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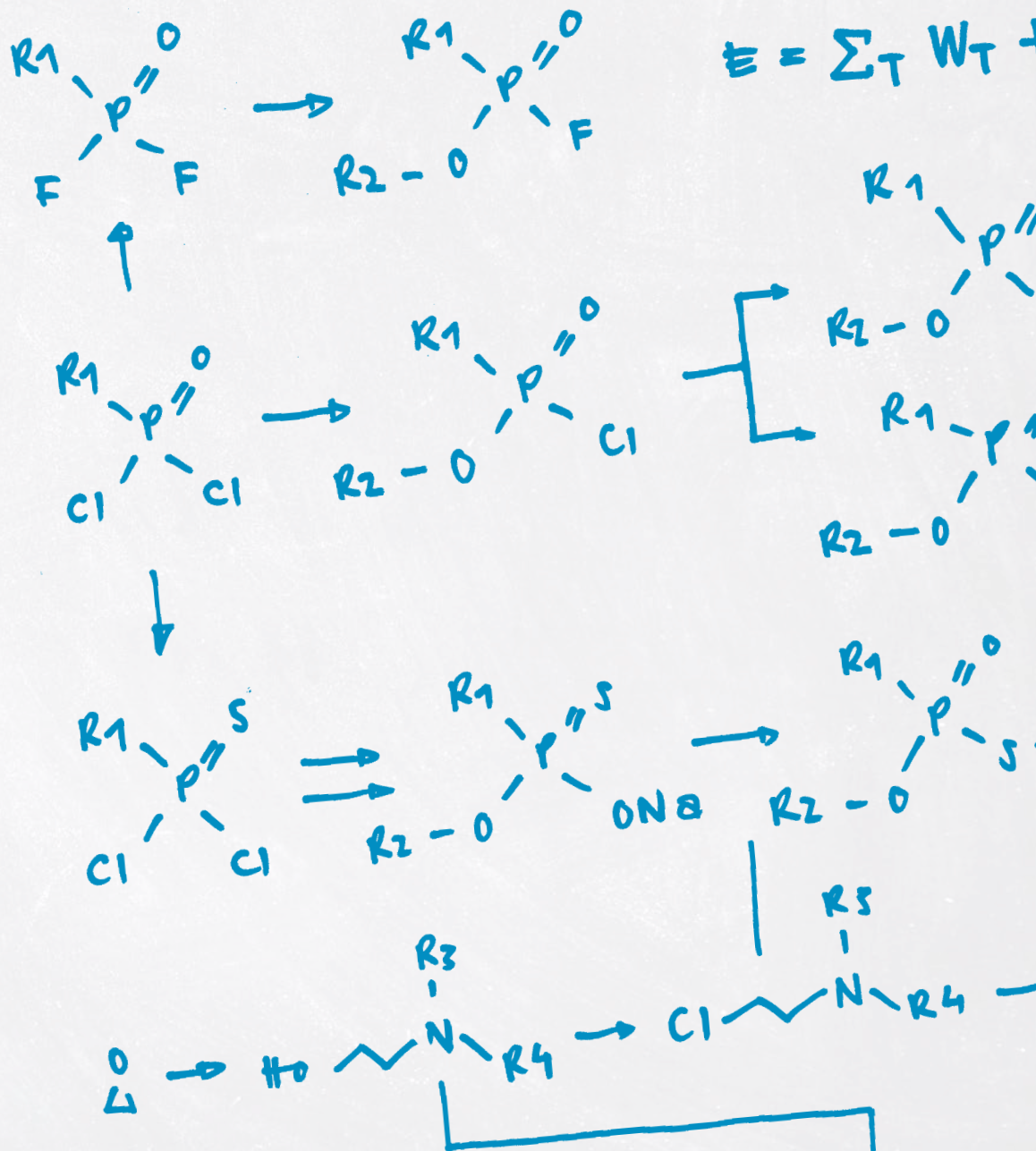
SPIEZ LABORATORY

Annual report 2016

SPIEZ LABORATORY



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Dear readers,

The *Bulletin of the Atomic Scientists* has moved its symbolic Doomsday clock from three to two and a half minutes to midnight. This group of experts considers the risk of a global catastrophe higher than ever since 1959 and it is not known for alarmism. The scientists justify their assessment as follows: *"Over the course of 2016, the global security landscape darkened as the international community failed to come effectively to grips with humanity's most pressing existential threats, nuclear weapons and climate change."*

The year 2016 was not just marked by stagnation and setbacks in nuclear and biological disarmament but also by an increase and escalation of violent conflicts, particularly in the Ukraine, in Iraq and Syria. The series of terroristic attacks by self-radicalised single actors and followers of the so called Islamic State continued from Paris and San Bernadino to Brussels, Nice, Cologne and Orlando. The world is experiencing a period of instability and a state of crisis seems to have replaced normality.

Nuclear weapons are experiencing a 'renaissance' in international security policy and chemical weapons continue to be used repeatedly in the Syrian civil war. The threat emanating from weapons of mass destruction therefore remains an important challenge for Swiss security policy. Spiez Laboratory is an interface between science and politics and issues of national security. The expertise of our specialists remains, in particular for arms control and NBC protection, in high-demand.

We are doing the utmost to deliver the level of quality our customers have become accustomed to. This requires innovation in times of cuts in government spending. Developing new fields of expertise is increasingly only possible by strengthening our international cooperation. For the coming years we were able to join several European research projects focusing on issues of public health and bio-safety and security. Moreover, we are proud that in November 2016 Spiez Laboratory became a designated Collaboration Centre for the International Atomic Energy Agency (IAEA). This new role allowed us to integrate two international fellowships for projects in our Physics Division.

Dr. Marc Cadisch
Director Spiez Laboratory

Science and technology based initiatives focusing on arms control are becoming ever more important and are well received, in particular if they stem from a neutral country such as Switzerland. We remain committed to supporting the goals of the Chemical Weapons Convention as well as the Biological Weapons Conventions and the treaties keeping pace with advances in science and technology: Our workshop series on the growing convergence in chemical and biological sciences (Spiez CONVERGENCE) continued successfully last year (p. 30). Progressing well is also our initiative to strengthen the United Nations Secretary Generals' Mechanism (UNSGM) – a project to establish a network of biological laboratories developing quality assurance and reporting criteria – with the aim that investigations into the alleged use of biological weapons would receive undisputed scientific and political acceptance (p. 37).

With regards to national security we support initiatives for the prevention of nuclear terrorism. We thus operate a mobile detection unit equipped with sensitive detector systems for gamma- and neutron radiation, and, we cooperate with customs, federal police and intelligence services to prevent illegal transfers and illicit trafficking of radioactive materials. The capability of our mobile detection systems was tested in an international exercise (p. 8).

Our core business remains detection, identification and diagnostics – in order to adequately respond to new and complex challenges, we must continuously adapt and develop our technical capabilities accordingly. In a time of staffing limitations, this necessitates expert personnel and state of the art laboratory instrumentation. Through participation in international round robin testing we benchmark our analytical capabilities and receive an objective assessment. For toxin analysis this is a European project under the name EQuATox (p. 19). For the verification analysis of chemical warfare agents it is the official proficiency testing program of the Organisation for the Prohibition of Chemical Weapons (OPCW), for which Spiez Laboratory prepared the test samples in 2016 and received the maximum score (p. 34). 'Readiness to respond' remains a top priority, which we maintain through the development of new testing methodologies (p. 12), as well as in the evaluation of new analytical approaches, such as in the field of bioinformatics (p. 22).

A prerequisite for conducting our work is a modern and well-functioning laboratory infrastructure. In 2016 we finalised the renovation of our chemical safety laboratory for the synthesis and handling of highly toxic chemicals (p. 40). Furthermore, we were able to put our new sample reception unit into operation. This is a unique facility in Switzerland to receive and process radiological, chemical and biological samples (p. 44).

