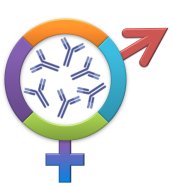
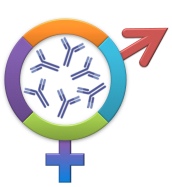
Title	  **SOP 07-Faecal sampling for analyses of microbiota analyses**
Document type	SOP
Version N.	2.0
Date	October 7th 2019

	NAME	**DATE**
PREPARED BY	Hanna Eriksson	May 15th 2019
REVIEWED BY	Nele Brusselaers	
APPROVED BY		
PURPOSE: *Description of faecal sampling procedure andmicrobiota analysis in the G-DEFINER project.*		

Acronyms:	

Related SOPs:	**SOP 3 – Study Conduct**

Appendices:	

Title	  **SOP 07-Faecal sampling for analyses of microbiota analyses**
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1. Scope and applicability

This SOP applies to the collection of faecal specimens at baseline (before treatment start T0), prior to second infusion (T1) and at the first instance of any immune related adverse event grade ≥3 (T2)for microbiota analysis andfor further RNA/DNA sequencing analysis in the G-DEFINER study. The main purpose of this SOPis to ensure the faecal samples from patients included in G-DEFINER are processed in a consistent manner at all study sites.

2. Glossary/definitions

KS: Theme Cancer, Patient Area Head/Neck, Head, Lung and Skin, Karolinska University Hospital, Stockholm, Sweden.
CTMR, KI: Centre for Translational Microbiome Research, Dept. of Microbiology, Tumor and Cellbiology, Karolinska Institutet, SciLifeLab, Stockholm, Sweden.

3. Responsibilities

KS: Hanna Eriksson, responsible PI at KS, responsible for this SOP and SOP writer.
CTMR, KI: Nele Brusselaers, Head of the Epidemiological Team.
PIs at G_DEFINER study sites: responsible for implementing SOP at own study site

4. Procedure for collection of blood specimens for SNP analysis

In G-DEFINER, faecal specimens will be collected at baseline (before treatment start, T0),prior to second infusion(T1)and at the first immune related adverse event grade ≥ 3(T3) for microbiota analysis and for further RNA/DNA sequencing in the G-DEFINER study. Collection and processing will be done according to this SOP. All samples will initially be biobanked at the sampling hospital, and transferred to the CTMR, KI, Sweden in batches at agreed timepoints as per study regulations.

4.1. Equipment list

- TheZymo RNA/DNA shield kit will be used and are provided by the CTMR to each participating center in the G-DEFINER project upon agreement.
- These kits are designed for the collection and preservation of nucleic acids from stool specimens. The stool collection tubes take a microbial snapshot of a sample while inactivating viruses making samples safe and ready for transport.

4.2. Sample collection

- Patients may deposit and collect their stool specimen within 72 hours of the planned study timepoints T0 and T1 and as soon as is appropriate for timepoint T2.
  The patients collect the stool specimens at home according to the instructions for the Zymo RNA/DNA shield kit and the samples should be shaken vigorously to ensure proper stabilization. The patient should store the stool sample ambiently at home. Samples stored in the stool collection tubes are stable at ambient temperature for a maximum of 4 weeks
  - The samples will be stored at -80°C until processing
  - The date the sample is taken and length of time sample is stored at ambient temp and -80°C should be documented.
  - See section 4.4 for instruction on dates registration in the study database.

4.3. Extraction and RNA/DNA sequencing (by the CTMR, KI):

- The extraction and analyses by 16S or multigenomics will be performed at the CTMR for all centres participating in the G-DEFINER project.
- See section 4.4 for instruction on dates registration in the study database.

4.4. Registration of the dates in the database

- Register in the study database the following dates: date of stool collection, date of freezing, date of defrost, date site lab received stool sample, date of RNA/DNA isolation, date of analysis.
- The paper DCFs "CRF 07 - Study specific blood and stool samples" may be used to collect data for a later entry in the study database.

5. Dissemination

The SOP will be disseminated according to the G-DEFINER"SOP 01 - Development, Approval and Review documents", and the PI at each site will ensure implementation of the SOP.

6. References

7. Document history

Version No.	Date	Reviewer	Details of changes
0.1	May 15th2019	ÅH	First version
0.2	June 5th 2019	RM	Graphical adjustments, modified n. According to the SOP sequence
1.0	July 30th 2019	RM	Modification "cycle 2/3 (i.e. week6)" to "second infusion" as in the study protocol
2.0	October 7th 2019	RM	Added section 4.4: Registration of dates on the database