
Center for Clinical Studies (ZKS)

User Guidelines

(Version: March 2023)

**Established by the Veterinary Clinics of the Vetsuisse Faculty and Institute of
Veterinary Pathology, University of Zurich**

Under the direction of Prof. Dr. Regina Hofmann-Lehmann

Laboratory Managers: Dr. Katrin Helfer-Hungerbühler, Dr. Marina Meli

URL: www.zks.uzh.ch

Email: info-zks@uzh.vet

Phone: +41 44 635 88 51

Content

Content	2
General Overview	3
Laboratories	3
Code of Conduct: General Overview	4
Workspaces	4
1. Cell Culture Work:	4
2. PCR workflow:	5
3. DNA/RNA purification & quality control:	6
4. Gel Electrophoresis:.....	6
5. Western Blot and ELISA:	6
6. Flow Cytometry:	7
7. Fluorescence Microscopy:	7
8. Workspace for Cloning:	7
9. NGS workflow:.....	7
10. Alternative Workspaces:.....	7
Clean-up	7
Safety Precautions and Obligations of ZKS Users.....	8
Research Output.....	9
Laboratory Manager	10
Supplies	11
Permanent Equipment	11

General Overview

The Center for Clinical Studies (Zentrum für Klinische Studien, ZKS) is an institution primarily available to researchers of the Vetsuisse Faculty and dedicated to supporting laboratory-based clinical research. The ZKS is predominantly financed by the Vetsuisse Faculty but, due to increasing operating costs, as of 2023, there is a yearly basic charge for using the ZKS facility/equipment. Some machines and equipment originate from various institutes and are generally available to all ZKS users. The ZKS Laboratory Manager has a list of all machines and equipment and the Institutes to which they belong. The owners reserve the right to withdraw their machines/equipment from the ZKS at any time. The ZKS has a Homepage (www.zks.uzh.ch) and is new also on Teams.

During the time of the COVID-19 pandemic, the ZKS lab management had to implement measures to guarantee the safety of its users. As of August 9th, 2022, there is no obligation to wear masks in the ZKS except for room TFA 00.01 (DNA Lab). The reason to keep this restriction in the TFA 00.01 room is that currently SARS-CoV-2 diagnostics is performed in the centre, therefore this measure aims to avoid possible contaminations of the samples. Researchers running SARS-CoV-2 molecular diagnostic assays are advised to wear a mask during their entire workflow (sample preparation, master mix preparation, PCR setup) also in the other labs.

The ZKS lab management implemented an online room booking system on the ZKS homepage: <https://www.zks.uzh.ch/en/ZKS-Booking-System.html>. This link only works if you are in the University network or using VPN to get into the University network. In this room booking system, you will find three areas/calendars: one for booking the equipment (ZKS-Instruments), one for CO₂ incubators and one for reserving ZKS-Workspaces.

Booking of the equipment is obligatory for the ABI 7500 Fast instruments and recommended for all other instruments. Routine diagnostics and exceptional projects will have some priority.

Laboratories

The following laboratories are currently available: TFA 00.01 (DNA Lab), TFA 00.02 (Histology Lab), TFA 00.03 (PCR Lab), TFA 00.04 (Immunology Lab), TFA 00.05 (Bone Lab), TFA 00.06 (RNA Lab), TFA 00.08 (Cell Culture Lab), TKL 01.08 (Gel Electrophoresis Lab) and TKL 01.09 (Molecular Biology Lab).

Code of Conduct: General Overview

The ZKS is used by several users simultaneously on most working days. On individual days, however, certain users or work groups will have to cope with an increased workload. To avoid parallel use of fixed equipment such as extraction robots or PCR thermal cyclers, users should register in advance in the available agendas (on paper and/or online). The ZKS must function as a cooperative work area. It is therefore of great importance, not only for the allocation of workstations but also when ordering materials, that individual users seek to talk to and contact other users of the ZKS if problems arise. In the case of double occupancy, the users should therefore coordinate by mutual agreement. The laboratories of the ZKS are biosafety level 2 (BSL2), therefore access is only permitted to authorized persons via batch. Researchers should send an application and, after completion of a lab introduction about biosafety and GLP rules, access will be granted.

