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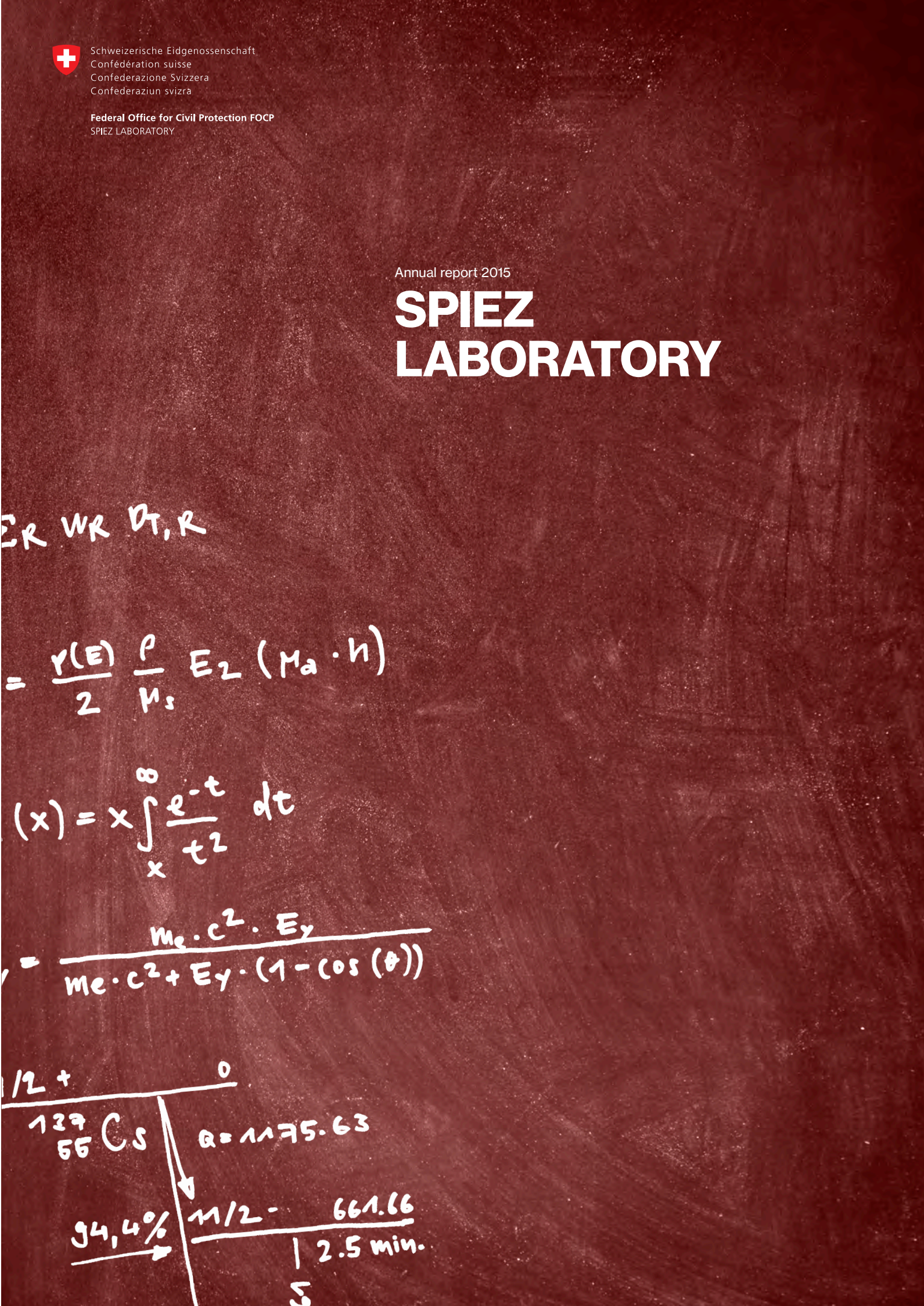
SPIEZ LABORATORY

Annual report 2015

SPIEZ LABORATORY



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Dear readers,

The latest risk report by the World Economic Forum again assigned to weapons of mass destruction the potential of causing, although with low probability, globally the worst consequences – right after the failure of agreeing on a climate policy.

Repeated confirmed use of chemical weapons in the Middle East as well as meticulously planned terrorist attacks by the Islamic State have in our opinion increased the likelihood of NBC-threats in Europe.

The proliferation of weapons of mass destruction remains one of the major problems for our security, and considering global developments, the corresponding challenges for Swiss security policy have not become any easier. This is a reality for intelligence services and police authorities alike, as well as for civil protection or NBC protection – a core objective of Spiez Laboratory.

Our resources were utilised to capacity in 2015: Our biosafety laboratory (operational at BSL-4 level since 2014) is the only facility in Switzerland set up for work with highly pathogenic microorganisms. Specific research projects are conducted inside the biosafety laboratory, such as studying the Ebola vaccine VSV-ZEBOV (page 18), as well as diagnostic activities as part of our reference functions for the Swiss health sector. Mandated by the Federal Office of Public Health, we operate the Reference Centre for Anthrax, thus ensuring a monitoring of highly pathogenic bacteria (page 22).

At Spiez we strive to establish and maintain state of the art, accurate and efficient analysis and diagnostic capability to meet our responsibilities in civil protection. We evaluate new developments, and where necessary we establish new procedures in-house, for example with the latest DNA sequencing methods. This is a very rapidly developing technology for which we have included an overview in this annual report (page 26).

This past year, we were again able to support international organisations with chemical analysis. And like every year, we had to demonstrate our analytical capability to the Organisation for the Prohibition of Chemical Weapons (OPCW) in their proficiency testing program. In their latest inter-laboratory test, we correctly identified all chemicals that are relevant for the Chemical Weapons Convention in the test samples and thus received the highest possible score. The OPCW proficiency testing program ensures a network of certified laboratories that is also available to the UN for investi-

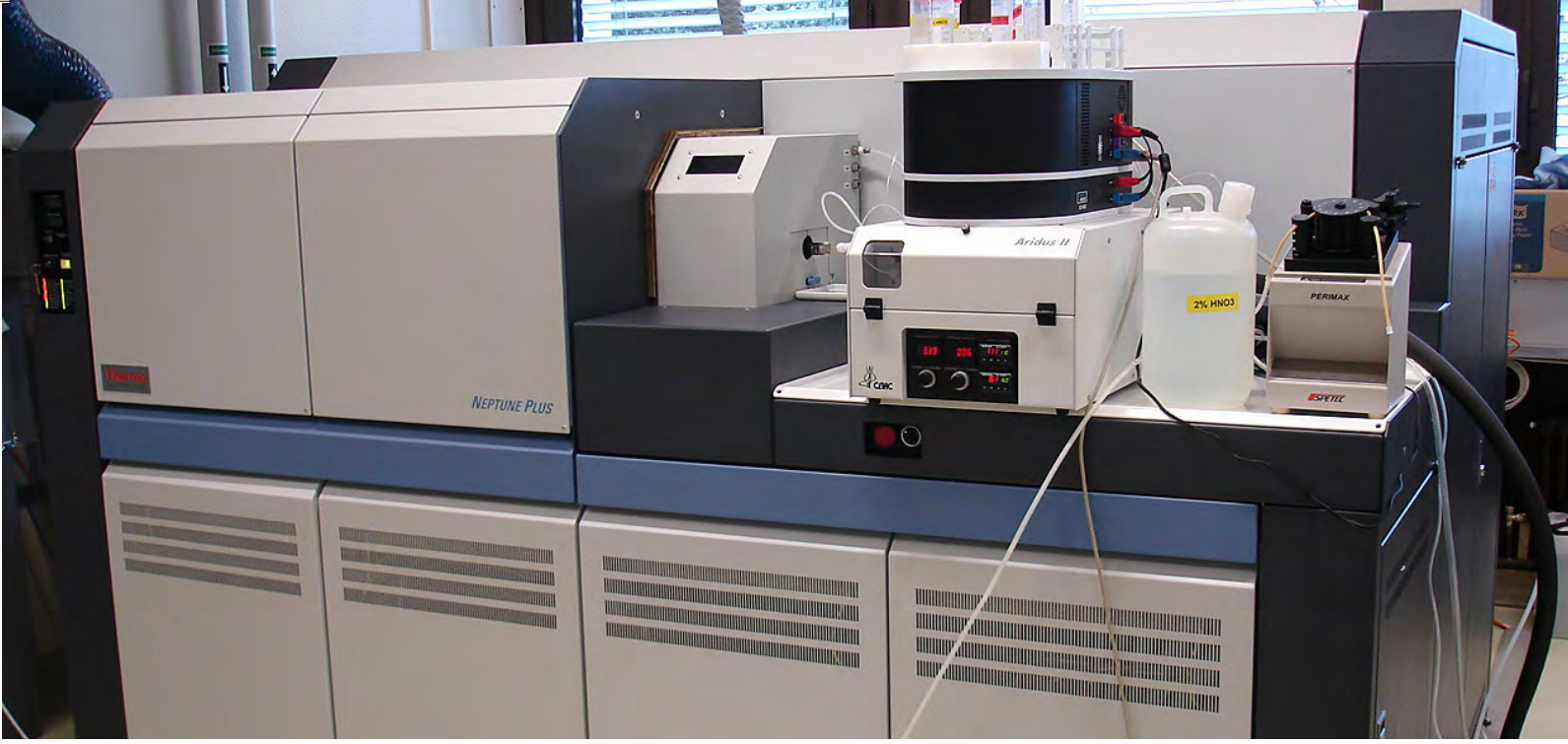
Working in the BSL-3 environment

Dr. Marc Cadisch
Director SPIEZ LABORATORY

gations under the Secretary General's Mechanism (UNSGM), as was the case in the investigation about Sarin use in Syria in 2013. There is, today, no similar network for the investigation of the use of biological weapons. This is the reason why Switzerland decided to organise a series of expert workshops to discuss the necessary steps to establish a network of designated laboratories in the field of biological weapons (page 34).

Not only are we active within our laboratory facilities: mobile measuring systems form an integral part of our legal mandate for national NBC protection. Spiez Laboratory response teams are well equipped and fully integrated into national measurement organisations (page 10). Our capabilities are put to the test regularly in challenging exercises with competent partners abroad (page 36). Close cooperation with the NBC Defence Laboratory 1 of the Swiss armed forces guarantees operational readiness over a long period of time, particularly during major incidents (page 48).

Our expertise is inevitably linked with the possibility of dual use, i.e. it could be applied to peaceful as well as non-peaceful purposes. We closely follow related discussions in policy and science communities and we are fully aware of our corresponding responsibility. In order to describe our responsible behaviour, we developed a code of conduct for the handling of dual use goods, data and information (page 14). Spiez Laboratory's commitment to international arms control and NBC protection also attracts great interest from the general public: The high number of visitors at last summers' open house day is proof of that (page 46).



Nuclear forensics - high end analyses

Dr. Stefan Röllin

Nuclear forensic methods are used to monitor both legal transport of nuclear material as well as increasingly illegal transport and contraband. The use of nuclear material can be linked to terrorist or extortionate intentions, right up to the intention to build nuclear weapons. The determination of isotope ratios when specifying the material is very important. Using the isotopic composition of uranium, for example, it can be determined whether a certain uranium is depleted or enriched and whether it would be suitable for the production of a nuclear weapon.

In order to determine the origin of nuclear material or at least to narrow down the origin, a kind of "fingerprint" is created using the radioactive substances and impurities. For example, very specific isotopic ratios of natural neodymium impurities in uranium samples can make it possible to draw conclusions about the origin of the uranium ore. In order to do this, the isotopic ratios have to be measured accurately at least in parts per thousand or even more precisely. This measurement accuracy can be achieved with a multi-collector mass spectrometer. Initial measurements in Spiez Laboratory using two natural neodymium solutions show that based on the isotopic ratios, a distinction can be made between the solutions. For nuclear forensics an accurate age determination of fissile material is crucial. In addition, the age of a sample can provide important information about the origin of a material. The Radioactivity Branch at Spiez Laboratory has developed a method of age determination for uranium and has applied it to various uranium samples. The method was validated by participating in an inter-laboratory test to determine the age of uranium.

Finding the origin of the uranium ore

The Radioactivity Branch has been using mass spectromic methods since 1999. These allow significantly lower detection limits than conventional radiometric methods, in particular for long-lived heavy isotopes such as uranium, plutonium, thorium and neptunium. Isotopic ratios of uranium and plutonium can be measured with an uncertainty of approx. 1% with the ELEMENT 2 ICP-MS. This level of accuracy suffices in distinguishing between depleted and enriched uranium or global fallout plutonium and plutonium from the Chernobyl reactor. The newly procured NEPTUNE PLUS multi-collector mass spectrometer (MC-ICP-MS) is also

NEPTUNE PLUS
multi-collector mass spectrometer (MC-ICP-MS)

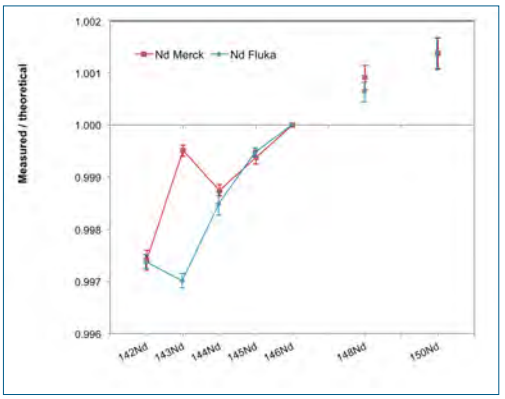


Figure 1:
Neodymium isotopic ratios of two different neodymium salts. The ratios of the Nd isotopes to the ^{146}Nd were measured and divided by the theoretical ratios of «natural Nd» according to the Karlsruhe table of nuclides.

an inductively coupled plasma mass spectrometer. The substance dissolved in diluted nitric acid is nebulised and ionised in the argon plasma. The ions are accelerated by magnetic and electric fields and separated according to their mass-to-charge ratio. However, instead of one detector 5 electron multipliers and 9 Faraday detectors are installed in the NEPTUNE PLUS, which can simultaneously measure different masses. Isotopic ratios can therefore be measured with an accuracy of < 0.01%.

With the Faraday detectors, isotopic ratios of well-ionisable elements, such as lanthanides and actinides, can be very accurately determined (< 0.01%) for concentrations 10 ng/ml or higher. However, a mass discrimination already occurs in the argon plasma, where space-charge effects result in a preferential transmission of the heavier isotopes. The mass discrimination is approx. 1% per mass difference. The measured isotopic ratio of ^{150}Nd/^{142}Nd would therefore be approx. 8 % too high. This effect was corrected by adding europium to each solution. The mass discrimination was determined using two europium isotopes. The influence of the mass difference between the europium and neodymium isotopes was corrected with an exponential function. Two different 10 ng/ml neodymium solutions were measured (Merck and Fluka). The information from the Karlsruhe table of nuclides was used as the theoretical natural neodymium isotopic composition. The isotopic ratios to the natural isotopic ratios are shown in Figure 1. The isotopic ratios to the isotope ^{146}Nd were each measured.

Figure 1 shows that the ratio ^{143}Nd/ ^{146}Nd clearly differentiates the two solutions. The ratio of ^{143}Nd to other Nd isotopes depends on the Sm/Nd ratio as well as the age in different geological formations, since ^{147}Sm decays with a half-life of 1.06x 10^{11} years in ^{143}Nd. The ratio ^{143}Nd/ ^{144}Nd and its deviation from the geological normalisation standard CHUR (Chondritic Uniform Reservoir) is given in literature. Using the remaining Nd isotopes, Table 1 shows the corrected ^{143}Nd/ ^{144}Nd ratio. Typical values of ε_{Nd} for the old continental crust are in the range from -10 to -36 and for volcanic rock layers in the range from 0-10.

The normalisation to a neodymium standard with a certified isotopic ratio is required to measure absolutely valid isotopic ratios. As the next step, an attempt is made to separate the neodymium impurities in uranium and draw conclusions about its origin by using the isotopic ratio ^{143}Nd/ ^{144}Nd and the element ratio Nd/Sm as well as the uranium deposition type.

Sample	^{143}Nd/^{144}Nd		ε Nd
	Ratio	±1σ	
Nd Merck	0.5123	0.0001	-7
Nd Fluka	0.5111	0.0001	-21

Table 1:
Measured ^{143}Nd/ ^{144}Nd ratios and their deviation from the isotopic standard CHUR.
ε_{Nd} = ((^{143}Nd/ ^{144}Nd)measured/(^{143}Nd/ ^{144}Nd)CHUR – 1)x10'000 where (^{143}Nd/^{144}Nd)CHUR = 0.512638.

Determining the age of uranium

The term 'age determination' here refers to the determination of the time of the last chemical separation of uranium from the daughter products. With the decay of uranium, the concentration of daughter products in the material increases again after the chemical separation. The ratio of daughter product to parent nuclide is used to determine the age. Ideally the half-lives of the measured nuclides are long when compared to the age of the specimen, which is typically between 0.5 and 70 years old. This is the case for the two following parent-daughter pairs:

$^{234}U \rightarrow {}^{230}Th$
$^{235}U \rightarrow {}^{231}Pa$

^{230}Th/^{234}U Chronometer

A complete separation of the daughter nuclides from the parent nuclide is assumed, i.e. N_{Th230}/N_{U234} is 0 and then increases steadily. As the time periods considered here consist of a few decades, it can be assumed with reasonable accuracy that the activity of ^{234}U remains constant and that of ^{230}Th only changes due to the decay of ^{234}U. This results in the following linear relationship:

$$A_{Th230} = \lambda_{Th230} \cdot A_{U234} \cdot t \text{ where } \lambda_{Th230} = 9.19 \cdot 10^{-6} y^{-1}$$

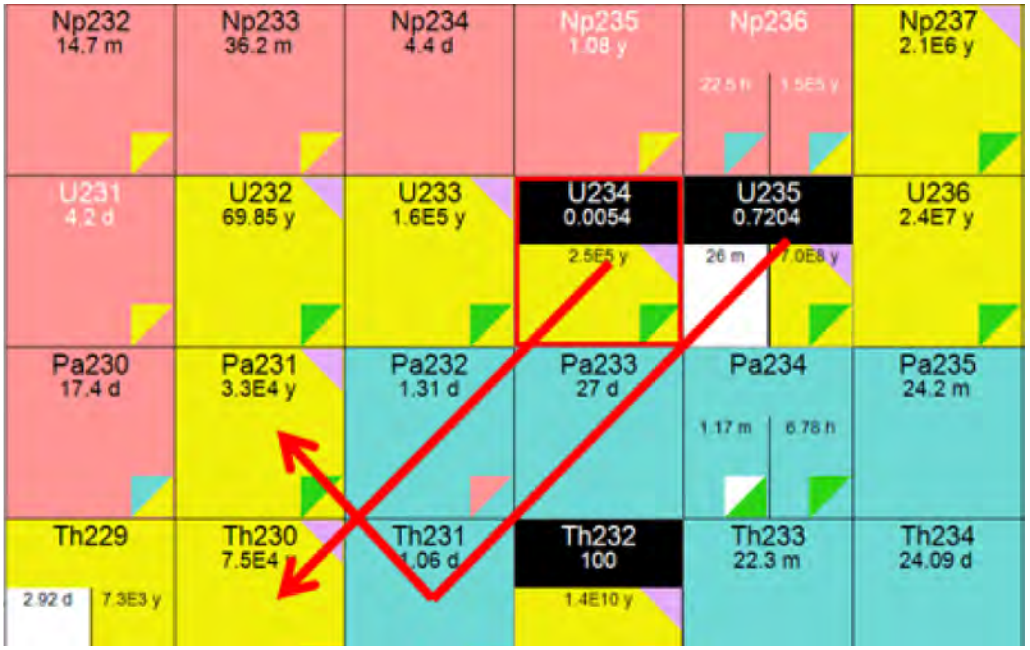


Figure 2:
Detail of the Karlsruhe table of nuclides with the two decay paths for uranium age determination.

