IOCB Emergency Plan For GMO Laboratories

(Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences - Research Areas: Biochemistry and Molecular Biology[a], Biochemical Pharmacology[b], and the Laboratory of Structural Biology[c])

pursuant to § 9 of Act No. 209/2004

English version[*)]

a) Addresses for contained use of GMOs

Place of business and ID of the Institute:

Institute of Organic Chemistry and Biochemistry of the CAS (IOCB)
Flemingovo nám. 542/2, Prague 6, 160 00
ID: 61388963
VAT: 006-61388963

Director of the Institute:

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Expert advisor:

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[*)] Wording of the document "DRAFT: L29 The Genetically Modified Organisms (Contained Use) Regulations 2014: Guidance on regulations" (http://www.hse.gov.uk/pubns/priced/l29.pdf) is used where topical; the meaning of certain terms is also explained therein.

b) Specification of workplaces and facilities for contained use of GMOs

Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, v.v.i., Flemingovo náměstí 542/2, Prague 6, 160 00

City: Prague, municipal district: Prague 6, cadastre: Dejvice

Parcel numbers: 638/1, 641/2; land registry number: 542

Address: Flemingovo náměstí 542/2, Bechyňova 542/2, Stavitelská 542/1, Zelená 542/19

c) Workplace maps

(see Addendum 2)

IOCB research areas: Biochemistry and Molecular Biology, Biochemical Pharmacology, Laboratory of Structural Biology; work with bacteria *Escherichia coli,* yeasts, baculoviruses, and mammalian cell lines is performed in laboratories A.01.11, A.01.13, A.01.55, A.01.57, A.01.62, A.01.63, A.01.64, A.01.78, A.01.79, A.1.06, A.1.21, A.1.22, A.1.23, A.1.24, A.1.34, A.1.47, A.2.28a, A.2.28b, A.2.29, A.2.48, A.2.92a, A.2.97 in building A and in laboratories C.1.03, C.1.04, C.1.05, C.1.06, C.1.20, C.2.05, C.2.07, C.2.08, C.2.09, C.2.10, C.2.16, C.2.17, C.2.18, C.2.20, C.3.05, C.3.07, C.3.08, C.3.09, C.3.10, C.3.16, C.3.17, C.3.18, C.3.20, C.4.05, C.4.07, C.4.08, C.4.09, C.4.10, C.4.16, C.4.17, C.4.18, C.4.20 in building C.

d) Description of potential accidents in contained-use workplaces

An accident is any significant unintentional release of a GMO from containment. In such cases, appropriate steps must be taken immediately. The various uses of GMOs are categorized according to their risk assessments, and evaluation of incidents depends on the actual risk assessed. There is no danger of harm in a release of a GMO from containment if the contained use falls under Risk Class 1, and cleanup of the released GMO may be carried out with common laboratory techniques. A small release of a GMO is not considered an accident if the released GMO is thoroughly eliminated within a locked workplace; however, the spread of a GMO beyond the premises of an IOCB Biochemistry and Molecular Biology research area designated for contained use of GMOs must be considered an accident.

The following types of accidents may potentially occur on IOCB premises while working with GMOs:

(i) a spill of a larger quantity of microbial culture containing plasmid DNA: wearing protective gloves, workers must dry the affected site and subject the waste to steam-sterilizer inactivation; the site must then be thoroughly treated with two different disinfectant solutions, e.g. Ajatin followed by Chloramin;

(ii) when glass or other cultivation vessel with in vitro cultures containing transgenes breaks, the procedure is the same as that described above in section (i). Potential harm relates to bacteria and yeast only, since tissue cultures cannot survive after spreading into nonsterile conditions.

After inactivation with a disinfectant agent (SAVO, Ajatin, or 2% solution of Chloramin) or in a steam sterilizer, the waste must be further processed by specialist company SITA Bohemia a.s. as waste per catalogue number 18 01 03: 'waste of special concern for prevention of infections during collection and elimination' pursuant to Act No. 381/2001 Coll. and Act No. 185/2001 Coll.).

e) Survey of potential impacts of accidents on human health, animal health, environmental and biological diversity, including methods for assessing impacts and effective prevention

Scientific research at IOCB is carried out solely with genes known to not have adverse consequences for human health, animal health, or biological diversity. Among the selected

genes, those conferring resistance to antibiotics are used. Nonetheless, infection laboratory practices are appropriate.

f) Validated techniques for detecting presence of GMOs

The basic method for detecting transgene DNA is amplification of a segment of the relevant gene with PCR in the presence of specifically designed primers, followed by DNA sequencing. Directions for this type of detection are provided in Addendum 1. The affected site must be monitored by means of microbiological cultivation and assessment of the presence or absence of plasmid vectors. If they are determined to be present, the site must be cleaned with an alternative disinfectant over a larger area.

g) Validated methods and techniques for inactivating GMOs or genetic products and decontaminating the containment workplace

In the event of a release of GMOs, the affected site must be treated with Ajatin followed by sodium hypochlorite. Following chemical decontamination, the room must be exposed to the UV light of a germicidal lamp. Small items must undergo decontamination in a steam sterilizer. Monitoring of the decontaminated site is performed as described above in section f) as well as in Addendum 1.

h) Methods for isolating premises and equipment affected by an accident and methods for assessing isolation efficacy

Under the present circumstances, there is no reason to expect an accident requiring isolation of premises or equipment. The absence of GMOs is assessed by means of a sample collection and PCR evidence of the absence of certain transgenes.

i) Description and illustration depicting placement of decontamination agents for inactivating GMOs or genetic products as well as for decontaminating workplaces

Decontamination agents: 2% solution of sodium hypochlorite; SAVO; Ajatin
Placement: in properly labelled chests in all GMO workplaces
Because placement varies from one room to the next, an illustration is not provided.

j) Measures for protecting human and animal health and the environment in cases of adverse impacts of an accident or, if need be, methods for eliminating or sanitizing plants and animals present in the accident site at the time of the accident pursuant to relevant regulations

N/A.

k) Description of procedures for monitoring premises and areas following sanitation

The absence of GMOs must be assessed by means of a sample collection and PCR evidence of the absence of certain transgenes.

l) Municipalities and/or persons notified about this Emergency Plan pursuant to § 20, part 3 of Act No. 78/2004 Coll.

Copies of this Emergency Plan (original Czech version) have been submitted to:
- the Transportation and Environment Department of the Prague 6 municipal district
- the Population Protection and Crisis Management Division of the Prague fire rescue service
- the Security Department of Prague City Hall

m) Means of notifying the competent authorities listed in § 27 of Act No. 78/2004 Coll. in the event of an accident and means of warning the public depending on the site and potential consequences of the accident

In the event of an accident, the Ministry of the Environment, the Ministry of Health, and the Czech Environmental Inspectorate must be notified. Notifications must be conveyed by email and then confirmed in writing. No steps shall be taken to warn the public.

n) Opinion of the Expert Advisor
Agreed (original Czech version dated 7 February 2022).

This English version of Emergency Plan has the force of an IOCB internal regulation. It comes into force on the day of authorization by the Director and shall remain in force until retraction by the Director.

