IHMS – QUALITY PROTOCOL
International Human Microbiome Standards

http://www.**microbiome-standards**.org/

IHMS SOP 06 V3: STANDARD OPERATING PROTOCOL FOR FECAL SAMPLES

DNA EXTRACTION

Protocol Q

Authors (if you want to quote us please copy paste below)

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.

Summary

1. OBJECTIVE:

This SOP is an update of IHMS SOP 06 v2 as QIAGEN no longer makes QIAamp DNA stool kit commercially available. This update uses the QIASymphony DSP Virus/Pathogen Midi Kit and the QIASymphony SP instrument from the same supplier.

It aims to optimize data comparisons in the human microbiome field by the standardization of the protocol for fecal samples DNA extraction.

This SOP is of first interest for reliable fecal samples DNA extraction practice in order to characterize the fecal microbiota by metagenomic profiling.

2. PRINCIPLE:

This SOP aims to standardize fecal samples DNA extraction by giving a step-by-step description.

3. PERSONS ENTITLED TO USE THE PROCEDURE:

This SOP applies to any person involved in fecal samples DNA extraction, for reliable fecal sampleDNA extraction.

This person can be a trainee, fellow, technician or the engineer in charge of fecal samples DNA extraction.

4. 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.

For the preparation of the nucleic acids, aliquots were prepared under anaerobic conditions from appropriately identified and collected samples, with respect to IHMS SOP 01 V1 and IHMS SOP 02, 03, 04 or 05 respectively. Following collection and reception in laboratory faecal samples are aliquoted in 50 to 200 mg subsamples, transfered into tubes and store at -80 °C freezer.

Moreover, it must be kept in mind that the specific area of nucleic acids preparation does see constant evolutions and improvements, such that it is hardly conceivable to definitely "freeze" a protocol that will be considered as optimal in the long term.

5. CONDITIONS AND USAGE CONSTRAINTS TO FOLLOW:

1- Observation of hygiene and safety rules

2- Mandatory use of lab coat, gloves, glasses

3- Mandatory use of a Biosafety cabinet (BSC)

4- Disposal of biological waste in appropriate containers

5- Disposal of chemical waste in appropriate containers

6. MATERIALS, REACTIVES, PRODUCTS, KITS, SOLUTIONS:

Materials:

Materials	Risks	Prevention	Providers Names and references
0.1 mm glass beads			152016 DUTSCHER
Screw cap microtubes, 2 mL, steriles			73.693.005 SARSTEDT
Safelock Eppendorf tubes, 2 mL, steriles			033297 DUTSCHER
Toothpicks, steriles			046003 DUTSCHER

1000, 200, 100 and 10 µL micropipettes			**Eppendorf Research**
1000 µL filter tips			S1122-1830 **STARLAB**
200-100 µL filter tips			S1120-8810 **STARLAB**
100 µL filter tips			S1121-3810 **STARLAB**
10 µL filter tips			S1121-3810 **STARLAB**
Sample Prep Cartridges 8-well			997002 **QIAGEN**
8-Rod Covers			997004 **QIAGEN**
Filter-Tips, 1500 µl, Qsym SP (1024)			997024 **QIAGEN**
Filter-Tips, 200 µl, Qsym SP (1024)			990332 **QIAGEN**
Mixer mill (Retsch MM400)	 Hand crush hazard	Beware of balancing	20750001 **RETSCH**
Benchtrop microcentrifuge	 Hand crush hazard		**DUTSCHER**
Benchtop refrigerated centrifuge		Beware of balancing	5409000217 **Eppendorf**
70 °C dry bath	 Burn hazard		060917 **DUTSCHER**
Vortex mixer			250158 **DUTSCHER**
Biosafety cabinet			1046-2614 **FISHER SCIENTIFIC**

Kits:

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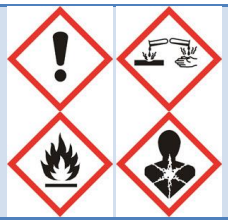
SEVENTH FRAMEWORK PROGRAMME

Materials	Risks	Prevention	Providers Names and references
QIAsymphony DSP Virus/Pathogen Midi Kit, version 1			937055 QIAGEN

Products/Reactives:

Materials	Risks	Prevention	Providers Names and references
Phosphate Buffer, 1 M			
Guanidine Thiocyanate 4 M		Health and environmental hazard (check SDS)	G6639 SIGMA
N-Lauroyl Sarcosine 10 % & 5 %		Health hazard (check SDS)	L9150 SIGMA
NaCl			S3041 SIGMA
EDTA			03690 SIGMA
PVPP			77627 SIGMA
Trizma® Base			T8524 SIGMA

7. STEP-BY-STEP PROCEDURE:

1. Add 250 µL Guanidine Thiocyanate to each tube containing frozen faeces (around 200 mg)
2. Add 40 µL N-lauroyl sarcosine 10 % and thaw.
3. Add 500 µL N-lauroyl sarcosine 5 %, homogenize the sample using a sterile toothpick and vortex to mix well.