The deviation due to the linear approximation is less than 3 days for an age of up to 50 years.

The uranium/thorium separation will never be complete. Assuming a separation factor of uranium and thorium of 10^6, the calculated age would be 58 days too old. However, the separation factors in newer systems are rather larger.

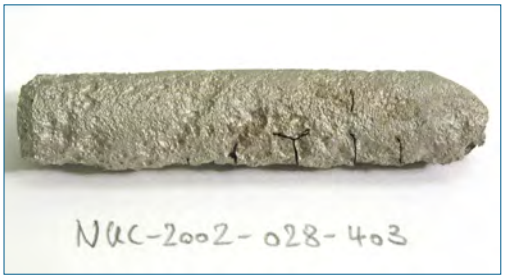
The samples of Table 2 were analysed: The uranium penetrator was used in the Bosnian War in 1995 in the bombing of the military barracks in Han Pijesak. The penetrator laid in the ground for 7 years and was first recovered during soil sampling in Bosnia-Herzegovina in 2002 by the UNEP mission. The corroded material was first removed mechanically and the penetrator was then washed with nitric acid. A few grams of the penetrator was leached in concentrated nitric acid.

The uranyl salts were old samples from the chemicals cabinet of the Radioactivity Branch. The uranyl nitrate sample REIMEP was prepared by Joint Research Centre of the EU as part of an inter-laboratory test to determine the age of uranium.

In each case 40 mg of uranyl salt was dissolved in 20 ml 3 M nitric acid. For the penetrator, an aliquot of the leaching solution was used, which corresponded to 20 mg of uranium. 0.2 ml of each solution was diluted for the determination of ^{234}U using ICP-MS. The rest of the solution was spiked with 20 mg of ^{232}Th and the thorium was separated by means of extraction chromatography and measured with the ELEMENT 2 ICP-MS.

Table 2:
Specimens for the age determination of uranium

Specimen	Origin of the uranium	Uranium preparation
Uranium Penetrator Bosnia-Herzegowina	NATO attack from 07 Sept. 1995	Before 07 Sept. 1995
Uranyl nitrate Fluka 94270 633141	Receipt at Spiez Laboratory 29 May 1972	Before 29 May 1972
Uranyl nitrate Fluka A58191	No information	
Uranyl nitrate B. Siegfried	No information	
Uranyl acetate Dr. Bender	Specification by Dr. Bender 29 May 1945	Before 29 May 1945
Uranyl nitrat REIMEP	Inter-laboratory test to determine the age of uranium	09 July 2012



Uranium Penetrator from Bosnia-Herzegovina. Corroded material was first scratched mechanically and then removed with acid.

Uranyl salts of various ages

Specimen	^{238}U		^{235}U		^{234}U		^{236}U	
	Comp. m%	±1σ m%	Comp. m%	±1σ m%	Comp. m%	±1σ m%	Comp. m%	±1σ m%
Uranium Penetrator Bosnia-Herzegowina	99.802	0.003	0.195	0.003	0.00067	0.00004	0.0028	0.00006
Uranyl nitrate Fluka 94270 633141	99.595	0.004	0.393	0.004	0.00261	0.00009	0.0085	0.00018
Uranyl nitrate Fluka A58191	99.564	0.005	0.431	0.005	0.00262	0.00004	0.0019	0.00012
Uranyl nitrate B. Siegfried	99.287	0.007	0.707	0.007	0.00545	0.00019	<	0.00003
Uranyl acetate Dr. Bender	99.287	0.008	0.707	0.008	0.00541	0.00027	<	0.00003
Uranylnitrat REIMEP	96.281	0.040	3.601	0.040	0.02816	0.00083	0.0896	0.00144

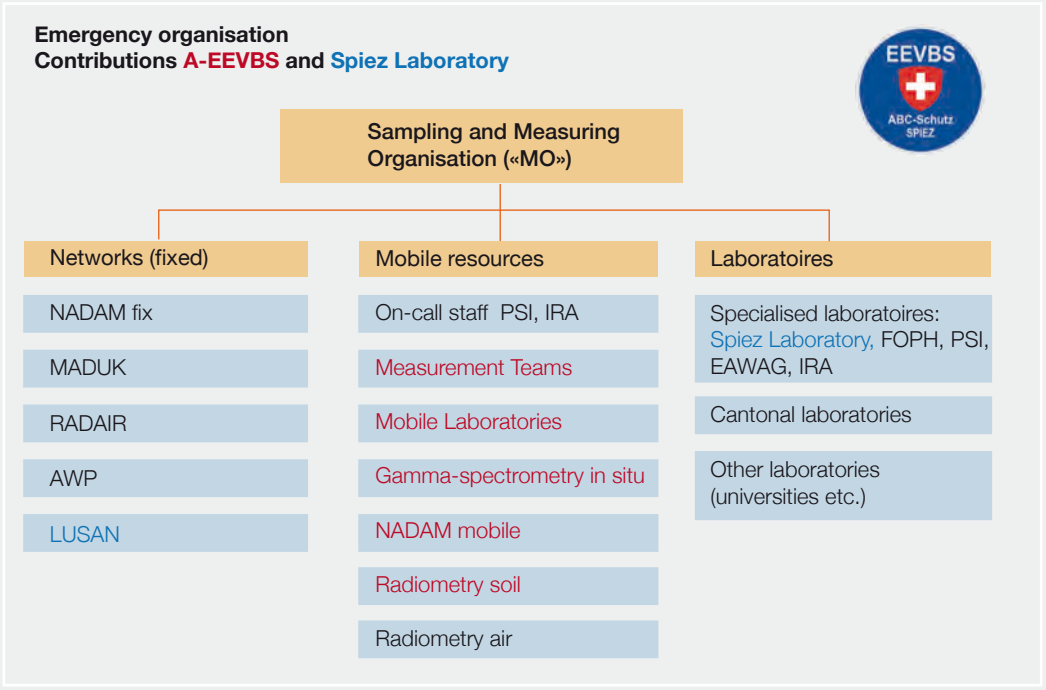
Table 3:
Isotopic ratios of uranium in per cent by mass (m%) with the ELEMENT 2 ICP-MS.

Specimen	^{234}U		^{230}Th		Uranium preparation	
	Active Bq/kg	±1σ Bq/kg	Active Bq/kg	±1σ Bq/kg	Separation Date	±1σ d
Uranium Penetrator Bosnia-Herzegowina	1'538'481	98'950	438	6	16.02.1983	± 740 d
Uranyl nitrate Fluka 94270 633141	2'839'787	101'404	1'054	10	20.09.1973	± 544 d
Uranyl nitrate Fluka A58191	2'963'563	63'316	1'408	17	06.06.1962	± 464 d
Uranyl nitrate B. Siegfried	6'107'926	232'808	50'515	340	-	-
Uranyl acetate Dr. Bender	7'446'718	398'270	5'660	65	10.06.1931	± 1653 d
Uranyl nitrate REIMEP	31'444'796	993'826	455	15	31.07.2012	± 20 d

Table 4:
Activity of U-234 and Th-234 and the resulting calculated time of uranium preparation

The experimentally determined age of the uranium for the five specimens was larger than or equal to the minimum age whilst taking uncertainty into account. For the uranyl nitrate of B. Siegfried, no age determination was possible, since the thorium daughters were not fully separated when the uranium nitrate was produced and therefore the ^{230}Th/ ^{234}U chronometer was not set to zero.

The accuracy of the age determination of uranium depends on the accuracy of the quantitative determination of ^{234}U and ^{230}Th. As the next step, the Radioactivity Branch will perform the quantitative determinations of ^{234}U and ^{230}Th with isotope dilution analyses. For this purpose, the isotopic ratios are measured with the multi-collector ICP-MS.



Mobile resources to be deployed in case of increased radioactivity levels

Dr. Béatrice Balsiger, Dr. Emmanuel Egger

In the event of incidents leading to increased radioactivity levels, the Federal Government establishes the so-called «MO», i.e. the sampling and measuring organisation.

The «MO» is based on 3 important pillars: Fixed networks supply the database to allow for large-scale decisions to be made. NADAM and MADUK networks constantly provide dose rate values (regardless of incidents). The RADAIR network operates continuously to monitor airborne radioactivity. In case of incidents, the NEOC (National Emergency Operations Centre) automatically deploys the «atomic warning posts» (police and firefighter measuring at predefined places) and LUSAN networks (aerosol collectors with gamma measurement at predefined locations).

Mobile resources are deployed either for minor incidents (picket services), or for major incidents to increase measurement points in the (potentially) affected area. For example, it is possible to place «mobile NADAM» sensors in an area, where the radioactive cloud is expected to move to following a nuclear plant accident.

The third pillar is provided by a few specialised laboratories, including Spiez Laboratory (if necessary, with the support of the NBC 1 defence laboratory of the Army), and some cantonal laboratories which in turn will be responsible for measuring food. These precise measurements in laboratories help determine the next measurement points for mobile resources.

Spiez Laboratory and the A-EEVBS (joint deployment team of Spiez Laboratory and the Centre of Competence NBC DEMUNEX of the Army) cover a large portion of the deployment resources, and especially those of a mobile nature. These are presented in the following.

In the context of nuclear forensics, a field of ever increasing relevance, Spiez Laboratory will work closely with the Federal Criminal Police (FEDPOL), the Border Guard, and the Intelligence Services of the Confederation to fight illicit trafficking of radioactive sources suitable for manufacturing dirty bombs, or nuclear material suitable for building explosive nuclear devices. For this purpose, a vehicle equipped with neutron and gamma ray detectors was commissioned and will be operational sometime in 2016.

Mobile laboratories serve to measure the radioactivity absorbed by people. To this end, we can measure the whole body or the thyroid gland. It is also possible to measure samples.
There are three available vehicles, which do not require a truck license.
Vehicles may also be employed for taking environmental samples.

Yellow mobile laboratories (3)

The new WBC allows incorporation measurements in five different positions. For this, the WBC is equipped with two removable HPGe detectors. To ensure optimal mobile measuring, the person being measured must be lying down.
These measurements serve to assess the incorporated radiation dose.

Whole body counter (WBC)

Sensors continuously measure the dose rate, and transmit it to NEOC to summarise the radiation situation.
The number of mobile NADAM sensors were recently increased from 20 to 30 units.
Thus, it became necessary to optimise the approach to storage and transport. Sensors are deployed to requested locations by the technical service of Spiez Laboratory.

Mobile NADAM resources

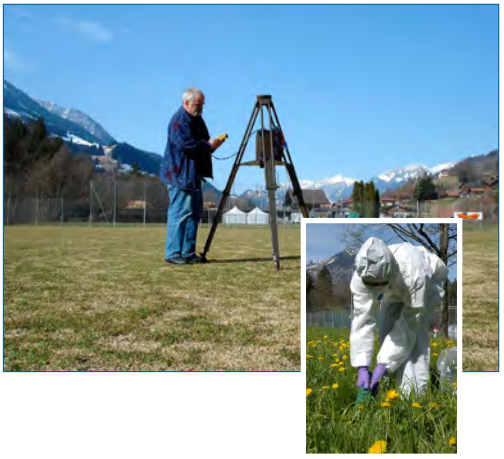
Portal monitors

Portal monitors are used for triage. This entails a rapid procedure whereby 2500 to 10000 people per day (depending on the chosen mode) can undergo an initial summary measurement.
Portal monitors are deployed with a trailer at the «Radioactivity Information Centre». The concept for deploying further portal monitors is currently in development.

Modularity

Mobile A-EEVBS resources must be deployable in different situations. This requirement has led to the creation of predefined modules which may be deployed with a vehicle and can be reloaded on pallets suitable for helicopter transport.
Special crates have been designed to meet the IAEA requirements for RANET (Response and Assistance Network) in international deployments.

Measurement and sampling



In situ gamma spectrometers (electrically-cooled HPGe detectors have been available for several years) are used to determine ground contamination. In November 2015, Spiez Laboratory organised a training course in this area for the IAEA.
Field samples must be taken in consistent manner. A national working group rewrote procedures and created new forms with the participation of Spiez Laboratory.

Outlook
New technologies have been acquired so that certain problems could be solved more effectively in the future.

Hexacopter

After a radiation accident e.g., Fukushima power plant, optimising the decontamination process and verifying the effectiveness of decontamination measures demands making dose rate measurement at many points including roads, trees, roofs etc. To access them quickly, we have acquired a hexacopter equipped with a dose-rate meter, which transmits its data by radio to the pilot on the ground. Thus, the latter can assess residual contamination on a roof, or on a tree without having to climb them.
As part of the fight against radiological and nuclear terrorism, Spiez Laboratory works closely

with the Federal Criminal Police, the Border Guard, and the Intelligence Services. An investigation vehicle fitted with gamma ray and neutron detectors will be used to monitor vehicles on our roads, or pedestrians attending mass demonstrations. This should help in detecting possible illegal transport of radioactive sources or of nuclear materials, which may be suitable for manufacturing explosive nuclear devices.

Series of neutron and gamma ray detectors on the manufacturer's test bench.

Acronyms

	french	german
NADAM (Automatic Dose Alarm and Monitoring Network)	**NADAM** (Réseau automatique de mesure et d'alarme pour le débit de dose)	**NADAM** (Netz für automatische Dosisalarmierung und –messung)
MADUK (Automatic dose-rate monitoring network)	**MADUK** (Réseau de mesure pour la surveillance automatique du débit de dose dans l'environnement de centrales nucléaires)	**MADUK** (Messnetz zur automatischen Dosisleistungüberwachung in der Umgebung der Kernkraftwerke)
RADAIR (Automatic Network for Air Radioactivity Monitoring)	**RADAIR** (Réseau Automatique de Détection dans l'Air d'Immissions Radioactives)	**RADAIR** (Automatisches Alarmierungsnetz für künstliche Radioaktivität in der Luft)
NEOC (National Emergency Operations Center)	**CENAL** (Centre National d'Alarme)	**NAZ** (Nationale Alarmzentrale)
AWP (Atomic Warning Posts)	**PAAT** (Poste d'alerte atomique)	**AWP** (Atomwarnposten)
LUSAN (Network of air and aerosol collectors)	**LUSAN** (Réseau de collecteurs d'aérosols)	**LUSAN** (Luftsammler-Netzwerk)
FOPH (Federal office of public health)	**OFSP** (Office fédéral de la santé publique)	**BAG** (Bundesamt für Gesundheit)
PSI (Paul Scherrer Institute)	**IPS** (Institut Paul Scherrer)	**PSI** (Paul Scherrer Institut)
Eawag (Swiss Federal Institute of Aquatic Science and Technology)	**Eawag** (Institut de recherche aquatique)	**Eawag** (Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz)
IRA (Institute of Radiation Physics)	**IRA** (Institut de radiophysique)	**IRA** (Institut für angewandte Radiophysik)

The dual use problem: A code of conduct for Spiez Laboratory

Dr. Cédric Invernizzi

Aspects of dual use are usually underestimated by researchers or even ignored, not least due to the lack of awareness and training. In order to prevent any deleterious legal scenario, it is important to tackle the problem at an early stage. This ensures that researchers take into account possible consequences and consider alternatives, and incorporate them into their thought process. A resonable approach that fosters responsible conduct in research with regard to the dual use problem has been a major concern for Spiez Laboratory for years.

«Killer mousepox virus raises bioterror fears» (New Scientist, 10 January 2001), *«Five easy mutations to make bird flu a lethal pandemic»*, (New Scientist, 21 September 2011). Disturbing headlines like these always make it into the daily press. Terrorist activities are not the triggers behind these headlines, but rather results from academic research, which have been published as scientific reports in journals or as contributions at conferences. In other words: new findings from basic research may not only be used for the benefit of society, but could also, under certain circumstances, be misused by state or non-state actors or individuals in their original or in an altered form to harm humans, animals, plants or habitats. This risk is described as the dual use problem in research.

An often-used example for the dual use problem is the mousepox experiment that was published by an Australian group of researchers in 2001.[1] Due to a plague of mice in Australia in the 1990s, some researchers studied genetically modified versions of the mousepox virus (organism listed as risk group 2 in Switzerland) with the intention of regulating the mouse population. In the process, an additional gene was

[1] Jackson RJ et al. Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. J Virol. 2001 Feb.; 75(3): 1205-10. Link: http://jvi.asm.org/cgi/reprint/75/3/1205.pdf

added to the genome of the naturally occurring virus, which should have acted as an immunological contraceptive. The desired immunological effect, however, remained well below expectations. The researchers therefore recombined the modified mousepox virus with an additional gene, which was supposed to increase the immune response of the mice. However, in the process they developed a mousepox virus that even killed those mice that were originally immune against the natural virus. Theoretically, this also paved the way to develop viruses that could cause smallpox in people (eradicated since 1980; organism listed as risk group 4 in Switzerland), against which today's vaccines could prove useless.

Another example that has been hotly debated since 2011 is based on the findings of a Dutch group of researchers that were published[2] in a scientific journal after a long controversy. The work was aimed at better estimating the pandemic potential of the avian flu virus H5N1, thereby possibly yielding important information for global influenza monitoring as well as for the future development of vaccines. For this purpose, researchers wanted to find out the minimal number of mutations required to make the avian flu virus H5N1 not only fatal to humans, but also highly contagious between humans. The Dutch authorities considered the results to be sensitive enough to subject the planned publication to export controls. The researchers therefore had to submit a licence application to export the data contained therein, which was also ultimately approved by the authorities. This highly controversial governmental intervention, however, proved to be a hardly relevant «emergency brake», since it may not be an adequate way of dealing with the dual use problem in research.

