Romania-Republic of Moldova
ENI-CROSS BORDER COOPERATION

GUIDELINE

for sample collection and handling

www.ro-md.net

A project implemented by
PMSI Institute of Oncology
Address: 30 Nicolae Testemitanu, street
MD-2025, Chisinau
Tel: + 373 22 852 - 303
Fax: + 373 22 733-363

This project is funded by the European Union

i. Project identification data	
Title of the Action:	Changes in human colonic microbiome in antibiotic generated stress
Project acromym:	COLONSTRESS
Lead Beneficiary:	PMSI Institute of Oncology, Republic of Moldova
EMS - ENI:	2 SOFT/1.2/105
Priority:	1.2 Promotion and support for research and innovation
Total implementation period:	24 months
Start Date:	10.10.2020
ii. Information concerning the cross border partnership	
Beneficiary no.1:	Regional Institute of Oncology Iasi
iii. Work plan by group of activities	
GA 3: Activity 3.5.	Generating a guideline for sample collection and handling
Type of deliverable:	Guideline for sample collection and handling

CONTENT

INTRODUCTION

This guideline intended to be the reference document for Colonstress project policies and procedures applies for human microbiome study. All members involved in harvesting and processing the microbiome samples should have access to the guide and be familiar with its contents.

SCOPE AND OBJECTIVE

The scope of the Colonstress project is characterization of human microbiome, focused on establishing the impact of antibiotic treatment on human colonic microbiome, in order to create a measurable proof of antibiotic induced changes.

SPONSORING/FUNDING

COLONSTRESS project is an Romania-Republic of Moldova ENI-Cross Border Cooperation Programme funded by the European Union

Responsibilities of the research team leader and core sampling sites

Both teams, from PMSI Institute of Oncology from Chisinau Moldova (PMSI IO) and Regional Oncology Institute from Iasi Romania (IRO Iasi) are involved in harvesting and handling the microbiome samples.

The protocol for sampling human microbiom was establish at the beginning of the project by collaboration between both teams and it will be respect by all team members.

The procedure it will be used only after the informed consent is signed by the patient.

PROTOCOL AMENDMENTS AND MODIFICATIONS

No changes to the study protocol will be allowed unless they have been discussed in detail and have received the concurrence of both teams' readers.

Any amendment/modification to the protocol will be adhered to by the participating center(s) and will apply to all subjects. Written approval of protocol amendments is required prior to implementation.

PUBLICATIONS AND CONFIDENTIALITY

Timely communication with the scientific community is one of the essential functions of the Colonstress project and is accomplished by the publication of manuscripts in scientific literature and oral or poster presentations at scientific meetings. Several publications and presentations will emanate from the Colonstress project.

The publication policy of this project is meant to be flexible and to facilitate rapid and accurate publication of results. Investigators are responsible for drafting the publications. Internal review of manuscripts and abstracts is necessary to ensure accuracy and consistent representation of concepts and data.

DISTRIBUTION AND UPDATES

This manual is prepared and distributed by both teams in collaboration.

This manual will be reviewed annually and updated as necessary. When the guide is updated, the new version will be posted on the project web site, accompanied by a memo describing the revisions.

REGULATORY REQUIREMENTS

STUDY CONDUCT

Colonstress project study will be conducted in accordance with its protocol, the policies and procedures described in this document, in compliance with Good Clinical Practice (GCP) as laid out in the International Conference on Harmonisation (ICH) E6 GCP Consolidated Guidance (ICH 1996), and in accordance with applicable regulatory requirements in both collaborating countries.

PROTECTION OF HUMAN SUBJECTS

Colonstress project will be conducted according to the principles of respect for persons, beneficence, and justice. This study will also embrace the principles set forth in the Declaration of Helsinki. Investigators will comply for the protection of the rights and welfare of human research subjects.

The study will also be conducted under Good Clinical Practice (GCP) as laid out in the International Conference on Harmonisation (ICH) E6 GCP Consolidated Guidance (ICH 1996).

