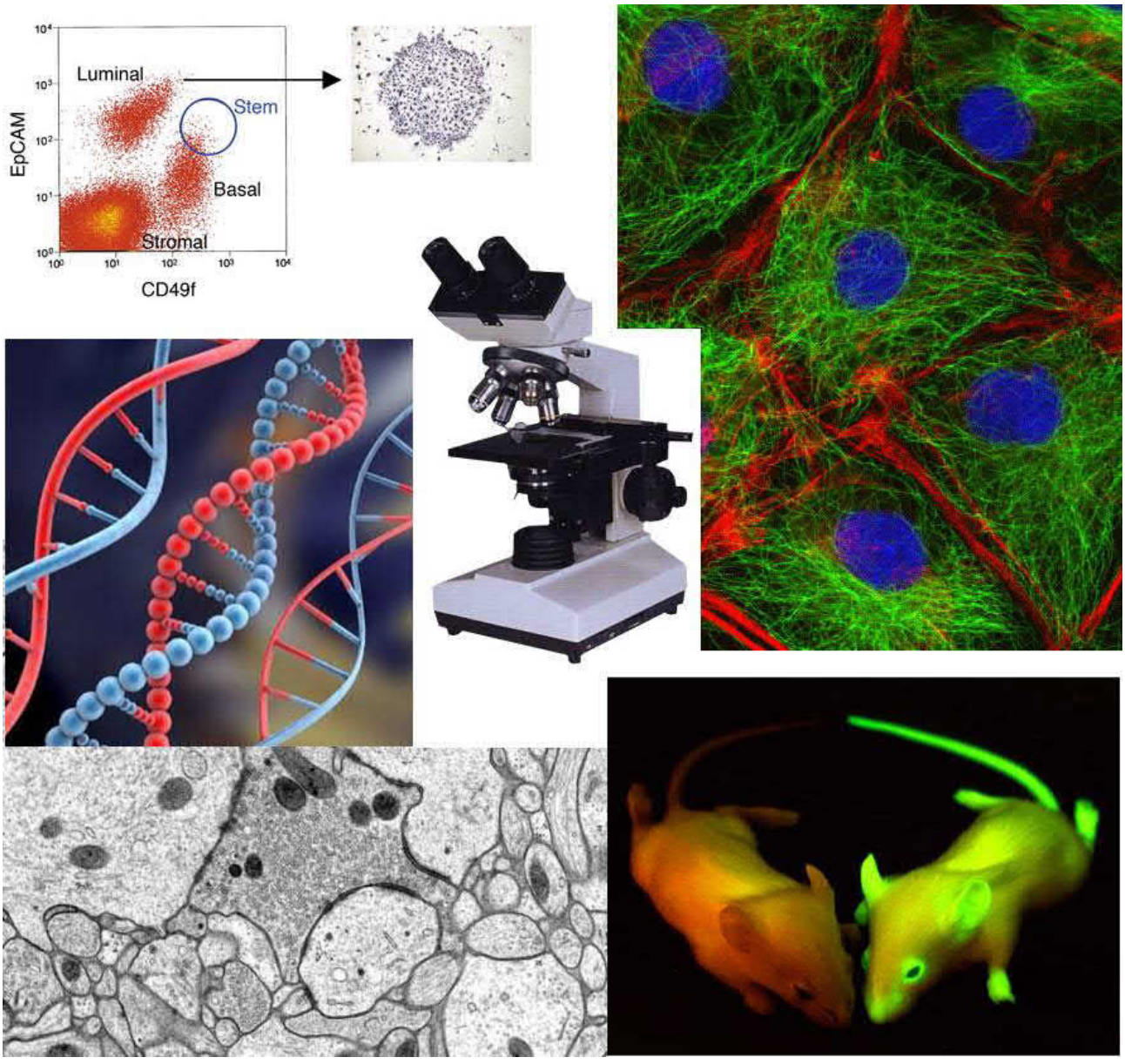
Lab Skills:

Cell Biology

Code: SKI2077



2017/2018

Period 4

Contents

General information

Introduction

Research-based learning is supposed to be the core of the new lab skills set-up. Students are required to find the theory linked to a practical assignment and to come up with their own protocols. The goal of this set-up is that students learn to think about the background of experiments and the practical execution. Every practical is annotated in a lab journal and preceded by an assignment for all students and a presentation by maximal 2 students.

The practicals take place at the Randwijck campus: Universiteitssingel 50, 6229 ER Maastricht. For exact locations, see course schedule on MyUM.

The appendix in this course manual contains information on how to work safely in the lab. Read this information prior to each practical and make sure you understand every detail and work according to the safety rules and regulations.

Aims of the skills training

The aim of this course is to develop competences in the planning and performance of experiments and evaluation of results using common techniques in molecular genetics and cell biology. The emphasis of this skills course lies on the visualization of cellular processes and cellular constituents. The course starts with an introductory lecture providing information on the assignments as well as an introduction into Good Laboratory Practice (GLP) and Safe Laboratory Practice (SLP). Students perform experiments on several different topics.

Learning outcomes

Understands the mechanisms of immunocytochemistry, immunohistochemistry, in situ hybridization.

Understands the background to the development of immunocytochemistry, immunohistochemistry, in situ hybridization.

Understands the general background of various visualization techniques, e.g. bright field/fluorescence microscopy, Confocal Scanning Laser Microscopy (CSLM), Electron Microscopy (EM) and Flow Cytometry (FCM).

Can cite the appropriate reference sources.