In order to avoid legal consequences, it is important to deal with the dual use problem at an early stage. Numerous states already go much further by implementing restrictive measures at the legislative level. In addition to the Dutch approach that made use of export controls, a moratorium is currently in force in the US, which prohibits certain research on potentially pandemic pathogens (influenza, SARS, MERS). Furthermore, measures regarding accessibility and personnel security are enforced in the context of licences and inspections for the storage of and work with certain pathogens. A similar picture can be seen in France or in Denmark where corresponding approval and security control regimes are in place in addition to the legal requirements on biosafety. Some states have also convened committees and commissions that are called on for the specific implementation of laws geared at dual use research.

Compared to these examples with sometimes questionable cost-benefit ratios, the approach to fostering a responsible conduct with regard to the dual use problem in research has been a major concern for us at Spiez Laboratory for years. For example in 2009, we organised a

2 Herfst S. et al. Airborne transmission of influenza A/H5N1 virus between ferrets. Science, 2012 Jun 22; 336(6088): 1534-41. Link: https://www.sciencemag.org/content/336/6088/1534.full.pdf

series of seminars by two British biosecurity experts (Professor Dando from the University of Bradford and Professor Rappert from the University of Exeter) at several research institutions in Switzerland. Accompanied by representatives of Spiez Laboratory as well as the International Relations - Defence (IB V), both experts were able to stimulate an open, sometimes intense dialogue on the subject. Furthermore, we regularly organise courses in cooperation with the Conférence Universitaire de Suisse Occidentale (CUSO) that focus on dealing with the dual use problem that may occur when handling highly pathogenic agents.

In the fall of 2015, the Swiss Academy of Sciences (SCNAT) also launched a project that develops a code of conduct in biological research in order to prevent a possible misuse of biological materials. Basic rules and recommendations for the responsible conduct in research with regard to the dual use problem are to be developed in the process together with the research community at public institutions. Spiez Laboratory is actively involved in this unique nation-wide project.

We at Spiez Laboratory have thought about how the responsible behaviour practised in the laboratory every day, especially in dealing with the dual use problem, can be made more understandable to the public. Due to their special tasks, a comprehensive implementation of international law (Biological Weapons Convention, Chemical Weapons Convention, Non-Proliferation Treaty, etc.) is particularly important for the scientists at Spiez Laboratory. We have therefore introduced a code of conduct geared at the dual use problem in order to prevent misuse. This code helps us to raise the necessary awareness to prevent misuse in the context of science as much as possible without unduly restricting the freedom of research.

Dual use code of conduct

1. Risk management

Risk management is integrated as a part of integral security (ISi) at Spiez Laboratory and is continuously improved. This also includes a reliable and reasonable risk assessment.

2. Access control

Substances with hazardous potential as well as sensitive expert knowledge are stored well-protected and access to them is controlled. This includes transfer (internal and external), packaging and transport.

3. Raising awareness

All employees are aware of the impacts of their activities. In particular, they take into account the possibility of misuse of their own research in the sense of the dual use problem.

4. Research and development

Research projects and development work are checked for dual use aspects. This process takes place continuously throughout the entire project duration.

5. Transfer of knowledge

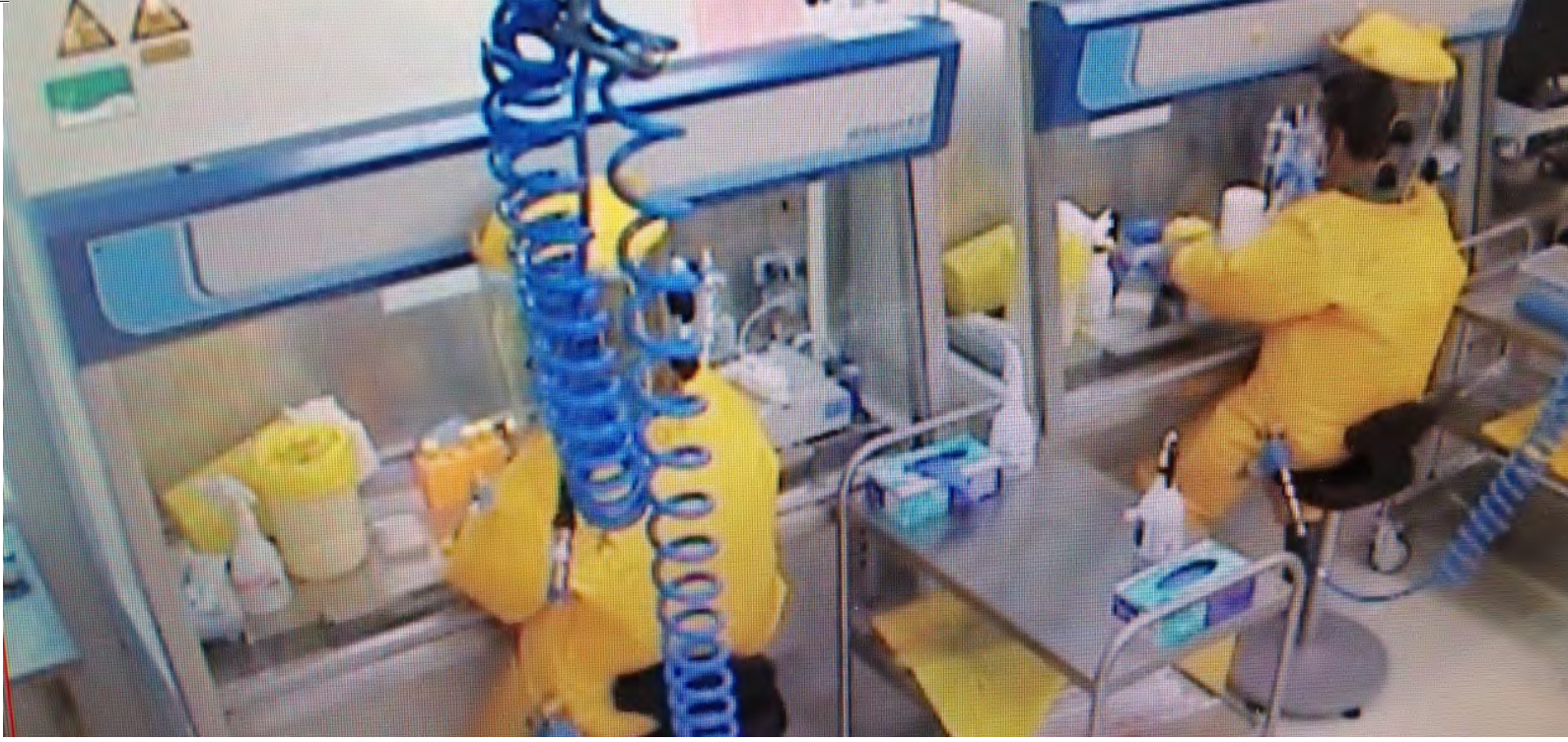
All employees pay attention to dual use aspects in their communication and are aware of the consequences. This applies mutatis mutandis to the transfer of «tacit knowledge».

6. Notification requirement

The observation or suspicion of misuse of substances or knowledge must be reported to the safety officer. Reporting persons are protected in every case.

7. Support

Spiez Laboratory supports national and international activities for the implementation of a responsible conduct in scientific research with regard to the dual use problem.



Determination of neutralising antibodies among subjects after vaccination with the Ebola vaccine rVSV-ZEBOV

Dr. Olivier Engler

Spiez Laboratory is participating in an international research project «VSV-EBOVAC», in which the promising vaccine VSV-ZEBOV to combat Ebola is being evaluated. As part of this project, the different aspects of the immune response are being analysed in 12 specialised laboratories in Europe, Africa and the US. At Spiez Laboratory, serum samples from immunised volunteers are being examined to determine the extent to which the serum of vaccinated individuals can neutralise the Ebola virus. Spiez Laboratory is Switzerland's only laboratory that is equipped to safely work with highly infectious pathogens like the Ebola virus.

Ebola was first identified in 1976 during outbreaks in Sudan and the Democratic Republic of Congo (DRC) and named after the Ebola River in the DRC. Infections with the Ebola virus were at that time characterised by a high case fatality rate of nearly 90 %. Since then, there have been a number of smaller and larger outbreaks in East Africa with a total of 2300 people infected and over 1500 deaths. The case fatality rate varied greatly from outbreak to outbreak, reflecting the fact that the term Ebola comprises of five types of viruses (Zaire ebolavirus, Budibugyo ebolavirus, Reston ebolavirus, Sudan ebolavirus and Tai Forest ebolavirus) with very different lethality rates.

The epidemic of 2013 differed from earlier outbreaks in many respects. For the first time, the origin of a chain of infection was in a West African country (Guinea) and due to the population density and the high mobility, rapidly spread to other countries (mainly Liberia and Sierra Leone). In less than one year, the infections increased to more than 9216 cases with over 4555 deaths. The extent of the epidemic was long underestimated, since many of the infected were initially treated outside of hospitals. By 3 January 2016, the WHO registered 28637 laboratory-confirmed cases with 11300 deaths.

Working in the BSL-4 environment
(Freeze frame of monitoring camera)

One of the international meetings of experts convened by the WHO deliberated in September 2014 for the need to introduce a well-tested and licenced vaccine to combat Ebola. Several vaccines had been evaluated in pre-clinical tests but only two formulations had shown good protection in non-human primates and had clinical grade material ready to start clinical testing. Both vaccines are based on recombinant viruses that are harmless to humans and were genetically modified so that they produce surface proteins of the Ebola virus in infected cells, thereby triggering an immune response against these proteins. The vaccine designated as «ChAd3-EBO» is based on a non-replicating chimpanzee adenovirus, which expresses the surface glycoprotein of Ebola Zaire and Sudan. The «rVSV-ZEBOV», developed by the Public Health Agency of Canada in collaboration with several international partners and licenced to Newlink Genetics and subsequently to Merck is the most advanced Ebola vaccine candidate and consists of a recombinant vesicular stomatitis virus (rVSV) where the virus's own glycoprotein was replaced with the Ebola Zaire surface glycoprotein (ref 1). Several pre-clinical and clinical studies with the vaccine rVSV-ZEBOV have shown promising results. At the end of 2014 and early 2015 clinical phase I/IIstudies were performed with the rVSV-ZEBOV vaccination under the patronage of the WHO. The vaccine induced a good immune response, which was stronger in applications with higher doses. However, transient vaccine side effects (ref 2) occurred in some of the test subjects. From April to July 2015, the effectiveness of the rVSV-ZEBOV vaccine was tested directly on Ebola-exposed persons in Guinea as part of a clinical phase III study (Ebola ca suffit; WHO and Wellcome Trust). Contact persons of Ebola patients either immediately received a high dose of rVSV-ZEBOV or first 21 days after a potential exposure. While none of the exposed persons with an Ebola vaccination became ill, 16 people in the control group became infected (ref 3). This data indicates a good vaccine efficacy, but the number of cases is too low for a definitive statement about the immunisation protection. There is also a need for further investigations with regard to the tolerance of the vaccine. As part of the VSV-EBOVAC project supported by the European Comission Innovative Medicines Initiative 2 joint Undertaking, the signature of the immune response, as well as the clinical symptoms that manifest as side effects upon progressive vaccination doses, are now (2015 to 2017) being characterised in great detail in a separate clinical phase I/II study. In addition to the classic immunological parameters, such as the antibody titre (amount and quality of antibodies produced) and the cellular immune response, changes to the gene expression in the immune cells as well as the effects on cell metabolism will be investigated.

The VSV-EBOVAC study is based on a vaccination concept with three different doses ($3x10^5$, $1x10^7$ and $5x10^7$ infectious viruses) and 10 consecutive blood samplings (Fig. 1). The immunisations were carried out at the end of 2014 in Switzerland, Kenya and Gabon and the clinical parameters were documented in detail by the test centres. The prepared blood samples were sent to ten specialised laboratories in Europe and the US according to an allocation formula. These laboratories will analyse in detail the key aspects of the innate and adaptive immunity over the next 2-3 years. Using activation markers and the release of mediators (cytokines) the activation of immune cells will be evaluated. Furthermore the effects of the vaccination on the gene expression pattern and the composition of the metabolites in the immune cells will be examined. An additional focus is on the establishment of a long-term immunity and the formation of corresponding memory B- and T-cells. Several studies indicate that the generation of antibodies is crucial for protecting against an Ebola virus infection (ref 4). The examinations of the rVSV-ZEBOV-induced production of antibodies is therefore of central interest. The neutralising capacity of these antibodies is being analysed in the BSL-4 facility of Spiez Laboratory.

Measurement of neutralising antibodies

Antibodies are proteins that are formed as effector molecules of B-cells and bind to surface proteins of viruses. Depending on the point of attack and strength of interaction, the viruses are only superficially bound with the potential to stimulate specific immune cells, or they cause the neutralisation of the viruses. This prevents the penetration of the viruses into the cells.

The neutralising effect of antibodies can be assessed with the serum neutralisation test (SNT). In this test a defined amount of infectious viruses is exposed to increasing dilutions of the test subject serums and the maximum serum dilution which has a neutralising effect

Fig. 1

VSV-EBOVAC Study Design

A clinical phase I/II dose finding, safety and immunogenicity study to identify the signature of an effective immune response to VSV-ZEBOV vaccine in correlation to different vaccine doses applied in volunteers.

Vaccinations were conducted in volunteers in Switzerland, Gabon and Kenya.

Vaccine doses

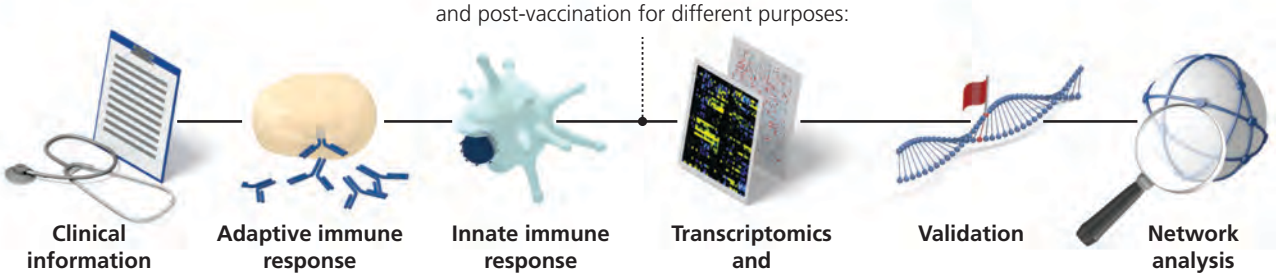
$3x10^5$, $1x10^7$ to $5x10Exp^7$ pfu (plaque forming units).

Vaccination day

Days

-90 -1 0 1 3 7 14 28 84 168

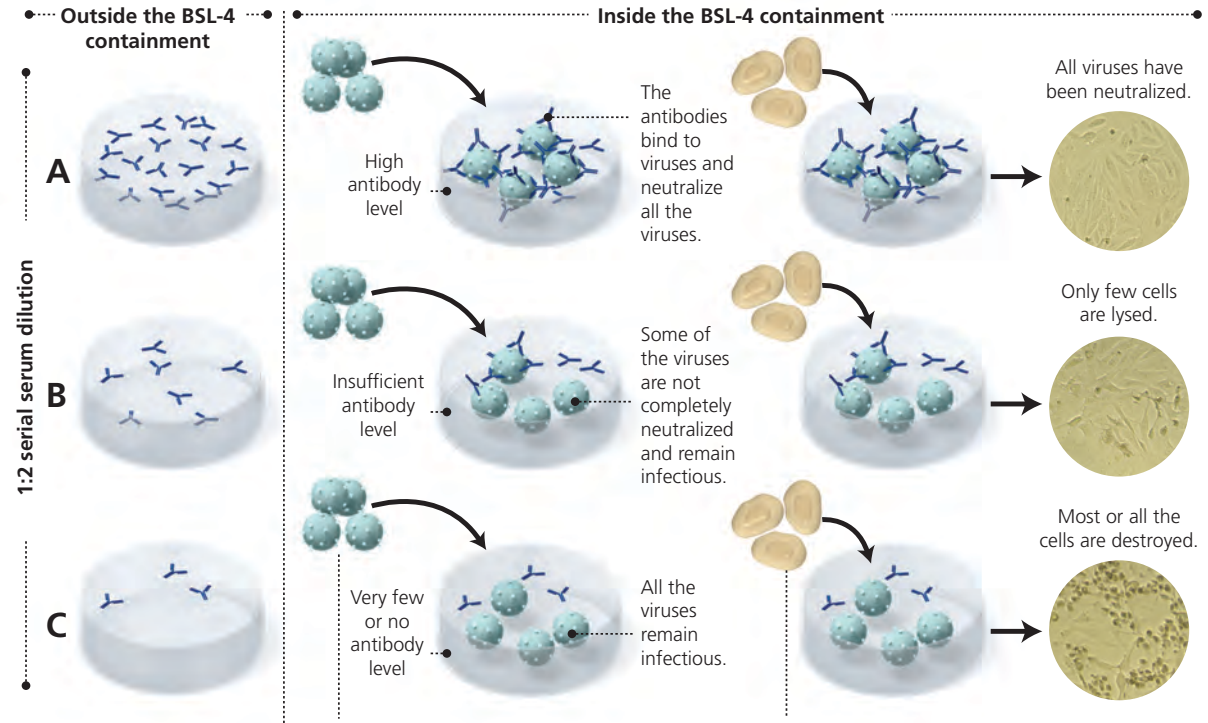
Blood samples were taken at 10 time points pre- and post-vaccination for different purposes:



Clinical information | **Adaptive immune response** | **Innate immune response** | **Transcriptomics and metabolomics** | **Validation** | **Network analysis**

Fig. 2

VSV-EBOVAC Neutralisation Test



1 Serum samples from vaccinees are diluted serially 1:2, starting from 1:8 to 1:1028 in 96 well plates.

2 A defined number of Ebola virus is added to each serum dilution.

3 The serum-virus mixture is then incubated for 1 hour at 37°C.

4 After the neutralisation time, cells are added to the virus-antibody mixtures and incubated for 6-7 days at 37°C.

5 Cell lysis is assessed by microscope after 6-7 days of incubation.

on the viruses is determined . Since Ebola viruses are highly pathogenic viruses of risk group 4, the procedures with infectious viruses must occur in a biological safety laboratory of level 4 (BSL-4 laboratory). The first step - the preparation of a dilution series of the serums from 1:8 to 1:1024 - is still possible in a normal microbiological laboratory of level 2 (Fig. 2, item 1). The serum dilutions are filled in a fourfold approach into a cell culture plate. From each vaccine recipient one serum sample taken before immunisation is analysed in parallel with a serum sample taken 28 days or 6 months after vaccination. In this way, the neutralising effects of individual serums that already existed before the immunisation can be detected. In each test series, a negative control serum and a standard antibody preparation (ZMapp) as a positive control are also introduced.