INFORMED CONSENT

Informed consent is an ongoing process that begins with the first contact with a prospective subject and continues until the study is completed. The consent form provides information about the study, including the rights of the subject and the risks and benefits involved in participating in the study. The consent form also documents the subject's agreement to participate. All procedures, subject obligations, and subject rights should be explained to the subject in easily understood language. During the explanation of the study and during the actual study, the subject is entitled to privacy and respect. The investigator or a designee may present the information and administer the consent. The

investigator/designee should be well versed in the protocol and able to answer questions about the study procedures. The investigator/designee presenting the study should encourage the prospective subject to ask questions during this introduction to the study and anytime during his/her participation. Following the information presentation, the administrator should feel confident that the subject understands the study before the consent form is signed and before final inclusion into the study.

The consent form must be signed by the subject before participation in any study-related activities. A copy of the signed and dated consent form must be provided to the subject. Signed consent forms must remain in each subject's study file and must be available for verification by study monitors at all times.

The study protocol, informed consent documents and all types of subject recruitment or advertisement information must be submitted to the ethical commission in each partner institution for review and must be approved prior to study initiation. Any amendments to the protocol and/or consent form must also be approved by the ethical commission prior to implementing any changes in the study.

To protect the privacy of study subjects, the Study ID code list should be maintained in a secure location that is separate from the regulatory binder. Copies of any identifying documents, e.g., documents containing the subject's name, should also be maintained in a secure location that is separate from both the regulatory binder and the subjects' individual study binders.

PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the study protocol, Good Clinical Practice (GCP), or protocol-specific Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

Each investigator must adhere to the study protocol as detailed in the study protocol document. Each investigator will be responsible for enrolling only those subjects who have met all protocol eligibility criteria.

The investigator must also keep the ethical commission informed of any serious unanticipated problems, or protocol deviations resulting in serious or severe adverse events.

STUDY SITE REGULATORY DOCUMENTS REQUIREMENTS

All required regulatory paperwork must remain at the study site (as indicated in the site registration packet received prior to study start) and must be accurately maintained and may be verified during study monitoring visits.

RETENTION OF RECORDS

Records and documents pertaining to the conduct of the study, source documents, consent forms, and laboratory test results must be retained by the investigator for a minimum of three years after the project is discontinued.

DATA MANAGEMENT

SOURCE DOCUMENTATION

The source document is defined as the first place the data are recorded.

All source documents should be completed by the clinician (or other appropriate study personnel). Data entries into source documents should be made in blue or black ink. Corrections should be made with a single line through the entry and the change initialed and dated. Original entries should remain legible (i.e., they should never be erased or covered with correction fluid to obscure the original entry). Late entries, e.g., laboratory results on the Eligibility Checklist, should be initialed and dated at the time entered.

CASE REPORT FORMS

Data will be entered electronically in statistical programs in institutional computers, developed and maintained by team's members.

DATA REVIEW AND QUALITY

Project committee reviews data for quality and provides a number of quality assurance reports to ensure that study data are clean and complete.

ARCHIVING OF DATA

Records and documents pertaining to the conduct of this study, source documents, consent forms, and laboratory test results, must be retained by the investigator for a minimum of 3 years after study completion. Electronic data including clinical information, LIMS (Laboratory Information System) and sequencing data will be kept in institutional servers. No study records shall be destroyed without prior authorization from project committee.

HANDLING OF DATA TO ENSURE CONFIDENTIALITY

In order to ensure confidentiality of data the following procedures will be followed:

- Label both the source documents and the specimens with code numbers.
- Enter data from the source documents in a controlled-access database identified only by code number. Only researchers who have been approved to look at the information will be able to see this information.
- Store all source documents and consent forms in a locked file cabinet. Only members of the study team will have access to this file cabinet.

UNANTICIPATED PROBLEMS AND SERIOUS ADVERSE EVENTS

DEFINITION OF AN UNANTICIPATED PROBLEM

The methods of specimen collection in the Microbiome Sampling study pose only minimal risk to the study subjects. As defined "Minimal risk means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests." The minimal physical risks associated with the sampling procedures are described in the protocol and in the informed consent document.