The users have the opportunity to receive support for the design and conception of their experiments. The implementation and financing of the projects are the responsibility of the users and their supervisors. As of 2023, there is a yearly basic charge for using the ZKS (<https://www.zks.uzh.ch/en.html>).

The user is asked to contact the Laboratory Manager also at the end of the research project and/or if the user wishes to not work in the ZKS any longer.

Workspaces

The ZKS was designed to include different fields of medical research, which include:

1. Cell Culture Work:

There are three laminar flow hoods available in lab TFA 00.08 for cell culture work: the one on the left and the one by the window must only be used for non-infected cell culture work;

the middle hood is equipped for infected cell culture work. Four CO₂ incubators are available. A Hettich (Rotina 420R) refrigerated centrifuge is available for centrifugation and must be balanced using a scale. Cell culture media should be stored in the cell culture refrigerator, which is immediately adjacent to the left laminar flow hood. The bottles must be labelled with the user's name, contents and the date when the medium was made. The bench top must be cleaned before and after each use, first with RNase Away followed by 70% ethanol, and then exposed to ultraviolet (UV) light for at least 20 minutes. The laminar flow hood shield must not be cleaned with cleaning solutions or 70% ethanol. Instead, it is cleaned only with RNase Away solution and double-distilled water (the container for the latter is located in lab TFA 00.04).

2. PCR workflow:

Several workspaces are reserved for PCR work. It is not permissible to use these spaces for any other type of work.

Sample preparation (RNA/DNA/TNA isolation) for PCR is done in labs TFA 00.06 (RNA) and TFA 00.01 (RNA/DNA/TNA). The designated workplaces must be used for each step of the process. All PCR Master Mixes (including the TaqMan Master Mixes) must be pipetted only in the laminar flow hood designated for this purpose (TFA 00.03). The laminar flow hood must be cleaned first with RNase Away, followed by 70% ethanol, and then exposed to UV light for 20 minutes before and after pipetting. The next step, which is the addition of the DNA or cDNA as well as RNA sample, must be carried out in the laminar flow hood designated for infected cell cultures (middle hood, TFA 00.08). RNA can also be pipetted in the laminar flow hood in the RNA lab (TFA 00.06). All materials used for pipetting the Master Mix (reagents and pipettes) must be cleaned away beforehand. Aerosol tips should be used for pipetting of samples.

PCR amplification is done in lab TFA 00.03 (real-time, TaqMan as well as conventional PCR). Reservations for the real-time PCR cycler must be made online in the room booking system area called: ZKS-Instruments. Please always add your name and phone number. In addition, lists are available to record any error messages for each real-time PCR instrument. This allows fast detection and thus troubleshooting of errors caused by a device.

3. DNA/RNA purification & quality control:

A workspace is reserved in lab TFA 00.06 (laminar flow hood) exclusively for manual RNA extraction and reverse transcription. Under no circumstances are the pipettes and heating block to be removed from this workplace. RNA extraction using the Silica method or any other method must be done in the RNA workspace.

DNA extraction using the Maniatis method or Qiagen extraction kits should be carried out in lab TFA 00.01 using the laminar flow hood.

In addition to conventional extraction methods, the ZKS also has two machines for automatic extraction of DNA/RNA and TNA from clinical samples: two MagNA Pure LC from Roche Diagnostics (lab TFA 00.01). Anyone wishing to use the devices for the first time must contact the Laboratory Manager beforehand.

A tissue homogenizer Precellys 24 instrument is available in the DNA lab (TFA 00.01) for the homogenization of tissues and possibly small arthropods, such as ticks, fleas and lice, before DNA or RNA extraction. Anyone using this machine for the first time must contact the Laboratory Manager beforehand.

For the determination of DNA and RNA concentration, there is a spectrophotometer (EPOCH2TC, 200 to 999 nm), which also measures protein concentration and runs ELISA and kinetic assays, in lab TFA 00.08. In addition, there is a NanoDrop 2000c spectrophotometer in lab TFA 00.20 and a Qubit 4 fluorometer in room TKL 01.08.