The tanker “Probo Koala” and environmental activists in the port of Abidjan

Ivory Coast Environmental Mission

Marc Stauffer

In 2016, a team from Spiez Laboratory were on a mission in the Ivory Coast on behalf of the United Nations Environment Programme (UNEP). The purpose of the mission was to investigate the long-term consequences of a grave toxic waste scandal: in 2006, the cargo ship *Probo Koala* shipped 500 tonnes of toxic waste into the port of Abidjan and dumped the material illegally at landfills, in canals and around the industrial zone of the city. Seventeen residents died of poisoning; tens of thousands had to be treated for respiratory problems. In order to make it possible to assess the long-term consequences of the contamination as well as the success of the remediation measures taken, Spiez Laboratory sampled air, drinking water, soils, fruit, sediment, seawater and mussels in Abidjan.

In the spring of 2006, the tanker *Probo Koala* flying the flag of Panama, had loaded on board petroleum coke, a residue from the distillation of mineral oil that contains a high amount of sulfur and metal. This petroleum coke had been treated with sodium hydroxide and processed further into Naphtha, a relatively light fraction of mineral oil. This process had been carried out directly on the ship, not in a petrochemical plant as usual. The residue from the processing contained dangerous substances such as caustic soda and sodium sulphide, a chemical that over time decomposes into the toxic and foul-smelling hydrogen sulphide gas. Other contaminants in the residue included heavy metals, phenols, and a range of other aliphatic and aromatic hydrocarbons.

The *Probo Koala* initially tried to unload the toxic residues in the port of Amsterdam; but the harbour police stopped this attempt. The Dutch authorities offered disposal of the cargo by specialised facilities in Rotterdam. However, the captain of the ship refused, as it would have incurred costs of around USD 250 000.

The Ivorian Minister of the Environment visits a sampling site, accompanied by his advisers and a crew of the Ivorian television

For several weeks, the freighter sailed to and fro across the Atlantic, but failed to find a port to get rid of the petroleum coke mixed with cleaning chemicals, benzene and crude oil residues. Finally, on 19 August 2006, the toxic waste was pumped into tanker trucks in the port of Abidjan.

Thereafter, and over a period of three weeks, the 500 tonnes of toxic waste were dumped across Abidjan and its surroundings - alongside roads, on landfills, in canals and in the industrial zone – in a stealth operation presumably conducted by Ivorian subcontractors of the operating company. Some of these areas were populated, leading to numerous cases of poisoning: By 2008, the Ivorian government had counted 17 fatalities, dozens of cases of poisoning, and 30 000 individuals who needed medical care. In addition, there were the incalculable consequences for the environment and the accumulation of poisonous substances in the food chain caused by the pollution of soil, ground water as well as the lagoon of Abidjan.

The case had judicial consequences in the Netherlands as well as in the Ivory Coast. Several persons involved were arrested, and the operating company had to pay compensation to the victims in Abidjan as well as to the Ivorian State. The scandal also had political implications. Worldwide attention was attracted by the fact that the 'Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal' had been breached by exploiting the situation in Western Africa.

The affected sites were cleaned up, and the waste treated directly on site and then shipped to Europe by a specialised hauler. However, even ten years after the disposal of the waste, rumours continued to circulate in Abidjan and some citizens complained about illnesses which they attributed to the residues from the *Probo Koala*. This led to the Ivorian government requesting the United Nations Environment Programme (UNEP) to conduct an independent investigation. Given its many years of experience with environmental sampling and analysis,

Spiez Laboratory was approached by UNEP with a request to join UNEP specialists in conducting this investigation and to coordinate the analytical work.

Preparations

Comprehensive preparatory work was needed to be able to provide answers to the question posed to the environmental analysis team: could remnants of the toxic freight of the *Probo Koala* still be detected ten years after the dumping and several years after the clean-up operations, or rather, in which way would it be possible to distinguish a kind of 'finger print' of these substances from that of other possible contamination possibilities?

For example, an attempt was made to reconstruct the composition of the waste in order to limit the range of substances that needed to be analysed. From the wide spectrum of possible compounds, the most important ones were selected and contractors from all over the world were hired to conduct the environmental analysis. The analysis included the determination of sulfur and so-called TPH fractions (total petrol hydrocarbons – aliphatic and aromatic fractions from the oil industry), polycyclic aromatic hydrocarbons (PAH), BTEX (a sum parameter for benzene, toluene, ethyl benzene and xylene) as well as heavy metals and other toxic metals and compounds. The inorganic analysis was assigned to the Spiez Laboratory's environmental analysis branch.

In addition, the dumping sites were surveyed by advance missions and by using satellite imagery. This data was used to draw conclusions about the types of materials to be investigated. In the majority of cases, these were soil, water and air samples. Foodstuff, mussels and sediment from the lagoon of Abidjan were selected as complementary matrices.

The inputs from the Centre Ivoirien Antipollution (CIAPOL) were of particular importance. Its specialists had detailed knowledge of the precise locations and circumstances of the dumped waste. This added contextual understanding with regard to each of the dumping sites.

Mission

On 2 July 2016, a three-person team from Spiez Laboratory flew to Abidjan. Together with the UNEP specialists the team was assembled. After preliminary consultations with CIAPOL and the Ministry of the Environment of the Ivory Coast, a base station was set up in order to prepare the daily sample collection, store the acquired samples, and to ensure the traceability of samples using unique sample codes and unambiguous geographical reference data and labelling.

Every morning, the analysts would drive in several vehicles under police escort through the heavy traffic of Abidjan to the affected sites. There, the precise quantity, location and type of sampling were determined. This was done in collaboration with the CIAPOL specialists, who were able to provide exact information about the localities and the way in which the waste had been disposed of. In addition, the CIAPOL – thanks to its policing authority and communication with the local population – facilitated the unhindered access to drinking water facilities, private wells and company premises. Subsequently, the samples were collected and filled into receptacles. The team had to make sure that none of the analytes would undergo any changes until arrival of the samples at the analytical laboratory. The fieldwork was vastly helped by the preparatory work that had been completed at Spiez Laboratory with regard to reconnaissance, coding and logging.

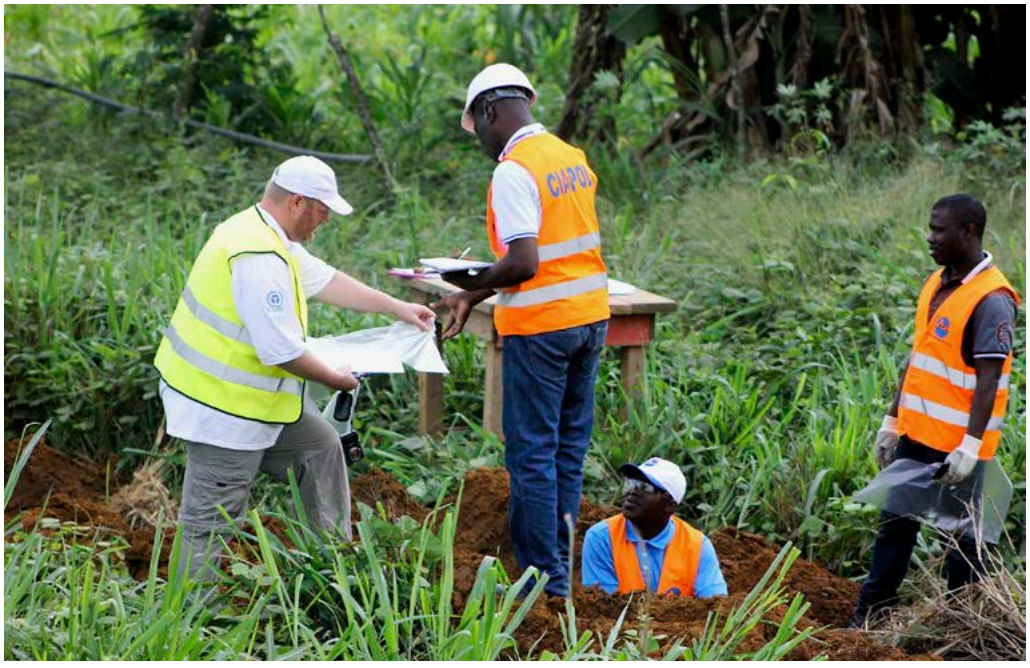
Every day, 2–3 sites were sampled in this manner. These sites were spread across the entire municipal area of Abidjan. The sampling sites were located in the industrial zone of Abidjan, alongside canals and roads, on a landfill site, in the city forest of Abidjan, in poor living quarters of the city, and in the lagoon of Abidjan. At all sampling sites, whenever possible, samples were taken from private drinking water wells and from water of the local drinking water distribution system. In addition, the team collected samples at two sites that had no contact with the waste from the Probo Koala. This was to establish the background pollution levels. During one of the last sample collections, the Minister of the Environment of the Ivory Coast together with a television crew visited the team. Amnesty International, also showed an interest in the mission and visited the team in the field.

