Biosafety Routines & Common Rules for PCR rooms at ANA Futura

Purpose

This document describes the general rules and routines for PCR workflow at ANA Futura level 7 (Room Numbers: 71402, 71401 and 71219) that applies to all departments at ANA8.

Lab Design

71219	71401	71402
PCR (Low copy number template)	RT (cDNA) and qPCR	Pre-PCR (Clean room)

General rules and responsibilities

- Only people that have received a formal introduction to the working routines and who have read and signed the guidelines for the respective room(s), can work in these laboratories.
- All people working in these laboratories have a responsibility to read and follow the risk assessments and the lab routines described in this guideline.
- All staff have a responsibility to take care of equipment and ensure that all rooms are kept clean and organized.
- Book and log your work in sterile hoods or common equipment according to the laboratory routines.
- Some instruments and equipment require special training or certification before use, which must be approved by appropriate personnel.
- All chemicals must be registered in Karolinska Institutes chemical inventory database (KLARA) including a risk assessment form for work with the chemical. Risk assessment is responsibility of each of the groups.
- A protective coat should be worn when working in the laboratories (only Textilia white lab coats or white/blue disposable lab coats).
- Use lab dedicated shoes inside all the labs.
- Gloves should be used in all laboratories, and safety glasses when necessary.
- No eating, drinking, smoking, handling contact lenses or applying cosmetics in any laboratory.
- When entering the lab enter your name, department, date and time in the room logbook.
- Always label reagents, containers and samples properly with your name, date and content.
- Clean up according to the cleaning guidelines when the work is finished.

Responsible Persons

In order to receive a formal introduction, please contact:

Department	Division	Group Leaders	Lab-Responsible Persons	Contact Phone	Contact e-mail
LABMED	Clinical Microbiology	Ujjwal Neogi	Shuba Krishnan	0720414017	shuba.krishnan@ki.se
MedH	Hematology	Evren Alici	Alamdar Hussain	0721207911	alamdar.hussain@ki.se
MedH	CIM	Jonas Klingström	Marina Garcia	0722716843	marina.garcia@ki.se

After the introduction, send your card number to the person who introduced you along with the signed and scanned (or printed) copy of this document.

Routines

Pre-PCR room (71402)

In the Pre-PCR room, you prepare aliquots and PCR master mixes, that means everything you do BEFORE you add the template (DNA/RNA).

Procedures

- Do not bring in any DNA/RNA template in the Pre-PCR room.
- Do not bring in unnecessary materials to the Pre-PCR room.
- Do not bring in big bottles with MQ-water into the Pre-PCR room. Instead, aliquot MQ-water in smaller tubes to bring in.
- Use pens and tubes already provided in the Pre-PCR room. Do not bring it from outside.
- Clean the bench with 70% ethanol before use.
- The plastic waste cup should always have a plastic bag in it.
- When the plastic bag is full, seal it and throw it in bins outside the PCR rooms (in the big lab, 71418 for example).
- After finishing your work, remove EVERYTHING from the working area and clean with 70% ethanol.
- Use dedicated material/pipette sets for individual groups. If you want to borrow other group's pipette sets, talk to people from the respective group.
- The reagent stock should be opened inside the lab and items can be kept in the dedicated freezer inside the room. Do not bring opened reagents (which were used in another area) into the room.
- **Do not bring ice in the room from the ice machine. Use strata coolers kept in the freezer instead.**
- If you by mistake removed anything from the lab, do not bring it back without proper cleaning; for example, clean thoroughly with 70% ethanol before bringing it in again. The idea is to keep this a nucleic acid free room.

RT and qPCR room (RNA/DNA ONLY! NO Plasmid) (71401)

This room is dedicated for reverse transcriptase PCR for cDNA conversion and qPCR. Each division/group has dedicated PCR and qPCR machines.

Procedures

- The samples tubes should be opened inside the DNA and RNA workstations **ONLY** for the addition of DNA and RNA.
- If you want to perform qPCR using a plasmid DNA, use another area (like your own bench space), for adding your plasmid in the plate, and bring the plate sealed to put in the machine.
- Use dedicated material/equipment/pipette sets for individual groups. If you want to borrow other group's equipment/pipette sets, talk to people from the respective group.
- After finishing your work, remove EVERYTHING from the working area and clean with DNAzap and RNAzap, followed by 70% EtOH and switch on the UV light in the workstation.

PCR room "Low copy number DNA, NO PLASMID" (71219)

This room is dedicated to low template PCR for DNA extracted from mammalian or bacterial origin which requires nested PCR. **Amplification of plasmids is NOT ALLOWED in this room.**

Procedures

- The sample tubes should be opened inside the DNA workstations **ONLY** for the addition of DNA template.
- Use dedicated material/equipment/pipette sets for individual groups. If you want to share other group's equipment/pipette sets, talk to people from respective group.
- After finishing your work, remove EVERYTHING from the working area and clean with DNAzap, followed by 70% EtOH and switch on the UV light in the workstation.

Maintenance

In order to keep track of the number of users for each room, please fill in the logbook when you enter the room.

It is each user's responsibility to keep the rooms clean and tidy.

All instruments in the PCR rooms will be maintained by the groups' responsible for the machines.

- Refill labware supplies, such as plastics, and throw the empty boxes and plastic bags in the recycling bins.
- Each group is responsible for ordering their own reagents and kits.
- Always write date and 'sterile'/'non-sterile' on PBS and water bottles you bring into the lab. Use opened bottles before bringing new ones. If you want to have your own bottle, keep it labelled on your bench/shelf. Empty bottles should be recycled.

Waste handling

- The risk assessment, which must be completed prior to starting new experiments, shall include a description of how any hazardous waste that will be generated shall be handled. KI's rules for laboratory waste management dictate how the major types of hazardous waste from laboratories shall be handled.
- Each room has small plastic waste containers and all containers must always have waste bags.
- Laboratory waste that is not hazardous and that is not contaminated with hazardous material shall be sorted for recycling (e.g.: glass recycling, plastic recycling, combustible, etc.) (access this link: recycling at KI).

Incident report

All laboratory incidents / near accidents, with or without bodily injury, have to be reported using Karolinska Institutet's internal incident reporting system (https://ki.se/en/staff/reporting-incidents) and sometimes also to the Swedish Work Environment Authority (*Arbetsmiljöverket*) and/or to the Swedish Social Insurance Agency (*Försäkringskassan*) (www.anmalarbetsskada.se).

I hereby confirm that I have been introduced to the above described rules, that I have understood these rules and that I will follow them during my work in the ANA Futura laboratories/PCR related rooms.

Date	Name	Signature

Card number:

Responsible person for the introduction. Name:

I want to have access to the following PCR-related rooms: "check boxes"

☐ Pre-PCR room "clean room" (71402)

☐ RT & qPCR room (71401)

☐ PCR room "Low copy number DNA, no plasmid" (71219)