4. Gel Electrophoresis:

After amplification, the PCR products are separated by gel electrophoresis in lab TKL 01.08. Under no circumstances are the gels to be stained with ethidium bromide: Other staining methods such as EZ-Vision and Red-safe are recommended. Two gel documentation systems (Ugenius 3 and a Fusion Solo 6S EDGE V.070) are available for imaging of DNA and protein gels as well as western blots.

5. Western Blot and ELISA:

The workspace for western blot and ELISA is in the Immunology lab (TFA 00.04). The spectrophotometer (EPOCH2TC, 200 to 999 nm) is in TFA 00.08. The imaging system for western blot (Fusion Solo 6S EDGE V.070) is located in TKL 01.08

6. Flow Cytometry:

Sample preparation for flow cytometry is also done in lab TFA 00.04. Flow cytometers are located in the Institute of Virology (ask for the person in charge) or can be booked in the cytometry Facility in the Irchel Campus (<https://www.cytometry.uzh.ch/en.html>).

7. Fluorescence Microscopy:

A fully automated fluorescence microscope (EVOS FL Auto) for the analysis of cells in cell culture is located in the darkroom inside the immunology laboratory (TFA 00.04A).

8. Workspace for Cloning:

Cloning, plasmid replication and purification, restriction analysis, preparation of DNA standard curves and their addition to PCR Master Mixes are carried out exclusively in lab TKL 01.09. **Materials required for this lab are prepared separately, marked „MB“ and are not to be used in any other ZKS labs.**

9. NGS workflow:

Qubit 4 Fluorometer and an Agilent Fragment Analyzer 5200 provide reliable quality control, especially for Next Generation Sequencing (NGS) applications as well as accurately measure DNA, RNA and protein quantity. Both devices are located in the TKL 01.08.

10. Alternative Workspaces:

Work that does not fall into any of the previously mentioned categories can be carried out in two workspaces reserved for this purpose: TFA 00.08 and TFA 00.01 by the window. Users must contact one another in person or by telephone to avoid double bookings of the work areas.

Clean-up

The *workplace must be cleaned* before leaving. Glassware must be washed, rinsed, dried and placed in the cupboard. For this purpose, there is a dishwasher and drying cabinet in the lab TFA 00.08. The sink is not to be used as a receptacle for lab materials.

Reusable plasticware such as racks should be decontaminated by placing them in the bleach bath overnight in lab TFA 00.08 or TKL 01.09. Once decontamination is complete, plasticware must be rinsed with deionized water from the appropriate tap and washed in the dishwasher. A container for glass waste is located in lab TFA 00.06. Each workplace is equipped with garbage pails and bags as well as paper towels and disposable gloves. When paper towels and disposable gloves run out, they must be restocked by the user; supplies are located in the hall cupboard (gloves) and in the PCR-lab (TFA.00.03) first cupboard to the left (paper towels).

Lab workspaces and laminar flow hoods must be cleaned as thoroughly as possible after use (RNase Away, 70% ethanol, UV light). However, the hood shields must be cleaned with RNase Away solution and deionised water. The shields are not to be cleaned with solvents or 70% ethanol.

Biowaste (everything associated with clinical samples, infected cell cultures and microorganisms) must be disposed of in the „Biohazard“ containers, which are white waste containers located in labs TFA 00.01, TFA 00.06, TFA 00.08 and TKL 01.09. These containers are not emptied by the cleaning staff. Everyone who uses them has a financial responsibility for their proper disposal. Biowaste disposal is very expensive. Therefore, only true biological waste belongs in these containers.

Touching telephones and door handles while wearing disposable gloves is not allowed.

At the end of your project, please plan permanent storage for potentially important samples with your group leader. Before leaving, all your samples and supplies/materials must be removed and all data deleted from the computers.

Safety Precautions and Obligations of ZKS Users

Future ZKS researchers must register their project and follow-up projects (short description) on the homepage (www.zks.uzh.ch) to use the ZKS. The Laboratory Manager keeps a list of the projects running in the ZKS. Every user of the ZKS is obliged to contact the Laboratory Manager before using the premises and equipment for the first time. The most important points regarding the use of the laboratories and equipment are shown during a mandatory ZKS introduction. After the introduction access will be provided by

batch. The user should again contact the Laboratory Manager at the end of the research project.