In a second step, the prepared serum dilutions in the cell culture plates are brought to the BSL-4 laboratory. Here, in a biological safety work bench, a standardised virus solution is made from a frozen Ebola virus stock and 100 infectious Ebola viruses are added to each serum dilution (Fig. 2, item 2). The virus serum mixtures are incubated at 37°C for 1 hour so that the antibodies bind to the viruses and can neutralise them. (Fig. 2; item 3). If there are a sufficient number of antibodies in the serum dilution, there is a complete neutralisation of the viruses (Fig. 2; situation A). If however, the antibody concentrations (titre) are too low, the Ebola viruses are not fully neutralised (Fig. 2; situation B) or are not neutralised at all (Fig. 2; situation C). In another step, a suspension with cells is added to the virus-serum mixture and this is incubated at 37°C for 7 days. During this incubation period the cells sink to the bottom of the cell culture plate and, if all Ebola viruses have been completely neutralised, they form a regular cell layer (situation A, item 5). In the event of an incomplete virus neutralisation, some of the cells are infected by the remaining infectious viruses. The viruses multiply in the cells, destroy them and infect other cells, which manifests in the cell culture as a microscopically perceptible change in the cells (cytopathic effect) (situation B, item 5). If no viruses or hardly any viruses were neutralised, the cytopathic effect appears as a nearly complete destruction of the cell layer (situation C, item 5).

For each serum in the serum neutralisation test, the dilution is determined and given as the vaccine titre that is able to substantially reduce the cytopathic effect. The vaccine titres are compared to the antibody titres of those Ebola patients who were able to successfully fend off the infection. This gives a good indication of whether the neutralising effect of the induced antibodies would provide sufficient protection. However, it has not at present been clarified with certainty which parts of the immune response are necessary and in what extent to eliminate the viruses. For this reason, the detailed evaluation of the immune response induced by the vaccine is of great interest in the overall context and should make it possible - in comparison to the immune responses induced in patients - to define a protective vaccine dose with low side effects. For this purpose, the results from the neutralisation test are being centrally merged with the analyses from other expert laboratories. The results obtained from the project enhance the development of a safe and efficascious vaccine to counter Ebola infections in humans and should contribute to a scientifically sound basis for recommending a vaccination dose (ref 1).

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The National Reference Centre for Anthrax: Secured laboratory diagnosis of reportable bacterial pathogens

Bacteriology Branch, Spiez Laboratory

On behalf of the Federal Office of Public Health (FOPH), the National Reference Centre for Anthrax (NANT) ensures the reference diagnostics and monitoring of highly pathogenic, bioterrorism-related bacteria. This includes the reportable pathogens of anthrax, plague, tularaemia, brucellosis and botulism. Both clinical samples as well as isolates are confirmed at Spiez Laboratory, typed and stored in a national culture collection. The NANT contributes to the quality assurance of primary diagnosis in the regional laboratories by using method transfer, control materials and inter-laboratory tests. External laboratories are increasingly invited to take advantage of NANT's services.

In the wake of the terrorist attacks following 11 September 2001 as well as the anthrax letters in the US, numerous powder-containing letters also popped up in Switzerland, which had to be tested for anthrax spores. At that time, there was still no concept for the analysis of suspicious bio-terrorist samples in Switzerland and there was a lack of resources, expertise and biosafety. In response to these deficits the National Reference Centre for Anthrax (NANT) was founded on 1 November 2001, which also explains the name. At this time, it was found by the Swiss Conference of Cantonal Health Directors and the FOPH that laboratory diagnostic evidence is crucial for detecting and controlling highly pathogenic agents, regardless of whether they are naturally occurring, accidentally or deliberately released. It was therefore requested that sufficient capacities for ensuring an adequate primary analysis must be available in the case of an event. From this requirement, the concept for creating a Swiss network of regional laboratories (RL) was established in 2003, which today is divided into six geographical regions involving all cantons. The six designated regional laboratories all have an infrastructure of biosafety level 3 (BSL-3) and are commissioned by the FOPH to provide an adequate primary diagnosis for highly pathogenic agents, especially in biological emergencies. Thanks to this decentralised structure, the consolidation of the dia-

Figure 1
Spiez Laboratory has two functions in the regional laboratory network (RLN): it performs the primary diagnosis of the central west regional laboratory on behalf of the Cantonal Laboratory of Bern and carries out the tasks of the NANT reference centre on behalf of the FOPH.

gnostic capacities as well as the creation of a certain redundancy, it was possible to strengthen Switzerland's crisis-resilience in the event of exceptional biological events. (see Figure 1)

Services and diagnostic capabilities

NANT is responsible for the secure laboratory diagnosis of *Bacillus anthracis, Brucella spp., Yersinia pestis, Francisella tularensis* and the botulinum toxin, as well as providing consultation for questions. After advance notification by telephone, the regional laboratories and other diagnostic laboratories can send primarily diagnosed isolates to the NANT for confirmation. NANT- according to the process for reportable pathogens - verifies the primary laboratory notification via the FOPH and the respective cantonal medical officer. Suspicious clinical samples are also accepted after consultation and then cultivated and confirmed in the biological safety laboratory of Spiez Laboratory under BSL-3 conditions (see cover image). The isolates are transferred into a national culture collection. This procedure is especially recommended for laboratories without an adequate security level. The corresponding examination applications and information about the sampling, proper packaging and transport can be found online at: http://www.labor-spiez.ch/en/the/bs/enthebsnant.htm

The NANT develops and validates detection methods for the pathogens mentioned. It has modern bacteriological diagnostic and identification methods based on cultivation and molecular biological, immunological and mass spectrometric analysis. Molecular epidemiology methods, including NGS ("next generation sequencing") are available for the typing and other clarification of isolates. If desired, clinical isolates can also be tested for sensitivity to select antibiotics. All the methods based on Polymerase Chain Reaction (PCR) are optimised for primary direct diagnostics. They are accredited (test body STS 0054) and provided to the regional laboratories together with the required control materials. This offer is also open to other diagnostic laboratories who require a validated method for primary diagnostic detection.

National quality assurance

Every year, the NANT organises an inter-laboratory test to verify the primary diagnostic capacities of the regional laboratories. For this purpose, 5-10 inactivated, quality-assured samples are provided together with a report template. Each year a different pathogen is tested (see Figure 2). The evaluation occurs anonymously and the results are analysed within the regional laboratory network and, if necessary also bilaterally. These inter-laboratory tests are used for the self-assessment of the regional laboratories and to optimise their processes.

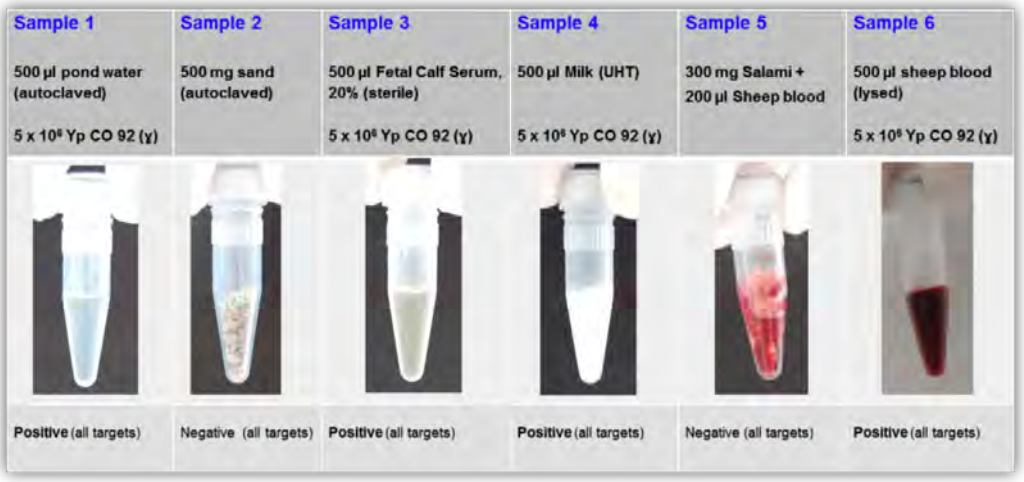


Figure 2
For the plague inter-laboratory test (October 2014), the challenge was to detect the inactivated pathogen *Y. pestis* (Yp, strain C092, inactivated by gamma irradiation) directly and with comparable sensitivity in different, sometimes disturbing matrices. All regional laboratories managed a correct interpretation of the samples. However, sensitivity differences showed a potential for optimisation.

They also verify the ongoing operational readiness of the RLN.

International cooperation

The diagnostic capabilities of the NANT are reviewed by participating in international inter-laboratory tests. Spiez Laboratory has been involved in the most important network of the European reference laboratories since 2009. Around 40 institutes from over 30 countries are represented in this network. The name of the network has been changed multiple times due to its status as an EU project (from EQADeBa to QUANDHIP to EMERGE starting in 2016). The main objective is to provide a long-term, integral European infrastructure to protect the population against highly pathogenic agents. This is to be achieved by developing, validating and standardising methods, through demanding inter-laboratory tests and training as well as by setting up a common reference repository for internal quality control. Spiez Laboratory plays an active role in the network and, thanks to its participation in inter-laboratory tests, has an independent performance verification.

Epidemiological monitoring

The NANT describes the variability of circulating pathogens for the FOPH. In addition to genetic and phenotypic properties, the temporal and geographical distribution is also documented. However, infections with anthrax (splenic fever), plague and brucellosis have become very rare in Switzerland. There has not been a case of plague in 30 years and an infection with skin anthrax was confirmed in 2014 for the first time in 23 years (see box). The situation is different with tularaemia: The number of cases has increased steadily in recent years to several dozen cases per year. Already in 2009, Spiez Laboratory placed its research focus on this pathogen and performed a national screening of the tick population. In an extensive epidemiological study, eleven tick isolates as well as 19 human and 15 animal isolates were fully sequenced in 2015. A profound bioinformatic analysis of the genome data now allows the representation of the phylogenetic relationship of the isolates (see Figure 3). A good cooperation with the initially reporting laboratories and the FOPH is crucial for the continuance of the study, since the understanding of the occurrence of infection in Switzerland can only be improved with the involvement of human isolates.

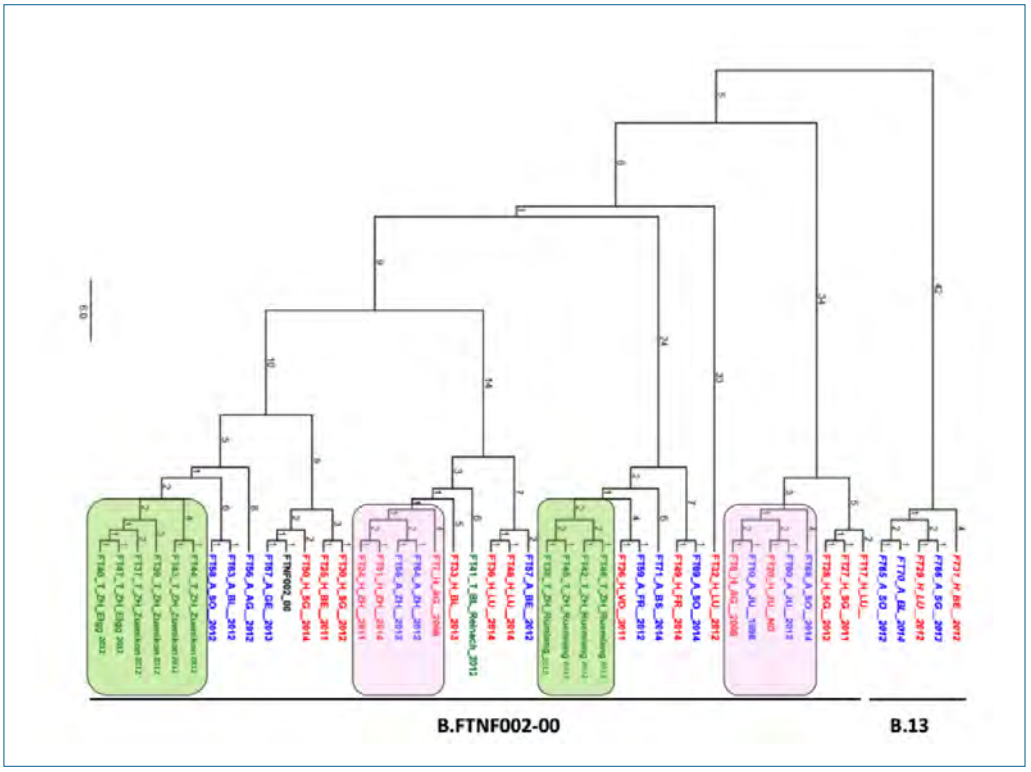
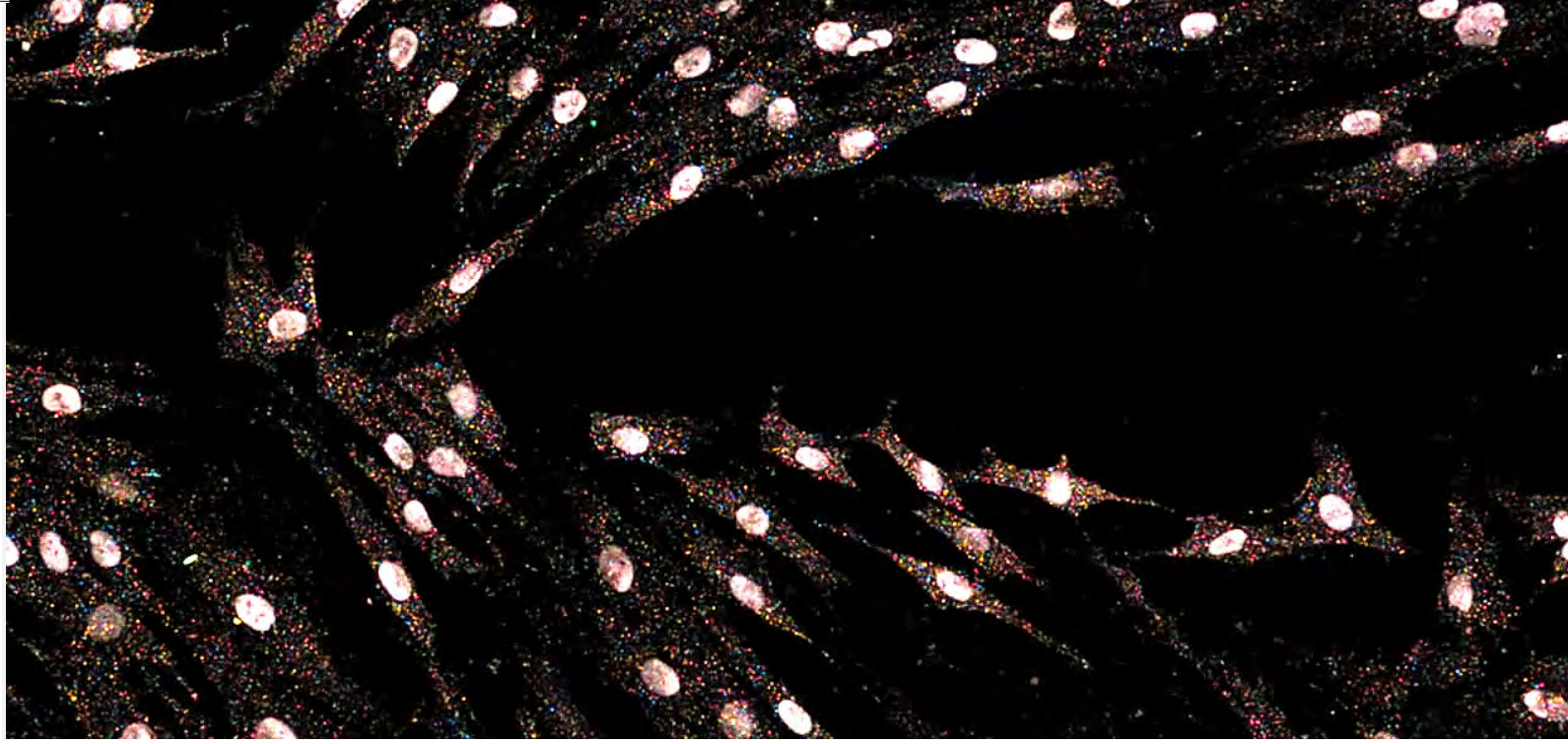


Figure 3:
Phylogenetic relationship of human isolates, animal isolates and tick isolates based on a genome-wide SNP (small nucleotide polymorphism) analysis. The numbers show the relative number of SNP mutations. The font colour indicates the origin of the isolates: red: human; blue: wild and zoo animals; green: ticks.

An unusual Turkish souvenir*

In August 2014, a Swiss citizen of Turkish origin returned back to Switzerland after staying with her family in a small Turkish village. Several cows that had died had been slaughtered and consumed in this village. The woman went to her doctor with a cutaneous ulcer on her finger and reported that other family members who were involved in the slaughter were suffering from skin ulcers. Some had even been admitted to the local hospital with nausea and vomiting. Turkey is an endemic area for anthrax (splenic fever), i.e. natural disease outbreaks among animals are not uncommon. The suspected cutaneous anthrax was substantiated by the isolation of the pathogen and the primary identification as *Bacillus anthracis* at the University Hospital of Basel. The NANT took care of the confirmation diagnostics and genome sequencing. Turkish anthrax isolates have been of particular interest for microbiologists for several years, because they have a close relationship with isolates from lesions of heroin users (so-called «injection anthrax»). It is believed that the drugs are unintentionally contaminated with anthrax spores on their trade route, whether by stretching with infectious animal meal or by packaging in contaminated animal hides.
Therefore an isolate with a complete match to the injection isolates would lead to a better geographical localisation of a drug trafficking route. The molecular epidemiological analysis of the isolated strain has shown that no closer relationship to injection isolates exists.

*Osthoff et al., Swiss Medical Forum, 2015;15(25):611-613



Next Generation Sequencing The latest developments in the field of sequencing

Dr. Christian Beuret

The unambiguous detection of a micro-organism occurs by the identification of its genes. Therefore, DNA sequencing methods are used to determine the sequence of the individual nucleotides in a DNA. Spiez Laboratory uses methods from three different generations for the sequencing of Biothreat-related pathogens.

Introduction

The unambiguous detection of a micro-organism occurs by the identification of its genes. To do this, DNA sequencing methods are used to determine the sequence of the individual nucleotides in a DNA. The first DNA sequencing method was described in 1975 (1.2) and the most common dideoxy method according to Sanger permitted the first publication of the human genome (3.2 giga bases for 2.7 billion US dollars) by 2003 in the 13-year long «Human Genome Project». Since then with increasing importance in research and development, faster and more cost-effective sequencing methods of the second, third and soon the fourth generation have been developed.