Knowledge

- Uses immunocytochemistry, immunohistochemistry, in situ hybridization, PCR, DNA isolation and DNA gel electrophoresis terminology appropriately
- Knows cellular structures and their function.
- Knows principles of cell cycle (regulation), hybridization and PCR amplification
- Knows the use of immunocytochemistry, immunohistochemistry, in situ hybridization and PCR in prognostic diagnostics
- Knows main difference in image formation in light microscopy (LM) and EM and the consequences for tissue handling
- Knows principles of the main method for immuno-EM
- Knows sensitivity of the different approaches

Able to describe the following:

- Antibody/antigen interactions
- DNA probe/target interactions
- DNA probe labeling systems
- The effect of fixation and processing on various tissue antigens and nucleic acids
- The use of different detection systems
- The use of different visualisation systems
- The importance and use of appropriate controls

Competence

Is able to:

- recognize cellular organelles in cell cultures stained with specific antibodies
- stain tissue and cell preparations using immunohisto- and cytochemistry, and in situ hybridization
- use different amplification systems
- use different visualization systems
- use different intensification systems
- assess stained slides/cell preparations for quality

- assess stained slides/cell preparations for artefacts
- assess staining of cellular structures (light microscopy and EM)
- perform and interpret cell cycle analysis
- assess subcellular structures on EM pictures

Assessment

In this course there are several elements of assessment:

1. written assignments (in pairs of two students) prior to each practical (30%) – for more information, see below;
2. presentation (in pairs of two students) for each practical (30%) – for more information, see below;
3. a written test at the end of the course (40%)
4. Professional behavior (attendance, motivation, participation). (Formative)

Attendance requirement

The attendance requirement for the tutorial meetings, practicals and presentations is 100%. If you miss a meeting, you will have to apply for an additional assignment (request forms are available at the Office of Student Affairs). In order to qualify for an additional assignment you have to have valid reasons for the missed session. If you don't meet the attendance requirement, you are not eligible for a resit.

Writing Assignments

Protocol proposals

Each practical team (2 students) needs to hand in their assignment (preferably about two A4 pages) before each practical. The assignment can be a protocol proposal or a specific assignment as a preparation for the subsequent practical. In case of a protocol proposal, the document should include a short section on the theory behind the experiment, the aim, and a clear and concise practical protocol containing all steps taken during the experiment. For deadlines see the schedule. All students receive feedback on their assignment. Students, who fail the assignment, are allowed a second chance (for deadlines, see schedule). In case of a pass, students are allowed to join the practical. However, if students fail the second proposal, they are not allowed to take part in the practical. Exclusion of a practical can be compensated by an additional assignment.

Assignments (1st and 2nd attempt) should be uploaded via safe assign. You will receive feedback on your first attempt. You will not receive extensive feedback on your second attempt, just a GO or NO GO, before Tuesdays at noon.

Presentations of practical reports

Each practical is rounded off by a presentation. For each practical one team of two students will be assigned to give the presentation (max 20 min). The other teams will prepare two questions for the

discussion. The content of the presentation will be posted on EleUM (for deadlines see schedule). Questions have to be posted on EleUM prior to the presentation.

The practical descriptions in the course manual contain some guidelines as to what should be included in the presentation.

In general, the presentation should contain the following sections:

- Front page: *name, ID#, experiment, experimental date, report date, tutor*
- Title
- Abstract/summary: *Summarize your experiment here. Repeat the hypothesis, and your findings.*
- Introduction
    - Theory: *What is the theory behind this experiment?*
    - Aim of the experiment
    - Hypothesis: *the (theoretical) result that you expect to find*
- Experiment
    - Materials
    - Methods: *the protocol in (academic) scientific language;*
    - Apparatus: *In official reports, it is essential to indicate the manufacturer and the registration number of the apparatus. Every apparatus of the university has a number. When a strange result is found in an experiment, the used apparatus can be traced, and it can be checked whether the problem lies in the apparatus.*
- Results: *including observations, data, calculations, error calculations, etc.*
- Discussion & Conclusion: *What did you find? Do the results meet the expectations of the hypothesis? How can the difference between hypothesis and actual findings be explained? Explain the observations and data.*
- Literature: *When you use information from a scientific article, university textbook or internet source, make sure you include the reference. Not doing so, is a form of plagiary. Do not take this lightly. Copying and pasting pieces of text from the internet is as much a form of plagiary as is copying from a book!*

General Literature

1. Alberts et al. Molecular Biology of the Cell, 6th edition, 2015; Garland Science, NY 4th edition available at: http://http.pubmed.com Search in Books instead of Pubmed (default)

2. Protocols on line available at: http://www.protocol-online.org/

5 International Chemical Safety Cards available at: http://www.ilo.org/dyn/icsc/showcard.home

6 Microscopy at: http://www.ammrf.org.au/myscope/

7 Flowing software at: http://www.flowingsoftware.com/

8 Flow cytometry at: http://flowbook.denovosoftware.com/

9 Introduction to Flow Cytometry Web-based training at: http://www.bdbiosciences.com/eu/s/training/e-learning

10 Online literature available at: https://www.accessmedicine.com or https://www.clinicalkey.com (needs login via UM; VPN client or Athena desktop)

Course coördinators

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Program and Timetable

For detailed information of the course time table MyUM is advised.

Date	Activity	Topic
Wednesday Feb 7th	Introduction UNS50 5.111 Cellular organelles	Introduction Practical (Schutte/Broers)
Friday 9th		Deadline submission assignment (2
	Carnaval	
Wednesday Feb 21st	Immunocytochemistry (part 2A)	Presentation practical (group 1) Practical Broers
Friday Feb 17th		
Wednesday Feb 28th	Confocal Scanning Microscopy (part2B) Starting In situ hybridization (part 3A)	Practical (Broers/ van Zandvoort Practical (Hopman)
Friday March 2nd		Deadline submission assignment (3)
Wednesday March 7th	Finishing In situ hybridization (part 3B) UNS40 4.570 8p	presentation (group 2) Practical
Friday March 9th		Deadline submission assignment (5B)
Wednesday March 14th	Presentation UNS50 5.111 Electron Microscopy (part 4A)	presentation practical (group 3) Practical
Friday March 17th		Deadline submission assignment (6)
Wednesday March 21nd	Electron Microscopy (part 4B)	Practical
Wednesday March 28th	Final exam	

Assignments

See Student portal

Available chemicals/solutions/materials:

- Glass cover slips containing fibroblast cells fixed with
    - Methanol
    - Formaldehyde
- Bovine serum Albumin (1 mg/ml in PBS)
- 1 x PBS
- 10 x PBS
- Methanol (-20 °C)
- Mouse anti-MPM2 IgG (concentration will be given later)
- Mouse anti-cytokeratin IgG (concentration will be given later)
- Mouse anti tubulin IgG1 (concentration will be given later)
- Mouse anti-vimentin IgG1 (concentration will be given later)
- Actin binding Phalloidin-TexasRed (concentration will be given later)
- PBS containing 3% BSA
- Mouse anti-bromodeoxyuridine IgG (concentration will be given later); bromodeoxyuridine is a thymidine analog, that is not naturally available.
- Rabbit anti-mouse IgG (concentration will be given later)
    - FITC-conjugated
    - R-PE-conjugated
    - TxRed-conjugated
- Goat anti-rabbit IgG (concentration will be given later)
    - FITC-conjugated
    - R-PE-conjugated
    - TxRed-conjugated
- Mouse anti-rabbit IgG (concentration will be given later)
    - FITC-conjugated

  - R-PE-conjugated
  - TxRed-conjugated
- Propidium Iodide, (concentration will be given later)
- DAPI (concentration will be given later)
- Spectral characteristics fluorochromes
  - FITC Exmax 488 nm, Emmax 540 nm
  - R-PE Exmax 488 nm, Emmax 580 nm
  - TxRed Exmax 595 nm, Emmax 613 nm
  - DAPI Exmax 360 nm, Emmax 460 nm
  - Propidium Iodide Exmax 488 nm, Emmax >580 nm
- DNAse (100 mg/ml stock solution)
- RNAse (100 mg/ml stock solution)
- Fluorescence mounting medium containing DAPI, antifading agent and glycerol
- Tubes of all sorts and sizes
- Microscope slides
- Confocal microscope, excitation lines 405 nm, 488 nm, 532 nm and 635 nm
- Fluorescence microscope
- FACSort equipped with air-cooled Argon laser (Em 488 nm), Fluorescence detectors: 520-540 nm (green); 565-585 nm (orange); >600 nm (donkerrood)
- Centrifuges

Practical 1 Cellular organelles

In contrast to prokaryotic cells, eukaryotic cells contain specialized compartments, each with a unique function, called organelles.
In this microscopy session you will examine immunocytochemical stainings of different organelles, the nuclear membrane, nucleoli, the ER, and the cellular membrane.
Also, three diffferent types of cytoskeleton proteins, i.e keratin, (intermediate filaments), actin and tubulin (microtubules) in cultured cells need to be examined. While initially, many structures may look alike, more extensive recognition of characteristics of each strucutre will allow the identification of each of the organelles.

Literature

- B. Alberts *et al.*, Molecular Biology of the Cell, 6th Ed., 2015, Chapter 9
- Anthony L. Mescher. Junquiera's Basic Histology 14th ed 2016,

To do's

- Describe the structures, that you stained with the specific antibodies (in brown). Compare what you see with drawings and/or stainings in the handbooks provided.Next, make a drawing of the structure as you think it looks like.

Practical 2: Immunocytochemistry

Immunocytochemistry is a powerful tool in the determination of the localization and quantification of proteins and other antibody generators (=antigens) in cells. This technique makes use of the possibility to generate specific antibodies against antigens carrying specific antigenic determinants (= epitopes). The classic method of generating antibodies is performing immunization with purified antigens in rabbits or other animals of a different species than the antigen source. This will result in polyclonal antibodies, specifically directed against one antigen, but recognizing different epitopes of the purified proteins. If the antigen source is not pure, antibodies will be generated also against the impurities, resulting in non-specific antibody binding.

Alternatively, antibodies can be generated by immortalizing activated B cells, derived from immunized animals. These so-called monoclonal antibodies, derived from one single cell per definition, recognize a single epitope, and thus are in general more specific than polyclonal antibodies. A combination of antibodies can be used to detect more than one protein specifically.

In general, binding of antibodies to antigens is visualized by so-called secondary antibodies. These secondary antibodies, binding to the first (primary) antibodies are labeled with fluorescent molecules (immuno-fluorescence) or enzymes (e.g. immunoperoxidase), which allow detection of the presence of a protein in subcellular structures.

For light microscopy, you will set up your own immuno-fluorescence procedure to visualize both actin and tubulin structures within preparations from a (in vitro) cell culture.

Assignment: protocol proposal

1. Define the term ‘antibody’. How are specific antibodies made?
2. How does the indirect immunofluorescence method work, how do you visualize Ag-Ab complex formation?
3. For microscopy cells and tissue have to be fixed. Describe the underlying mechanism of fixation by formaldehyde and (m)ethanol.
4. Define the term ‘titer’.
5. Write a complete immunofluorescence protocol for the detection of tubulin or actin or vimentin. Describe and find arguments for performing each step.

Practical

The practical contains two main parts;

1. Execution of the protocol.

Literature

1. L. Benerini Gatta, M. Cadei, P. Balzarini, S. Castriciano, R. Paroni, A. Verzeletti, V. Cortellini, F. De Ferrari, P. Grigolato Application of alternative fixatives to formalin in diagnostic pathology Eur J Histochem. 2012 June 29; 56(2):

2. William E. Grizzle Models of Fixation and Tissue Processing Biotech Histochem. 2009 October ; 84(5): 185–193.

3. Van der Loos CM: Multiple Immunoenzyme Staining: Methods and Visualizations for the Observation With Spectral Imaging. Journal of Histochemistry & Cytochemistry Volume 56(4): 313–328, 2008

4. Alberts B. et al. Molecular Biology of the Cell, Chapter 9: Visualizing cells. Garland; 6th ed, 2015.

5. DAKO Handbook Immunochemical staining methods. 1989, 2006. http://www.agilent.com/cs/library/technicaloverviews/public/ihc-guidebook-detection-methods-chapter6.pdf

Practical 3: CSLM

Assignment: protocol proposal

Discuss extensively the principles of one- and two photon confocal laser scanning microscopy (CSLM). Discuss also the topics of resolution and penetration with respect to these two techniques. Which technique gives the best result? Why?

Minimum lengt of assignment two written pages

Practical

The slides which were prepared during the previous practical (immunocytochemistry) will be analyzed using one and two photon CSLM. During the session the students will be split into two groups. During the first half of the practical one group will attend the one photon CLSM session and during the second half the two photon session and vice versa.

