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| <b>IHMS Consortium</b> | IHMS - QUALITY PROCEDURE<br>SOP FOR FECAL SAMPLES<br>PRESERVED IN STABILIZING<br>SOLUTION SELF-COLLECTION<br>laboratory analysis<br>handled within 24 hours to 7 days<br>(24 hours < x ≤ 7 days) | Code : IHMS_SOP 05 V2<br>Version : 2<br>Date : 2015-04-12<br>Number of pages : 16<br>Page n° : 1 | Last Contributor :<br>Sebastian BURZ<br>Approved by: IHMS<br>CONSORTIUM<br>Date : 2015-01-31 |
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## IHMS – QUALITY PROCEDURE International Human Microbiome Standards

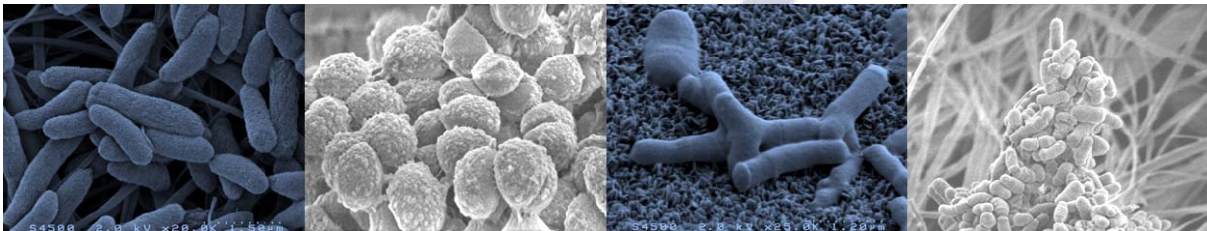
<http://www.microbiome-standards.org>

### **IHMS SOP 05 V1: STANDARD OPERATING PROCEDURE FOR FECAL SAMPLES**

### **PRESERVED IN STABILIZING SOLUTION 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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## **AUTHORS/CREDITS: IHMS CONSORTIUM**

| No | Name   | Short Name           | Country                      | Authors  |
|----|--|----------------------|------------------------------|--|
| 1  | INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE                  | <u>INRA</u>          | France                       | Joël Doré, Florence Levenez, Christel Béra-Maillet, Fabienne Béguet-Crespel, Patricia Lepage, Maxime Rochet, Angélique Doré, Thierry Vanduyvenboden, Sebastian Burz, Charlene Fouquéray        |
| 2  | INSTITUT CATALA DE LA SALUT                                    | <u>HUVH</u>          | Spain                        | Francisco Guarner, Chaysavanh Manichanh, Alba Santiago et al.  |
| 3  | BAYLOR COLLEGE OF MEDICINE                                     | <u>BCM</u>           | United States                | James Versalovic, Ruth Ann Luna, Joseph Petrosino et al.   |
| 4  | SHANGHAI JIAO TONG UNIVERSITY                                  | <u>SJTU</u>          | China (People's Republic of) | Liping Zhao, Jian Shen, Chenhong Zhang et al.  |
| 5  | COMMISSARIAT A L'ENERGIE ATOMIQUE ET AUX ENERGIES ALTERNATIVES | <u>CEA Genoscope</u> | France                       | Eric Pelletier, Adriana Alberti, Laurie Bertrand, Karine Labadie, Céline Orvain  |
| 6  | BEIJING GENOMICS INSTITUTE AT SHENZHEN                         | <u>BGI Shenzhen</u>  | China (People's Republic of) | Huanming Yang, Songgang Li, Jun Wang et al.  |
| 7  | EUROPEAN MOLECULAR BIOLOGY LABORATORY                          | <u>EMBL</u>          | Germany                      | Peer Bork, Paul I. Costea, Shinichi Sunagawa, Sean Powell, Julien Tap, Tomas Larsson, Alison Waller, Kalliopi Trachana, Pablo Minguez Paniagua, Jaime Huerta Cepas, Samy Deghou, Tobias Doerks |
| 8  | THE UNIVERSITY OF WESTERN ONTARIO                              | <u>Western</u>       | Canada                       | Emma Allen-Vercoe, Greg Gloor, Bhagirath Singh et al.  |

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|------------------------|--|--|--|