Nucleic acids

Deoxyribonucleic acid (DNA) forms the genome (genetic make-up) in all forms of life and in certain types of viruses (DNA viruses). It consists of a sequence of four nucleotides, which are composed of a phosphoric acid residue, a ribose sugar and one of the four nucleobases, adenine (A), guanine (G), cytosine (C) and thymine (T). Thymine is replaced by uracil (U) in the single-stranded ribonucleic acid (RNA), which, amongst other things, allows for the conversion of genetic information into proteins in biological cells. The stable DNA double helix consists of two DNA single strands twisting around each other in opposite directions. The DNA single strands are held together by hydrogen bonds, two between A=T and three between C=G. In molecular biological detection methods, the complementary sequence of the opposing single strand can therefore be derived from the sequence of a single strand template.

With Fluorescent in situ sequencing (FISSEQ), living cells are fixed on a glass slide and their mRNA is reverse transcribed in the fixing medium in cDNA.

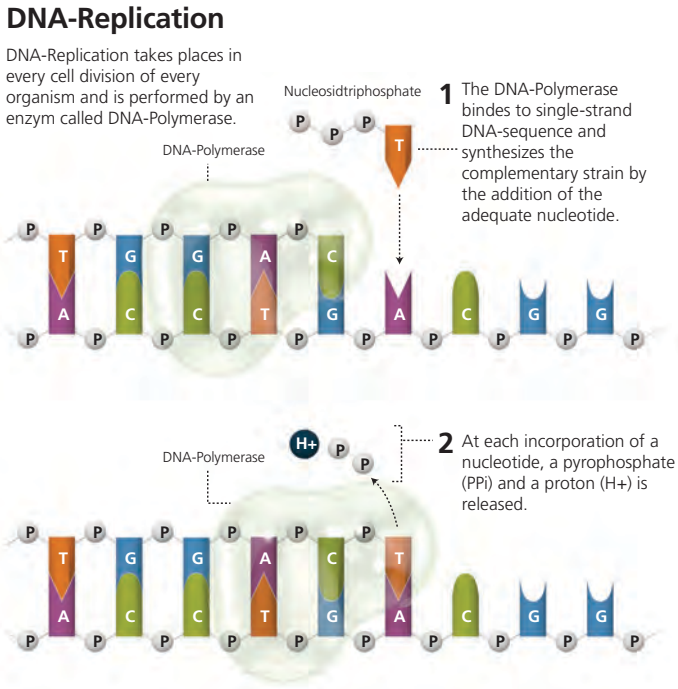


Fig. 1

DNA Replication

In any organism, the genes are replicated in the cell division by DNA polymerases (4). These enzymes bind to DNA single strands and synthesise the complementary strand by inserting the complementary nucleoside triphosphates (dNTPs). In the process, one pyrophosphate (PPi) and one proton (H+) are split during each insertion of a nucleoside triphosaphate (Fig.1).

In molecular biological detection methods, the short double-stranded DNA area required for the binding of the enzyme is made possible by the addition of one or two sequence-specific oligonucleotides (20-30 bases) (Polymerase Chain Reaction (PCR) 5,6.

In general it should be noted that modern DNA polymerases with an insertion rate of 1000 nucleotides have a biological insertion error rate (amplification bias). In NGS methods, a modified ϕ(phi)-29 DNA polymerase is therefore usually used, which is able to split a DNA double strand («stand displacement» activity) and which has an additional corrective «proofreading» activity (7). Since DNA polymerases require DNA as a template, their genome must first be rewritten by a reverse transcription in **cDNA** (complementary DNA) for the detection of RNA viruses. For this purpose, «RNA-dependent» DNA polymerases, so-called **reverse transcriptases**, are required.

Sequencing of the second (2nd) generation

The 2nd generation methods have replaced the previously most widely used dideoxy method according to Sanger (1). They are referred to as «**sequencing by synthesis**» (**SBS**) methods, since the sequencing occurs already during the replication. As «massive parallel or **whole-genome sequencing**» methods, they enable for the first time the investigation of entire genomes (genomics) and all genes present as transcribed mRNA in a cell (**transcriptomics**). In the 2nd generation method, the amplification bias induced by polymerases is minimised by repeated sequencing of a target sequence. A minimum 30-fold coverage of a sequence is the norm. The analysis of the data output was decisive for developments in the field of **bioinformatics** and IT infrastructure.

Massive parallel Sequencing (MPS)

The first SBS method was presented as «**pyrosequencing**» (8) in 1996 by a Swedish group of researchers. By 2006, it was further developed into an MPS method by 454 Life Sciences (US) (9) and starting in 2008 marketed by ROCHE. A further development by **454 technology** was published in 2007 as **semiconductor sequencing** (11) (IonTorrent Systems Inc.) and has been commercialised by LifeTechnologies (US) since 2010.
At the same time in 2008, today's «reversible terminator method» (10) from Solexa, UK (since 2007 Illumina, US) was already commercialised as the first NGS device under the name «Genome Analyser».

Fig. 2a

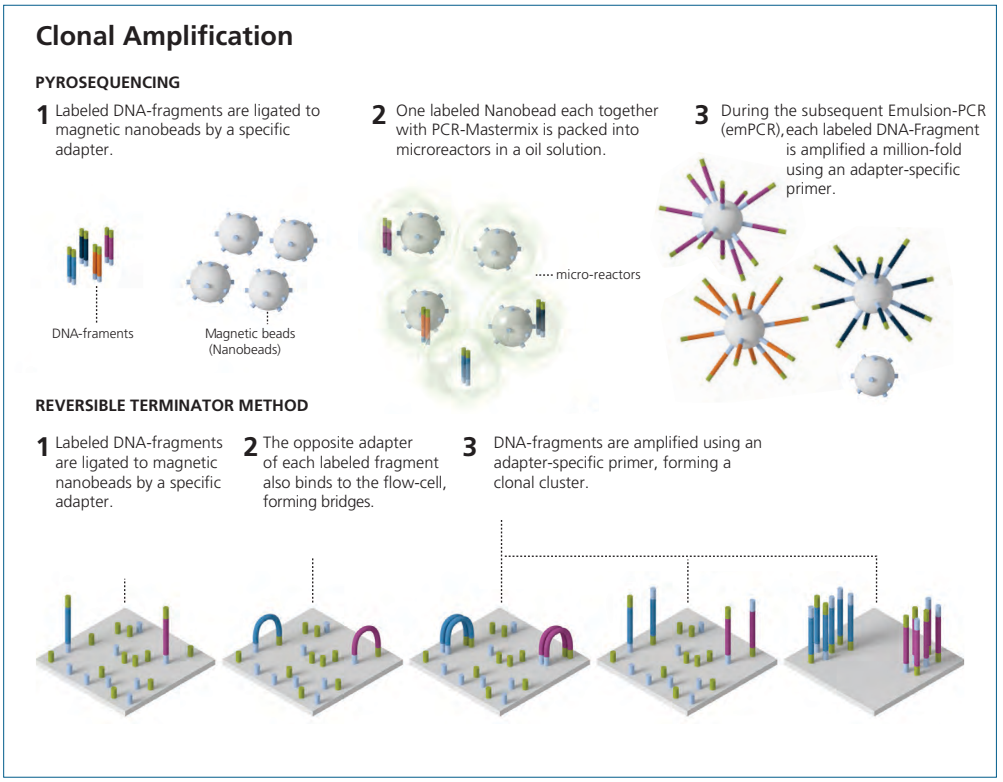


Fig. 2b

Clonal Amplification

In the aforementioned MPS method, the entire DNA of a sample is sequencend instead of a single previously amplified PCR fragment. In order to allow a parallelisation of this method, the entire DNA of a sample is first enzymatically, or using ultrasound, sheared into small DNA fragments (100-1000 bp). Since the detection of individual DNA fragments is not possible, these are amplified (cloned) before the sequencing. In the process, an adapter with a known «primer sequence» is ligated (appended) to both ends of all DNA fragments in order to allow an amplification without «DNA fragment-specific» primers. This «labelled» DNA library is then replicated in the «template preparation» via a clonal amplification:

a) For the methods based on **pyrosequencing** (Fig. 2a), the labelled DNA fragments are coupled specifically to a magnetic **bead** (nanospheres) via the adapter. Then the loaded beads are packaged in an oil-containing buffer in micro bubbles together with a PCR reaction mix. In the following **emulsion PCR (emPCR)**, the DNA fragments are amplified millions of times on each bead using an adapter-specific primer.

b) For the **reversible terminator method** (Fig. 2b), the labelled fragments are specifically bound via their adapters to an object slide size «**flow cell**» studded with matching adapters. However, the adapter at the other end of the fragment also binds to the flow cell, which results in bridge-type arcs.

These DNA fragments are cloned millions of times into a **clonal cluster** with an adapter-specific primer via a «**bridge PCR**». This «cluster technology» was primary developed by the Swiss company Manteia Predictive Medicine (Serono at the time) and acquired by Solexa in 2004.

In both methods, the template strand is finally enzymatically degraded and the amplified complementary strand is hybridised with a sequencing primer. This single strand template is used for the «sequencing».

Sequencing

a) **454 Technology** (2008) - Pyrosequencing

For the parallelisation of the sequencing reactions, the loaded beads from the emPCR are distributed together with polymerases on a picotitre plate (75 cm x 70 cm) with 1.7 million reaction vessels (Ø 29 µm). During **pyrosequencing**, the picotitre plate is flooded with one of the four nucleotides with cytosine). If a base is incorporated by the polymerases, the released **pyrophosphates** (PPi) generate a measurable bioluminescence through the enzymatic degradation of luciferin. The GS FLX titanium system mentioned thus allows for an output of up to 0.7 gigabases, or 1 million reads (700 bp), in 24 hours and for about CHF 7000. The sequencing of the human genome (30 x 3.2 Gb) was possible in 5 months and for about CHF 1 million. (Fig.3)

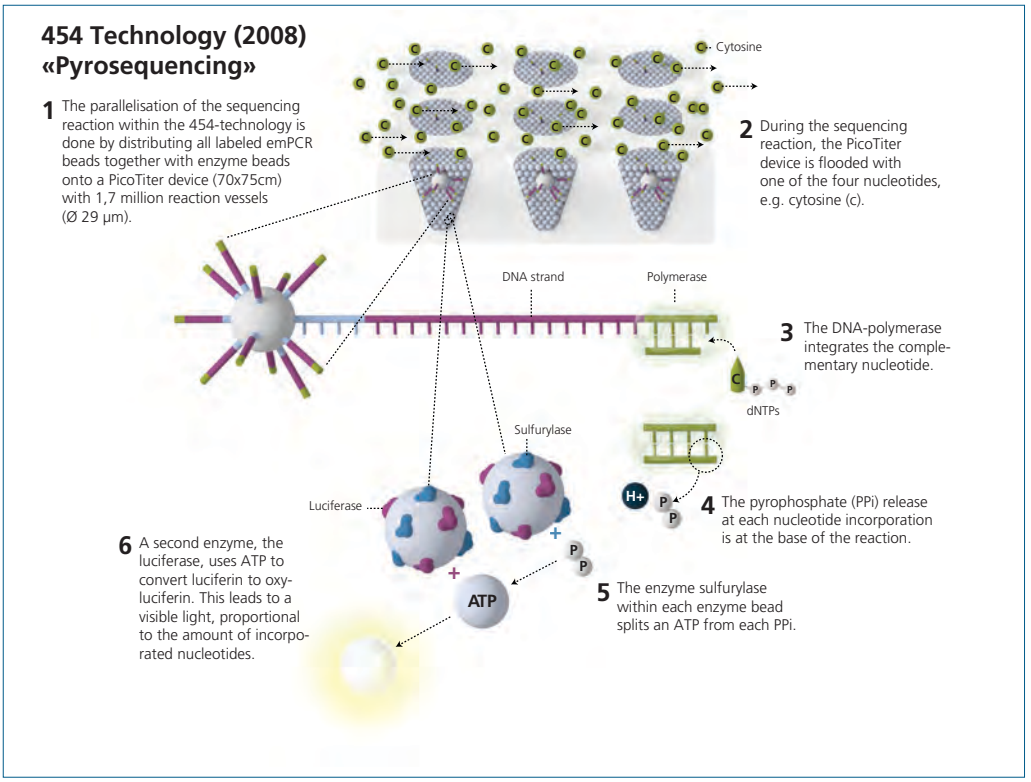


Fig. 3

b) Semiconductor sequencing (2010) – IonTorrent technology
With the advanced semiconductor sequencing, the previous bioluminescence detection of the pyrosequencing is replaced by the «**post-light sequencing technology**». This is based on the measurement of a short-term pH difference, which comes about through the **proton (H+)** that is released with each nucleotide insertion. This technology was previously packed in complementary «metal oxide semiconductor reaction chips (CMOS)» with 148 million reaction vessels. The underlying ion-sensitive coating passes on the pH change as a positive electrical signal and ultimately the sequence of a DNA fragment. Advantages of the technology are the measuring speed and the lower acquisition costs thanks to the lack of an optical and a laser device. The big disadvantage lies in the error-susceptible distinction of **homopolymers**, a sequence of the same nucleobases that, however, plays a subordinate role in the detection of micro-organisms.

The new ion S5 system (at Spiez Laboratory) delivers an output of up to 80 million reads (200 bp) in 7.5 hours, or 15 Gb for CHF 1500. The acquisition costs are relatively low at CHF 100,000. The sequencing of the human genome (30 x 3.2 Gb) is possible in 24 hours and for about CHF 7,000.

c) Reversible terminator method, the Solexa method
The reversible terminator method is based on the **simultaneous** addition of four **reversible-competitive nucleotides** on the already described flow cell occupied with «fragment clusters» These are each coupled to a fluorescent marker and a reversible **3'-synthesis blocker**, which prevents the insertion of additional nucleotides. If a DNA polymerase inserts one of the nucleotides (Fig. 4), the cluster is excited by a laser and the nucleotide-specific luminescence per cluster is registered. The **fluorescent marker** and synthesis blockers are enzymatically degraded and further synthesis is enabled. Using the resulting colour patterns, the DNA sequences of up to 300 million clusters can be simultaneously photographically evaluated.

In 72 hours, Illumina's new HiSeq X system produces a data output of up to 5.5 billion (2 x 150 bp, paired-end) reads, i.e. 1.7 terabytes of data for USD 1000. The acquisition costs of CHF 1 million, however, are high.

Sequencing of the third (3rd) generation
The 3rd generation sequencing methods first determine single long DNA molecules (20-60 kB) and are summarised as "single molecule sequencing" technologies. Since the amplification step is eliminated within the preparation of the template, the previous amplification bias of NGS methods of the second generation is

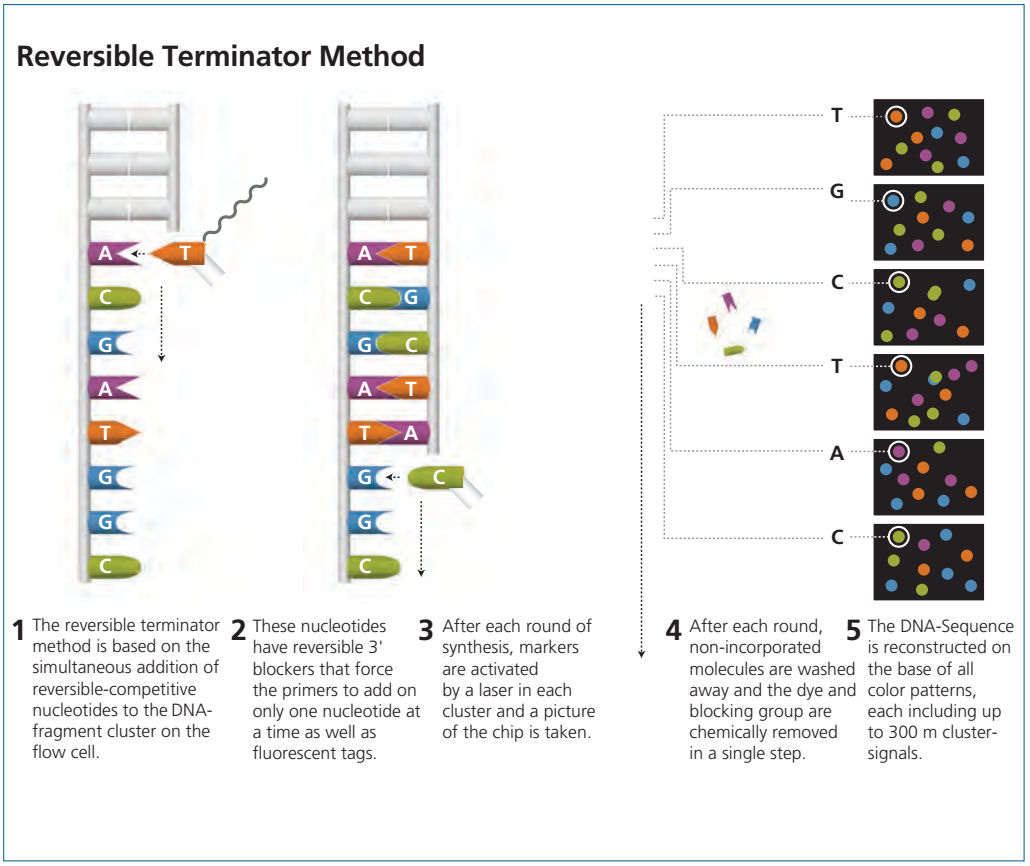


Fig. 4

avoided. In this way, the sample preparation time and, thanks to longer reads, also the bioinformatic analysis time is significantly shortened. For the first time, complex gene regions can be characterised by low bioinformatic effort.

Single Molecule real time Sequencing (SMRT), Pacific Biosciences (2010) The first commercial 3rd generation technology was presented in 2011-2012 by the US company Pacific Biosciences. The technology used is called «**single molecule real time sequencing (SMRT)**» (12) and for the first time, the sequencing of single, up to 20 000 bp (20 kb)-long DNA fragments become possible. For this purpose, the insertion of nucleotides using a DNA template is still measured, but the ϕ29 DNA polymerases are newly fixed to the bottom of «**zero mode waveguides (ZMW)**» using a biotin streptavidin link. The ZMWs are 70 nm x 100 nm large holes in an aluminium-coated silicate (SiO_2) with a capacity of about 20 zeptolitres (10-21 litres).

The second change lies in the use of nucleotides that are connected with a fluorophore via a «natural» phosphate bonding and therefore do not interrupt the DNA replication during their insertion. If one of the four nucleotides is inserted during the sequencing, the fluorophore is excited for a few milliseconds by a laser (600 nm) through the glass bottom of the ZMWs and the sequence can be continuously read. Since the laser merely reaches 30 nm through the ZMW base, only the nucleotide found in the DNA polymerase will be illuminated. For the sequencing, a SMRT bell library from a DNA sheared to 20 kb has to be created. Subsequently, «hairpin adapters» are coupled to both ends of the DNA fragments, which contain a sequence complementary to the sequencing primer. During the sequencing, the strand-displacing ϕ29 polymerase can sequence both DNA strands using the double-stranded circular template. Together with the deep signal-to-noise ratio, the SMRT technology achieves a previously amazing precision (system measuring deviation: >99.999 % (QV50).