REPORTING OF UNANTICIPATED PROBLEMS

Institutions engaged in human subjects research must have written procedures for ensuring prompt reporting to appropriate institutional officials, and any supporting department or agency head of any unanticipated problem involving risks to subjects.

Incidents or events that meet the criteria for unanticipated problems require the completion of an unanticipated problem report form. Investigators should include the following information when reporting an adverse event, or any other incident, experience, or outcome as an unanticipated problem:

i. appropriate identifying information for the research protocol, such as the title, investigator's name, and the project number;
ii. a detailed description of the adverse event, incident, experience, or outcome;
iii. an explanation of the basis for determining that the adverse event, incident, experience, or outcome represents an unanticipated problem;
iv. a description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the unanticipated problem.

If a local investigator at one institution engaged in the research independently proposes changes to the protocol or informed consent document in response to an unanticipated problem, the investigator

should consult with the study staff regarding the proposed changes because changes at one site could have significant implications for the entire research study.

DEFINITION OF A SERIOUS ADVERSE EVENT

An Adverse Event (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. A Serious Adverse Event/Experience (SAE) is any adverse event/experience that meets any of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in congenital anomaly/birth defect
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

REPORTING REQUIREMENTS

SAEs that are related to study participation will be reported immediately to the project staff. The ethical committee may convene an ad hoc meeting for review of the SAE and consideration of corrective actions. Other supporting documentation of the event may be requested by the ethical committee and should be provided as soon as possible. A summary of the ethical committee meeting and proposed actions will be provided to the study staff. The study staff will then submit the ethical committee review and any protocol changes to the protocol.

Serious adverse events that are considered unrelated to study participation will be provided to the ethical committee in a line listing to be reviewed at regularly scheduled meetings.

STUDY OVERSIGHT AND SAFETY MONITORING

ROLES AND RESPONSIBILITIES OF R&D TEAM

The R&D team will define its deliberative processes. These may include event triggers or a process that would call for an ad hoc review, milestones expectations, endpoint analysis, and voting

procedures. The R&D team is responsible for maintaining the confidentiality of its internal discussions and activities as well as the contents of reports provided to it.

The R&D team will review the protocol, including the oversight and risk monitoring plan, and identify any major concerns prior to implementation. During the study the R&D team will review:

1. Real-time and cumulative safety data for evidence of procedure-related serious adverse events
2. Unanticipated problems involving risks to subjects or others (for additional information
3. Adherence to the protocol
4. Factors that might affect the study outcome or compromise the study data, such as protocol violations, losses to follow-up, breach of subject confidentiality
5. Unexpected barriers, if any, to study progress or completion, such as slow enrollment, new data or findings, other milestones, change in resources, futility of endpoints.

The R&D team will process all the project steps, from the harvesting of samples to the DNA extraction, NGS testing and data analysis.

In the and R&D team has to integrate all the obtained data and process the statistical interpretation in order to provide final results.

STUDY REPORTS OVERVIEW

Reports will be prepared by the study investigators.

The teams will periodically issue a written summary report that identifies topics discussed by the inverigators and describes their individual findings, overall safety assessment, and recommendations. This would generally occur after each meeting. The rationale for recommendations will be included when appropriate. This report will not include confidential information. The project staff or designee is responsible for preparing and distributing the report.

ENROLLMENT AND STUDY ID ASSIGNMENT

ENROLLMENT

Inclusion criteria:

- Adult patient with any type of surgery involving antibioprophylaxis, including patients undergoing neoadjuvant treatment (chemotherapy, radiotherapy) after a break of 21 days

- Patient agreement and signing of informed consent for faecal sampling for processing

Exclusion criteria:

- Systemic and oral antibiotic therapy in the last 30 days (for infectious pathologies – eg. urinary tract infections)

- History of mechanical preparation of the colon in the last 30 days

- The presence of ileostomy at the time of hospitalization or if the operating protocol requires the formation of an ileostomy

- Surgery without antibioprophylaxis (eg. breast surgery, plastic surgery)

- Late resumption of intestinal transit (more than 10 days after surgery) or occlusive syndromes

SUBJECT STUDY ID

Subjects who have documented informed consent by signing a study Consent Form will be assigned study IDs sequentially as they are screened. The Study ID includes a protocol identifier, a site identifier, and a subject identifier.