4. Incubate at 70 °C in a dry bath for 1 hour.

5. At the end of incubation, add 750 mg glass beads (0.1 mm) in each tube and vortex vigorously.

6. Shake for mechanical disruption:
   a. with Bead Beater™:
      i. Turned on (medium speed) for 5 min
      ii. Stopped for 10 min
      iii. Turned on again (medium speed) for 5 min
   b. with MixerMill MM400 (Retsch): run Program 1, 10 min at 25 s^{-1} (Hz)

7. Add 15 mg PVPP (powder) per sample and vortex vigorously.

8. Centrifuge at 18,200 ×g for 5 min, 4 °C.

9. Recover the supernatant in a sterile 2 mL tube and set aside.

10. Add 500 µL TENP (resuspend before use) to the pellet and vortex vigorously. Use a toothpick if necessary.

11. Centrifuge at 18,200 ×g for 5 min, 4 °C.

12. Recover the supernatant and pool with the first.

13. Dilute sample by adding 640 µL TEN to 160 µL sample in a Sarstedt 2 mL tube. The remaining lysate can be stored at -80 °C for at least 3 months.

The dilution ratio used here 1/5 (v/v) can be modified depending on biomass concentration.

14. Turn on, prepare, and run the QIASymphony according to the manufacturer's instructions and use the protocol **COBL1200_CR23506_ID4502**. Elution will be performed in 110 µL buffer, in 0.5 mL Matrix tubes contained in a 96-well SBS rack (*« Deep well »* then *« TS#3744 2D storage tubes* »).

The QIAGEN client can install the protocol used on demand. One cartridge is enough to extract 110 samples. Users will need to plan a training with QIAGEN to learn how to use the QiaSymphony.

15. Mesure DNA concentration with a fluorometer (e.g. Qubit) and check for DNA quality with a 1 % Agarose gel.

Annex. Preparation of solutions

Phosphate buffer pH 8, 0.1 M

- 9.32 mL Na_2HPO_4 solution (14.2 g in 100 mL H_2O, dissolve on heating stirrer)
- 0.68 mL NaH_2PO_4 solution (12 g in 100 mL H_2O)
- H_2O q.s. 100 mL

Check pH with pH paper.

EDTA, 2 H_2O pH 8, 0.5 M

- 9.305 g EDTA
- H_2O q.s. 50 mL (dissolve by heating)

Adjust to pH 8 with NaOH pellet (approximately one) using a pH meter

Tris-HCL (pH 7.5, 1 M or pH 8.0, 1 M)

- 6.05 g Trizma base
- H_2O q.s. 50 mL

Adjust to pH 7.5 or 8.0 with concentrated HCl using a pH meter

NaCl 5 M

- 14.6 g NaCl
- H_2O q.s. 50 mL

TEN (50 mM Tris pH 8, 20 mM EDTA pH 8, 100 mM NaCl) / TENP (TEN + 1 % PVPP)

- 1.5 mL Tris-HCl pH 8, 1 M
- 1 .2 mL EDTA pH 8, 0.5 M
- 0 .6 mL NaCl, 5 M
- H_2O q.s. 30 mL
- For TENP, add 0.3 g PVPP powder (will not dissolve)

N-Lauroyl Sarcosine 10 %

- 2 g N-Lauroyl Sarcosine
- H_2O q.s. 20 mL

N-Lauroyl Sarcosine 5 %

- 1 g N-Lauroyl Sarcosine
- Phosphate buffer 0.1 M q.s. 20 mL

Guanidine Thiocyanate 4 M

- 23.635 g Guanidine Thiocyanate

- 5 mL Tris-HCl
- H_2O q.s. 50 mL

Shake overnight on a rocking agitator in closed flacon, protected from light by aluminum foil. Heat in dry bath or in an oven at 60-70 °C for 10 minutes if not totally dissolved. Filter through 0.2 microns Millipore filter and store at 4 °C protected from light.