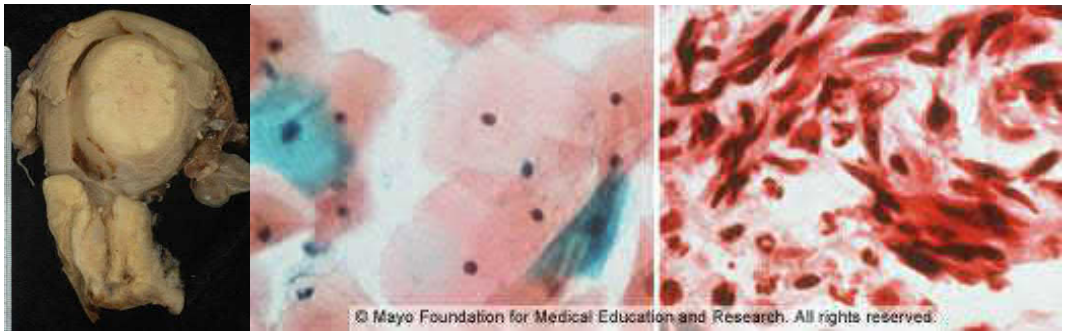
Literature:

1. Paddock SW, Principles and practices of laser scanning confocal microscopy. Molecular Biotechnology
2. Volume 16, (2), 2000, 127-149, DOI: 10.1385/MB:16:2:127 Van der Loos CM: Multiple Immunoenzyme Staining: Methods and Visualizations for the Observation With Spectral Imaging. Journal of Histochemistry & Cytochemistry Volume 56(4): 313–328, 2008
3. Alberts B. et al. Molecular Biology of the Cell, Chapter 9: Visualizing cells. Garland; 4th ed, 2002.
4. Nikon Microscopy http://www.microscopyu.com/articles/confocal/
5. Leica Microsystems http://www.leica-microsystems.com/science-lab/topics/confocal-microscopy/
6. Kurt Thorn (UCSF): Confocal Microscopy . http://www.youtube.com/watch?v=YRQsjPAx9UU

Practical 4: HPV In situ hybridization

Background

The human papillomavirus (HPV) is the most important risk factor in the development of uterine cervical cancers (UCC) (see Figure below, left side) and other anogenital cancers, and has even been detected in a subset of head and neck tumors. All UCC are HPV-positive or have been positive in at least one phase of the disease.



UCC *normal cytology* *aberrant cytology*

In the nineteen seventies researchers already postulated the possible role of HPV in cervical cancer. It was shown that the appearance of koilocytes (specific cells) in cervical smears (cytology) indicate the presence of a papillomavirus infection. It was suggested that it might be possible to differentiate between 'benign, warty' lesions that do not progress to cervical cancer and dysplastic precursor lesions that do progress to cervical cancer. The identification of typical papillomavirus particles in uterine cervical (pre)malignancies by means of nucleic acid hybridization procedures (in situ hybridization (ISH) and polymerase chain reaction (PCR) in the nineteen eighties was the definite proof of the involvement of papillomavirus infections in these lesions.

The first cloned HPV types (HPV16, HPV18) were isolated directly from cancer biopsies of the cervix by the research group of the latter Nobel laureate Harald zur Hausen. HPV types that are found preferentially in cervical and other anogenital cancers have been designated as 'high-risk' types. Conversely, those found primarily in genital warts and non-malignant lesions have been called 'low-risk' types. High-risk HPV types, particularly HPV16, are widespread within all human populations. Infection is commonly transmitted by sexual contact and results initially in inconspicuous squamous intraepithelial lesions (SIL) in women. Most of these lesions are cleared 6–12 months after appearance, probably due to immunological intervention. A small percentage, however, persists, progresses to high-grade SIL, carcinoma in situ and without surgical intervention, to UCC.

Screening for uterine cervical (pre)malignancies

In order to prevent UCC, women of 35 years and older have the opportunity to join a (inter)national screening program, so that they can check if they are carrier of "relatively innocent" precursor lesions of

the disease as determined by cytology. For this purpose, brush preparations of uterine cervical cells will be Papanicolaou-stained and examined by a pathologist for (pre)malignant cells (see Figure above, middle and right image). Often only a few morphologically altered cells are detected, which complicates the analysis. The gynaecologist will, if required, remove the lesions by colposcopy (surgical resection). But in some cases the lesions will recur and progress to invasive cancer. Cytological screening as well as colposcopic analysis by a gynaecologist has resulted in a dramatic reduction of cervical cancer incidence.

HPV screening by hybridization technologies

The detection of high-risk HPV DNA by **ISH** or **PCR** technology has been developed to assess the risk of a low-grade precursor lesion to progress to cancer. These approaches have recently been recommended to replace or be combined with conventional cytological screening procedures. If competently performed, these techniques clearly improve the fast and accurate detection rate of high-risk HPV DNA in women, and also avoid some false-negative diagnoses.

Visualization of HPV by means of *(fluorescence) in situ hybridization* ((F)ISH)

The (F)ISH is a powerful technique for localizing specific nucleic acid sequences (DNA, RNA) in microscopic preparations of tissues, cells, chromosomes, and linear DNA fibers. To date, a wide variety of research and diagnostic applications of (F)ISH have been described to detect the presence or absence of specific nucleic acids (such as virus sequences), deletion of sequences, gene translocations or gene amplifications.

As discussed in practical 5, the human papillomavirus (HPV) is the most important risk factor in the development of uterine cervical cancers (UCC) and other anogenital cancers, and has even been detected in a subset of head and neck tumors. The identification of typical HPV signal patterns in uterine cervical (pre)malignancies by means of FISH is playing an important role in deciding if virus infection is present in biological samples and if lesions are at risk for cancer development.

We are going to analyze two HPV-positive tumor cell lines (CaSki and SiHa) by means of FISH. We have stored the cells in a cell suspension containing 70% ethanol. For this purpose, we will use biotin- or digoxigenin-labeled DNA probes specific for oncogenic HPV type 16. For the development of a useful (F)ISH method it is important to find a balance between tissue pre-treatment on the one hand, making the tissue nucleic acids accessible for nucleic acid probes, and preservation of tissue morphology on the other hand, ensuring visualization of detected nucleic acid targets in their natural histological or cytological context.