Identification of samples and anonymization of patients

- Assigning an anonymized ID to each patient included in the study according to the model

- A label containing the specific ID of each patient will be applied to each sample collected.

- After harvesting, complete the technical sheet

- Anonymization will be done according to the following model O-NN-ZZLLAA-P, where:

O = city where the samples are taken, the usable letters being C (for the center of Chisinau) or I (for the center of Iasi)

NN = initials of the patient's first and last name (if the patient has several first / last names, only the first letter of the first and last name will be used, according to the order in the identity card)

ZZ = patient's birthday (if the patient is born on a single-digit day, this will be preceded by the number 0 - zero)

LL = month of birth of the patient (if he is born in a month with a single digit, this will be preceded by the digit 0 - zero)

AA = patient's year of birth (last two digits of the year will be used)

P- test number (TEST M / TEST 0 / TEST T)

Example: I-PI-17.02.79-M C-PI-17.02.79-M

Subject names or other personally identifying information should NOT be recorded on any documents.

BIOLOGICAL SPECIMENS

This section provides information to the investigators and study personnel regarding the collection and processing of clinical specimens to the proper destination. It is essential that these guidelines be followed without deviation to ensure that specimen integrity is maintained.

Biological specimens are to be collected in accordance with protocol specifications, as outlined in the Study Procedures in the protocol.

COLLECTION OF CLINICAL SPECIMENS

Taking samples

Rectal level sampling:

- With the help of a glove, fecal matter is collected from the rectum, which is applied on a 5 * 3cm filter paper (on an area of at least 2cm2). The filter paper must first be annotated with the patient's ID (directly on paper with a pencil or pen).

Samples from the colostomy:

- With the help of a glove, fecal matter is collected from the colostomy which is applied on a 5 * 3cm filter paper (on an area of at least 2cm2). The filter paper must first be annotated with the patient's ID (directly on paper with a pencil or pen).

- The samples of feces taken on the filter paper are left to dry for at least 30 minutes at room temperature (avoid positioning the samples in areas with strong air currents).

- The filter paper is then bent so that the sample is inside.

- The folded filter paper, with the dry sample, is stored in the sealable plastic envelope, specially destined for transport.

- A sticker is affixed on the plastic envelope with the patient's name and the type of sample (M, 0 or T) written on it.

- fill in the collection form

- the completed form and the dry envelope plastic envelope are placed in an A6 paper envelope which in turn is annotated with the patient's name and sample type (M, 0 or T)

- 3 fecal samples will be collected as follows:

1. control sample collected at the time of inclusion in the study (TEST M)
2. intraoperative sample collected before anesthetic induction (TEST 0)
3. test sample collected at 7-10 days postoperatively (TEST T)

METHODOLOGY FOR SAMPLING, STORAGE, DOPING AND TRANSPORT OF SAMPLES.

a. Sealable plastic bag 7 - 10cm / 4-5cm non-sterile

b. 5/3 cm non-sterile filter paper stick

c. Paper envelopes

d. Foils with self-adhesive labels with 10 boxes.

e. 1 pair of gloves, which are not mentioned in the non-sterile data sheet

METHODOLOGY FOR SAMPLING, DRYING, STORAGE, TRANSPORT OF SAMPLES:

a. Collection of 3 fecal samples, each sample being spread on a single filter paper, placed in a single sealable plastic bag and then placed in a single paper bag. A self-adhesive label will be applied over the paper envelope.

b. The sample is dried at room temperature for 15-30 minutes. After drying, it will be placed in the sealable plastic bag.

c. The envelopes will be stored in a dry storage area with normal temperature.

How to send evidence:

A copy of the data sheet will be sent with each sample

SPECIMEN PROCESSING FOR EXTRACTION OF DNA

The DNA will be extracted xith NucleoSpin Soil – Macherey Nagel protocol optimized:

Overnight incubation at 37°C on water bath with 1 mL SL2 and 40uL Proteinase K followed by 1-hour incubation at 37°C on water bath with 50 uL Lysozyme (10mg/mL) and 3 uL Lysostaphin (1mg/mL ~ 3 KU/mL) and continued with the mechanical lysis step on FastPrep Instrument 6m/sec for 40sec.