In the latest sequel system (2015), up to 16 SMRT cells, each with 1 million ZMV, can produce up to 10 Gb of data in six hours, which corresponds to three human genomes.

Nanopore Sequencing (1996)

A very promising NGS method of the third generation is referred to as «Nanopore sequencing». Nanopore sequencing is based on a **potential** change in an artificial membrane that is generated during the flow of molecules (RNA, DNA, proteins) from other artificially-made nanopores (13). Biological transmembrane proteins as well as synthetic and semi-synthetic pores are also used as nanopores. Mainly two transmembrane proteins made of bacteria and one made of a virus are used as biological nanopores: the heptameric Ø-**haemolysin** toxin made of *Staphylococcus aureus*, the oct-

americ **MspA** porin of Mycobacterium smegmatis and the dodecamer «connector channel» from the «DNA-exciting motor» of the bacteriophage φ**29** or X174. The nanopores are embedded in a biological or artificial membrane, which has a particularly high electrical resistance. Unlike ordinary ion channels, nanopores are permanently open and allow for a constant molecular flow through the membrane after the application of a potential. The sequence can be read from the obtained data set through the specific potential changes for each of the four nucleotides.

Oxford Nanopore Technologies (ONT, 2009)
The company «**Oxford Nanopore Technologies (ONT)**» - an Oxford University spin-off - was founded in 2005. ONT managed the breakthrough in 2009 with the publication of the first validated nanopore technology (15). At the end of 2013, ONT opened a **MinION** Access Programme (MAP) where Spiez Laboratory was able to successfully bid for the evaluation of a revolutionary «USB stick size sequencer», the MinION. At the end of 2015, an early access programme was also opened for a future high-throughput system, **PromethION**. This system is operated with up to 48 ASIC chips, each for four samples. With a reading speed of 500b/s, the MinION allows a data output of up to 500 Gb. The PromethION promises up to 6.4 terabytes (1900 human genomes) per day.

Both devices are based on the patented «**strand sequencing technology**». For this purpose, the DNA of a sample is sheared to 8 kb beforehand and both sides are in turn ligated with adapters. The 5' adapter is a phi29 polymerase. The 3' adapter is a hairpin sequence that holds the 3' ends of both DNA single strands together. For sequencing, the polymer membrane with up to 3000 MspA nanopores is put under slight voltage within an ASCI chip and the DNA fragments are deposited on the nanopores with the 5'-phi29 polymerases. With its large single strand affinity, the **phi29 polymerase** ensures that only one DNA single strand flows through the nanopore caused by the charge. Thanks to the 3' **hairpin** sequence, both DNA strands are sequenced with up to 500 bases per second.

One of the challenges for the sequencing of DNA molecules using biological nanopores lies in the size ratio between the nucleotide and transmembrane protein, since up to four nucleotides simultaneously occupy a nanopore (14). The associated high error rate of 26.6 % in the sequencing of various bacterial species (16) and the increased difficulty in GC-rich gene regions could also be confirmed in Spiez Laboratory. The latest developments (2016) which are not yet confirmed should significantly reduce the error rate.
Another evaluation programme concerns the development of a sample preparation unit with the name «**VolTRAX**», which in the future will be combined with newly developed ASIC chips and should allow for a sequencing of non-prepared samples.

Sequencing of the fourth (4th) Generation
The term «4th generation sequencing» is first in its infancy, but could be used for the following methods:

Solid State sequencing
In the field of nanopore sequencing, synthetic pores are also being researched at the same time in order to achieve a «single base solution». To this end, nanoholes are shot into artificial membranes using various technologies. These artificial membranes consist of graphene, silicon oxide / nitride (Si_3N_4), aluminium oxide (Al_2O_3) and new molybdenum sulphate membranes (MoS_2). This technology has been researched by ONT since 2010 (18) among other things and is called «**solid state sequencing (SSS)**». In summary, voltage is also applied to the SSS membrane provided with nano-holes and in the future it should be possible to sequence DNA strands without additional preparation and without the use of enzymes. Further developments in nanopore technology concern the characterisation of entire proteins and other macromolecules for the passing of nanopores.

ROCHE - Genia - Stratos Genomics (2014)
Since 2014, the company ROCHE has also been active with respect to nanopore sequencing. On the one hand, the US company Genia with nanotag technology was acquired and, on the other hand, significant investments were made in the US company with the «Sequencing by Expansion™» (SBX) method. Both methods should be combined in order to achieve a «1-base solution». Genia's nanotag sequencing technology was developed in collaboration with Columbia University and Harvard University (17). In the process, a complementary strand with four tag-labelled nucleotides is synthesized from a DNA single strand template using a modified polymerase. During its replication, the larger tags and not the nucleotides fall through a biological nanopore and a base-specific voltage drop is measured. With this SBX method, the «1-base solution» is achieved by extending the DNA strand and the use of large nucleotide replacement tags. A polymerase is used for this purpose, which performs a replication with xNTPs using a DNA single strand template. These are a combination of four nucleotides, whose sequence is

shown in an attached hairpin structure with four colour differentiated tags. Once all xNTPs are inserted, the single strand template is enzymatically broken down and the xNTPs are enzymatically split in the middle. The distance between the bases is thus enlarged by 38-times, whereby so-called xpandomers are generated. These can be drawn through any nanopore induced by the charge and the sequence of the tags and thus the nucleotides can be read out.

In-situ Sequencing

New methods of sequencing aim at the investigation of the spatial distribution of the transcriptome of cell types or tissues. Previously, the examination of the transcriptom of a sample was dependent on an appropriate lysis step using subsequent DNA sequencing methods. With 3D imaging, the study of the gene expression takes on a completely new dimension.

With «Fluorescent in situ sequencing (FISSEQ)» (19), living cells are fixed on a glass slide and their mRNA is reverse transcribed in the fixing medium in cDNA (RT). To avoid that the cDNAs are not spatially displaced, a soluble «primary amine-coupled nucleotide» is used for the RT. The cDNAs are ampified on the spot with a «rolling circle amplification». With an isothermal «sequencing by ligation» method (20), the cDNAs are sequenced with fluorescent-labelled primers and photographed using confocal microscopy after each addition of one of these primers. Other developments should allow for the «online measurement» of resulting mRNA transcripts.

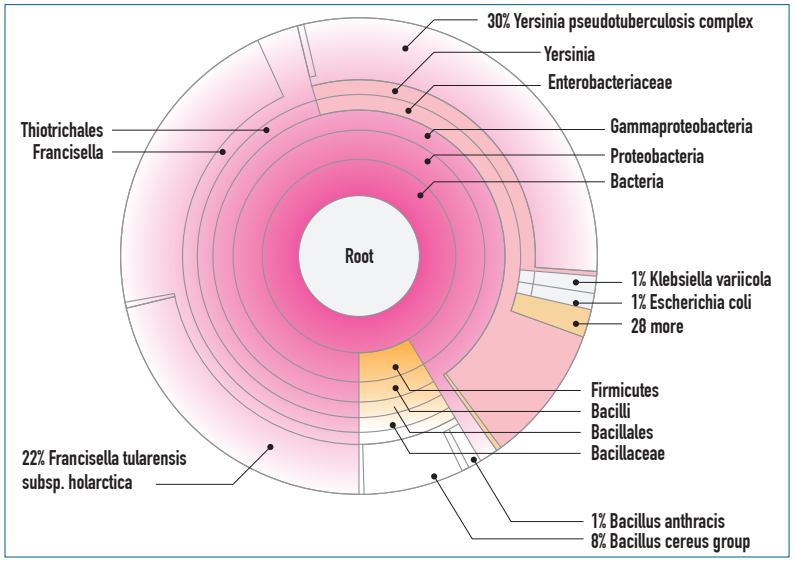


Fig. 5
Krona plot of a metagenomic analysis of an unknown BSL-3 bacteria mixture

NGS at Spiez Laboratory

Spiez Laboratory uses methods from three generations for the sequencing of biologically-related pathogens. With the Sanger sequencing method (1st generation), viruses are identified via DNA fragments from pan-virus family specific PCRs. Another application is the genotyping of bacteria using PCR-amplified 16S ribosomal RNA gene regions.

In the field of whole-genome sequencing, the already mentioned «Ion S5 NGS system» platform is used, which can generate up to 16 giga bases of raw data with up to 80 million and 200 bp long reads (DNA fragments). The nanopore technology in the aforementioned USB stick-sized MinION is the last method that is evaluated at Spiez Laboratory. Since this technology does not yet provide the desired quality of DNA sequences, two Ion S5 analyses are presented.

The first example is a metagenomic analysis of an unknown BSL-3 bacteria mixture. To identify the bacteria, 60 million 200 bp reads (IonS5) were generated with the extracted DNA of an «unknown» bacterial mixture and bioinformatically analysed with the new high-performance computing (HPC) server at Spiez Laboratory. To do this, all reads were compared to an international micro-organism database with a Linux-based bioinformatic tool called Kraken (21), i.e. they were «blasted» (English BLAST: Basic Local Alignment Search Tool). All reads with an identity were graphically represented according to the percentaged taxonomic affiliation with the hierarchical data browser Krona (22) (Fig. 5). The BSL-3 bacteria Bacillus anthracis, Francisella tularensis and Yersinia pseudotuberkulosis were successfully identified in the mixture.

In the second analysis, Spiez Laboratory wanted to determine the exact identity of an Ebola virus Zaire (RNA virus). To this end, the nucleic acids were extracted from a culture supernatant of the virus cultivation and non-specifically reproduced using isothermal multidisplacement amplification (phi29 polymerase).

After the Ion S5 sequencing, the reads obtained were «mapped» on an Ebola virus /H.sapiens-wt/CHE/2014/Makona-GE1 (KP728283) reference strain sequence. 54 000 of 1.2 million reads were mapped on the reference strain. From a virus culture with 1 million Ebola viruses/ml, 4.5 % of all reads are therefore specific to the virus. The remaining 95.5 % of the reads map to the genome of the cells (host) that were used for the virus cultivation. Nevertheless, the virus cultivation and the isothermal amplification were necessary to characterise the entire genome of the virus. If the whole genome of a weakly concentrated virus in a clinical sample has to be sequenced, considerable sequencing costs will result. Methods are therefore being developed at Spiez Laboratory to reduce the unwanted host genome sequences even from an unknown sample.

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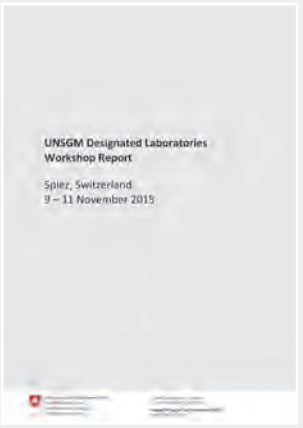


Designated laboratories for the United Nations Secretary General's Mechanism

Stefan Mogl, Dr. Cédric Invernizzi, Dr. Beat Schmidt, Dr. Nadia Schürch

The UN has invited member states to designate analytical laboratories that are able to support an investigation of alleged use of chemical or biological weapons in accordance with the United Nations Secretary-General's Mechanism (UNSGM). An international workshop in Spiez discussed the necessary steps to establish a global and functional network of analytical laboratories for biological weapons. To gain full acceptance, such a network must meet similarly stringent requirements as are existing for analytical laboratories for chemical weapons.

In the case of chemical weapons, a network of designated laboratories has been established by the Organisation for the Prohibition of Chemical Weapons (OPCW); this network is available to Secretary General Mechanism (SGM) investigations and in 2013 it confirmed the use of Sarin in Syria. As for toxins, the OPCW has begun developing a capacity for conducting analyses of environmental samples containing toxins but the number of OPCW designated laboratories capable of undertaking such analysis, and the range of toxins tested, are still limited. There is, today, no similar network for the investigation of the use of biological weapons.

This was the reason why Switzerland decided to organise a series of expert workshops to discuss the necessary steps to establish a network of designated laboratories in the field of biological weapons. The objectives of this first of three workshops were to:

- Clarify the tasks of designated laboratories in an investigation of alleged use of biological weapons;
- Discuss how the designated laboratories can fulfil these tasks; and
- Identify steps to ensure that designated laboratories meet international requirements in order to gain full scientific and political acceptance.

52 participants from 15 countries (Australia, Canada, China, Denmark, Finland, France, Germany, Norway, Portugal, the Russian Federa-

tion, Singapore, Sweden, Switzerland, the United Kingdom of Great Britain and Northern Ireland, and the United States of America), the United Nations Office for Disarmament Affairs (UNODA) and the OPCW attended the workshop. They included arms control and technical experts from a range of laboratories with relevant scientific competence. The following summarises the findings of the workshop and sets out the next steps that the participants considered necessary for the development of a trusted international laboratory network to investigate allegations of the use of biological weapons.

Workshop Summary

SGM Guidelines and Procedures require from designated laboratories the identification and characterisation of agent(s) used – in environmental and clinical samples – as well as other information that may assist an investigation in attributing a possible release. To date, a few dozen laboratories have been designated by UN Member States. Little is known however about their capabilities as well as capacities. Laboratories submitted information as part of their designation process. But based on this information only, Member States are unable to assess, whether designated laboratories meet the high standards that are required so that the findings of an investigation will be trusted. The network of off-site laboratories of the OPCW, may serve as example.

Worldwide, there are many high-quality laboratories covering human, animal and plant pathogens as well as toxins. However some biological agents of interest in the context of biological weapons investigations are of little interest to public health. What is missing is a dedicated network of laboratories that maintains the scientific competence for the analysis of samples related to a possible use of biological weapons as well as meets the forensic and procedural requirements and is able to face the scrutiny that accompanies such an investigation. The experience of a number of national, regional and international networks and initiatives mentioned in the workshop report[1] could offer a starting point. Laboratories that take part in SGM investigations cannot afford to report false positive or negative results. For this type of investigation quality assurance and validation of methods and procedures is of utmost importance. Furthermore, laboratories must adhere to rigid administrative and reporting requirements, and demonstrate a strict chain-of-custody of samples. Advances in life sciences are expected to increase the capacity for biological analysis and create new opportunities for investigating biological incidents. Automated commercial systems however frequently operate as «black boxes» rendering an assessment of obtained results difficult; a disadvantage in a political context.

At a fundamental level, there is the question of what «identification» actually means in the context of a biological weapons investigation. An important issue is also, how reliable and comprehensive reference data libraries on biological agents are, and how easily designated laboratories can access them. A peer-to-peer network of designated laboratories carrying out confidence building exercises would enhance mutual trust in the validity, accuracy and traceability of reported results. Such a network must be approached step-by-step with a long term view: starting by the sharing of information about existing capabilities and capacities and continuing with a whole range of benefits for the laboratories, such as opportunities for collaboration and sharing of best practices.

This process will, to a considerable extent, rely on the resources and expertise of Member States and on the willingness of their laboratories to engage in the formation of a trusted laboratory network on a voluntary basis. Switzerland and Spiez Laboratory stand ready to provide a platform to further progress on these issues.

1 http://www.labor-spiez.ch/de/akt/pdf/UNSGM_Bericht_LOW.PDF

Current training exercises of C-EEVBS

Dr. Beat Aebi

In 2013, the population in Ghouta, Syria was attacked with sarin. More recently, the vesicant agent yperite and the lung toxic chlorine gas were used in this region. In order to be prepared in Switzerland for threats and attacks with chemical warfare agents, the Swiss chemical response team (C-EEVBS) conducts intensive training, also with specialists abroad.

Training exercises together with the German Analytical Task Force ATF 2011 in Sonthofen and 2015 in Münster

The Analytical Task Force (ATF) consists of NBC specialists from the professional fire brigades of Hamburg, Mannheim, Dortmund, Cologne, Leipzig and Munich as well as the State Office of Criminal Investigation of Berlin. From these locations, the ATF is able to reach any NBC event in the district in a radius of 200 km within a maximum of three hours. The ATF supports the operational command of the local fire brigade with measurement technology and expertise. The German Federal Office of Civil Protection and Disaster Assistance (BBK) outfits the ATF and coordinates the NBC training.

During the 2015 exercise in Münster, five specialists from C-EEVBS had the opportunity to train together with the ATF for the second time under the leadership of the BBK. On the first day, C-EEVBS and the ATF together investigated an illegal basement laboratory. In the afternoon, the C-EEVBS was able to practice handling of suspicious substances in a mail room, while the ATF handled a radiological scenario. On the second day, the ATF and C-EEVBS trained together the handling of spilled and leaking chemicals in a high-bay warehouse of a small chemical company. The teams discovered during the exercise that the spills were mixtures of chemical waste, containing highly toxic substances. In addition to using proper personal protection and the right procedures for detecion and sample collection, exercise participants had to discover hidden clues and link information between the different scenarios.

The training facility was impressive and the scenarios were realistic and well-prepared. Not just the detection and sample collection team, but also the team leader was challenged by the exercise management. Through realistic scenarios, the exercise permitted to utilise the expertise of all participating organisations and their

specific resources at the training centre of the Institute of Fire Brigades in North Rhine-Westphalia. The C-EEVBS and the ATF worked well together right from the start. The close cooperation of the C-EEVBS with the BBK and the ATF has been beneficial for all parties involved and should be continued in this form.

Training exercises with the bomb disposal engineers from the Scientific Research Service (WFD) of the Forensic Institute of Zurich (FOR) 2013, 2014 and 2015

Situations where hazardous chemicals are combined with explosives pose particular challenges for emergency services. So far, three joint training exercises between the chemical specialists of the EEVBS and the bomb disposal engineers of the Scientific Research Service WFD have taken place and were very beneficial. The challenges for the C-EEVBS were increased with each exercises. During the first joint exercise in 2011, two sub-scenarios were run separately involving explosives as well as chemical warfare agents simulants. In the second exercise in 2014, chemical equipment was secured with a simulated booby trap. For the third exercise in 2015, an abandoned factory with many rooms was selected. According to the szenario, construction workers had found explosives and shortly thereafter exhibited symptoms of poisoning. The exact location of the explosives was not known. The joint WFD and C-EEVBS team had to search the rooms of the abandoned factory, always monitoring their position and watch out for possible hazards - not an easy task. While the team was performing its search, a small explosive charge was set off in an adjacent building to test the team's reaction. It correctly decided to retreat immediately. Inspite of this disruption, the team remained calm and resumed work after a short briefing. This third joint exercise with the WFD Zurich was completed successfully without major difficulties. All parties involved collaborated professionaly. This successful cooperation will be continued. Both teams feel well-prepared for deployment.