Assignments (3A/B)

In principle (F)ISH is quite similar to immunocytochemistry (Practical 4), with the difference that not proteins but nucleic acids are detected in cells and tissues. We have split the practical in part A en part B. In part A we will start the hybridization and in part B we will finish the hybridization and at the end we will have a look at the detection of the formed hybrids microscopically. Two series of questions are raised that are answered (assignment practical A and B) and which are the basis for both practical's.

Assignment (3A)

What are cell lines and what are the characteristics of the cell lines as e.g. Hela, CasKi and SiHa?

For what HPV type are these cell lines positive?

Where are the HPV sequences localized?

What is a FISH and try to make a flow chard for the entire procedure?.

What is a DNA probes for e.g. HPV16 and how are these probes labelled to enable detection a microscopically (question for practical A en B)?

How can we handle the cells in the cell line to enable a (F)ISH analysis?

What is an in situ hybridisation mixture and what is meant with a denaturation and hybridization reaction?

How long will you hybridize?

Try to provide a practical protocol to start with.

(Your answers on these questions will serve as starting point for the hybridization practical A).

Provide a detailed (practical) description/protocol, step by step, for the FISH

Assignment (5B)

We will start with a post hybridization washing. What is a post hybridization wash?

What is meant with a "stringent" post hybridization wash?

What is the primary incubation step you need after the hybridization reaction to detect the formed hybrid?

How can you detect e.g. a digoxigenin labelled probe?

What does it mean detection of a biotin labelled probe with an avidin-biotin detection system?

Make a schematic drawing of the immunological and immunochemical network to detect the hybridized probes.

Have you some idea how the FISH signals will look through the fluorescence microscope?

Practical

The practical 5A starts with sample preparation for FISH, starting the hybridization and the practical 5B with ending the hybridization reaction and immunochemical detection.

The students can use from the following available reagents to carry out the FISH practical:

- glass slides
- cell lines in 70% ethanol
- pepsin dissolved in 0.01 M HCl (different concentrations)

- coplin jars
- PBS
- 2 x SSC
- 1% formaldehyde/PBS
- H_2O
- an alcohol series (70%-96%-100% ethanol)
- HPV16 DNA non labelled; DNA labelling kit with digoxigenin or biotin by nick translation.
- a hybridization mixture containing 60% formamide, 10% dextrane sulfate, carrier DNA, RNA, 2 x SSC
- a metal plate at 80^{o}C
- incubator at 37^{o}C
- water bath to be programmed at different temperatures
- 4 x SSC, 0.05% Tween.
- 5% non-fat dry milk
- fluorochrome-labelled avidin, biotinylated goat anti-avidin
- sheep anti-digoxigenin
- fluorochrome-labelled secondary antibodies
- Vectashield (anti-fading reagents) containing 0.2 ug/ml DAPI (blue fluorescent nuclear counterstaining)
- any chemical or reagents needed (for your protocol suggestion)

Presentation

Prepare a presentation according to the outlines described in the 'Presentations of practical reports' section including 5A and 5B.

For the pairs of students that are not presenting: prepare two questions each for the discussion. The presentation is schedule on Tuesday March 22nd.

Literature

Speel EJM. Detection and amplification systems for sensitive, multiple-target DNA and RNA in situ hybridization: looking inside cells with a spectrum of colors. Histochem Cell Biol 112, 89-113, 1999.

Hafkamp HC et al. A subset of head and neck squamous cell carcinomas exhibits integration of HPV 16/18 DNA and overexpression of p16^{INK4A} and p53 in the absence of mutations in exons 5-8. Int. J. Cancer 107, 394-400, 2003.

Practical 5: Electron Microscopy

In the electron microscope, fixatives for preservation of cells and tissues are different from those commonly used for light microscopy (LM). Also the processing of the samples, the embedding methods, the sectioning and staining techniques of the sections are different. These are related to the difference in image formation in the light and electron microscope, respectively.

Immunocytochemistry is the tool in the determination of the localization and quantification of proteins and other antibody generators (=antigens) in the electron microscope. The labeling of the antigenic sites occurs via the use of electron dense markers such as horseradish peroxidase substrate precipitates and particularly gold particles

For EM, you will be asked for to determine the specificity of image formation in the electron microscope and the consequences for this in processing tissue samples. Further, you will be asked for to differentiate subcellular organelles on electron microscopic pictures and to give clues for the different steps described for instance in the Tokuyasu method for immuno-EM.

Assignment: protocol proposal

1. What is the difference in image formation between the light microscope and the electron microscope?

2. By knowing the difference in image formation between the light microscope and the electron microscope, what are the consequences then for fixation, embedding, cutting and staining the samples for observation in the electron microscope? Give the rational for using each of these steps.

3. What is image resolution? When you compare EM with LM which structures can be distinguished, which are not visible with LM?

EM workshop

Literature

1. Junqueira's Basic Histology http://accessmedicine.mhmedical.com/ online Chapter 1, 2 and 3.

2. Sadava 9th ed. Life the Science of Biology Chapter 5

3. Alberts B. Molecular Biology of the Cell. Chapter 9: Visualizing cells Garland; 6th ed, 2015.

Appendix

Elementary laboratory rules

- The practical work takes place at UNS 50 (Randwijck) in room 4.101.
- Lab coats are available and should be worn **at all time** while present in the laboratory.
- It is **not** allowed to eat or drink in the laboratory.
- Safety glasses should be worn when working with chemicals and biological material.
- Gloves should be worn when working with chemicals and biological material. It is **not** allowed to wear gloves outside of the practicum room.
- Volatile liquid chemicals should only be handled in a fume-hood.
- Be present on time.
- Coats and bags should be stored in the lockers.
- Only the participating students are allowed access to the laboratory.
- Coffee or tea breaks are only allowed in consultation with the laboratory staff.
- At the end of each practical, laboratory tables need to be cleaned.

Safe Laboratory Practice (SLP)

In laboratories you always find a high concentration of hazardous substances. Materials that are poisonous (toxic) for humans, animals and plants, aggressive (caustic/corrosive) – like strong acids and bases – infectious (pathogenic/contaminated) – meaning that they contain micro-organisms that could cause disease in humans, animals or plants – inflammable and/or explosive, emitting radiation (UV, IR, electromagnetic, radioactive), very hot or very cold, or carcinogenic, teratogenic (causing damage to posterity) or mutagenic can all be present in a laboratory. Therefore it is essential to work safely. In order to be able to work safe, you need to be familiar with safety rules and regulations. Safe Laboratory Practice does not concern the experiment itself, but the person who is conducting it and everybody around. When you work in a safe way, you will be able to prevent many dangerous situations. A few guidelines can help to achieve SLP.