A thorough mechanical lysis step is essential to break up the crumbs, to free the cells and to break up cells and spores. Ceramic beads have proven to be most effective in combination with a bead mill, a FastPrep®-24 instrument (MP Biomedicals). The lysis time should be as short as necessary to

avoid shearing of DNA and to minimize the release of humic acids. Depending on the sample, however, it might be advantageous to increase the lysis time to 10, 20, or 30 min.

Homogenization and cell disruption should be performed at room temperature (18–25 °C) to avoid SDS precipitation in the lysis buffers. Overheating the sample, for example by prolonged bead beating in a bead mill or the FastPrep®-24 instrument, should be avoided to minimize liberation of humic acids.

Repeated extraction

For sample materials containing a high amount of microorganisms a single extraction step might not be sufficient to disrupt every cell and to release all DNA. Extracting the sample twice may help to increase DNA yield significantly. Therefore, follow the protocol until the first centrifugation in step 4. But instead of adding SL3 directly to the MN Bead Tube Type A, transfer the supernatant to a new collection tube (not provided) and complete step 4 with this supernatant.

Then repeat steps 1–4 with the same soil sample in the MN Bead Tube Type A. Filter both final supernatants of step 4 through a NucleoSpin® Inhibitor Removal Column as described in step 5. Add Binding Buffer SB to both filtrates according to step 6 and finally load both samples on one NucleoSpin® Soil Column according to step 7 in multiple loading steps.

Elution procedures

It is possible to adapt the elution method, temperature, and volume of elution buffer used for the subsequent application of interest. In addition to the standard method where an increase of DNA concentration can be achieved by reducing the elution volume from 100 to 30 µL, there are two options to increase the DNA yield:

• Heat the elution buffer to 80 °C.

• Perform two subsequent elution steps with fresh elution buffer.

How to interpret DNA yield and purity from UV-VIS

The most common method to determine the DNA yield is UV-VIS spectroscopy. The DNA concentration in the final eluate can be calculated from its absorption maximum at 260 nm (A260) based on the fact that an absorption of A260 = 1 corresponds to 50 µg/mL double stranded DNA. However, this calculation assumes the absence of any other compound that absorbs UV light at 260 nm. Any contamination with, for example, RNA, protein, or especially humic substances significantly contributes to the total absorption at 260 nm and therefore leads to an overestimation of the real DNA concentration.

Protocol – purification of DNA

Prepare sample

Cut the paper in small pieces and put them in a 1.5ml tube. Incubate overnight at 37°C on water bath with 1 mL SL2 and 40uL Proteinase K followed by 1-hour incubation at 37°C on water bath with 50 uL Lysozyme (10mg/mL) and 3 uL Lysostaphin (1mg/mL ~ 3 KU/mL). Centrifuge 2 min/2000g and transfer the supernatant into a fresh MN Bead Tube Type A containing the ceramic beads.

Important: Do not fill the tube higher than the 1.5 mL mark.

Perform mechanical lysis step on FastPrep Instrument 6m/sec for 40sec.

Precipitate contaminants

Centrifuge for 2 min at 11,000 x g to eliminate the foam caused by the detergent.

Note: The clear supernatant can be transferred to a new collection tube (not provided) prior to the following precipitation. This might result in more consistent yields from prep to prep and is highly recommended for carbonate containing samples.

Add 150 µL Buffer SL3 and vortex for 5 s.

Incubate for 5 min at 0–4 °C.

Centrifuge for 1 min at 11,000 x g.

Filter lysate

Place a NucleoSpin® Inhibitor Removal Column (red ring) in a Collection Tube (2 mL, lid).

Load up to 700 µL clear supernatant of step 4 onto the filter.

Centrifuge for 1 min at 11,000 x g.

Discard the NucleoSpin® Inhibitor Removal Column.

If a pellet is visible in the flow through, transfer the clear supernatant to a new collection tube.