In just under two weeks, all planned sites had been successfully sampled. The team took a total of 150 samples. The samples were dispatched by courier to the different laboratories that had been commissioned to conduct the analyses. ALCONTROL laboratories (UK) were responsible for the organic parameters, ALS-Laboratory in Sweden for the analysis of the mussels. After final administrative work, the team from Spiez Laboratory left the Ivory Coast and subsequently, the extensive analyses began at Spiez Laboratory. All data generated by the various laboratories were consolidated and interpreted at Spiez Laboratory.

The final report, which will be prepared by UNEP, will be published in the spring of 2017.

Clean-up work at one of the contaminated sites

The local residents show much interest in the investigation



Soil sample collection at different depths at one of the cleaned-up sites

Detectors for gamma and neutron radiation

Mobile Measurement System Radioactivity MMR

Dr. Emmanuel Egger

Since 2016, the A-EEVBS (Nuclear Emergency Response Team VBS) operates a mobile measurement system with sensitive detectors for gamma and neutron radiation. In collaboration with the Directorate General of Customs, the Federal Criminal Police or the Federal Intelligence Service, the system can be deployed for the detection of illegal nuclear materials – not only in stationary mode in buildings but also for the identification of vehicles carrying radioactive sources. During an international measurement exercise, the capabilities of this new equipment were thoroughly tested and confirmed.

Several initiatives have been started in recent years to prevent the use of nuclear materials for terrorist purposes, for example the Global Initiative to Combat Nuclear Terrorism or the Nuclear Security Summit. These meetings have defined measures for the secure storage of radioactive sources and nuclear materials as well as for the interdiction of their illicit trafficking. In the context of these initiatives as well as in the context of the International Convention for the Suppression of Acts of Nuclear Terrorism, Switzerland has also made commitments for the implementation of measures. To this end, Spiez Laboratory acquired the Mobile Measurement System Radioactivity (MMR). The purpose of this acquisition is to search for deliberately smuggled or orphan radioactive sources, including nuclear materials in collaboration with the Federal and Cantonal investigation authorities dealing with customs controls, goods transportation, mass events and similar issues.

The MMR is equipped with 64 neutron detectors filled with He-4 gas. There are also six

gamma detectors (three on either side) for the detection of gamma rays. For the verification of detected sources, two portable, highly sensitive measurement instruments which can confirm the presence of specific nuclides are aboard the MMR. The vehicle is equipped with eight batteries to ensure the autonomy of operation for approximately 8 hours even with the air conditioning running. In addition, the vehicle carries a petrol-driven electricity generator to further extend its time of operation. If an external power supply is available (230 V) the MMR can remain operational for an unlimited period of time. The vehicle can take measurements whilst in motion to detect stationary or moving sources, or it can be used in stationary mode to screen vehicles or persons passing by. The latter measurement configuration requires that the vehicles pass fairly slowly alongside the measurement instruments, as directed by the police. The measurement system yields optimum results when the vehicles to be measured pass at a velocity of no more than 10 km/h. However, even at higher speed it will still be sufficiently sensitive for detection purposes.

Figure 1 shows the way the measurement is conducted. The autonomy of 8 hours makes it possible, without extensive preparations and in collaboration with the partner organisations (Directorate General of Customs, Federal Criminal Police, Cantonal Police), to conduct measurements of vehicles passing by. When a predetermined level of radiation is exceeded, an alert is triggered. The system stores a series of photographs of the passing vehicle that has triggered the alarm. These data will be transmitted by radio to the partner organisations, which will stop the vehicle. The vehicle can then be examined for specific nuclides by a second A-EEVBS Team, using one of the portable specialised instruments.

The MMR can also be deployed to search for a source whilst driving, or to map the local dose rate (LDR). The co-driver of the MMR uses a tablet or laptop computer to log the current location and LDR. The MMR is also equipped with GPS. In search mode, the current location of the vehicle will be shown every second as a dot on the map (figure 2). The colour of the dot represents the actual LDR. In addition, four monitors display the current gamma and neutron LDR. Once a predetermined dose rate is exceeded, an alert is triggered and the map representations will be saved to document the current location.

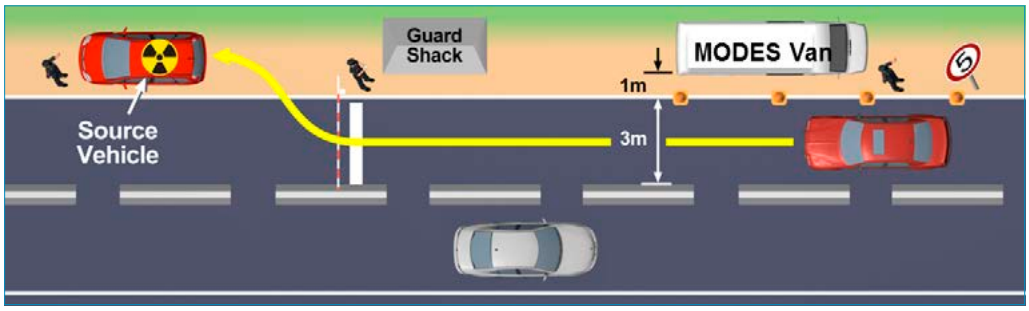


Figure 1: Schematic of the operations of the MMR (MODES Van) in stationary mode.

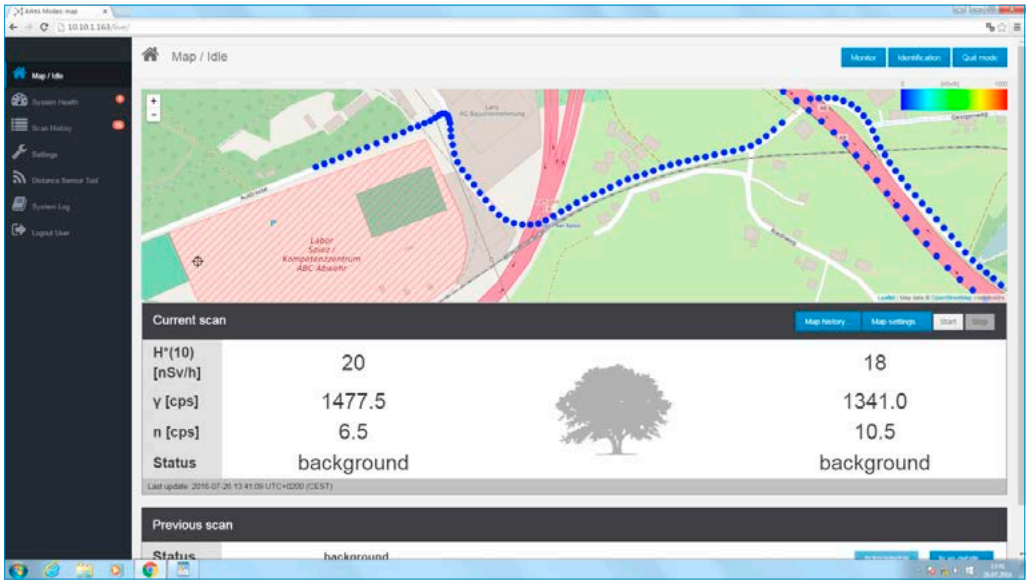


Figure 2: Map generated in search and map mode. The blue dots represent the position of the MMR at different times. The colour of each dot represents the LDR measured at that location (blue: LDR < 100 nSv/h).