Personal conduct

- Eating, drinking, smoking, mobile chatting or using make up in the laboratory is **strictly forbidden**.
- Materials which are not required for the experiment should be kept in lockers. This includes mp3-players and mobile phones.
- Wearing a white laboratory coat is **obligatory**.

- Always keep you coat closed.
- Make sure the sleeves of you lab coat cover your own clothes.
- **Never** wear your lab coat outside the laboratory.
- Remove rings, watches, bracelets, etc.
- Long hair should be put together.
- **Always wear gloves** when working with chemicals or biological materials – **remember**: gloves do not offer complete protection, so keep working as careful as if you were not wearing them.
- **Always wear safety glasses** when working with chemicals or biological materials.
- Instructions from the laboratory staff should be followed immediately at all times.
- In case of accidents, immediately notify the laboratory staff; this includes accidents that seem harmless.
- In case of accidents involving eyes (e.g. chemicals in the eye), first flush with water, then warm the laboratory staff: "**first water, then bother**".
- **Always wash your hands** before leaving the laboratory; use disinfecting soap and disposable drying paper.

Work area

- **Clean and disinfect the working area** before each experiment.
- Put **only the necessary equipment** in the working area.
- **Always clear away unused materials**, not only at the end of the practical but also during the practical.
- **Immediately remove and clean spilled solutions or chemicals**.
- **Always close** bottles or jars immediately after using it.
- **Never** lift a bottle or a jar by its stopper, lid or neck.
- When using aggressive, volatile or inflammable substances, **always work inside a fume hood**.
- **Always make sure** that people around you know what you are working with.
- **Always** work in a logical, tidy manner, **minimizing risks**.
- **Always label or mark glassware** - write down the content (even if it is merely water) and the concentration.
- **Always remain focused** on your work. Carelessness produces accidents!

Waste handling

Proper segregation of laboratory waste is essential to good chemical hygiene and a safe workplace environment. You should aim to minimize the quantity of waste products and correctly dispose of all waste products. Many researchers often tend to put all of their wastes into the same cabinet or fume hood. Doing so can have disastrous results!

The guidelines for temporary storage of chemical wastes in the laboratory are really no different than those that you use for the storage of your usual lab chemicals. There are 6 main categories in chemical waste:

Category 1 **Acidic and neutral inorganic waste in solution**
Acidic laboratory waste (mixtures)
Fixative
Bleach fixative
Sulfuric acid (diluted)
Phosphoric acid (diluted)
Other inorganic acids

Category 2 **Alkaline inorganic waste in solution**
Alkaline laboratory waste (mixtures)
Inorganic bases
Ammonia solution

Category 3 **Halogen-deficient organic waste**
Waste water containing organic substances
Waste oil
Paint
Medicine waste
Solvents

Category 4 **Halogen-rich organic waste**
Laboratory waste, organic, halogen-rich
Methylene chloride
Chlorinated aromatics

Category 5 **Special waste**
Waste containing heavy metals and metalloids
Laboratory waste containing heavy metals
Batteries
Mercury-containing objects

Category 6 **Waste with exceptional risks**
Asbestos-containing objects
Extremely corrosive waste (e.g. concentrated acids)
Explosive substances
Gas cylinders
Organic peroxides

Liquid alcohol	Extremely toxic substances (beryllium/selenium/arsenicum) PCB/PCT-containing objects or substances

The most important rule is to make sure that any chemicals or wastes that stored together are **compatible** with each other. Therefore, proper segregation of wastes involves making sure that wastes within a bottle are compatible. Only chemically compatible waste can be mixed together and placed in a common container for disposal.

NEVER store the following types of wastes near each other:

- Acids and bases.
- Organics and acids.
- Cyanide, sulfide or arsenic compounds and acids.
- Alkali or alkali earth metals, alkyllithiums etc. and aqueous waste.
- Powdered or reactive metals and combustible materials.
- Mercury or silver and ammonium containing compounds.

If a bottle broke in a waste storage area where incompatibles were present, the results could be disastrous. Remember: incompatible bottles of wastes should be stored in separate cabinets, preferably as far apart as possible.

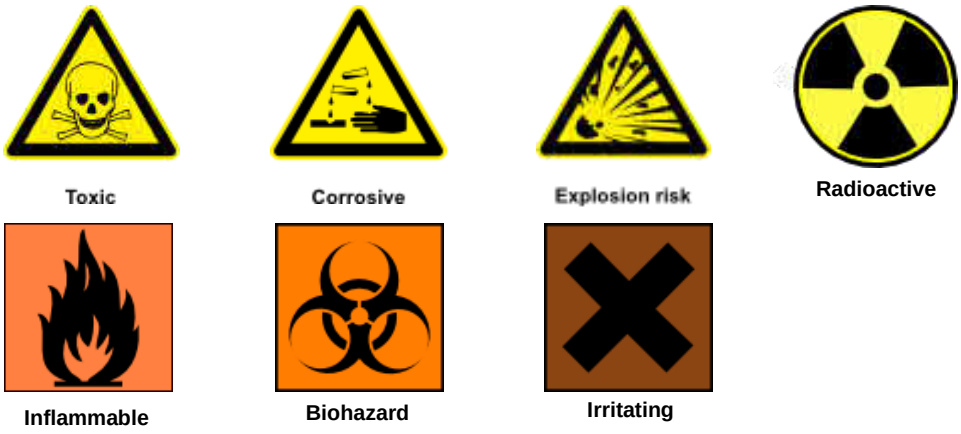
The rules and regulations regarding various categories of waste products are presented below.

- All glassware must be rinsed with tab water and labels and marks must be removed before placing it into the white container.
- All reaction tubes must be rinsed with tab water. All contents must be removed – if necessary by using a tube brush – and put upside-down in the baskets.
- All stoppers must be rinsed and deposited into the containers marked with “VUIL”.
- All glass pipettes must be rinsed with tab water and put upside-down into the pipette-holders.
- Chemical waste products must be stored in the appropriate containers (ask the laboratory staff).
- Risk waste products and all materials contaminated with those substances are disposed in special yellow bags (ask the laboratory staff).

