PROCESSING OF BAL, BLOOD AND PBMCs FROM COVID-19 SUSPECTED OR INFECTED PATIENTS FOR FLOW CYTOMETRY

Handling of blood and BAL (Bronchoalveolar lavage)

Refer to document "HANDLING OF SPECIMENS FROM COVID-19 SUSPECTED OR INFECTED PATIENTS"

Handling of PBMCs

- In principle, plasma and mononuclear cells obtained from blood may contain transmissible infectious agents, and should be handled in a Class II biological safety cabinet (BSC) within a Biosafety Level (BLS)-2 laboratory throughout all preparation and staining processes.
- Refer to indications given by the International Society for Advancement of Cytometry (ISAC), with the recent updates (https://isac-net.org/page/Biosafety).

Sample preparation

- Handling of any unfixed samples from patients with suspected or confirmed COVID-19 must be done in a certified Class II biological safety cabinet (BSC) within a Biosafety Level (BLS)-2 laboratory.
- All procedures with a high likelihood to generate aerosols or droplets, e.g., vortexing, mixing, sonication must be performed in a certified Class II Type A1 or A2 BSC.
- Personnel involved in sample preparation and handling of BAL, blood and/or PBMCs specimens are requested to wear a disposable laboratory coat, gloves, surgical mask, and eye protection.
- The use of two pairs of gloves is mandatory to work in BSC, so that at the end of the procedure the external layer of gloves is removed and discarded into the waste located inside the BSC containing 0.5% bleach (1/10 volume dilution of household bleach in tap water, made fresh daily).

Sample fixation

- Samples from patients with suspected or confirmed Covid-19 to be run on the cytometer should be fixed according to a process known and documented to inactivate SARS-CoV-2 and other biohazards
- E.g., treatment with 1% formaldehyde for at least 2 min resulted in 3 log reduction in infectivity in suspension tests (Kampf, Todt, Pfaender, & Steinmann, 2020).
- Fixation of SARS-CoV-infected Vero E6 cells with a fixative including formalin, glutaraldehyde, methanol and acetone for 5 min or longer eliminated all infectivity (Kariwa, Fujii, & Takashima, 2006).

Sample acquisition at the flow cytometer

- Fixed samples may be acquired only using an instrument that is located in a BSL-2 room (Cossarizza et al., 2020).
- Personnel involved in sample acquisition must wear a laboratory coat, gloves, surgical mask, and eye protection.

Instrument and workplace decontamination

- After acquisition, wash the flow cytometer for 15 min with 0.5% bleach (1/10 volume dilution of household bleach in tap water, made fresh daily), 15 min with cleaning solution and finally 15 min with deionized water.
- Clean the entire working area by using 0.5% bleach for 10 min, then 70% ethanol for 2 min.
- Discard all disposable materials (collection tubes, gloves, pipettes, tips) into appropriate biohazard containers with 0.5% bleach.
- Wipe off all work areas.

Laboratory Waste Management

- Discard the laboratory waste from testing suspected or confirmed COVID-19 patient specimens as all other biohazardous waste in the laboratory.

References

Cossarizza, A., Gibellini, L., De Biasi, S., Lo Tartaro, D., Mattioli, M., Paolini, A., … Mussini, C. (2020). Handling and Processing of Blood Specimens from Patients with COVID-19 for Safe Studies on Cell Phenotype and Cytokine Storm. *Cytometry Part A*, (3). https://doi.org/10.1002/cyto.a.24009

Kampf, G., Todt, D., Pfaender, S., & Steinmann, E. (2020). Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. *J Hosp Infect*, *104*(March), 246–251. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7132493/#

Kariwa, H., Fujii, N., & Takashima, I. (2006). Inactivation of SARS coronavirus by means of povidone-iodine, physical conditions and chemical reagents. *Dermatology*, *212*(SUPPL. 1), 119–123. https://doi.org/10.1159/000089211