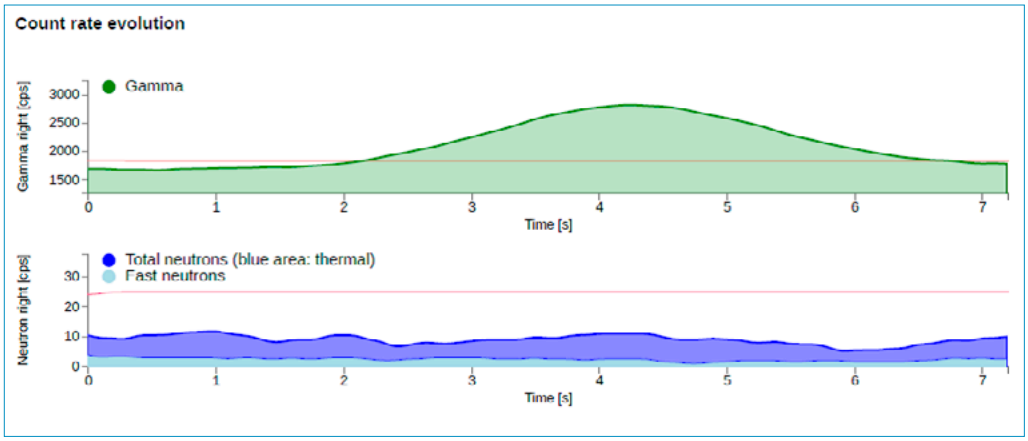


Figure 3: Count rate evolution over time for gamma and neutron radiation, case of a gamma alert being triggered.

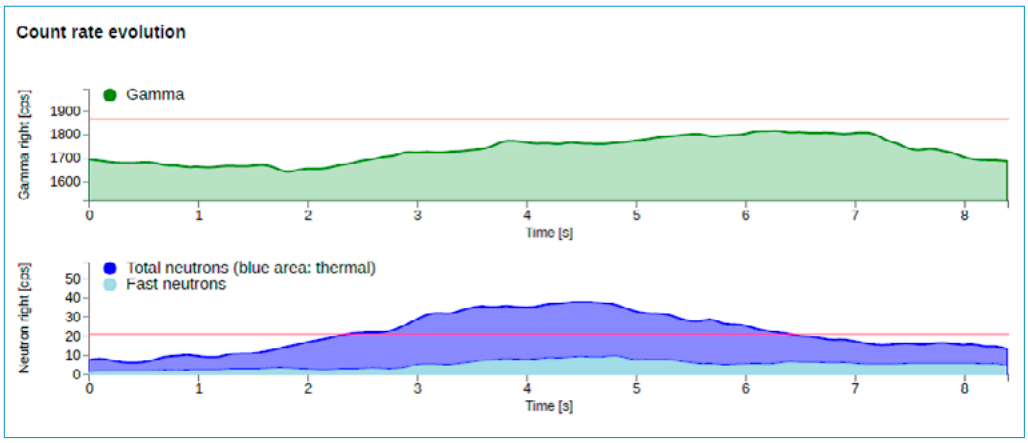


Figure 4: Gamma and neutron count rate evolution over time, case of a vehicle passing that triggered a neutron alert.

Initial experiences

In the summer of 2016, the Mobile Measurement System Radioactivity MMR was deployed for the first time in an international exercise: During a priority check at a customs control post the MMR was to be assessed for its capability to detect radioactive materials in transit traffic. Firstly, all transiting vehicles that passed through the customs control post were measured. Secondly, colleagues from the neighbouring country played the role of smugglers who were transporting a source into Switzerland. The task of the A-EEVBS Team was to screen all passing vehicles with the MMR and to inform the Swiss customs when an alarm was triggered. The customs officials then immediately stop the transit traffic and divert the suspect vehicles into an adjacent area before allowing the traffic to continue. A second A-EEVBS Team then conducts in the adjacent area detailed measurements of the vehicles that had triggered the alarm. During this exercise, a total of 320 vehicles were screened. Twelve of the vehicles stopped triggered a gamma alert, one a neutron alert.

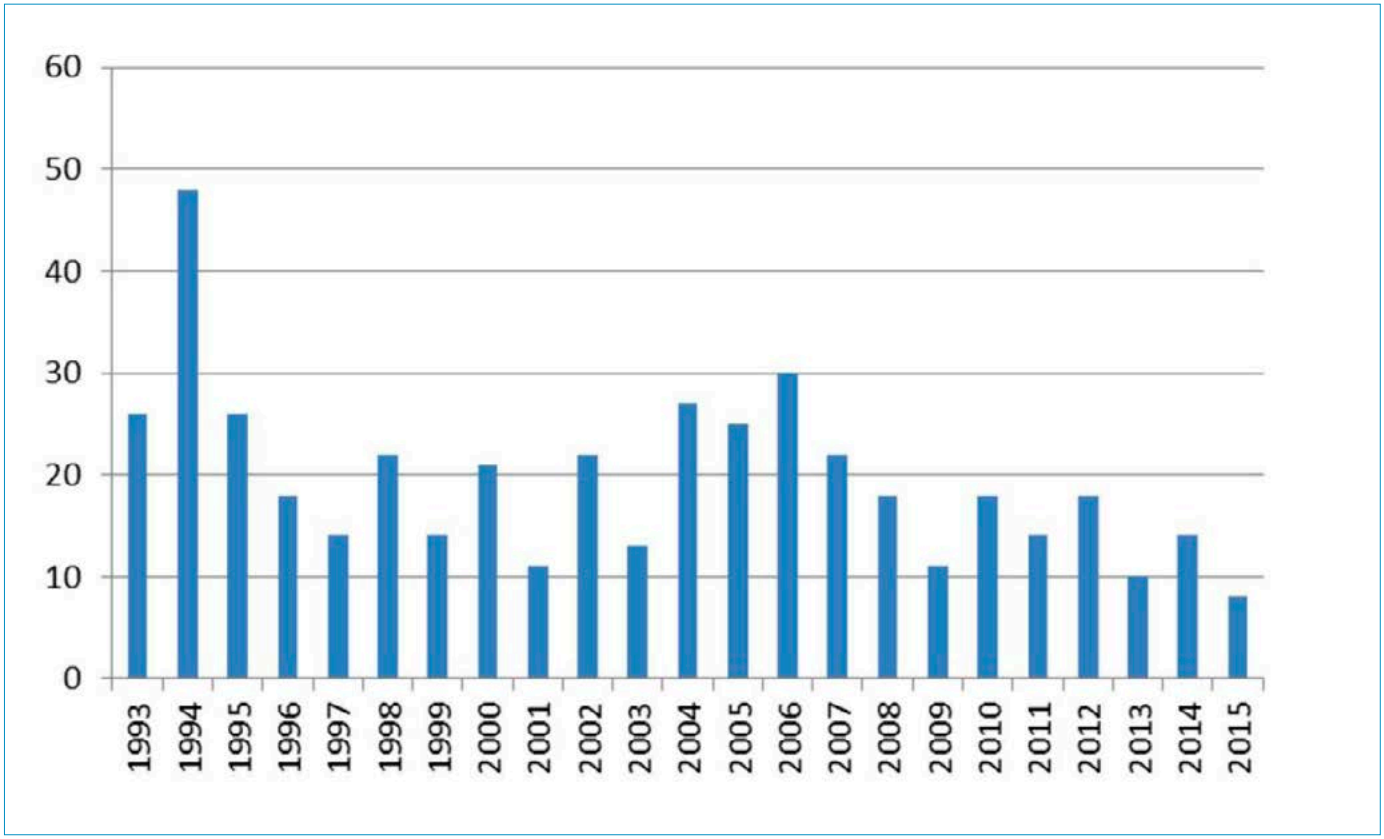
The car that had triggered the neutron alarm was identified as the 'smugglers vehicle'. The gamma alarms could all be ascribed to NORM (Naturally Occurring Radioactive Material, such as sanitary ceramics, granite, fly ash, fruit rich in potassium such as bananas) or a correctly declared and labelled transport of a class 7 dangerous goods (radioactive material).

Figure 3 shows the gamma count rate evolution over time that triggered a gamma alert. The lorry concerned was stopped and investigated in detail by the second A-EEVBS Team.

At 08:00:34 CET, a vehicle passed the MMR which triggered a neutron alert (figure 4). The passing vehicle was automatically photographed.

The team in the MMR alerted the Swiss customs officers who stopped the vehicle and directed it to the adjacent area. The second A-EEVBS Team received information that this vehicle had caused the neutron count rate to increase four-fold, and that the large percentage of thermic neutrons measured was indicative of a shielded neutron source inside the vehicle.