All activities at the ZKS are subject to the **work and biosafety regulations** (BSL2).

This includes:

- Wear gloves and lab coat (and safety glasses when required) - remove gloves/lab coat and wash/disinfect hands when leaving the laboratory
- No food/drinks and pets in the lab
- Decontaminate work surfaces
- Use the biosafety cabinet to contain aerosols
- Use centrifuge safety buckets and aerosol-tight caps or lids to contain aerosols when working with patient samples
- Biohazard (clinical samples, infected cell cultures & microorganisms) needs to be placed in designated „Biohazard“ containers
- Access only to registered personnel – access by batch will be provided after the ZKS introduction

Laboratory incidents and accidents must be reported to the Laboratory Manager immediately.

Research Output

To guarantee further financial support by the Vetsuisse Faculty and to enable future investment in new technologies, the Center must provide a scientific justification.

Therefore, the users of the Center for Clinical Studies are required to **include the Center for Clinical Studies in their affiliation** in addition to their department in scientific publications that arise from work performed completely or in parts at the Center for Clinical Studies. Authors should also include the following sentence in the acknowledgement section:

“The laboratory work was (partly) performed using the logistics of the Center for Clinical Studies at the Vetsuisse Faculty of the University of Zurich”.

The users should **deliver a list of all such publications to the Laboratory Manager**, who maintains a list of publications of the Center for Clinical Studies. Publications with ZKS affiliation must be listed in ZORA

(<https://www.zora.uzh.ch/view/subjectsnew/11434.html>), and the ZKS needs to be added as Communities & Collections. ZKS lab managers will check the ZKS ZORA entries and if necessary, ask the ZORA redaction to link the ZKS affiliation to the publication.

Laboratory Manager

The Laboratory Manager is hired to run the ZKS and has the following duties:

1. The Laboratory Manager provides technical and scientific *information and advice* for all users of the ZKS with regard to research planning, work protocols, materials, supplies, etc. The Laboratory Manager is the contact person for individuals involved in clinical research.
2. The Laboratory Manager is responsible for *introducing new users* to the ZKS. The introduction includes the provision of a copy of the ZKS Guidelines, an explanation of biosafety rules and good labor practice (GLP), introductions to other users of the ZKS, instructions for use of the necessary permanent equipment and allocation of storage rooms for personal materials. In addition, the Laboratory Manager keeps a list of all ZKS users and their telephone numbers.
3. The Laboratory Manager organises the basic supplies for the ZKS. This includes materials that do not have to be supplied or purchased by individual users (Tela paper towels, gloves, rolls of absorbent paper, pipette tips, etc.) and general cleaning actions involving all the users.

4. The Laboratory Manager is responsible for the *permanent equipment/machines* in the ZKS. This includes equipment maintenance (provided that this is possible) and arranging for a service call when a malfunction occurs. The Laboratory Manager is also responsible for coordinating work on the larger machines, such as the ABI TaqMan 7500 Fast, Corbett Rotorgene 6000, Roche MagNA Pure LC, Agilent Fragment Analyzer 5200, Laminar Flow Hoods, thermocyclers and scales.
5. The Laboratory Manager is responsible for maintaining safety and biosecurity (BSO) in the ZKS and serves as the contact person for questions concerning chemical and biological waste.
6. The Laboratory Manager is responsible for the ZKS Homepage (<https://www.zks.uzh.ch/en.html>).
7. The Laboratory Manager keeps a list of projects running in the ZKS.
8. The Laboratory Manager maintains an inventory of current equipment.
9. The Laboratory Manager is responsible for evaluating new equipment purchases for the center. These new acquisitions need to be discussed with the steering committee.

Supplies

The ZKS runs on the principle that users provide all their reagents and kits including supplies/materials needed for their experiments. Users are responsible for purchasing all the materials needed for their project and for all refills.