## CONTRIBUTORS/CREDITS: IHMS WP2 PARTICIPANTS

| Name   | Short Name | Country         | Authors  |
|--|------------|-----------------|--|
| Laboratory of Microbiology Wageningen University                 | WUR        | The Netherlands | Erwin Zoetendal, Hans Heilig, Ineke Heikamp-de Jong, Willem de Vos et al |
| University College Cork  | UCC        | Ireland         | Paul O'Toole, Jenifer Deane, Eibhlis O'Connor et al                      |
| Merieux NutriSciences, Nantes                                    | Biofortis  | France          | Françoise Le Vacon, Thomas Carton et al                                  |
| University of Helsinki, Faculty of veterinary Medicine           | UH         | Finland         | Anne Salonen, Outi Immonen, Laura Huuskonen et al                        |
| University of North Carolina, Kannapolis                         | UNC        | USA             | Ryan Balfour Sartor, Melanie Spencer et al                               |
| University of North Carolina, Chapel Hill                        | CGIBD UNC  | USA             | Ryan Balfour Sartor, Ian Carroll et al                                   |
| Statens Serum Institute, Copenhagen                              | SSI        | Denmark         | Soren Persson, Joan N Jensen et al                                       |
| University of Florida, Gainesville                               | UF         | USA             | Volker Mai, Maria Ukhnova et al  |
| INRA Micalis Jouy-en-Josas                                       | INRA       | France          | Philippe Langella, Jean-Pierre Furet et al                               |
| University of Tokyo & Azabu University                           | UniT       | Japan           | Masahira Hatori, Hidetoshi Morita et al                                  |
| Medical University Graz  | MUG        | Austria         | Ingeborg Klymiuk et al   |
| Maastricht University Medical Center                             | EPID       | The Netherlands | John Penders et al   |
| University fo British Columbia, Vancouver                        | UBC        | Canada          | Brett Finlay, Marie Claire Arrieta, Lisa Thorson et al                   |
| Rowett Institute of Nutrition and Health, University of Aberdeen | RINH       | Scotland, UK    | Harry Flint, Karen Scott, Petra Louis et al                              |
| Danone Research, Palaiseau                                       | Danone     | France          | Johan van Hylkama Vlieg, Tamara Smokvina, Anne Druesne et al             |
| German Institute of Human Nutrition, Potsdam Rehbruecke          | DIfE       | Germany         | Michael Blaut, Kathleen Slezak, Delphine Saulnier et al                  |



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## Summary

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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 in order to characterize the fecal microbiota by metagenomic profiling.

### 2. PRINCIPLE:

This SOP aims to standardize the fecal samples self-collection by giving a step-by-step description in case of the samples will be handled by the “laboratory” within 24 hours to 7 days, and preserved in a stabilizing solution during transportation 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, preservation in the stabilizing solution 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 by the “laboratory” in charge of the analysis, and transported in a stabilizing solution.

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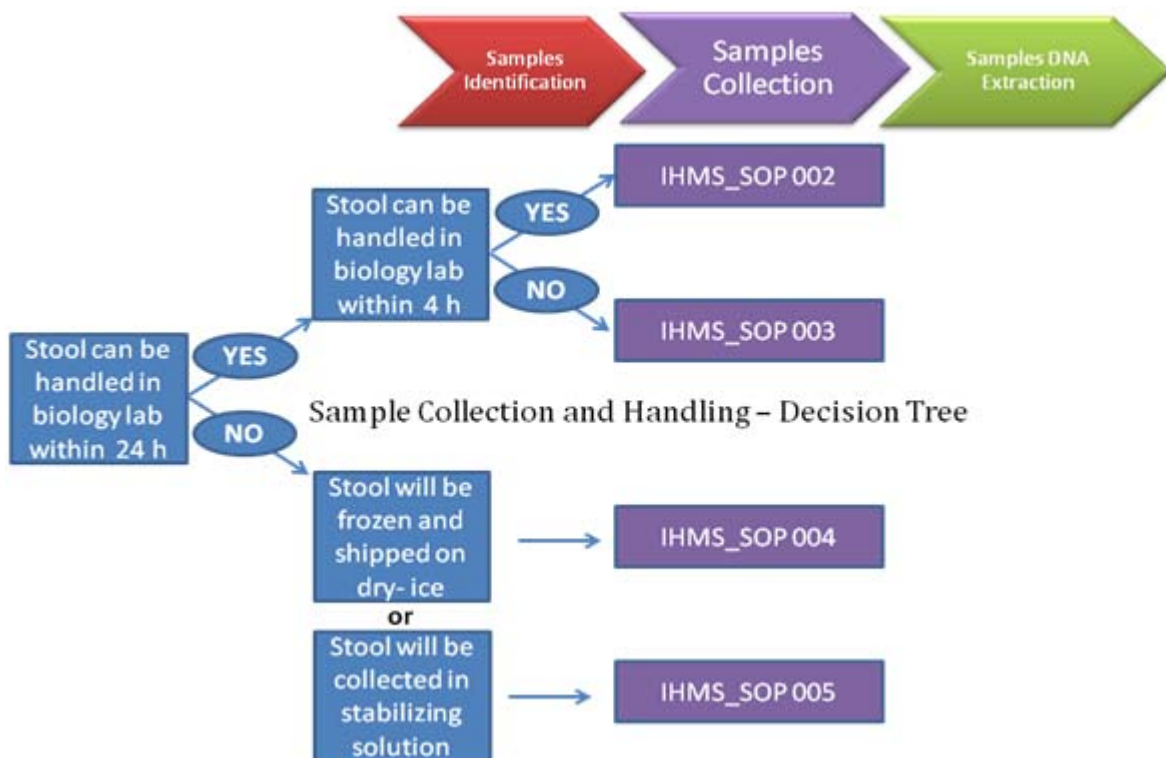
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**If it is not your case, please refer to the decision Tree below** to select the appropriate SOP for your fecal sample collection:



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## 6. Kit:

| Materials  | Picture 1   | Suppliers Names and references   |
|--|---|--|
| <b>A plastic lining to cover the toilet (i.)</b>   |    | Care Bag® protège WC by 20 pces.<br>Supplier in France: Cleanis<br>Ref 9538534   |
| <b>A tissue lining to put inside the plastic lining (ii.)</b>                              |   | Care Bag® protège WC by 20 pces.<br>Supplier in France: Cleanis<br>Ref 9538534   |
| <b>Two plastic tubes containing the stabilizing solution with a brown screw-cap (iii.)</b> |  | Plastic vessel by 1000.<br>Supplier in France: Starstedt<br>Ref 80 623 022<br><br>RNAlater® Stabilization Solution Ambion by 500mL.*<br>Supplier in France: Life technologie<br>Ref AM7021 |
| <b>Two spoons in a small transparent plastic bag (iv.)</b>                                 |  | Plastic spatula by 1000.<br>Supplier in France: Starstedt<br>Ref 80 623 022  |

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Two plastic protecting containers with a yellow screw-cap (v.)



Transport tube by 250.  
 Supplier in France: Starstedt  
 Ref 78 898

A safety plastic bag (vi.)



NA\*

A series of labels (vii.)



NA\*

An envelope (viii.)



NA\*

A small transparent plastic bag (ix.)



Plastic bags by 1000.  
 Supplier in France: VWR  
 Ref 129-0297

NA\* Non Available

\* RNAlater® Stabilization Solution Ambion was validated for the proposed procedure. Other solutions may apply similarly but require specific validation.

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## 7. Conditions and usage constraints to follow:

- 1- Hand washing is mandatory and essential
- 2- The fecal sample minimal volume must be at least the volume of a clementine
- 3- The maximal volume is determined by the volume of the plastic bag and white pot
- 4- The plastic bag must NEVER be closed
- 5- Close the pot tightly with the plastic lid
- 6- Stool samples should be kept at room temperature
- 7- Stool samples should be brought to the laboratory within 4 h to 24 hours maximum after collection for aliquoting and storage at -80°C
- 8- The white anaerobic generator paper bag must never be open!  
Keep it CLOSED during shipment for appropriate use!

## 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 containing the stabilizing solution with a brown screw-cap
  - iv. Two spoons in a small plastic bag
  - v. Two plastic protecting containers with a yellow screw-cap

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- vi. A transparent plastic safety bag
- vii. A series of labels
- viii. An envelope
- ix. A small transparent plastic bag



2) Stick the same identification label (vii.) on both tubes (iii.), on their both protection containers (v.) and on the small transparent plastic bag (ix.)

**cf. movie SOP 005 V1 part 1**



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**Please urinate (if possible) before fecal sample collection**

**Then please wash your hands**

- 3) Place the plastic lining over the toilet bowl. Then place the tissue lining inside the plastic lining, and flip down the toilet seat for defecation



**Then please wash your hands**

- 4) Keeping it vertical on the yellow screw-cap, open one of the plastic tubes containing the stabilizing solution (iii.)

**cf. movie SOP 05 V1 part 2**



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- 5) After defecation (bowel movement), fill up a first spoon flat with feces  
**Do not overfill the spoon: it is calibrated to contain 1 gram**



- 6) Keeping the plastic tube containing the stabilizing solution (iii.) vertical, Insert the filled spoon in its tube before closing the brown screw-cap.

**Attention:**

**Avoid spoiling any stabilizing solution.**

**Rinse any surface exposed to the stabilizing solution with water.**

**Make sure the spoon is entirely submerged in the stabilizing solution.**

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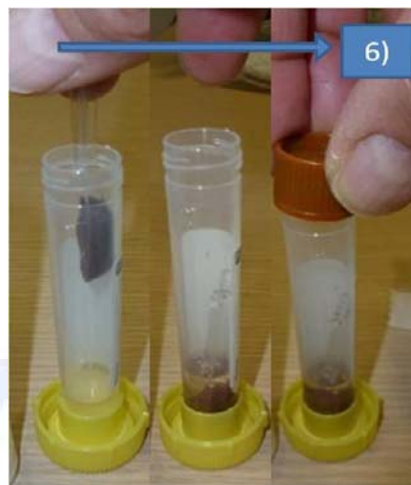
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**Close firmly iii. with the brown cap**



- 7) If possible, fill up a second spoon (repeating steps 4, 5 and 6)
- 8) Maintain tubes at room temperature, vertically, the spoon submerged in the stabilizing solution for **at least one hour**



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9) Insert tubes (iii.) in their protection containers (v.) and put them in the small plastic bag (ix) before putting them in the safety plastic bag (vi.)  
**cf. movie IHMS SOP 05 part 3**



10) Insert the safety plastic bag (vi.) in the envelope (viii.) supplied and mail it to the “laboratory” for analysis **within 7 days**



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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](mailto:contact@human-microbiome.org)

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