- "Biohazard" waste products, i.e. biological substances, and all materials contaminated with those substances disposed in stainless steel sterilization jars, equipped with transparent plastic bags.
- All needles and scalpels are discarded in the small yellow/red containers present on the laboratory tables.
- Lab tables must be thoroughly cleaned and dried – when hazardous materials or bio-materials have been used, tables should be disinfected with 70% ethanol.
- All lab tables must be left neat and tidy (electrical instruments can remain plugged-in).

Chemical safety

You need to know what you are doing when working with chemicals. Therefore, it is essential to know where to find chemical information, risk indicators and safety advice. The category or risks of substances can be judged from the pictogram of logo displayed on bottles or jars. A few of the most frequently used logos are shown below:



In addition to theses logos, bottles and jars usually contain safety risk codes, referring to Risk and Safety Statements. Theses statements contain information on risks (R-phrases) and safety (S-phrases). Additional information on chemical characteristics, risks and safety can be found in the "Merck Index".

R-phrases

R-phrases

R1	Explosive when dry
R2	Risk of explosion by shock, friction, fire or other sources of ignition
R3	Extreme risk of explosion by shock, friction, fire or other sources of ignition
R4	Forms very sensitive explosive metallic compounds
R5	Heating may cause an explosion
R6	Explosive with or without contact with air
R7	May cause fire
R8	Contact with combustible material may cause fire
R9	Explosive when mixed with combustible material
R10	Flammable
R11	Highly flammable
R12	Extremely flammable
R14	Reacts violently with water
R15	Contact with water liberates extremely flammable gases
R16	Explosive when mixed with oxidizing substances
R17	Spontaneously flammable in air
R18	In use, may form flammable/explosive vapor-air mixture
R19	May form explosive peroxides
R20	Harmful by inhalation
R21	Harmful in contact with skin
R22	Harmful if swallowed
R23	Toxic by inhalation
R24	Toxic in contact with skin
R25	Toxic if swallowed
R26	Very toxic by inhalation
R27	Very toxic in contact with skin
R28	Very toxic if swallowed

R29	Contact with water liberates toxic gas.
R30	Can become highly flammable in use
R31	Contact with acids liberates toxic gas
R32	Contact with acids liberates very toxic gas
R33	Danger of cumulative effects
R34	Causes burns
R35	Causes severe burns
R36	Irritating to eyes
R37	Irritating to respiratory system
R38	Irritating to skin
R39	Danger of very serious irreversible effects
R40	Limited evidence of a carcinogenic effect
R41	Risk of serious damage to eyes
R42	May cause sensitization by inhalation
R43	May cause sensitization by skin contact
R44	Risk of explosion if heated under confinement
R45	May cause cancer
R46	May cause heritable genetic damage
R48	Danger of serious damage to health by prolonged exposure
R49	May cause cancer by inhalation
R50	Very toxic to aquatic organisms
R51	Toxic to aquatic organisms
R52	Harmful to aquatic organisms
R53	May cause long-term adverse effects in the aquatic environment
R54	Toxic to flora
R55	Toxic to fauna
R56	Toxic to soil organisms
R57	Toxic to bees
R58	May cause long-term adverse effects in the environment

R59	Dangerous for the ozone layer
R60	May impair fertility
R61	May cause harm to the unborn child
R62	Possible risk of impaired fertility
R63	Possible risk of harm to the unborn child
R64	May cause harm to breast-fed babies
R65	Harmful; may cause lung damage if swallowed
R66	Repeated exposure may cause skin dryness or cracking
R67	Vapors may cause drowsiness and dizziness
R68	Possible risk of irreversible effects

Combinations of R-phrases

R14/15	Reacts violently with water, liberating extremely flammable gases
R15/29	Contact with water liberates toxic, extremely flammable gases
R20/21	Harmful by inhalation and in contact with skin
R20/22	Harmful by inhalation and if swallowed
R20/21/22	Harmful by inhalation, in contact with skin and if swallowed
R21/22	Harmful in contact with skin and if swallowed
R23/24	Toxic by inhalation and in contact with skin
R23/25	Toxic by inhalation and if swallowed
R23/24/25	Toxic by inhalation, in contact with skin and if swallowed
R24/25	Toxic in contact with skin and if swallowed
R26/27	Very toxic by inhalation and in contact with skin
R26/28	Very toxic by inhalation and if swallowed
R26/27/28	Very toxic by inhalation, in contact with skin and if swallowed
R27/28	Very toxic in contact with skin and if swallowed
R36/37	Irritating to eyes and respiratory system
R36/38	Irritating to eyes and skin
R36/37/38	Irritating to eyes, respiratory system and skin

R37/38	Irritating to respiratory system and skin
R39/23	Toxic; danger of very serious irreversible effects through inhalation
R39/24	Toxic; danger of very serious irreversible effects in contact with skin
R39/25	Toxic; danger of very serious irreversible effects if swallowed
R39/23/24	Toxic danger of very serious irreversible effects through inhalation and in contact with skin
R39/23/25	Toxic; danger of very serious irreversible effects through inhalation and if swallowed
R39/24/25	Toxic; danger of very serious irreversible effects in contact with skin and if swallowed
R39/23/24/25	Toxic; danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed
R39/26	Very Toxic; danger of very serious irreversible effects through inhalation
R39/27	Very Toxic; danger of very serious irreversible effects in contact with skin
R39/28	Very Toxic; danger of very serious irreversible effects if swallowed
R39/26/27	Very Toxic; danger of very serious irreversible effects through inhalation and in contact with skin
R39/26/28	Very Toxic; danger of very serious irreversible effects through inhalation and if swallowed
R39/27/28	Very Toxic; danger of very serious irreversible effects in contact with skin and if swallowed
R39/26/27/28	Very Toxic; danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed
R42/43	May cause sensitization by inhalation and skin contact
R48/20	Harmful; danger of serious damage to health by prolonged exposure through inhalation
R48/21	Harmful; danger of serious damage to health by prolonged exposure in contact with skin
R48/22	Harmful; danger of serious damage to health by prolonged exposure if swallowed
R48/20/21	Harmful; danger of serious damage to health by prolonged exposure through inhalation and in contact with skin
R48/20/22	Harmful; danger of serious damage to health by prolonged exposure through inhalation and if swallowed
R48/21/22	Harmful; danger of serious damage to health by prolonged exposure in contact with skin and if swallowed
R48/20/21/22	Harmful; danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed
R48/23	Toxic; danger of serious damage to health by prolonged exposure through inhalation
R48/24	Toxic; danger of serious damage to health by prolonged exposure in contact with skin