Supplies must be clearly marked with name and date. Unlabeled and old reagents will be disposed of by the lab managers from time to time.

The ZKS organizes the basic supplies including Tela paper towels, latex and nitrile gloves, rolls of absorbent paper, and pipette tips.

Permanent Equipment

The following are considered permanent equipment:

- **Real-time PCR-Cyclers:**
 - **ABI PRISM 7500 Fast TaqMan, SDS Software Version 2.3. (No. 275011639, owned by ZKS):** TFA 00.03, left by the window. Real-time PCR machine. Reservations for the real-time PCR cyclers must be made online: area in the room booking system called: ZKS Equipment
 - **ABI PRISM 7500 Fast TaqMan, SDS Software Version 2.3 (No. 275010813, owned by VetMedLab):** TFA 00.03, middle in the room. Real-time PCR machine. Reservations for the real-time PCR cyclers must be made online.
 - **ABI PRISM 7500 Fast TaqMan, SDS Software Version 2.3 (No. 275014998, owned by ZKS):** TFA 00.03, right in the room. Real-time PCR machine. Reservations for the real-time PCR cyclers must be made online.
 - **Corbett Rotorgene 6000 (owned by VetMedLab):** TFA 00.03. Real-time PCR machine. A sign-up sheet is available for reservations.
- **PCR-Thermocyclers:** TFA 00.03. 1x gradient thermocycler (Biometra Tgradient, owned by VetMedLab); 2x thermocycler (Biometra Tpersonal, owned by ZKS). It is a good idea to consult with other users of the thermocyclers to ensure they are not needed by more than one party at a given time. There is also a sign-up sheet to make a reservation.
- **Roche MagNA Pure LC (2.0) (owned by ZKS):** TFA 00.01. DNA/RNA extraction robot for 8 to 32 samples. A sign-up sheet is available for reservations.
- **Roche MagNA Pure 96 (owned by ZKS):** TFA 00.01. A high-throughput instrument for automated nucleic acid purification of 24 to 96 samples.
- **Corbett CAS 1200 (owned by ZKS):** TFA 00.01. Pipetting robot.
- **Tissue homogenizer Precellys 24:** TFA 00.01. For homogenization of tissues and small Arthropods before RNA/DNA/TNA purification.
- **Lorrca® MaxSis Osmoscan (owned by ZKS):** TFA 00.06. Laser Optical Rotational Red Cell Analyzer

- **Spectrophotometer EPOCH2TC (owned by ZKS):** For measurement of DNA, RNA and protein concentration, ELISA and Kinetics. The spectral range is 200-999 nm.
- **Spectrophotometer Synergy H1 (owned by Vetlab):** multi-mode Microplate Reader (UV-Vis absorbance; Fluorescence intensity; Luminescence).
- **Qubit 4 Fluorometer (owned by ZKS):** TKL 01.08. For measurement of DNA, RNA- and protein concentration and quality.
- **Spectrophotometer NanoDrop 2000c (owned by VetMedLab and Pharmacology):** TFA 00.20. For measurement of DNA, RNA- and protein concentration.
- **Fluorescence-Microscope EVOS FL Auto (owned by ZKS):** TFA 00.04A
- **Various Refrigerators (owned by ZKS and VetMedLab):** TFA 00.01, TFA 00.04, TFA 00.06, TFA 00.08, TLKL 01.08., TKL 01.09. In TFA 00.08 the refrigerator beside the laminar flow hood is reserved for cell culture.
- **1 combination refrigerator/freezer (owned by Pathology):** TFA 00.02. The freezer portion belongs exclusively to Pathology.
- **Various Freezers (-20°C) (owned by ZKS and VetMedLab):** TFA 00.01, TFA 00.03, TFA 00.04, TFA 00.06, TFA 00.08, TKL 01.08, TKL 01.09
- **BioBenches and Laminar Flow Hoods:** BioBenches and laminar flow hoods should be reserved online. It is important to remember that the ultraviolet light must be turned on for 20 minutes before the start and after the completion of work. The workspace must be left absolutely clean. Only the minimal equipment needed should be used in the flow hood to keep the airflow working correctly, otherwise, protection from contamination (in both senses) is not guaranteed. Especially the air grids should always be kept free. Coordination directly among users is required when more than one research group wants to use one of these workspaces.
- **CO₂ Incubators (owned by ZKS and small animal medicine):** TFA 00.08. A total of four incubators are available for infected and non-infected cultures. All incubators can be booked on the ZKS homepage (<https://www.zks.uzh.ch/en/ZKS-Booking-System.html>).