Compiled by:

RNDr. Milan Kožíšek, Ph.D.

Authorized on 18th January 2023

Prof. RNDr. Jan Konvalinka, CSc.

Addenda:
1. Procedure for detecting the presence of GMOs
2. Workplace maps

Addendum **1:**
Procedure for detecting the presence of GMOs

Detection of air contamination

The method is used to monitor the release of genetically modified organisms (GMO) into the space. The recommended interval for conducting the procedure is once every 6 months as well as immediately following any extraordinary event. It is carried out by distributing open Petri dishes with an agar medium in a closed area of the GMO workplace. The composition of the medium in the agar dishes should be specific to the tested microorganisms that are being worked with and for which there is suspicion of a release with an antibiotic selected for GMOs with a plasmid carrying a genetic modification. Open dishes with the agar medium must be placed in the closed area where the suspected release has occurred. After 15 minutes, the dishes must be covered and left to incubate for 1 day in a thermostat at 37 °C or 30 °C depending on the microorganism. If colonies are present, all surfaces must be thoroughly disinfected with disinfectants.
In the event of a repeated presence of colonies in the area where Category 2 GMOs are being handled, genetic modification of microbial isolates grown on Petri dishes must be ruled out using the PCR method and appropriate primers. In the case of a positive PCR reaction, effective inactivation and disposal of the detected GMO must be achieved.

Detection of contamination of surfaces

The method is carried out by wiping the monitored surface with sterile gauze and then spreading the smear on Petri dishes with a suitable agar medium (just like when detecting air contamination). If colonies are present, the procedure is the same as when detecting air contamination.
It is also possible to directly inoculate the nutrient LB medium with a suitable antibiotic with a smear. A medium inoculated in this way is subsequently incubated at 37 °C overnight with shaking in an incubator. If a bacterial suspension is grown, the presence of GMOs is determined by PCR. In the case of a positive PCR reaction, the surface must be thoroughly disinfected with disinfectants.

Detection of GMOs by polymerase chain reaction (PCR)

The presence of GMOs is detected by polymerase chain reaction using specific primers derived from sequences of inserted DNA inserts.

Sample test for the presence of modified DNA using PCR:

Reaction mixture composition

Component	**Volume (µl)**
sterile water	31
5x conc. buffer for Phusion High-Fidelity DNA Polymerase	10
10 mM dNTP mix	1
DMSO	1.5
10 µM primer 1 for detection of GMO DNA ("forward primer")	2.5
10 µM primer 2 for detection of GMO DNA ("reverse primer")	2.5
Phusion High-Fidelity DNA Polymerase	0.5
suspension of the tested colony in sterile water	1

Automatic cycler program

1) 98°C/30 s
2) (98°C/10 s – 65°C/30 s – 72°C/90 s) repetition 30x
3) 72°C/8 min

The PCR amplification product is visualized and examined by means of electrophoretic analysis. Amplified sections of DNA can be further precisely characterized via sequencing and comparison with the sequence of the predicted modified section in the GMO.

Example of basic primers for detection of certain GMOs by PCR:

Primers for genes cloned into bacterial vectors:

T7 prom: 5′ TAATACGACTCACTATAGGG 3′
T7 term: 5′ GCTAGTTATTGCTCAGCGG 3′

Primers for genes cloned into vectors for Pichia pastoris:

5' AOX1: 5′ GACTGGTTCCAATTGACAAGC 3′
3' AOX1: 5′ GCAAATGGCATTCTGACATCC 3′

Primers for genes cloned into vectors for Saccharomyces cerevisiae:

Matchmaker 5′ADL: 5′ CTATTCGATGATGAAGATACCCCACCAAACCC 3′
Matchmaker 3′ADL: 5′ GTGAACTTGCGGGGTTTTTCAGTATCTACGAT 3′

Primers for genes cloned into Leishmania tarentolae vectors:

sat F: 5′ CCTAGTATGAAGATTTCGGTGATC 3′
ssu R: 5′ CTGCAGGTTCACCTACAGCTAC 3′

Primers for genes cloned into baculovirus vectors:

Bac1: 5′ AACCATCTCGCAAATAAATA 3′
Bac2: 5′ ACGCACAGAATCTAGCGCTT 3′

Primers for amplification of DNA segments cloned into the SV40 vector:

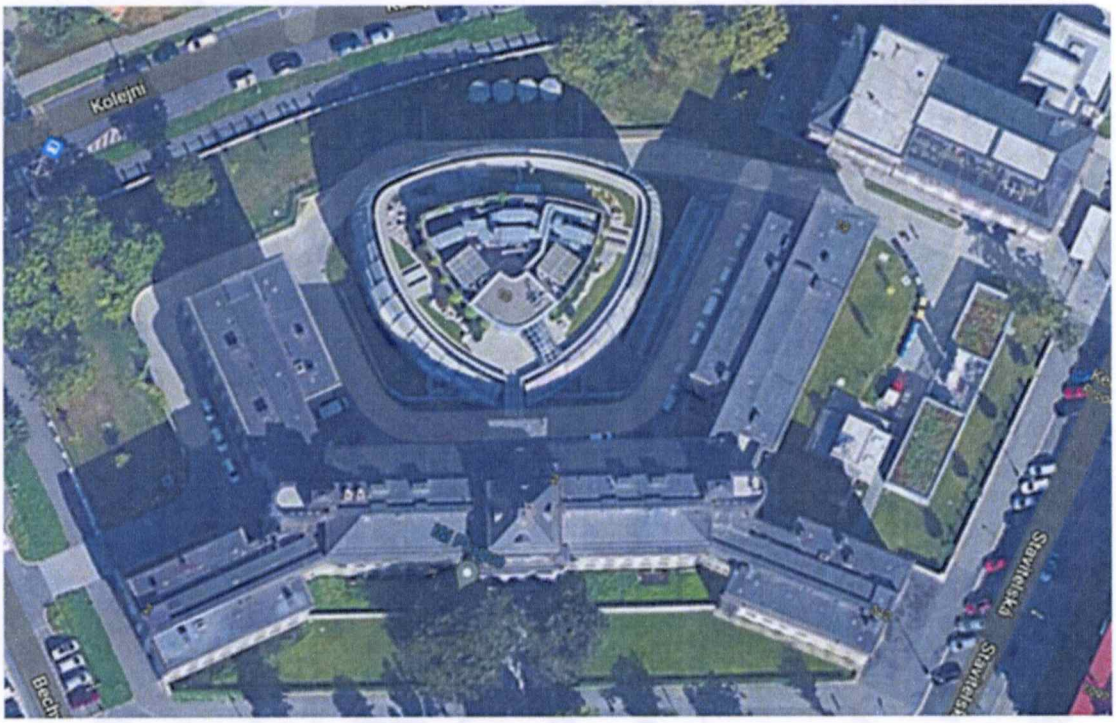
E1a-1 forw: 5′ CCGAAGAAATGGCCGCCAGTC 3′
E1a-2 reverse: 5′ GGACGCCGGGTAGGTCTTGC 3′