C-EEVBS

The special unit C-EEVBS has been ready for deployment since January 2010. It was formed from the VBS rapid intervention team (EEVBS) that has existed for 10 years. The C-EEVBS is composed of about 20 volunteer specialists, all professionals from Spiez Laboratory and the Centre of Excellence NBC-EOD of the army. In the event of suspected chemical warfare agents or highly toxic chemicals, the C-EEVBS shall provide assistance to the cantonal response units on request, the services include:

- rapid consultation by phone
- deployment of a field response team of 4 specialists with an emergency reponse vehicle, or airborne by an army helicopter
- on-site consultation, measurements and sampling
- provision of nerve agent antidotes for about 500 people (for 1 day)
- operations centre at Spiez Laboratory

Testing of hand-held detectors for chemical warfare agents

Dr. Anna-Barbara Gerber

Chemical warfare agents are highly toxic already in small quantities, generally invisible and recognised late. Rapid detection and identification of an agent using detection devices is of great importance, in order to prevent intoxication and initiate necessary decontamination measures. Furthermore, a timely identification of the agent provides valuable information to support correct medical treatment of persons exposed.

Chemical warfare agents or other toxic substances may occur in a variety of ways. Corresponding to a wide range of possible scenarios, a large number of different detection devices are commercially available as chemical agent-specific detectors. These devices are based on physical, chemical or enzymatic technologies and can detect either a group of chemical warfare agents or provide a concrete indication of a specific agent within a short period of time. In case of an event, it is important that onsite detection is done quickly and that it can be achieved with non-expert personnel. Large laboratory instruments are not suitable for this purpose. While such instruments offer a high degree of sensitivity and measurement accuracy, they are difficult to transport and their operation requires sound training. That is why small, so-called hand-held detection devices, are commonly used on site. Hand-held detectors must meet the following requirements:

- Lightweight and small device
- Quick and reliable analyses
- Simple operation, even for non-specialists
- Low price compared to laboratory devices
- Long battery life

While we do not develop hand-held detection devices at Spiez Laboratory, we have the possibility to test commercially available detectors and establish their limitations. The chemistry division operates a laboratory for activities with highly toxic chemicals which facilitates the work with chemical warfare agents. The Organic Synthesis Branch supplies the necessary chemical warfare agents, precursor chemicals, degradation products as well as relevant impurities. We perform this detector testing for our operational benefit – in the case of our chemical incident response team – and to provide expert advice to the armed forces, response organisations of cantons and international or-

Chemical warfare agent enrichment equipment

ganisations. The Detection and Decontamination Branch has developed testing procedures to comprehensively evaluate all types of chemical warfare agent detectors.

Important criteria for testing are the sensitivity as well as the selectivity of a device. The sensitivity refers to the detection limit of a device, a high selectivity ensures that the device will correctly detect and identify a particular agent also in the presence of other chemicals that might interfere with the measurement. Detectors, that in spite of the presence of a toxic chemical fail to display an alarm, are very dangerous.

Other factors that are considered during testing are the handling of a device under aggravated conditions, such as working in protective equipment, the scope and quality of the integrated database as well as costs, both, for procurement as well as for maintenance including spare parts. Spiez Laboratory evaluates devices for vapour detection as well as for solids and liquids.

Detection of gaseous substances

A number of different technologies are available for the detection of gaseous warfare agents. Widespread for example are ion mobility spectrometry (IMS) or flame photometry. We maintain a large selection of substances to perform qualitative «sniff-tests» for all types of detectors. This selection not only includes the traditional warfare agents but also their precursors, degradation products as well as analogues. Through such sniff-tests, the selectivity and (if present) the integrated database can be evaluated.

We perform quantitative measurements for a few substances using our warfare agent enrichment equipment. The heart of this system is a so-called permeation cell. This is a plastic tube that is filled with chemical warfare agents and then welded shut. After a period for stabilisation and under constant temperature, the agent permeates out of the cell at a constant rate, where it can be diluted with a calibrated flow of air of a selected humidity. This method permits to generate agent concentrations in the range from 20-1000 µg/m³, depending on the substance.
In order to simulate conditions close to reality, substances interfering with a detection such as gasoline, diesel or cleaning solutions can be added to the defined air/agent mixture.

A small selection of detectors evaluated in Spiez

1. Chempro 100i, IMS
2. TruDefender FTX, IMS
3. Raid, IMS
4. GDA, IMS
5. AP4C, FPD
6. Chemsentry 150c, SAW
7. RAID-M100, IMS
8. LCD 3.3., IMS
9. Gemini, FTIR/Raman
10. CNG 97, IMS
11. FIDO C1, Colorimetrie

Detection of liquids and solids

There is also a wide selection of technologies for liquid and solid detectors. Known systems include Fourier transformation infra-red spectrometers (FTIR) and Raman devices. The evaluation is largely the same for all systems. The bases for the testing is the extensive substance library at Spiez Laboratory. Especially for FTIR and Raman devices, the scope and quality of the integrated database are an important criteria. This database is best evaluated by testing the detector against many different substances. Factors such as the substrate of a sample and, for Raman detectors, the packaging material as well as the glass thickness of a sample vial are also taken into consideration. Mixtures of liquids or solids are used for quantitative measurements. This permits to determine the minimum detectable quantities as well as the influence of interfering substances.

Based on our evaluation methods we are able to assess the performances of all commercially available chemical detectors. Futhermore, we offer our services to detector manufacturers to test their devices and prototypes. Due to our testing activities we are able to maintain an overview of the technical capabilities of chemical detectors on the market.

The ideal detection device unfortunately does not exist. It is therefore all the more important to test devices specifically and to provide customers with expert advice that meets their needs.

Type of substance	Technologies of detection devices
Gaseous	• Ion mobility spectrometry (IMS) • Flame photometry (FPD) • Surface Acoustic Wave (SAW) • Mass spectrometry (MS) • Fourier transformation infra-red (FTIR) • Colorimetry
Liquid	• Raman • Fourier transformation infra-red (FTIR) • Colorimetry • IMS and FPD with liquid detection sets
Solid	• Raman • Fourier transformation infra-red (FTIR)

8th National NBC Protection Conference

Pia Feuz, Dr. César Metzger, Dr. Giuseppe Testa

The 8th National NBC Protection Conference of the Federal Commission for NBC Protection was marked by preparedness and incident management in 2015. The focus was on the cantons, which bear the main responsibility in certain areas of civil protection and therefore also bear the burden of incident management. The topic of preparedness was addressed with presentations about psychological emergency relief in the event of an incident or about NBC protection of the water supply. Remarks about the Ebola patients treated in Geneva in 2014 and about the chemical accident in Daillens in April 2015 placed an emphasis on current events in Switzerland.

«In the field of NBC protection, the cantons should be even more effective, which can only be achieved together with partners», said Norman Gobbi, president of the Governmental Conference of the Military, Civil Protection and Fire Services (GC MCF) and senior civil servant in the canton of Tessin, in his opening speech. The governmental conference has proved to be the central political platform for preparedness in civil protection in the federal system of Switzerland and specifically in the security network of Switzerland.

Dr. Ralf Trapp, international consultant for arms control of biological and chemical weapons, presented the sad hundred-year history of chemical weapons in an impressive presentation and pointed out how topical these weapons unfortunately still are today.

Overall, a record-setting 162 people took part in the conference. The exchange of information on topics related to civil protection and the opportunity for networking served both the purposes of preparedness as well as incident management, according to the motto: «In crises: know the experts and their expertise!»

NBC Preparedness

Our modern society relies on a superior, yet vulnerable infrastructure. In Switzerland, the preparation to manage potentially catastrophic events is based on so-called reference scenarios. During the preparation phase, processes are being defined, material is being acquired and the readiness of response organisations are being trained and tested. The revised NBC

Dr. Ralf Trapp, international consultant for arms control of biological and chemical weapons, gives a talk on the history of chemical weapons.

and Natural Disaster reference scenarios of the Federal Office for Civil Protection (FOCP), respectively Spiez Laboratory, were presented at the conference to a broad audience by Dr. Marc Cadisch, Director of Spiez Laboratory. Presentations on NBC protection of the drinking water supply by Dr. Andreas Peter, or on the risk of measles by Dr. med. Edith Betschart provided a practical reference for preparedness: In Switzerland, cases of drinking water contaminated with bacteria were reported in 2015. The temporarily affected drinking water supply of St. Maurice (VS), Zweisimmen (BE) and Le Locle (NE) as well as in some areas of Lugano (TI) was discussed at length in the media. In the case of Le Locle, the consequences were clearly felt and the health of over 1000 people was affected. The topic of «measles» also underlined the importance of preparedness in civil protection. This often underestimated threat could be eliminated by vaccinating a significant part of the population. What is missing, however, is the willingness of the entire population to be vaccinated as recommended by the medical profession.
Efforts to increase preparedness in the nuclear sector have been the focus of interest since Fukushima. In 2014, work was completed on the nuclear power plant reference scenarios, which are used as the basis for a zoning concept. The scenarios were presented by Dr. Cyrill von Arx from the Swiss Federal Nuclear Safety Inspectorate (ENSI).

Incident Response
In his presentation, Gerald Scharding, head of the National Emergency Operations Centre (NEOC), demonstrated how the NEOC coordinates incident responses within Switzerland. Had it unfolded somewhat differently, the railway accident in Daillens in 2015 could have had catastrophic consequences. The chemicals which leaked out from a railcar tank fortunately only caused localised damage. However, the secondary consequences of the accident, for example the passenger transportation within the swiss railroad network, were of national proportions. The presentations by Dr. Sylvain Rodriguez and Bertrand Dubey from the Directorate for Industrial, Urban and Rural Environment, Canton of Vaud about this rail accident showed which human and material resources were used and how favourable circumstances made it easier to handle this accident.

Using an example of a decommissioned factory in Avully contaminated with hazardous waste, Claire Walenda, department head of the OCPPAM, Canton of Geneva, illustrated the challenges that incident response teams may face when confronted with a chemical contamination. Because of the complexity of the situation, authorities expect to be dealing with this plant for years to come.

Professor Dr. med. Laurent Kaiser, chief physician for infectious diseases at the Geneva University Hospitals) reported on Ebola and the globalisation of emerging viruses. He demonstrated how viral infections may threaten our society. Today, several major hospitals in Switzerland are now ready to treat Ebola patients. They have developed corresponding concepts for this purpose, tested them and trained their employees. The successful treatment of a patient in Geneva in 2014 shows how Switzerland can respond to this threat today.

Responding to an incident always means high psychological stress for the involved responders. However, responders are trained on how to deal with these situations. But what about the affected population? The psychological emergency assistance service provides important and necessary support to victims by dispatching care teams to the stage of any serious event. For instance, the evacuation under emergency in Fukushima and the fear and stress experienced by the population will burden the affected persons for years to come. Through adequate psychological support, in part over years, such effects can be relieved to a certain degree. The psychological dimension is therefore to be taken into account both in responding to an incident as well as in preparedness as Dr. Urs Braun from the National Network of Psychological Emergency Relief pointed out.

On behalf of the Federal Commission for NBC Protection, Spiez Laboratory will host the 9th National NBC Protection Conference on 22 September 2016 in Berne.

Filed study on the effects of weather conditions on activated carbon filter performance

Andres Wittwer, Markus Gurtner

The performance of new or spare NBC protective materials must be tested according to standard methods. These methods must follow up-to-date practices and be appropriate for current hazard and threat scenarios. In 2015, the impact of weather conditions on the performance of NBC protective filters was investigated in a field study. Whilst actual Swiss standard methods allow the simulation of real field conditions when filters are used outdoors or in underground shelters, they do not correctly simulate indoor conditions. Therefore, the Swiss standard methods should be revised for (newer) indoor protection concepts such as filters in vehicles or in buildings.

Activated carbon is a typical sorbent used in NBC protection filters. Water molecules also adsorb on activated carbon. Consequently, the NBC filter sorption capacity can be dramatically reduced for some air pollutants in cases of high humidity. Accordingly, depending on the final use of the filters, this effect must be taken into consideration in the test requirements.

In this field study, the effects of weather conditions on filter performance were investigated for four common activated carbon types, at three different sheltered locations at Spiez Laboratory. A winter and a summer semester campaign were also carried out to study seasonal variations. The activated carbon samples were ventilated similarly to a precautionary protective filter operation.
After the two campaigns, the sorption performance of each filter was measured with five relevant test substances and compared with standard methods (e.g. test simulation of weather conditions).

The three field locations were:

- Outdoor canopy cover: outdoor shelter, about 50 cm above the ground, shielded from rain, sun and wind;
- Underground shelter: ventilated but unheated, unoccupied shelter according to the technical directives of the Swiss Civil Defence for mandatory shelter construction;
- Indoor, heated room: work and storage room,

Three field-locations:
outdoor canopy cover (l),
underground shelter (m),
heated room (r)

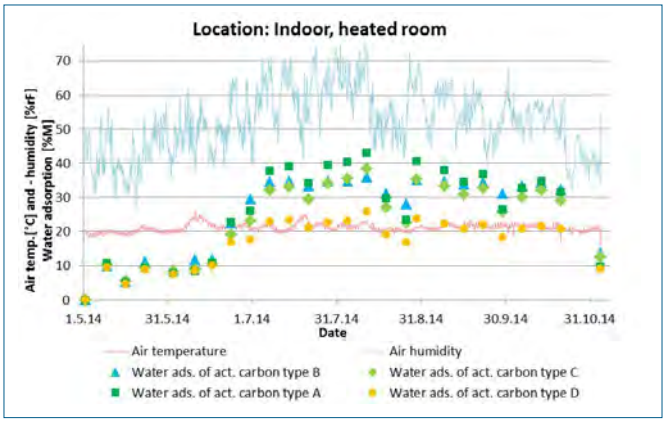


Diagramm 1:
Air temperature and humidity as well as water gain for the indoor location during the summer campaign

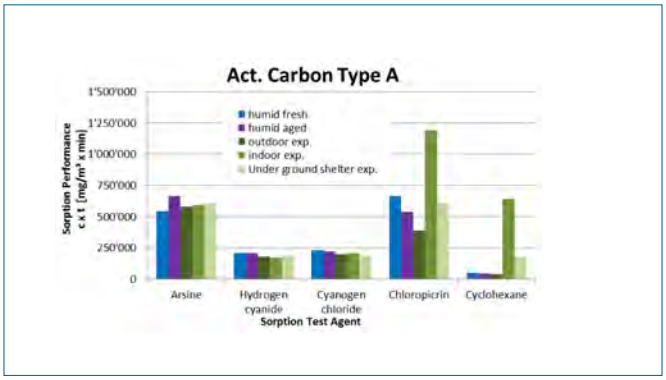


Diagram 2:
Results of one of the four activated carbon types during the summer campaign

radiators equipped with thermostatic valves, natural ventilation through doors and windows, no air conditioning, rare presence of people

The ambient air temperature and humidity were continuously recorded at these three locations. The activated carbon samples were weighed weekly and compared to their start weight to determine the amount of water gained.
It was interesting to observe how the water absorption was affected in the long-term by the weather and what maximum values were reached. Diagram 1 shows air temperature and humidity as well as water gain for the indoor location during the summer campaign.

The key results of this study is the sorption performance of the activated carbon after real weather exposure. Diagram 2 depicts the results of one of the four activated carbon types during the summer campaign (similar results are obtained with the three other types). Generally, the adsorption capacities during the field study (green) compare well with those obtained in standard test conditions (blue and violet), with some minor deviations resulting from measurement inaccuracy. An important exception is given by the samples located indoors, these show a higher performance with chloropicrin and cyclohexane compared with the samples tested under standard conditions. This effect was slightly higher during the winter campaign. Both agents are the only agents tested that adsorb purely physically, which could explain these discrepancies.

Main findings:

- Water adsorption on activated carbon after six months exposure is still reversible, even in the presence of highly humid air. Water saturated, activated carbon can dry out again if exposed to dry air.
- Sorption performance for substances prone to chemisorption (chemical deposition) is marginally affected by the presence of water on activated carbon surface and, in turn, by the weather conditions.
- Water adsorption on activated carbon in case of humid air (over 50 % RH) strongly reduces the sorption performance for substances prone to physisorption (filling of the micropore volume).
- The standard test conditions to simulate exposure to real weather conditions were found to be appropriate for locations "outdoor" and "underground" for all four activated carbon types, covering most of today's product range.
- Established standard test conditions need to be adapted for filters used indoors, such as in buildings and in vehicles, especially if the contaminants adsorb physically on activated carbon.

Testing of polymer materials for CBRN protection equipment

Thomas Friedrich

Spiez Laboratory runs an ISO 17025 accredited laboratory that is specialised in testing of polymer materials used for CBRN protection equipment. To verify that the materials in use meet specific requirements emerging from CBRN threats the following testing methods are regarded to be of prime importance.

Identification and fundamental characterisation of materials
Infrared-Spectroscopy FTIR allows rapid identification of materials by comparing the response spectra with those of known materials. Differential Scanning Calorimetry DSC reveals significant information about polymers such as glass transition temperatures, peak temperatures and heat of fusion of melting and crystallisation.
By Thermogravimetric Analysis TGA the content of plasticizers, elastomer, carbon black and inorganic filler of elastomer compounds can be determined.
Proof of optimal processing quality
Material properties of polymers are strongly dependant on the molecular weight. It has to be ensured that thermoplastics do not experience degradation due to shear and heat loads during final forming processes, e.g. injection moulding. Determination of molecular weight is preferably made by measurement of the Melt Flow Rate MFR or by Dilute Solution Viscosity. Measurement of the Compression Set CP is a reliable method for the determination of the processing quality of elastomers, i.e. level of vulcanisation (cross-linking).

Resistance to Chemical Warfare Agents
Materials used for CBRN protection equipment such as suits, masks, gloves, boots etc. are tested for resistance to penetration of chemical warfare agents by a fully automatic testing machine. Warfare agent (HD) can be applied in various ways, i.e. laid droplets or fallen droplets from heights up to 10 m, application of pressure and reaming or by gas flow through the sample. Breakthrough of the warfare agent is detected either by means of change in electrical conductivity of water (Conductivity Meth-

Fully automatic equipment for testing resistance to penetration of chemical warfare agents

od) or by discolouration of an indicator paper (FINABEL Method).

Investigation of long-term material stability

The performance of CBRN protection equipment has to be ensured even after storage of 10 to 20 years. Mechanical properties of new material samples are compared with samples that have been subjected to artificial aging. This approach allows the prediction of the service lifetime. To simulate the various aging processes the following test methods are applied:

- Storage at elevated temperatures, i.e. accelerated physical and thermo-oxidative aging
- Accelerated aging of elastomers at elevated ozone levels
- Accelerated artificial weathering at elevated UVA-radiation and cyclic rain, i.e. photo-oxidative aging
- Thermal analysis, i.e. Oxidation Induction Time OIT for investigation of the effectiveness of stabilisers
- Exposure to various expected substances as well as to chemicals which simulate chemical warfare agents

Artificial aging

Infrared-Spectroscopy

Thermal Analysis DSC

Open House Day 2015

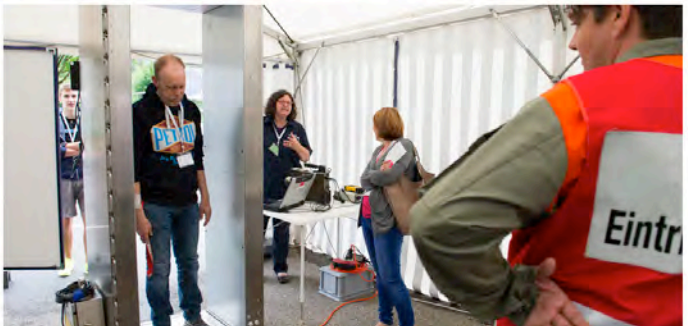
On 19 and 20 June 2015, we opened up our doors to a wide audience from the region and across Switzerland. More than 2000 visitors could inform themselves about our various tasks and activities. The response to this event showed us the significant need to comprehensively inform the public about our work. Specialists were able to impress the interested visitors with their expertise and open communication. The photos testify to the pleasant atmosphere on both days.