Adjust binding conditions

Add 250 µL Buffer SB and close the lid.

Vortex for 5 s.

Bind DNA

Place a NucleoSpin® Soil Column (green ring) in a Collection Tube (2 mL).

Load 550 µL sample onto the column.

Centrifuge for 1 min at 11,000 x g.

Discard flow through and place the column back into the collection tube.

Load the remaining sample onto the column.

Centrifuge for 1 min at 11,000 x g.

Discard flow through and place the column back into the collection tube.

Wash and dry silica membrane

- **1st wash**

Add 500 µL Buffer SB to the NucleoSpin® Soil Column.

Centrifuge for 30 s at 11,000 x g.

Discard flow through and place the column back into the collection tube.

- **2nd wash**

Add 550 µL Buffer SW1 to the NucleoSpin® Soil Column.

Centrifuge for 30 s at 11,000 x g.

Discard flow through and place the column back into the collection tube.

- **3rd wash**

Add 650 µL Buffer SW2 to the NucleoSpin® Soil Column.

Close the lid and vortex for 2 s. Centrifuge for 30 s at 11,000 x g. Discard flow through and place the column back into the collection tube.

- **4th wash**

Add 650 µL Buffer SW2 to the NucleoSpin® Soil Column.

Close the lid and vortex for 2 s. Centrifuge for 30 s at 11,000 x g. Discard flow through and place the column back into the collection tube.

Dry silica membrane

Centrifuge for 2 min at 11,000 x g.

If for any reason, the liquid in the collection tube has touched the NucleoSpin® Soil Column after the drying step, discard flow through and centrifuge again.

Elute DNA

Place the NucleoSpin® Soil Column into a new microcentrifuge tube (not provided).

Add 30 µL (for high concentration), 50 µL (for medium concentration and yield), or 100 µL (for high yield) Buffer SE to the column.

Do not close the lid and incubate for 1 min at room temperature (18–25 °C). Close the lid and centrifuge for 30 s at 11,000 x g.

Note: Quantify DNA not only by UV-VIS but also run an agarose gel to verify yield and DNA quality (see section

SOURCE DOCUMENT INSTRUCTIONS

SPECIFIC INSTRUCTION FORMS

INCLUSION CRITERIA:

- ✓ Adult patient with any type of surgery involving antibioprophylaxis, including those undergoing neoadjuvant treatment (chemotherapy, radiotherapy) after a break of at least 21 days
- ✓ Patient's consent and signing of the informed consent to take samples of faeces for processing

EXCLUSION CRITERIA:

- ✓ Systemic and oral antibiotherapy in the last 30 days (for infectious pathologies – e.g. urinary tract infections)
- ✓ History of mechanical preparation of the colon in the last 30 days
- ✓ The presence of ileostomy at the time of admission or if the surgical protocol requires the formation of an ileostome
- ✓ Surgery without antibioprophylaxis (e.g. breast surgery, plastic surgery)
- ✓ Late resumption of intestinal transit (over 10 days after surgery) or occlusive syndromes

IDENTIFICATION OF SAMPLES AND ANONYMIZATION OF PATIENTS

- Assigning an anonymized ID to each patient included in the study according to the model
- On each sample collected, a label containing the specific ID of each patient will be applied.
- After harvesting, the data sheet is filled in
- Anonymization will be done according to the following model O-NN-ZZLLAA-P, where:

O = the city where the samples are taken, the usable letters being C (for the center in Chisinau) or I (for the center of Iasi)

NN = initials of the patient's name and surname (if the patient has more than one name / surname, only the first letter of the first name and surname will be used, according to the order in the identity card)

ZZ = the patient's birthday (if he is born on a single-digit day, it will be preceded by the number 0 – zero)

LL = the month of birth of the patient (if he is born in a month with a single digit, it will be preceded by the number 0 – zero)

AA = year of birth of the patient (the last two digits of the year will be used)

P= sample number (SAMPLE M/SAMPLE 0/SAMPLE T)

example: I-PI-17.02.79-M C-PI-17.02.79-M

TAKING THE SAMPLES

Sampling from the rectal level:

- with the help of a glove, fecal matter is collected from the rectum, which is applied on a filter paper (on an area of at least 2cm2). In advance, the filter paper must be annotated on the back with the ID assigned to the patient and the type of sample (M,0 or T), the date of sampling (directly on the paper with a pencil or pen).