The aforesaid procedures are according to the following sources:
Sambrook J., Fritsch E. F., Maniatis T.: in *Molecular Cloning, A Laboratory Manual*. Second Edition, Cold Spring Harbor Laboratory Press, New York, (1989)
Ruml T., Rumlová M., Pačes in *Genové inženýrství*, VŠCHT Praha 2002
PCR protocol for Phusion High-Fidelity DNA polymerase according to the protocol available online (product of New England Biolabs, M0530, www.neb.com)
E. Gendra et al: *A Sequence Motif in the Simian Virus 40 (SV40) Early Core Promoter Affects Alternative Splicing of Transcribed mRNA*. J. Biol. Chem. 282, 11648-11657, 2007

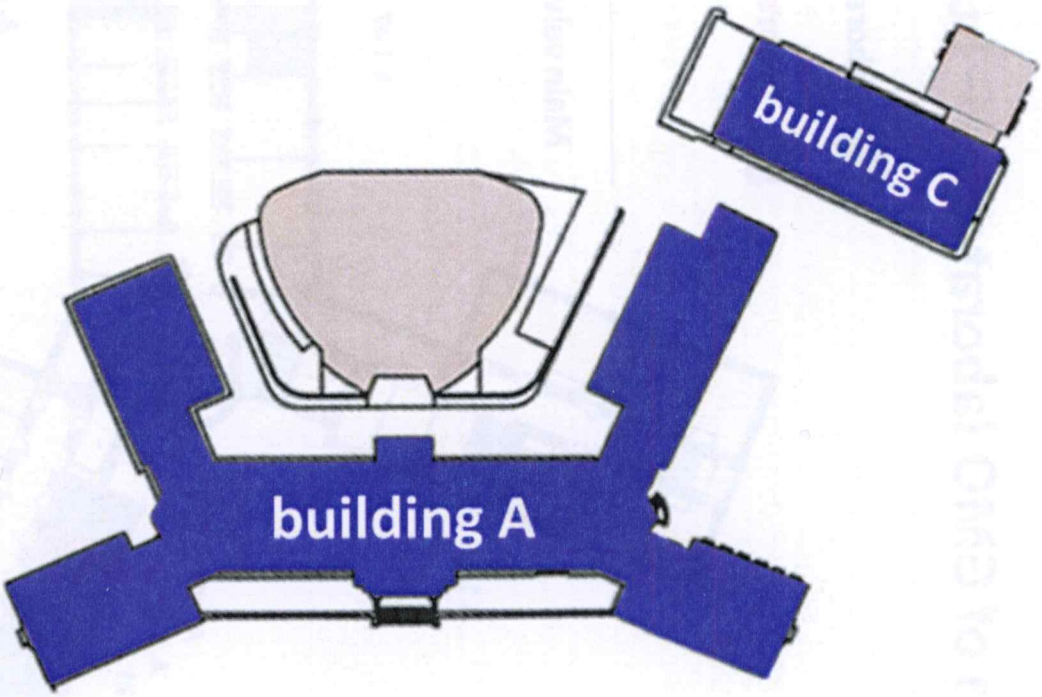
Note: Following isolation of DNA from bacterial or yeast cells, it is possible to determine whether the DNA contains a cloned fragment also by restriction analysis, i.e. by specific cleavage of DNA using restriction endonucleases. However, this analysis is no longer widespread today and has been superseded by far more sensitive and specific detection using PCR.

Addendum **2:**
Workplace maps

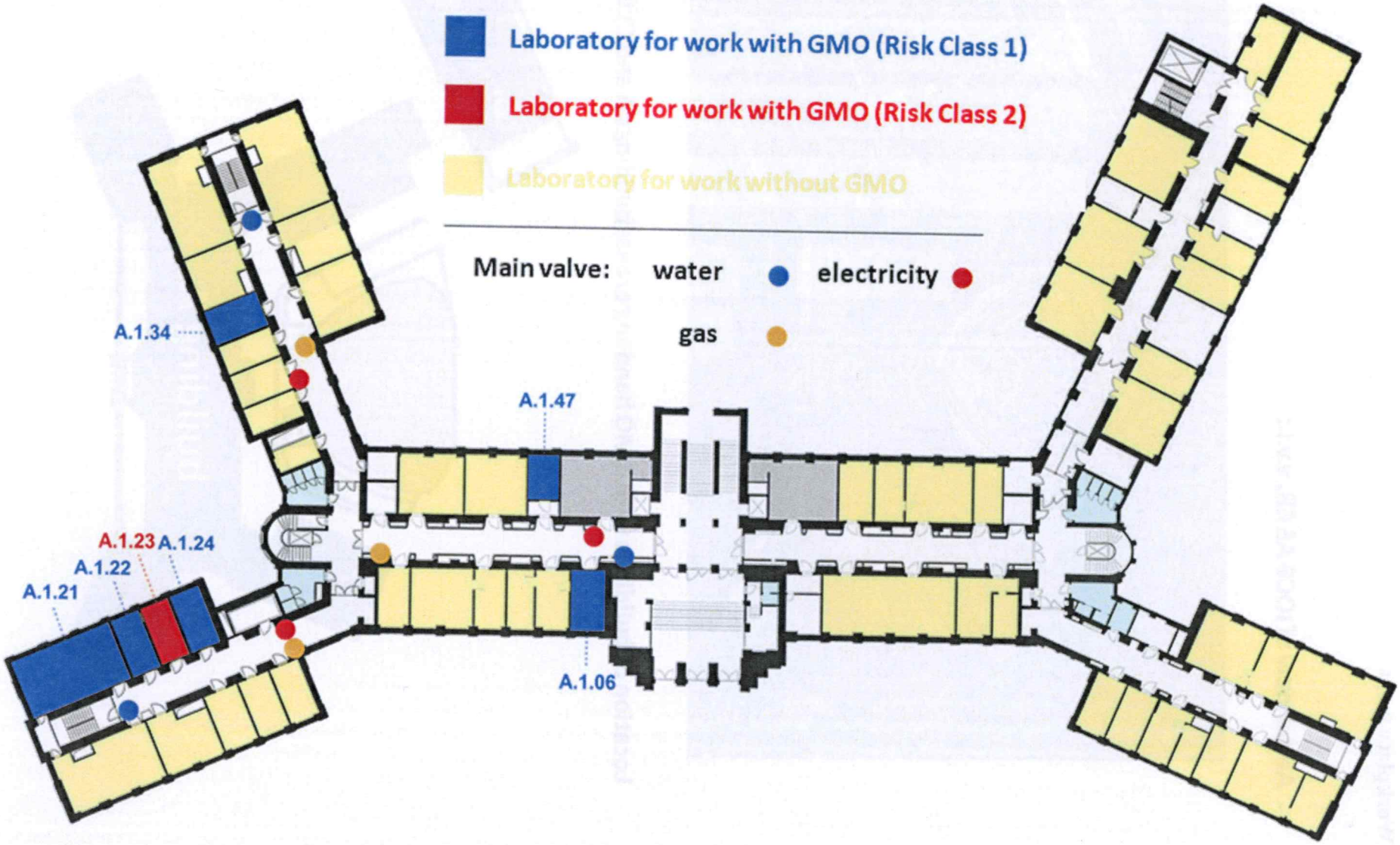
Aerial view of IOCB AS CR, v.v.i.:



Location of buildings with GMO handling areas (buildings A and C) in the campus:

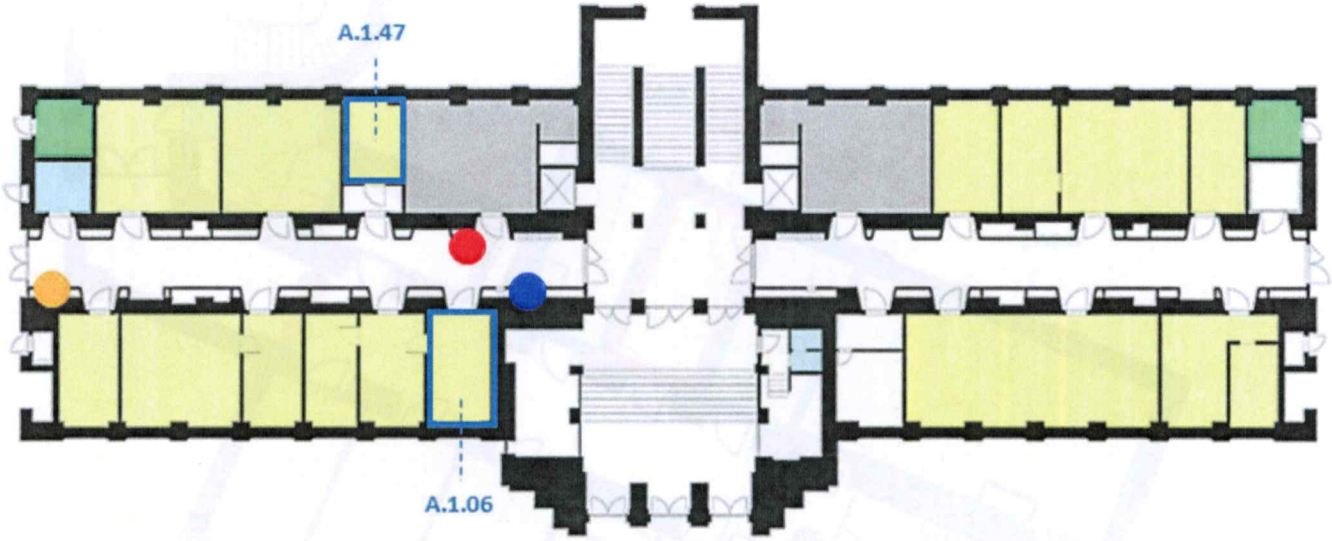


Plan of GMO laboratories – building A 1.NP

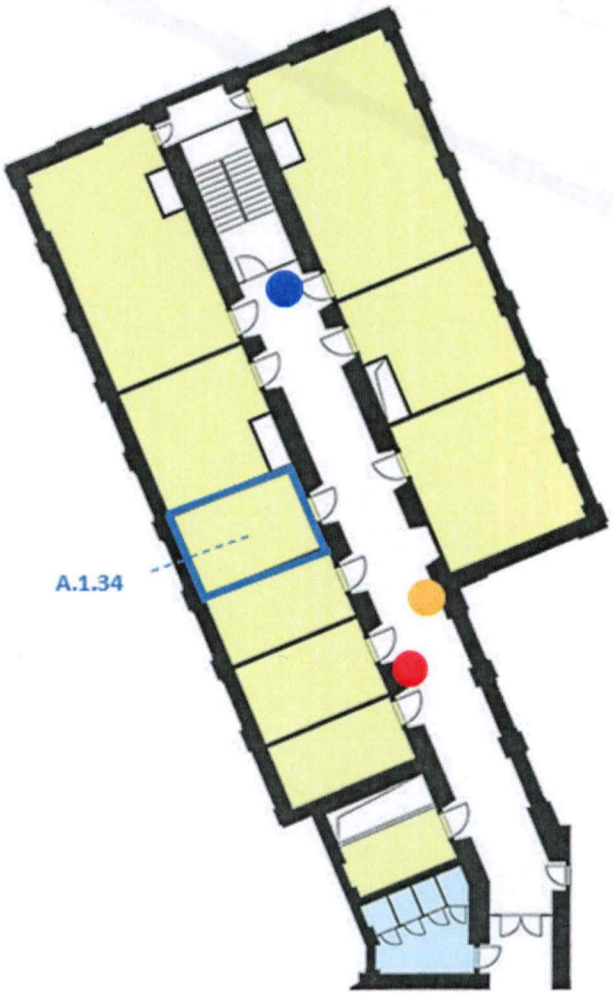


Detail of floor plans of GMO laboratories in building A 1st floor (1.NP)

- central part of building A, 1st floor (1.NP):



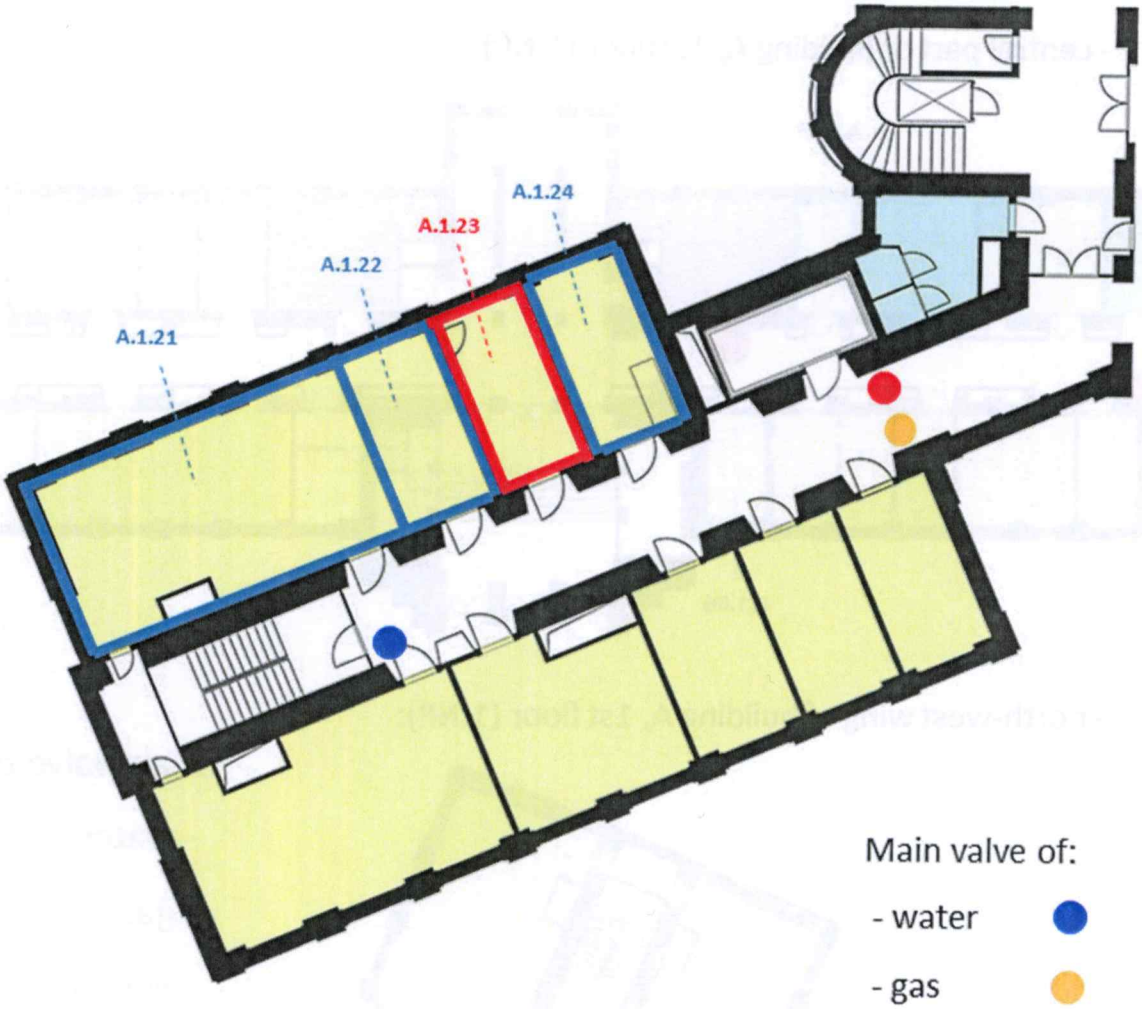
- north-west wing of building A, 1st floor (1.NP):