Tag der offenen Tür
LABOR SPIEZ

Eintr


Spiez Laboratory and the NBC Defence Laboratory 1 as Partners

Lt. Col. Roger Herger, Commanding Officer of NBC Defence Laboratory 1

Spiez Laboratory and the NBC Defence Laboratory 1 – a batallion of the Swiss armed forces – both deal with the risks from NBC events and their possible effects from a scientific and technical perspective. In response to today's hybrid threats, this collaboration is necessity- and operations- based and a model of civil-military partnership in the Swiss security architecture.

Hybrid threat scenarios are characterised by the deliberate denial of clearly separable states of peace and war. In such situations, the opposite side may be composed of both regular and irregular forces, supported by terrorists and criminal groups. In such a conflict environment, a well-planned path of action is to be anticipated and acts of subversion [1] and coup de main [2] can be expected. Direct confrontation or clearly attributable signatures are generally avoided. The opponent side makes use of the existing logistics infrastructure and possibly employs also high-tech means (e.g. cyberwar). It is therefore difficult to make a distinction between the opposite side and one's own population [3, 4]. The use of NBC agents during such scenarios is realistic.

Such hybrid threat scenarios involving NBC agents illustrate the need for a close cooperation between civil and military partners at federal government level. However, this is not the sole reason for this cooperation: The collaborative partnership between Spiez Laboratory and the NBC Defence Laboratory 1 has to be mobilised on short notice, when civil specialists require the support of military forces to cope with NBC events. An example of this is the active service of the army at Spiez Laboratory following the nuclear accident in Chernobyl in 1986, which was rendered by the laboratory ADSD 86, the military unit preceeding the NBC Defence Laboratory 1.

The NBC Defence Laboratory 1 is a military battalion, which comprises three identical deployment companies, each staffed with 222 military personnel, who can be mobilised within 24 hours and deployed in phases [5]. The companies are made up of 81 laboratory specialists, 36 members of the army (MOA) for sampling

Discussion of analysis strategy during the exercise SOLO DUE as part of the RC in 2015

A response team of the BLS railway leaving the incident area while a military specialist operates a chemical agent detector. In the background, the fire and rescue team 04 of the BLS railway.

and decontamination as well as 105 MOA for security, logistics and support.
The refresher courses (RCs) of the NBC Defence Laboratory 1 are conducted annually in two time windows in Spiez and the surrounding area. The military personnel undergoes demanding and high quality technical training by civilian experts from Spiez Laboratory. The training builds on information the military personnel acquired during their NBC defence recruit school. Applying a modular training concept, the military personnel are trained in laboratory methods and procedures in subsequent RCSs, where analytical expertise is gradually developed based on operational requirements. Furthermore, under the guidance of expert staff, sample collection is trained according to national and international protocols and the procedures for processing samples through the Spiez Laboratory sample handling facility are practised. At the end of each RC, the military personnel tests their skills in sample collection, in operating the sample handling facility and in sample analysis in multi-day practical exercises where they work in close cooperation with laboratory staff. This facilitates the collaboration of civilian an military actors as shown in the pictures from the sampling exercise during a recent RC in 2015.

The NBC Defence Laboratory 1, a battalion of the Swiss citicens army, embodies this collaboration in an exemplary manner: Many of the NBC specialists and of the cadre have expertise in physics, biology or chemistry, or, are in the process of acquiring such expertise through their ongoing studies and professional formation. The federal government thus benefits from this NBC-expertise, and as a result of the joint civil-military training, can deploy these resources expediently in case of NBC events. Due to the constant rejuvenation of the army, the NBC Defence Laboratory 1 will, however, continue to depend on voluntary lateral entry scientists willing to perform their military service in this challenging environment.

Literature and footnotes

1. Subversion: Action to weaken the military, economic or political power of a nation by undermining the morality, loyalty or reliability of its citizens.
2. Coup de main: Prepared attack with a limited goal. It can be used to take possession of important terrain parts, or objects.
3. D. Lätsch, D. Moccand, Military Power Review of the Swiss Army 2, 3 (2010).
4. M. Zäpfe, CSS Analyses in Security Policy 174, 1 (2015).
5. Planning state of further development of the army.

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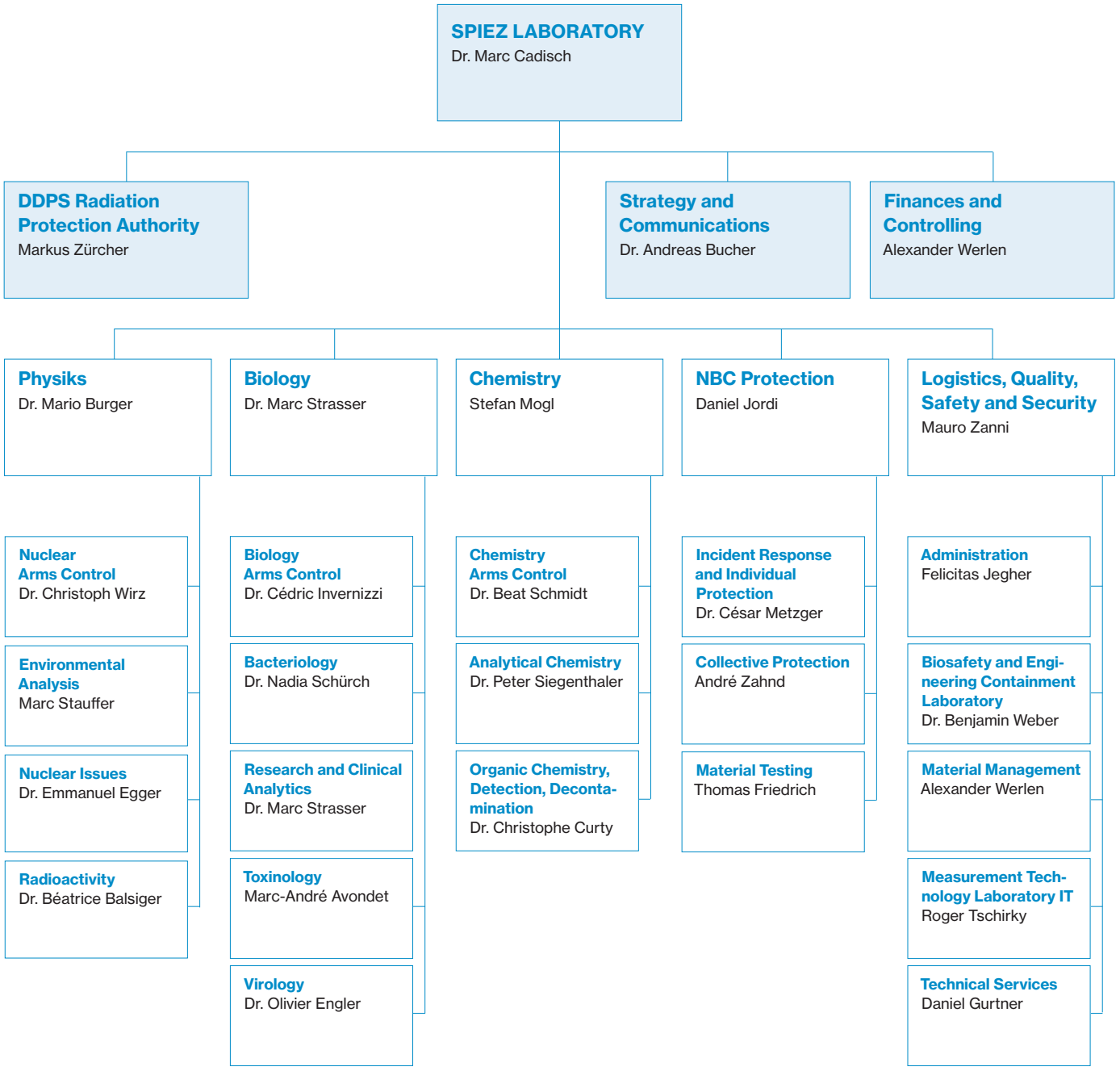
Organisation



Accredited activities

ISO/IEC 17025 accredited laboratories

STS 0019	Testing laboratory for the analysis of samples for chemical warfare agents and related compounds
STS 0022	Testing laboratory for adsorbents and respiratory protection filters
STS 0028	Testing laboratory for the determination of radionuclide concentration
STS 0036	Testing laboratory for polymers and rubber, and for the protection performance of polymers, rubber and textiles against chemical warfare agents
STS 0054	Testing laboratory for the detection of biological agents
STS 0055	Testing laboratory for NBC protection material, shelter equipment and shelter installations
STS 0101	Testing laboratory for the determination of main and trace elements and selected air-pollutants

Round Robin tests October 2014 – September 2015

Accredited laboratory		Quantity	Type and partner
STS 0019	Chemical Analysis/ Verification	1	35. Official OPCW Proficiency Test
STS 0022	Sorbent	–	
STS 0028	Radionuclides	7	IAEA TEL-2015-04-ALMERA-PT (Gamma, Beta, Alpha, ICP-MS) IAEA Characterisation of Rice Material (Gamma) FOI Sweden, Find Five Flaws (Gamma) IRA/BAG RV2015 (Gamma) BfS RV 2/2015 (LSC, Tritium, ICP-MS, U-isotopes) IAEA RV TEL-2105-PT Drinking water JRC, RV Cs-137
STS 0036	Plastics and Rubber	14	Round robin series, Kunststoffinstitut Lüdenscheid, Germany
STS 0054	Biological toxins	–	
	Medical biochemistry	–	
	Diagnostics of bacteria - drinking water	6	Food and Environmental Proficiency Testing Unit, Public Health England
	Diagnostics of bacteria - molecular biology	2	INSTAND, Society for Promoting Quality Assurance in Medical Laboratories e.V.
	Diagnostics of viruses - molecular biology		QCMD, Quality Control for Molecular Diagnostics, Glasgow, Scotland
	Diagnostics of viruses serology	2	INSTAND, Society for Promoting Quality Assurance in Medical Laboratories e.V.
STS 0055	Ventilation	–	
	Air blast effects	–	
	Ground shock effects	–	
STS 0101	Major and trace elements	5	Ielab Drinking Water I-III, Alicante, Spain International Soil Exchange (ISE), University Wageningen IAEA RV TEL-2015-PT Drinking water with STS 0028, Vienna
	Air pollutants	–	

Presentations

Our scientists attend and actively contribute to conferences and offer their input to training courses dealing with NBC protection issues. Below are some of the presentations, given by our specialists during 2015.

Date	Subject
13.02.2015	Dr. Andreas Bucher: Technik und Naturwissenschaft für Frieden und Sicherheit, TecDay, Gymnasium Thun, Thun
27.02.2015	Dr. Marc Cadisch: Das Labor Spiez und seine Aufgaben und Aktivitäten, Akademie der Generationen Spiez
23.04.2015	Dr. Marc Cadisch: Schweizer Engagement bei der Beseitigung von Chemiewaffen, Vorlesung UZH, Zürich
24.04.2015	Dr. Beat Schmidt: ICA contribution to GRULAC, Den Haag, Niederlande
20.05.2015	Dr. Marc Cadisch: Internationale Aktivitäten des Labor Spiez, Wirtschaftsbrunch Volkswirtschaft Berner Oberland, Interlaken
21.05.2015	Dr. Stefan Röllin: Erste Erfahrungen mit dem Multikollektor, ICP-MS, Brugg
04.06.2015	Dr. Christophe Curty: Bioaddukte, Doktorandentag UZH, Spiez
10.06.2015	Dr. Marc Strasser: Natürliche oder beabsichtigte Biologische Gefahren, Biologische Gefahren Hygieneforum, Zürich
16.06.2015	Dr. Patrick Wick: Persönliche ABC-Schutzausrüstung, 5. Branchentreff des swiss safety, Bern
12.08.2015	Dr. Cédric Invernizzi: Standing Agenda Item: Science & Technology, BWC Meeting of Experts UN, Genf
26.08.2015	Dr. Beat Schmidt: Briefing UN Fellowship CB-Disarmament and CB-Control, Swiss Day of UN Disarmament, Bern
02.09.2015	Dr. Marc Cadisch: Die neuen ABCN-Referenzszenarien im Überblick, 8. Nationale ABC-Schutz Konferenz, Bern
04.09.2015	Dr. Peter Siegenthaler: Einführung Verifikationslabor für Chemische Kampfstoffe und Untersuchung von Proben einer UNO Mission zur Abklärung von vermuteten C-Waffeneinsätzen, Besuch Absolventen des MAS HRM ZHAW 2009, Spiez
14.09.2015	Dr. Cédric Invernizzi: S&T-Trends-A-Personal-Perspective, Royal Society S&T Trends Symposium, Warschau, Polen
24.09.2015	Thomas Friedrich: Prüfung und Qualitätssicherung an Elastomerwerkstoffen, Dichtungstechnik heute und morgen, Winterthur
24.09.2015	Dr. Cédric Invernizzi: The relevance of an S&T review process, Compliance with the BWC, Wilton Park UK
02.10.2015	Dr. Emmanuel Egger: Forschungsprojekt Emergency Preparedness & Business Continuity Management after DBA Scenario, Wehrwissenschaftliches Institut für Schutztechnologien, Munster, Deutschland
13.10.2015	Dr. Martin Schär: Overview SPIEZ LABORATORY, Vorlesung ETHZ, Zürich
19.10.2015	Dr. César Metzer et Dr. Patrick Wick: Equipement de protection individuelle ABC, Officiers de l'EM du NEDEX, Spiez
19.10.2015	Dr. Peter Siegenthaler: Introduction Verification Laboratory for Chemical Warfare Agents and Analysis of Environmental Samples in Support of a United Nations Investigation of Alleged Use of Chemical Weapons, Besuch Chef der Armee und Kommandant Heer mit Gästen, Spiez
21.10.2015	Daniel Jordi: Collective Protection - The Swiss Approach, International Symposium on CBRN Defense Capabilities, Berlin, DE
22.10.2015	Stefan Mogl: Convergence in Chemistry and Biology, Vorlesung ETHZ, Zürich
02.11.2015	Dr. Béatrice Balsiger: Surveillance de l'environnement en situation d'urgence, ARRAD, Lausanne
04.11.2015	Dr. Benjamin Weber: Effluent Water Treatment, International Veterinary Biosafety Workgroup, Geelon, Australia
01.12.2015	Dr. Beat Schmidt: CNS acting chemicals CSP20, Den Haag, Netherlands
01.12.2015	Stefan Mogl: Australia/Swiss Side Event: Central Nervous System Acting Chemicals (CNSA-Chemicals) – Technical Perspective, OPCW Conference of States Parties The Hague, Niederlande

Publications

The list is not exhaustive. Some of the reports are classified.

General topics, interviews

Stefan Mogl
Chemiewaffen: Kampfstoffe der Massenvernichtung
Bayerischer Rundfunk, 20. Februar 2015

Stefan Mogl, Oliver Thränert
Die Rekonstruktion eines Pockenvirus
NZZ, 20. März 2015

Andreas Bucher
Spiez Laboratory aces chemical weapons test
SwissInfo, 17. September 2015

Mario Burger
Detektion: die Kontrolle in der Schweiz muss ausgebaut werden
Sonntagszeitung, 20. September 2015

Stefan Mogl
Biologische Labors für die Uno
Radio BEO, 5. November 2015

Physics Division

V. Putyrskaya, E. Klemt, S. Röllin, M. Astner, H. Sahli
Dating of sediments from four Swiss prealpine lakes with 210Pb determined by gamma-spectrometry: progress and problems
Journal of Env. Radioactivity 145 (2015) 78-94

José Corcho
Schnelle Bestimmung des Sr-89 und des Sr-90 in Milchproben mittels Flüssigszintillationszähler (LSC)
Labornotiz LN-2015-01-CORJ

J. Corcho, H. Sahli, Dr. B. Balsiger
RANET: IAEA Response and Assistance Network
Labornotiz LN-2015-02-CORJ

José Corcho
Détermination du tritium organiquement lié dans les sédiements: étude de faisabilité
Labornotiz LN-2015-03-CORJ

José Corcho
Bestimmung von Tritium und der Gesamt-Alpha und -Beta Aktivitätskonzentration in Wasserproben mittels Flüssigszintillationszähler (LSC)
Labornotiz LN-2015-04-CORJ

Alfred Jakob
Contaminated Military Training Grounds – Guidelines for Sampling, Sample Preparation and Analytical Methods for Heavy Metals
Labornotiz LS 2015-12

Nina Mosimann
«Find the flaws» (NATO CBRN Exercices in Gamma-Spectrometry)
Labornotiz LN-2015-01-SNIN

Jasmin Ossola
Validierung des Mikrowellendruckaufschlusssystems Multiwave PRO
Labornotiz LN-2015-01 OSJA

Jasmin Ossola
Validierung der Uranbestimmung mit dem ICP-Massenspektrometer NexION 300D
Labornotiz LN-2015-02 OSJA

Jasmin Ossola
Neues Extraktionsverfahren für Chromat auf imprägnierter Aktivkohle
Labornotiz LN-2015-03 OSJA

Jasmin Ossola
Validierung des optischen Emissionsspektrometers 5100 ICP-OES Dual View
Labornotiz LN-2015-04 OSJA

Christoph Wirz, Emmanuel Egger
Entwicklungen im Bereich nukleare Rüstungskontrolle
Labornotiz LN EGM-WIC 2015-01/2

Christoph Wirz
Uran- und Plutoniumisotopenverhältnisse
Gammaspektrometrieauswertung mit Multi Group Analysis MGA
Labornotiz LN WIC 2015-01

Christoph Wirz
CTBTO: Integrated Field Exercise Jordanien
ABC Bulletin, 1/15 März 2015

Biology Division

Werner Arnold
Nachweis von Saxitoxin mit LC-MS MS (MSQ-Plus)
Labornotiz LN AW-2015-2

Werner Arnold
Analytik von Tetrodotoxin (TTX)
Laborbricht LS 2015-09

Rahel Gäumann
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