The team undertook a detailed investigation of the vehicle and discovered a slightly elevated brutto gamma dose rate of 150 nSv/h (the background in the mission location was 80 ± 30 nSv/h), with the highest level being measured at the side door of the vehicle's loading compartment. Furthermore, an elevated neu-

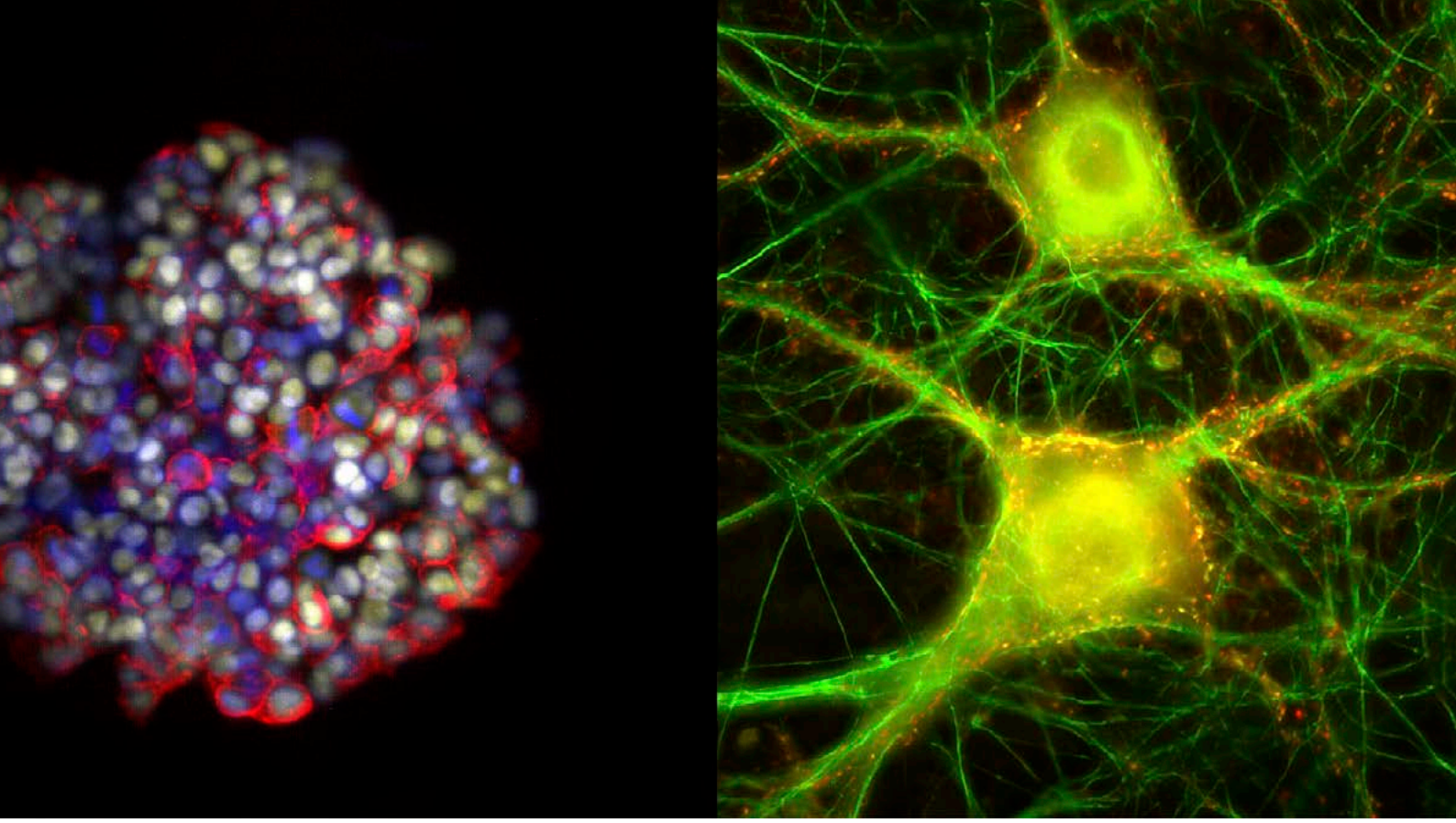


Confirmed incidents involving unauthorised possession and related criminal activities 1993–2015 (Source IAEA ITDB)

tron dose rate of 340 nSv/h was measured, with the highest level again at the side door (background < 70nSv/h). Using the portable instrument for nuclide specific measurements, traces of Cs-137 were detected.

The transport documents carried by the drivers showed a transport of a Cs-137 source exempted under UN2910. This appeared to be compatible with the gamma measurements but not with the elevated neutron dose rates, which was indicative of an illicit transport. Based on this evidence, the driver was denied continuation of his journey, and the vehicle as well as driver were transferred to the neighbouring customs office for further investigation. This customs office alerted the authorities responsible in such cases, who mobilised their on-call response team. Subsequently both response teams conducted the investigation of the suspect vehicle together. It was confirmed that there was indeed, a concealed neutron source shielded by mineral water bottles and Zirconium sand aboard the vehicle. This confirmed the initial suspicion of the MMR team. The smuggling of nuclear material – concealed alongside a correctly declared transport of a class 7 radioactive material – was successfully intercepted.

This exercise demonstrated that with the MMR, Switzerland has at its disposal an excellent tool for the detection of sources that emit neutron and gamma radiation. The collaboration between the Directorate General of Customs, the customs office and other federal partners, but also with authorities of the neighbouring country, had worked very well. Additional missions for the MMR are planned for 2017.



(Left) A Single colony showing pluripotent mouse embryonic stem cells. Visible are the known pluripotent markers Oct4 (yellow) and SSEA1 (red). The nuclei where identified with DAPI and are shown in blue.
(Right) Shown are two single neurons (green). In addition, the SV2 receptor is shown in red and yellow.

Embryonic stem cell-derived neuronal networks for *in vitro* toxicity screening of *Clostridium botulinum* neurotoxins

Dr. Stephen Jenkinson

To date, an ethical disputable in vivo mouse bioassay, introduced in the 1920s, is still considered as the gold standard detection method for *Clostridium botulinum* neurotoxins. Spiez Laboratory in collaboration with the Institute for Infectious Diseases has developed an *in vitro* stem cell-based bioassay based on multi-electrode arrays capable of detecting the biological activity of *Clostridium botulinum* neurotoxins. This assay could serve in minimising animal experiments as well as provide a physiological relevant platform for drug-screening of neuroactive compounds.

Botulinum neurotoxins (BoNTs) are produced and secreted by the spore forming, gram-positive bacteria *Clostridium botulinum*, *C. butyricum* as well as *C. baratii* and are ubiquitously found in terrestrially and aquatic environments as well as in anaerobic regions of the intestines of animals and insects (Johnson and Montecucco, 2008). By using a multi-step mechanism of cellular intoxication and inhibiting neurotransmitter release from cholinergic motor neurons in vertebrates BoNTs can cause severe and potentially life-threatening symptoms known as botulism (Rossetto et al., 2014; Rummel, 2016). Symptoms of botulism range from fatigability affecting cranial musculature leading to double vision, slurred speech and impaired swallowing to a generalised paralysis affecting limbs and the respiration (Lindstrom