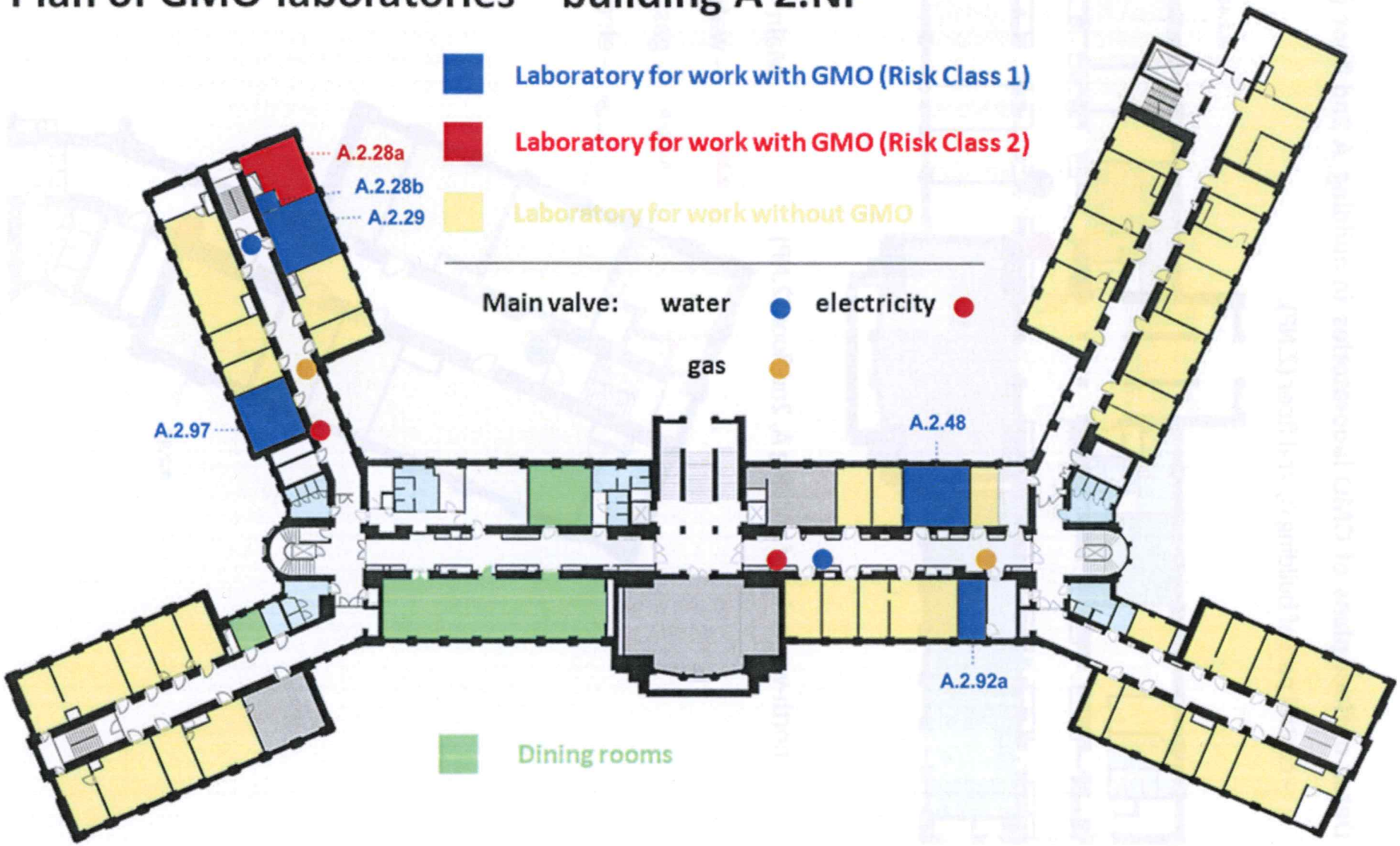
Main valve of:

- water
- gas
- electricity

- south-west wing of building A, 1st floor (1.NP):

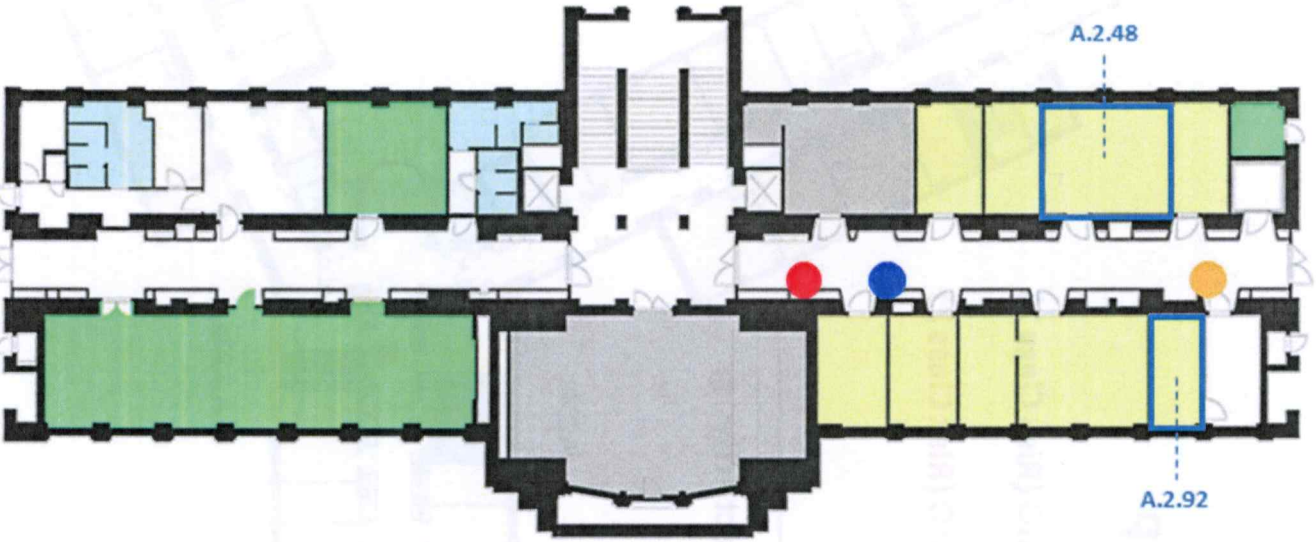


Plan of GMO laboratories – building A 2.NP



Detail of floor plans of GMO laboratories in building A 2nd floor (2.NP)

