fecal sample collection

Then please wash your hands

- 3) Place the plastic lining (i.) over the toilet bowl
 Then place the tissue lining (ii.) inside the plastic lining (i.)



Then please wash your hands

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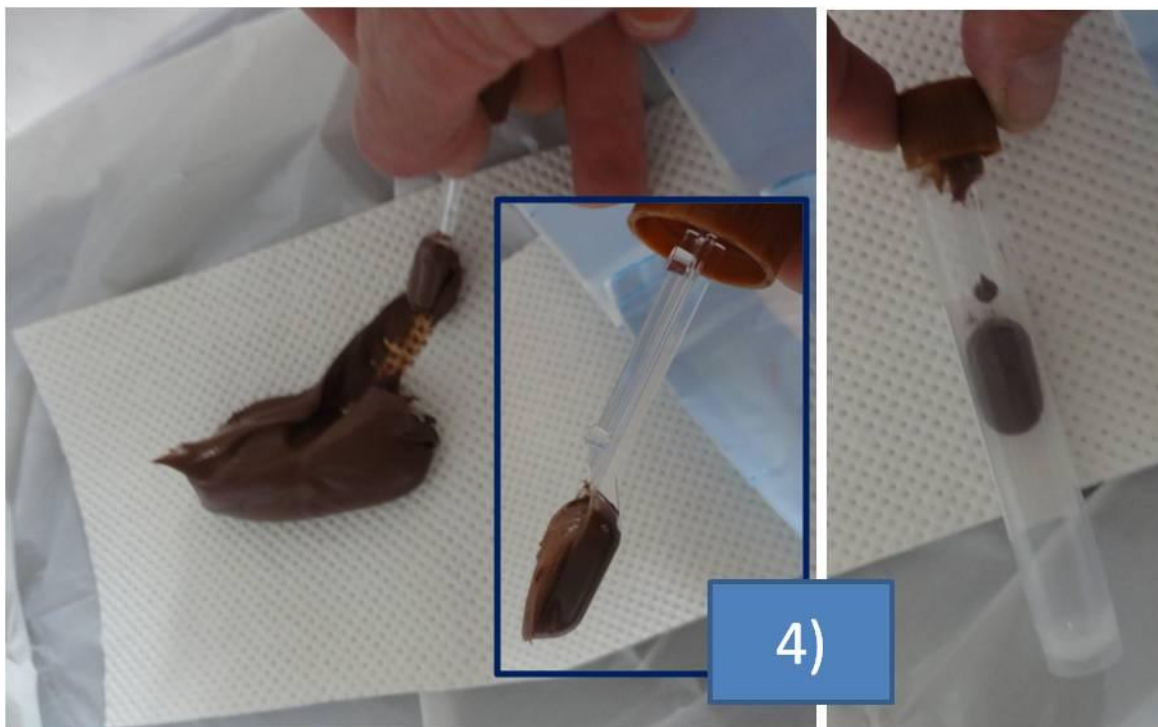




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- 4) After defecation, fill up a first spoon with feces. **Do not overfill**; insert spoon in its tube (iii.) and **DO NOT CLOSE** the tube (iii.)
cf. movie IHMS SOP 04 V1 part 2

Do not overfill the spoon



The fecal sample minimal volume must be at least 2/3 of the volume of the spoon. The maximal volume is determined by the volume of the spoon (cf. movie IHMS SOP 04 V1 part 2)

Do not close the tubes

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5) Repeat as for the first spoon with the second spoon



6) Insert the two plastic tubes (iii.) **UNCLOSED** inside the labeled small open transparent plastic bag (iv.)



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7) Insert the labeled small open plastic bag (iv.), containing the labeled open tubes (iii.) inside the labeled opaque bag (vi.)



8) Open the catalyst bag (vii.) and insert the sachet in the labeled opaque bag (vi.)



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- 9) Use the clipping system (viii.) to close the opaque plastic bag (vi.) tightly. The best way is to place the tube underneath and the clip above, and to press hard starting at one end.



- 10) Place the opaque plastic bag (vi.) containing tubes and catalyst in a deep freezer (-20°C or if possible -80°C) until collection and transportation to the 'laboratory' on dry-ice with the safety tag 3373



Then follow the instructions for your sample to be picked up and delivered to the "laboratory".

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- 11) As shown here, you may flush the remaining feces in the toilet and dispose of the plastic lining (i.) as regular garbage.



9. Contacts:

If you have any question regarding this SOP please contact us at:

contact@human-microbiome.org

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