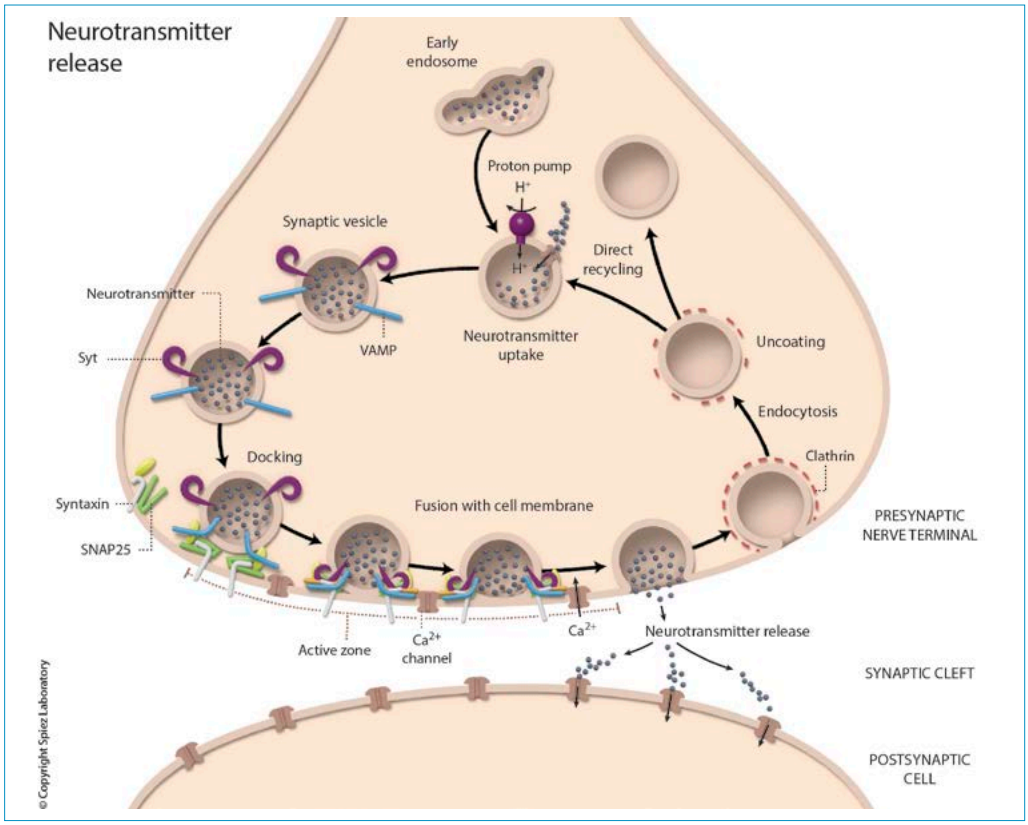


Figure 1: Schematic illustration of the signalling process at chemical synapses
Neurotransmitters that are released from the presynaptic cell mediate the signalling at chemical synapses. They are stored in synaptic vesicles. By using an electrochemical proton gradient, that is generated by the vesicular ATPase proton pump, the neurotransmitters are loaded into the vesicles. Thereupon, the synaptic vesicles bind to the active zones of the presynaptic membrane and the proteins VAMP, Syt, SNAP25 and syntaxin form with other proteins a complex known as SNARE (soluble N-ethylmalmeide-sensitive factor attachment protein receptor) complex. Upon depolarisation of the nerve terminal, Ca^{2+} channels open and Ca^{2+} ions bind to Syt, inducing the fusion of the vesicle with the membrane and neurotransmitter release. The neurotransmitter diffuse across the synaptic cleft and bind to specific receptors located on the postsynaptic cell.

and Korkeala, 2006; Johnson and Montecucco, 2008). It is estimated, that the human lethal dose is 0.1 – 2 ng/kg if applied intravenously and around 7 µg/kg by oral administration (Arnon et al., 2001; Simpson, 2004).

Structure and BoNT intoxication

To date, there are seven serotypically distinct BoNTs (referred to as BoNT/A–G) known. In addition, each serotype is further divided into subtypes on the basis of their amino acid sequence and over 40 different subtypes have been already described (Rossetto et al., 2014; Peck et al., 2017). All BoNTs are initially synthesised as 150 kDa polypeptides and processed by post-translational proteolytic cleavage yielding a 100 kDa heavy chain (H-chain) and a 50 kDa light chain (L-chain) linked by a disulphide bond (DasGupta and Sugiyama, 1972).

Using a dual receptor binding mechanism, which is mediated by the H-chain, enables BoNTs to bind with a high selectivity towards presynaptic membranes of peripheral nerve terminals (Rummel, 2016). In a first step, BoNTs bind via the H-chain towards polysialoganglioside receptors. Upon this initial anchorage, the neurotoxins bind towards specific synaptic vesicle protein receptors. In particular, BoNT/A, D, E and F bind to the SV2 receptor and BoNT B and G bind to synaptotagmin (Syt) (Berntsson et al., 2013; Rummel, 2016). After successful binding towards both receptors, the toxin is internalised into endosomes by receptor mediated endocytosis (Montal, 2010) (Fig. 2).

Upon internalisation the toxin must translocate across the membrane of the endosome. This step is mainly driven by a transmembrane pH gradient generated by the vesicular ATPase proton pump that serves for the co-transport of neurotransmitters and H^+ ions into the lumen of the endosomes (Ahnert-Hilger et al., 2003) (Fig. 1). It is proposed, that a lowering of the surrounding pH to 4.5–6 induces a conformational change within the H-chain followed by its insertion into the endosomal membrane thus forming a channel. The L-chain is thereupon translocated over the membrane and the

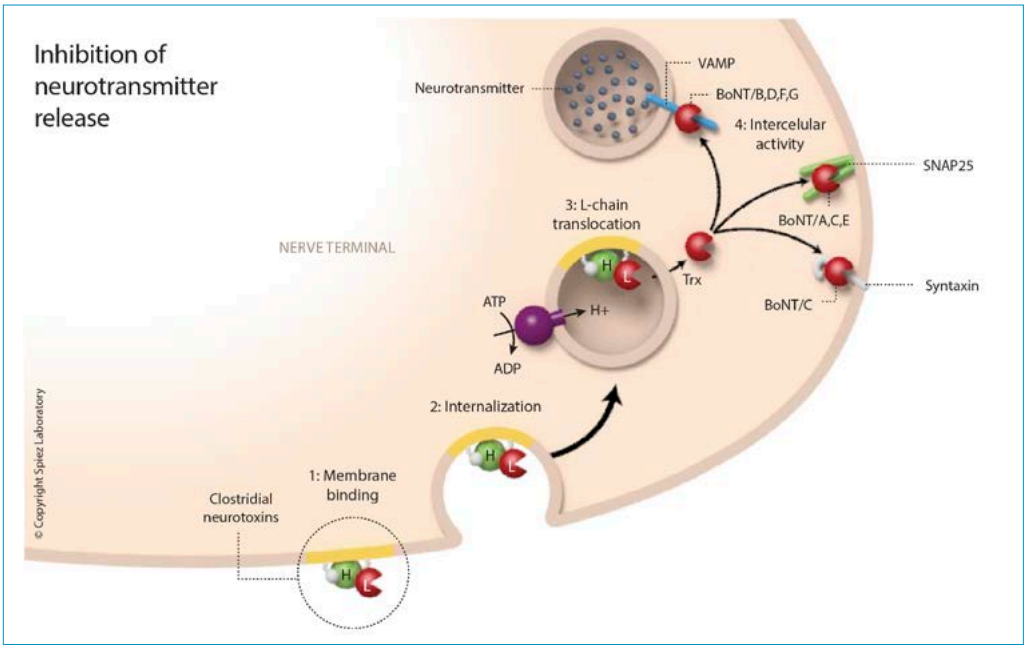


Figure 2: The four steps of BoNT intoxication: binding, internalisation, translocation and SNARE cleavage
The first step of intoxication is mediated by the binding of BoNTs via the H-chain domain towards specific receptors located on the nerve terminal membrane (step 1). In a second step, BoNTs are internalised into endosomes by receptor mediated endocytosis. Subsequently, the toxin exploits the vesicular ATPase proton pump, which drives the reuptake of neurotransmitters into the vesicle. A decrease of the pH to 4.5 – 6 protonates the toxin and initiates the translocation of the L-chain across the synaptic vesicle membrane into the cytosol (step 3). Upon successful translocation and reduction of the inter-chain disulphide bond (S-S bond) through a thioredoxin reductase-thioredoxin system (Trx) the L-chain cleaves specific SNARE proteins. In particular, BoNT/A and E cleave SNAP-25 and BoNT/C cleaves both SNAP-25 and syntaxin. BoNT/B, D, F and G cleave specifically VAMP (step 4). In all cases this results in the inhibition of neurotransmitter release and consequent neuroparalysis.

disulphide bond linking the H-chain with the L-chain is reduced by thioredoxins (Trx) resulting in the release of the L-chain into the cytosol of the neuron (Montal, 2010; Fischer, 2013) (Fig. 2).