- central part of building A, 2nd floor (2.NP):

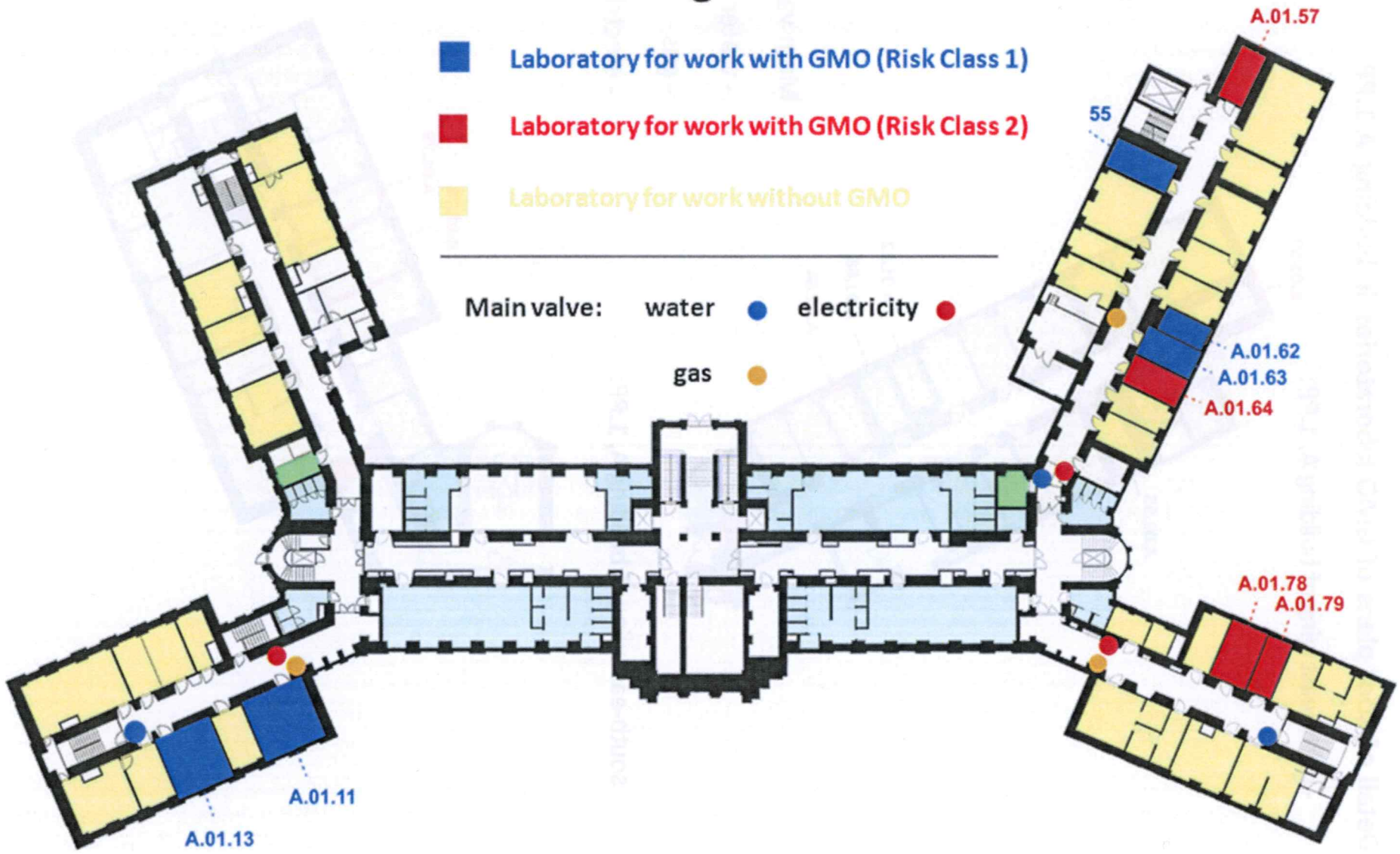


- north-west wing of building A, 2nd floor (2.NP):

Main valve of:

- water
- gas
- electricity

Plan of GMO laboratories – building A 1.PP



Detail of floor plans of GMO laboratories in building A 1.PP

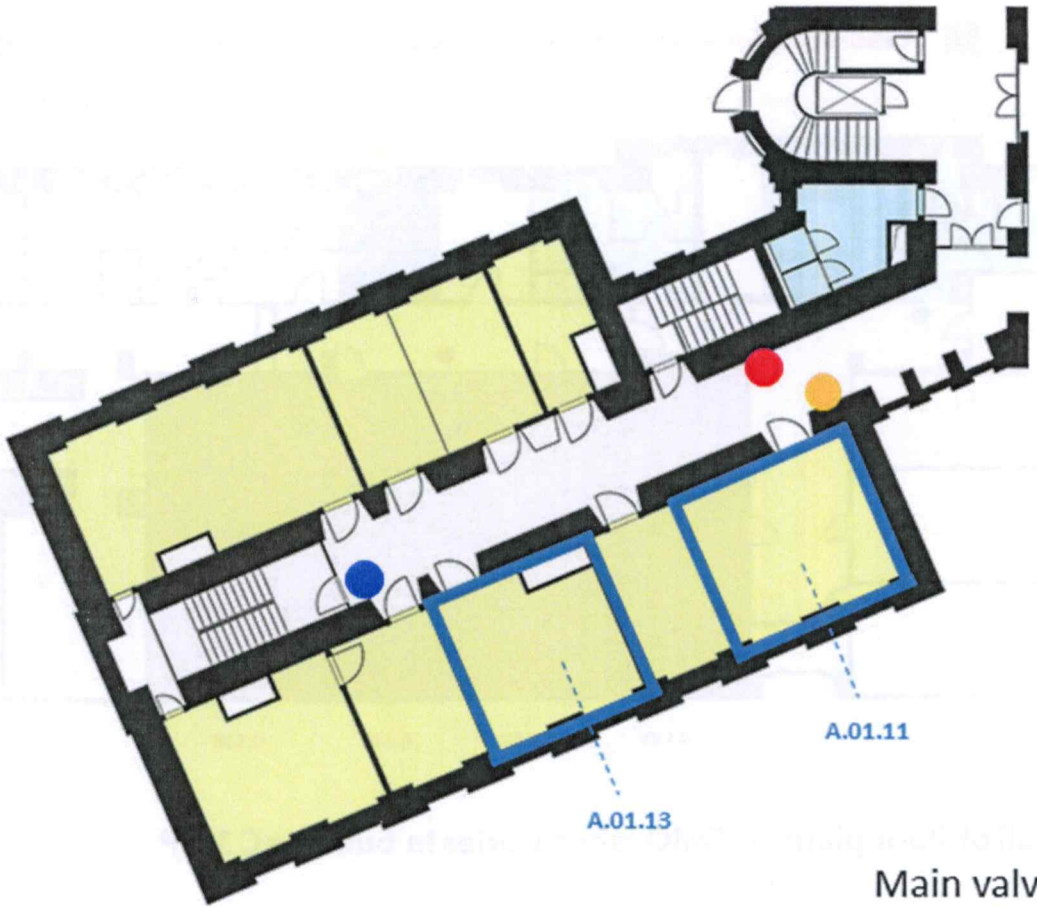
- north-east wing of building A, 1.PP:

Main valve of:

- water
- gas
- electricity

- south-east wing of building A, 1.PP:

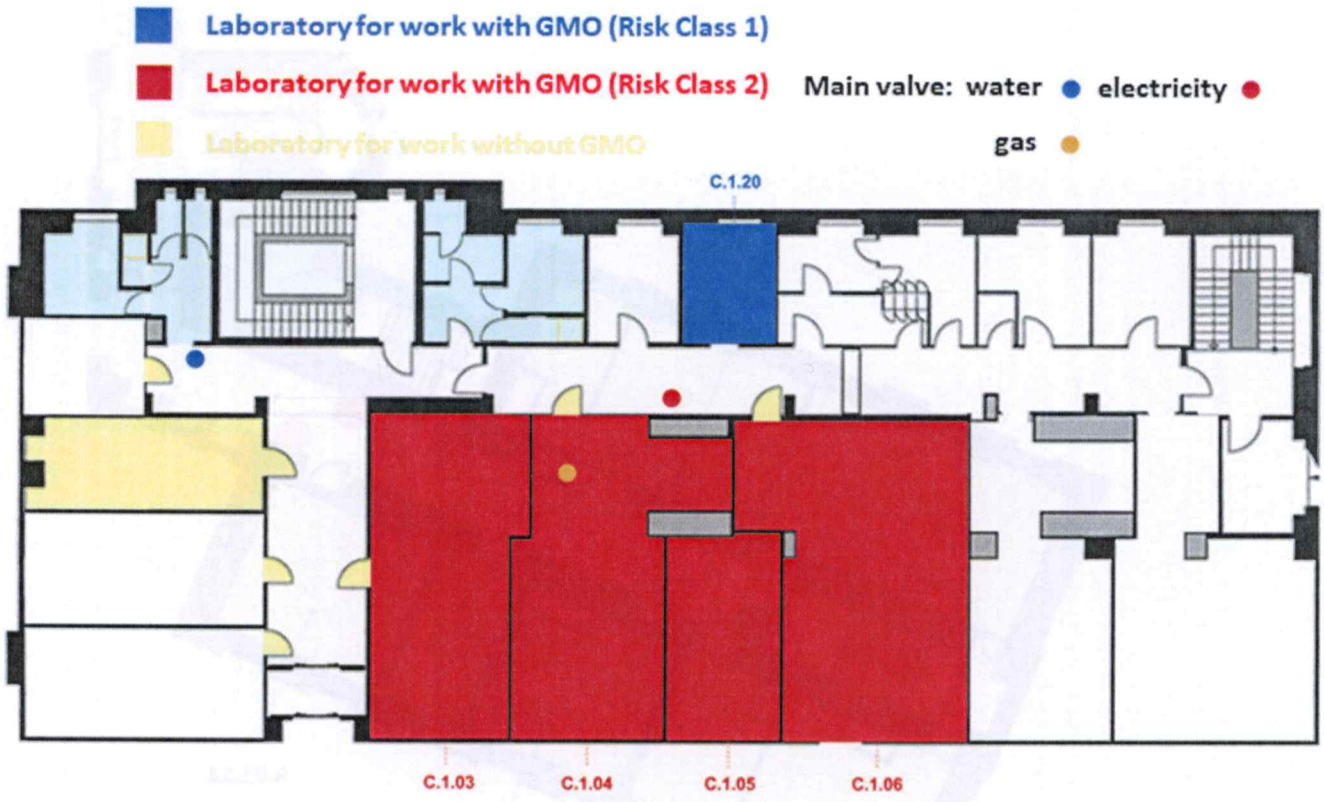
- south-west wing of building A, 1.PP:



Main valve of:

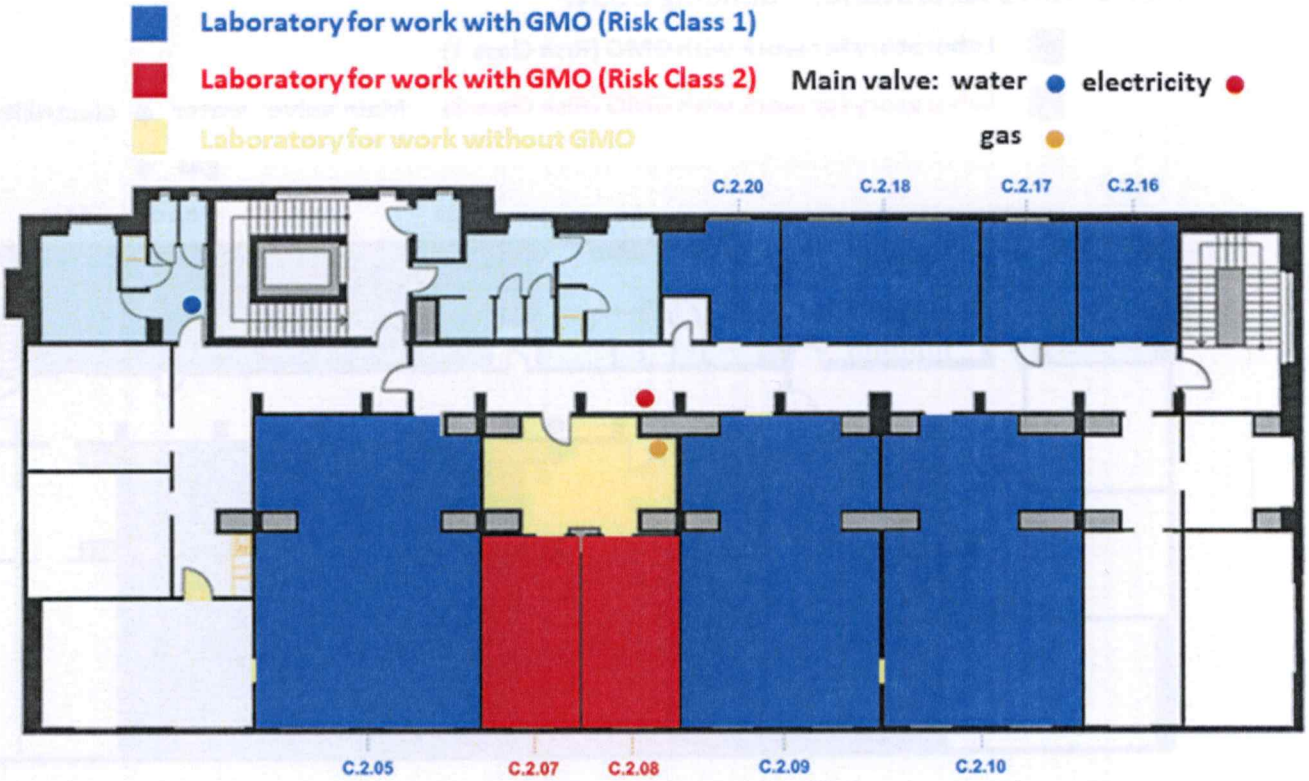
- water
- gas
- electricity

Plan of GMO laboratories – building C 1.NP



Detail of floor plans of GMO laboratories in building C 1.NP

Plan of GMO laboratories – building C 2.NP



Detail of floor plans of GMO laboratories in building C 2.NP

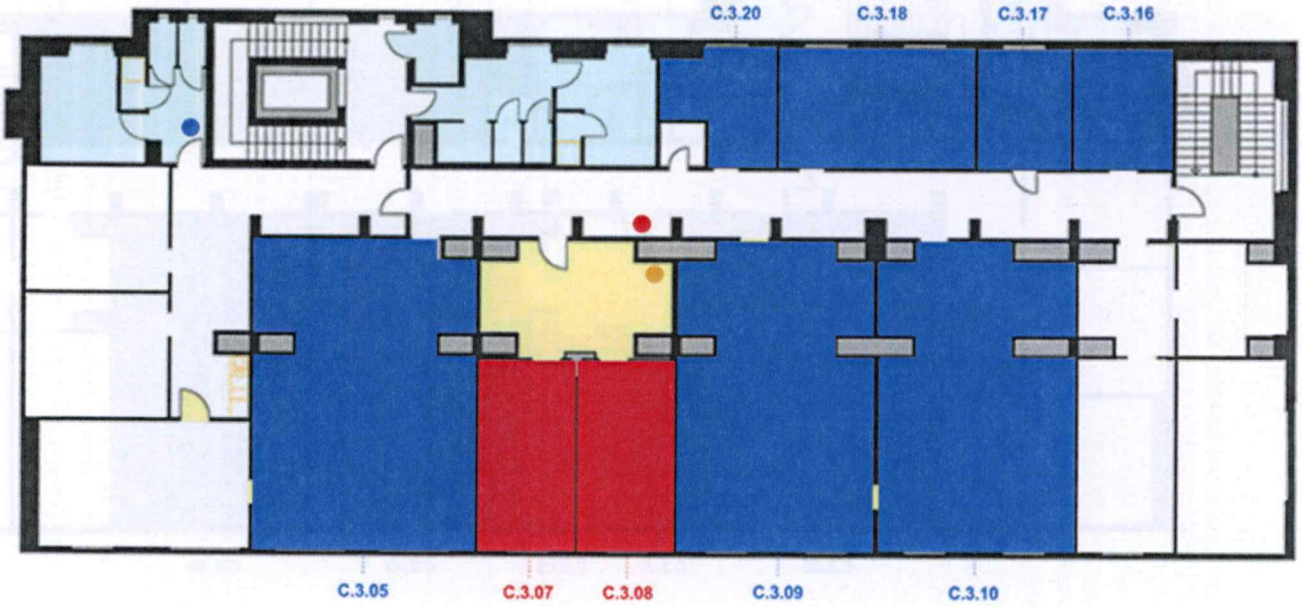
Plan of GMO laboratories – building C 3.NP

Laboratory for work with GMO (Risk Class 1)

Laboratory for work with GMO (Risk Class 2)

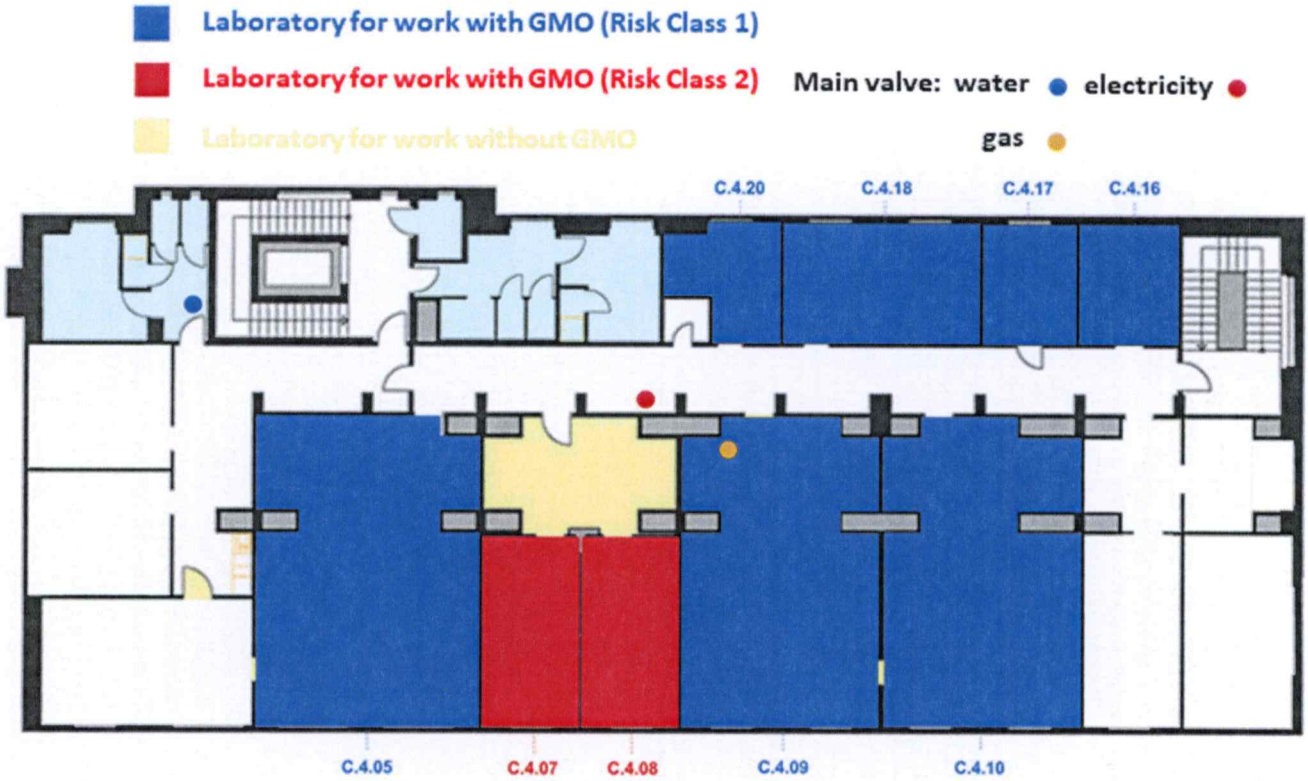
Laboratory for work without GMO

Main valve: water ● electricity ● gas ●



Detail of floor plans of GMO laboratories in building C 3.NP

Plan of GMO laboratories – building C 4.NP



Detail of floor plans of GMO laboratories in building C 4.NP