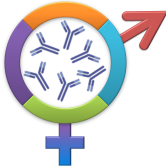




Title	  GENDER-NET Plus Promoting gender equality in H2020 and the ERA G-DEFINER  <small>This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 741874</small>
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SOP10 - Blood sampling and storing for cytokines

	NAME	DATE
PREPARED BY	Rosalba Miceli, Loris De Cecco, Licia Rivoltini	July 23rd 2019
REVIEWED BY		
APPROVED BY		
PURPOSE : Describe blood sampling and storing procedure for plasma collection to analyze cytokine/chemokine profile in future studies		

Acronyms:	
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Related SOPs:	
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Appendices:	
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1. Scope and applicability

This SOP applies to the collection of blood specimens at baseline (T0, before treatment start), at 2nd ICI infusion (T1) and at occurrence of the first irAE Grade ≥ 3 (T2) for future analyses of cytokine/chemokine profiling.

2. Glossary/definitions

3. Responsibilities

PIs at G_DEFINER study sites: responsible for implementing SOP at own study site

4. Procedure for collection of blood specimens

In G-DEFINER, blood specimens will be collected at baseline, T1, and T2 for cytokine/chemokine analysis. Collection and processing will be done according to this SOP. All samples will be biobanked at the sampling hospital.

4.1. Equipment list

- 1 x 10ml EDTA tubes (BD Vacutainer K2EDTA tube, lavender lid, Catalogue #367525).
- Centrifuge with swing out bucket rotor
- Sterile screw-cap cryotubes for plasma/buffy coat aliquots (1,5 or 2 ml) labelled according to CRF
- Filter pipette tips/Pasteur pipette
- General lab equipment

4.2. Sample collection


- Collect 7 ml blood
- Gently invert the EDTA tubes 8-10 times immediately following sample collection. Do NOT shake.
- Store the vacutainer tubes upright at room temperature (RT) until centrifugation.
- The blood samples should be centrifuged as soon as possible within 2.0 hours of blood collection, and processed into aliquots **immediately** due to cfDNA quality.
- See section 4.4 for instruction on dates registration in the study database.

4.3. Plasma preparation from the EDTA tube and storing

- Centrifuge the sample in a centrifuge with swing out bucket rotor at 1000 g for 10 min with the brake on 2, at RT.
- Collect the plasma layer with a Pasteur pipette without disturbing the buffy coat layer.
- Leave approx. 0.5 cm of the plasma layer to avoid contamination of the underlying buffy coat layer (with lymphocyte DNA).
- Centrifuge once again the plasma at 1700 g for 10 min at RT.
- Aliquot 1.0 ml plasma in 2-3 aliquots in labelled cryotubes
- Store the aliquots at -80°C.
- See section 4.4 for instruction on dates registration in the study database.

4.4. Registration of the dates in the database

- The dates of blood collection and of freezing must be registered in the study database.

Title	 <p style="text-align: center;">SOP10 - Blood sampling and storing for cytokines</p>
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- 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	July 25 rd 2019	RM,LR	First version (with Licia Rivoltini)
1.0	October 7 th 2019	RM	Added section 4.4: Registration of dates on the database