-
- **Fume Hoods:** Two fume hoods are available for work with volatile compounds and are located in labs TFA 00.06 and TFA 00.02. Before starting any work, ensure that the exhaust is functioning. After work is completed, close the glass shield of the fume hood.
 - **Centrifuge Use:**
 - **Hettich Refrigerated Centrifuge (owned by VetMedLab):** TFA 00.08. There are special inserts for this centrifuge, which are located in a box under the centrifuge bench. Every user is responsible for accurately balancing the centrifuge. The scale for ensuring this is located in lab TFA 00.06.
 - **Different Hettich and Eppendorf Universal and tabletop centrifuges:** Samples must be accurately balanced when using these centrifuges.
 - **Sorvall Discovery 90 Ultra-Centrifuge:** TFA 00.04. Requires very accurate balance.
 - **Pipette Sets (owned by ZKS):** At least 7 sets of pipettes are reserved for special work.
 - One set is reserved for the Master Mix Preparation of the PCR cocktail.
 - A second set of pipettes is located at the workspace called „Sample Preparation“ of PCR samples. This set must only be used with pipette tips that have special filters because they are for DNA/RNA work and there is the risk of contamination.
 - A third set of pipettes is located at the „Flow Cytometry“ workspace and can also be used for other work.
 - A fourth set of pipettes consisting of a 20 µl pipette and a 200 µl pipette are located beside the gel electrophoresis and are to be used for loading the gel. All pipette sets are in marked pipette stands, which are labelled by their workplace designations.
 - A fifth set of pipettes is reserved for cell culture and is located in the Biobench or the laminar flow hood. This set is to be only used for cell culture work.
 - A separate set of pipettes is available for RNA extraction and reverse transcription.
 - The pipettes are not to be removed from the ZKS or used for other work. Users are free to bring their own set of pipettes for their use.

- **Mettler Scale PB 303 and OHAUS Analytical Scale:** These scales are available for various uses. The measuring plate of the scale must be cleaned after every use; the glass lid must be removed for this purpose.
- **Agarose Gel Electrophoresis:** TKL 01.08. The combs, glass plates, chambers and other materials must be rinsed with deionized water and thoroughly dried immediately after use.
- **Gel Documentation System Ugenius3 (owned by ZKS):** TKL 01.08. For UV and VIS.
- **Multipurpose Imaging System Fusion Solo S (owned by ZKS):** TKL 01.08. A platform for Chemiluminescence-, Bioluminescence- and Fluorescence (SPECTRA Capsule-Blue-480 nm, SPECTRA Capsule-Green-530 nm, SPECTRA Capsule-NIR-640 nm).
- **Agilent Fragment Analyzer 5200 (owned by ZKS):** TKL 01.08. Parallel capillary electrophoresis instrument for nucleic acids quality control. A sign-up sheet is available for reservations.
- **Sink Area:** This area is available for washing used glass- and plasticware. Once the glassware is dry, it must be placed in the hall cabinet and not left to collect dust in the drying cabinet. A Dishwasher is available in the lab TFA 00.08.
- **Racks:** A variety of plastic and metal test tube racks are available for use in the ZKS. They are not to be removed from the ZKS or used for storage of samples in the refrigerator or freezer. After every use, the racks must be soaked in 3% bleach (grey container beside the sink in lab TFA 00.08 and TKL 01.09) to prevent contamination. Plastic racks are soaked overnight but metal racks are soaked only briefly because they rust otherwise.
- **Water Tanks and Distilled Water:** These tanks must be refilled by the user when the water level reaches the lower water line mark. The distillation apparatus (Water purification system: ELGA) is located in lab TKL 01.09.