The L-chain of all known BoNTs are zinc dependant metalloproteases that target distinct SNARE proteins which play a crucial role in synaptic exocytosis (Schiavo et al., 2000) (Fig. 1). Specifically, BoNT A, C and E cleave SNAP-25 and in addition BoNT C cleaves syntaxin whereas BoNT/B, D F and G cleave VAMP (Fig. 2). Proteolysis of any of these proteins prevents the assembly of the conserved SNARE exocytosis complex, therefore inhibiting neurotransmitter release and leading to the symptoms associated with clinical botulism (Binz, 2013; Pantano and Montecucco, 2014).

Usage and Detection of BoNTs

Despite the high toxicity of BoNTs, BoNT/A and to a lesser extent BoNT/B are used and approved by the national regulatory authorities as pharmaceuticals to treat an increasing number of neurological and non-neurological indications and in cosmetics (Bigalke, 2013; Walker and Dayan, 2014). Because these toxins are natural products and produced by bacteria, pharmaceutical batch-to-batch variations in concentration and activity occur. Therefore, each batch must be tested and the potency of biologically-active BoNT must be precisely determined. To date, the gold standard for BoNT detection and quantification is mainly based on an *in vivo* mouse bioassay (MBA). In this assay, different dilutions of the preparation containing BoNT are injected into mice and symptoms of paralysis are observed over several days (Schantz and Johnson, 1990). This leads ultimately to the death of the animals due to respiratory arrest and causes considerable ethical concerns. In addition, a large amount of animals is required, lab to lab variations can occur, high costs accrue, and up to 4 days are necessary to yield results. Therefore, there is a high demand for the replacement of the MBA and much effort has been made in the search for novel *in vitro* detection assays. To date, different functional, immunological and spectrometric assays have been developed which are capable of detecting BoNTs or BoNT catalytic activity (Dorner et al., 2013; Pellett, 2013).

Since BoNTs are extremely potent and use a multi-step mechanism of cellular intoxication, the detection is extremely challenging and novel assays must have sensitivities in the picogram (pg) range. Neuronal cell-based assays are currently the only *in vitro* alternative capable of detecting all steps of BoNT-intoxication, including binding to the cell surface, endocyto-

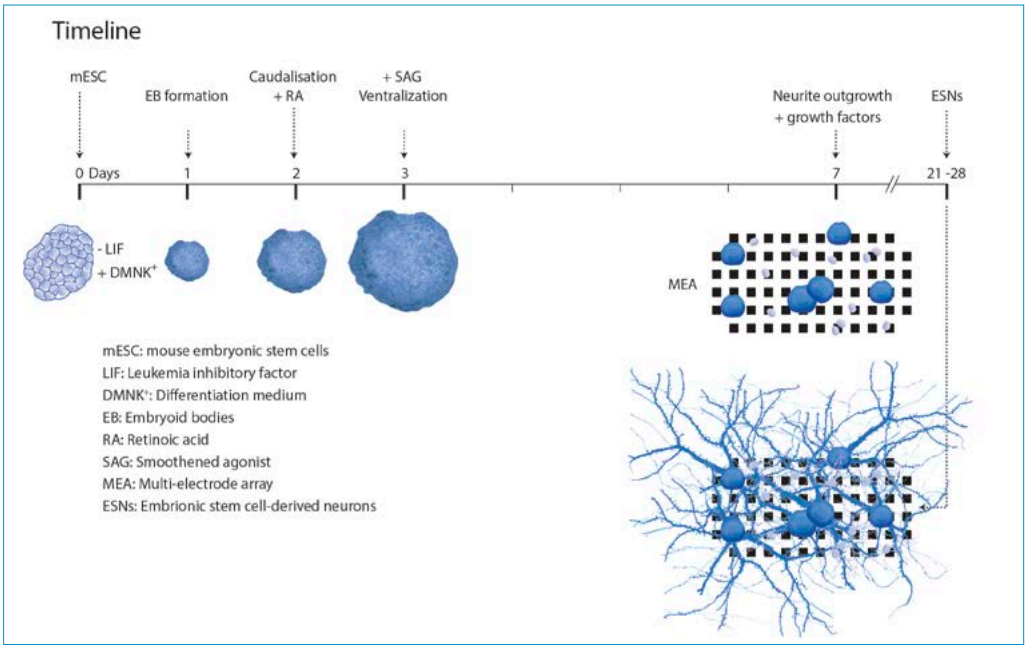


Figure 3: Timeline of neuronal differentiation
Upon removal of the leukemia inhibitory factor (LIF) and changing the medium to differentiation medium (DMNK+) the formation of embryoid bodies (EBs) is visible. These increase in size during the differentiation process. On day 2, the medium is supplemented with retinoic acid (RA) to induce neural differentiation and neural patterning. Adding smoothened agonist (SAG) on day 3 contributes to the caudalisation and formation of the neural plate. After 7 days, the EBs are dissociated and replated onto multi-electrode (MEAs) arrays. The medium is supplemented with growth factors and neurite growth is observed beginning day 8. At day 10 first single action potentials, recorded from individual electrodes are measured and at day 13 the cultures show first synchronous spontaneous activity over several electrodes reaching a plateau at day 28.

sis, translocation of the LC into the cytosol and enzymatic activity of the L-chain towards SNARE substrates, within one step. Several assays have been developed using primary or embryonic stem cell derived neurons that show similar or even greater sensitivity than the MBA (Pellett, 2013). In fact, Allergan Inc., the distributor of Botox®, published data for an alternative detection assay using a continuous cell line which has been approved by the Food and Drug Administration as a replacement method for potency testing of pharmaceuticals (Fernandez-Salas et al., 2012). However, most of these assays, including the assay developed by Allergan Inc., rely on destructive homogenisation of tissue to allow BoNT quantification and assessment, thus requiring additional methods and further hands-on time to yield a result. In addition, these methods do not allow a continuous monitoring of neuronal activity upon treatment with BoNTs.

Embryonic stem cell-derived neuronal networks grown on multi-electrode arrays can detect the biological activity of BoNT/A

In response to this high demand, a novel *in vitro* assay as an alternative to the widely used *in vivo* MBA has been developed at the Institute for Infectious Diseases in collaboration with Spiez Laboratory. By differentiating mouse embryonic stem cells towards neurons and culturing them on multi-electrode arrays (MEAs) a physiologically relevant cell-based method for the detection of BoNT/A holotoxin and complex (e.g. Botox®) has been established (Fig. 3). The cultivated neurons form functional networks and show high spontaneous synaptic activity resulting in the formation of bursts (Fig. 5) Further, the neurons express all necessary proteins for BoNT/A intoxication (Fig. 4). Treating the cultures with BoNT/A results in a decrease of burst activity in a time- and dose-dependent manner. Further, a complete silencing of synaptic activity is observed after incubating the cultures for 24 hours with 1.66 pM BoNT/A holotoxin or Botox® (Jenkinson et al., 2017) (Fig. 5).

The main advantage of the present work is the culturing of embryonic stem cell-derived neuronal networks on MEAs. Commercially available MEA systems allow for continuous recording in defined environments (e.g. 37°C and 5% CO_2) and do not require highly trained staff. Additionally, this approach can be easily upscaled to meet the requirements for high-throughput screening as shown in different studies in which the effects of several drugs have been assessed on cardiomyocytes (Gilchrist et al., 2015; Clements, 2016).