R48/25	Toxic; danger of serious damage to health by prolonged exposure if swallowed
R48/23/24	Toxic; danger of serious damage to health by prolonged exposure through inhalation and in contact with skin
R48/23/25	Toxic; danger of serious damage to health by prolonged exposure through inhalation and if swallowed
R48/24/25	Toxic; danger of serious damage to health by prolonged exposure in contact with skin and if swallowed
R48/23/24/25	Toxic; danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed
R50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
R51/53	Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
R52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment
R68/20	Harmful; possible risk of irreversible effects through inhalation
R68/21	Harmful; possible risk of irreversible effects in contact with skin
R68/22	Harmful; possible risk of irreversible effects if swallowed
R68/20/21	Harmful; possible risk of irreversible effects through inhalation and in contact with skin
R68/20/22	Harmful; possible risk of irreversible effects through inhalation and if swallowed
R68/21/22	Harmful; possible risk of irreversible effects in contact with skin and if swallowed
R68/20/21/22	Harmful; possible risk of irreversible effects through inhalation, in contact with skin and if swallowed

R-phrases no longer in use

R13	Extremely flammable liquefied gas.
R47	May cause birth defects.

S-phrases

S-phrases

(S1)	Keep locked up
(S2)	Keep out of the reach of children
S3	Keep in a cool place
S4	Keep away from living quarters
S5	Keep contents under … (*appropriate liquid to be specified by the manufacturer*)
S6	Keep under … (*inert gas to be specified by the manufacturer*)
S7	Keep container tightly closed
S8	Keep container dry
S9	Keep container in a well-ventilated place
S12	Do not keep the container sealed
S13	Keep away from food, drink and animal feeding stuffs
S14	Keep away from … (*incompatible materials to be indicated by the manufacturer*)
S15	Keep away from heat
S16	Keep away from sources of ignition - No smoking
S17	Keep away from combustible material
S18	Handle and open container with care
S20	When using do not eat or drink
S21	When using do not smoke
S22	Do not breathe dust
S23	Do not breathe gas/fumes/vapor/spray (*appropriate wording to be specified by the manufacturer*)
S24	Avoid contact with skin
S25	Avoid contact with eyes
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S27	Take off immediately all contaminated clothing
S28	After contact with skin, wash immediately with plenty of … (*to be specified by the manufacturer*)
S29	Do not empty into drains

S30	Never add water to this product
S33	Take precautionary measures against static discharges
S35	This material and its container must be disposed of in a safe way
S36	Wear suitable protective clothing
S37	Wear suitable gloves
S38	In case of insufficient ventilation wear suitable respiratory equipment
S39	Wear eye/face protection
S40	To clean the floor and all objects contaminated by this material use ... (*to be specified by the manufacturer*)
S41	In case of fire and/or explosion do not breathe fumes
S42	During fumigation/spraying wear suitable respiratory equipment (*appropriate wording to be specified by the manufacturer*)
S43	In case of fire use ... (*indicate in the space the precise type of fire-fighting equipment. If water increases the risk add - **Never use water***)
S45	In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)
S46	If swallowed, seek medical advice immediately and show this container or label
S47	Keep at temperature not exceeding ... °C (*to be specified by the manufacturer*)
S48	Keep wet with ... (*appropriate material to be specified by the manufacturer*)
S49	Keep only in the original container
S50	Do not mix with ... (*to be specified by the manufacturer*)
S51	Use only in well-ventilated areas
S52	Not recommended for interior use on large surface areas
S53	Avoid exposure - obtain special instructions before use
S56	Dispose of this material and its container at hazardous or special waste collection point
S57	Use appropriate containment to avoid environmental contamination
S59	Refer to manufacturer/supplier for information on recovery/recycling
S60	This material and its container must be disposed of as hazardous waste
S61	Avoid release to the environment. Refer to special instructions/safety data sheet
S62	If swallowed, do not induce vomiting; seek medical advice immediately and show this container or label
S63	In case of accident by inhalation; remove casualty to fresh air and keep at rest

S64	If swallowed, rinse mouth with water (only if the person is conscious)

Combinations of S-phrases

S2)	Keep locked up and out of the reach of children
S7	Keep container tightly closed in a cool place
S7/9	Keep container tightly closed in a cool, well-ventilated place
S3/9/14	Keep in a cool, well-ventilated place away from … (*incompatible materials to be indicated by the manufacturer*)
S3/9/14/49	Keep only in the original container in a cool, well-ventilated place away from … (*incompatible materials to be indicated by the manufacturer*)
S3/9/49	Keep only in the original container in a cool, well-ventilated place
S3/14	Keep in a cool place away from … (*incompatible materials to be indicated by the manufacturer*)
S7/8	Keep container tightly closed and dry
S7/9	Keep container tightly closed and in a well-ventilated place
S7/47	Keep container tightly closed and at temperature not exceeding … °C (*to be specified by the manufacturer*)
S20/21	When using do not eat, drink or smoke
S24/25	Avoid contact with skin and eyes
S27/28	After contact with skin, take off immediately all contaminated clothing, and wash immediately with plenty of … (*to be specified by the manufacturer*)
S29/35	Do not empty into drains; dispose of this material and its container in a safe way
S29/56	Do not empty into drains, dispose of this material and its container at hazardous or special waste collection point
S36/37	Wear suitable protective clothing and gloves
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection
S36/39	Wear suitable protective clothing and eye/face protection
S37/39	Wear suitable gloves and eye/face protection
S47/49	Keep only in the original container at temperature not exceeding … °C (*to be specified by the manufacturer*)