Sampling from the colostomy:

- with the help of a glove, fecal matter is collected from the colostomy that is applied on a filter paper (on an area of at least 2cm2). In advance, the filter paper must be annotated on the reverse with the ID assigned to the patient and the type of sample (M, 0 or T), the date of sampling (directly on the paper with a pencil or pen).
- faeces samples taken on filter paper are left to dry for at least 30minutes at room temperature (avoid positioning the samples in areas with strong air currents).
- the filter paper then bends so that the sample is located inside (on the reverse).
- bent filter paper, with dry sample, is stored in the sealable plastic envelope, specially designed for transport.
- the plastic envelopes with the samples are placed in a paper envelope type A6 on which is applied the sticker label with 10 boxes in which the patient's ID is annotated
- 3 samples of faeces will be collected as follows:

1. the control sample collected at the time of inclusion in the study (SAMPLE M)
2. intraoperative sample collected before anesthetic induction (SAMPLE 0)
3. test sample collected at 7-10 days postoperatively (SAMPLE T).

- fill in the harvesting form in the technical data sheet
- the envelopes will be stored in a dry storage area and with normal temperature

- as the study lots are completed, the envelopes with the collected samples will be delivered to the laboratory

METHOD OF SENDING EVIDENCE:

A copy of the data sheet will be sent together with each paper envelope, which will contain 3 samples (SAMPLE M, 0 and T) in the laboratory, another copy will be kept to the researchers who took the samples.

CONSUMABLES USED IN EACH PATIENT:

a. Sealable plastic envelope 7 – 10cm / 4-5cm, non-sterile (1 sachet for each sample, 3 pieces for each patient)
b. Stick of filter paper, nonsterile (1 stick for each sample, 3 pieces for each patient)
c. Paper envelopes (1 envelope per patient)
d. Foils with sticker labels with 10 boxes (1 foil for each patient)
e. pair of gloves, non-sterile / per sample.

DATA SHEET

INSTITUTION _____

1) WITHDRAW:

Sample barcode: 1. _ _ _ _ _ _ _ _ _ _ Anonymized patient code : In I- I_II_I I_II_II_II_II_II_I - I_I Center. Patient name date of birth (DD/MM/YY) sample type	Type of sample	Date of harvest
	SAMPLE M	
	TEST 0	
	PROBA T	

2) CLINICAL DATA :

1. Age :_____
2. Sex : _____
3. Smoker ❑NO ❑ YES (average number of cigarettes/day _____)
4. Diagnostic:_____

5. Type of surgery: _____ _____

6. Preoperative preparation:

❑ enema ❑ polyethylene glycol

7. Clostridium difficile status (for the IRO IASI center):

1. GDH ❑Positive ❑Negative
2. TOXIN A ❑Positive ❑Negative
3. TOXIN B ❑Positive ❑Negative

4. Screening status of resistant multidrug bacteria (ESBL) (for IRO IASI center):
❑ POSITIVE ❑ NEGATIVE ❑ SPECIFY TYPE.....

5. Antibioprofilaxiei:
6. Type and dose of antibiotics administered:.....
7. Antibioprophylaxiei duration.....
8. Method of administration.....

ASEPTIC TECHNIQUE

The collection and processing of all samples will be done utilizing Aseptic Technique. All collection materials will be cleaned and sterile or confirmed sterile upon purchase and use.

Collection of Subject Specimens:

Follow all safety guidelines of your local safety committee and/or institutional policies for handling biological specimens. Gloves, safety glasses or face shield, and a lab coat must be worn at all times when aliquoting sera. Observe Universal Precautions guidelines when collecting any biological specimens to include at the least wearing of gloves.

When handling the specimen collection tubes during sampling, care should be taken to maintain a cleanly gloved hand, so as to not contaminate the outer or inner tube areas.

Clean disposable gloves are recommended for all specimen collections.