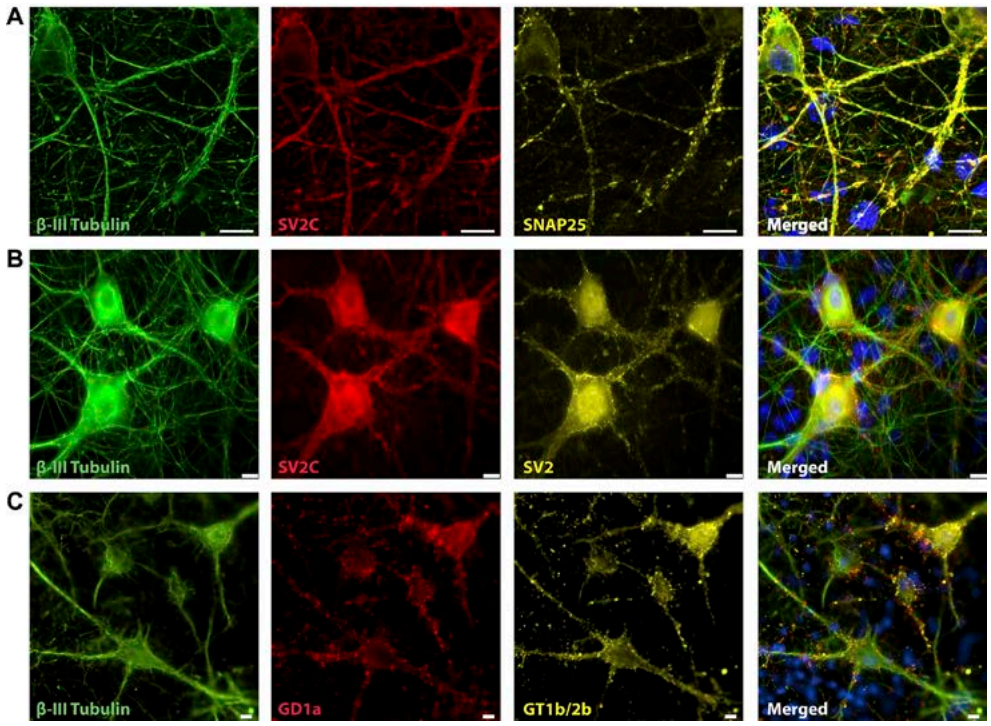


Figure 4: The neuronal cultures express all necessary proteins for BoNT/A intoxication
After 21 days in culture, the neuronal cultures were immunostained against the neuronal marker β-III Tubulin (A-C) and the BoNT/A target SNAP-25 (A). In addition, immunostaining against SV2 isoform A-C (A and B) and the polysialo gangliosides GD1a and GT1b/2b (C) showed the presence of the receptors necessary for the initial anchorage and cytosolic uptake of BoNT/A. Shown also are DAPI nuclear staining and the merged images. Scale bar is 10 µm. Figure adapted from (Jenkinson et al., 2017).

Conclusion

In summary, we have shown that electrophysiological detection of network activity provides a physiological relevant readout for BoNT/A intoxication. Unlike most cell-based assays which are customised for specific BoNT serotypes, mouse embryonic stem cell-derived neurons can be used for the detection of a range of BoNT serotypes (Beske et al., 2016). Though the presented assay holds a high sensitivity down to 1 pM of BoNT/A, further research has to be conducted to increase the overall sensitivity. However, the novel bioassay holds great potential to reduce animal use for BoNT detection and activity determination in pharmaceuticals. In addition, the assay can be expanded for other BoNT serotypes and for the drug-screening of possible neuroactive compounds with an emphasis on synaptic activity. The study has now been published in the journal “Frontiers in Pharmacology”.

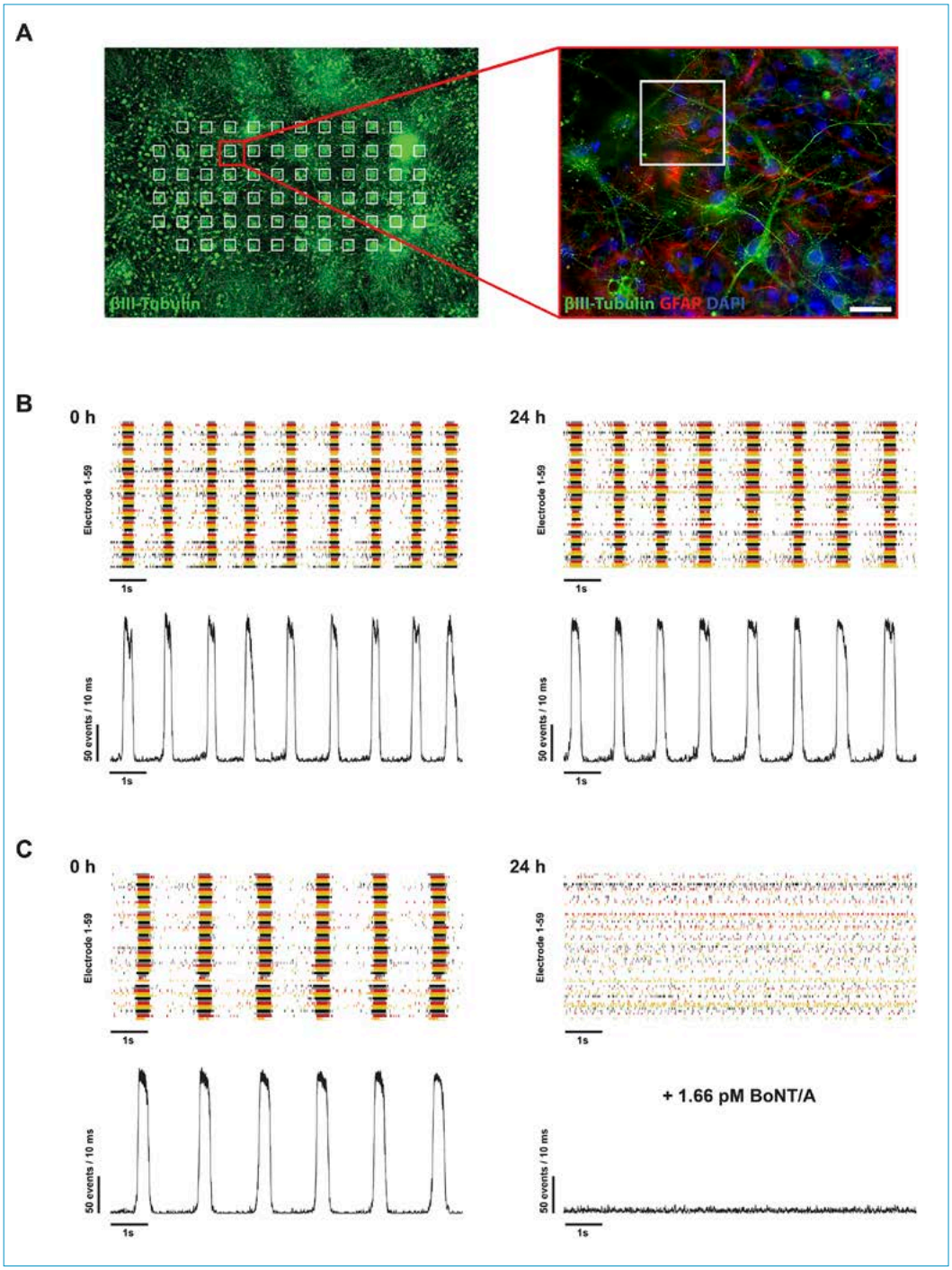


Figure 5: Reduction of synaptic transmission upon addition of BoNT/A
(A) Schematic representation of embryonic stem cell-derived neurons cultured for 21 days on a MEA immunostained for the neuronal marker β-III Tubulin (green), the glial fibrillary acidic protein GFAP (red) and DAPI (blue). Highlighted in white are the positions of each electrode. Scale bar is 20 µm.

(B, top panels): Raster plot of 60 single electrodes (represented by different coloured lines) showing high spontaneous neuronal activity of a culture 21 days after plating prior **(left panel)** and 24 h after receiving 10 µl of PBS **(right panel)** Each coloured event represents a detected action potential of one or multiple neurons recognised by the detector of the MEA setup. The formation of bursts (visible by the bands over all electrodes) is based on spontaneous intrinsic activity and recurrent excitation of the neuronal network through synaptic transmission. **(B, lower panels)** Network activity plot visualising the amount of activity in the whole neuronal culture network during the time illustrated in the raster plot. It shows the number of events detected from all active electrodes with a sliding window of 10 ms shifted by a 1ms step.

(C) Upon treatment and incubation with 1.66 pM BoNT/A for 24 h a complete loss of synaptic activity is visible. This is represented in the loss of burst activity. However, asynchronous intrinsic activity, originating from spontaneous neuronal action potentials, is still visible **(right panel)**. Seen on the left is the same culture prior to addition of the toxin serving as the base activity at 0 h **(left panel)**.

Figure adapted from (Jenkinson et al., 2017)

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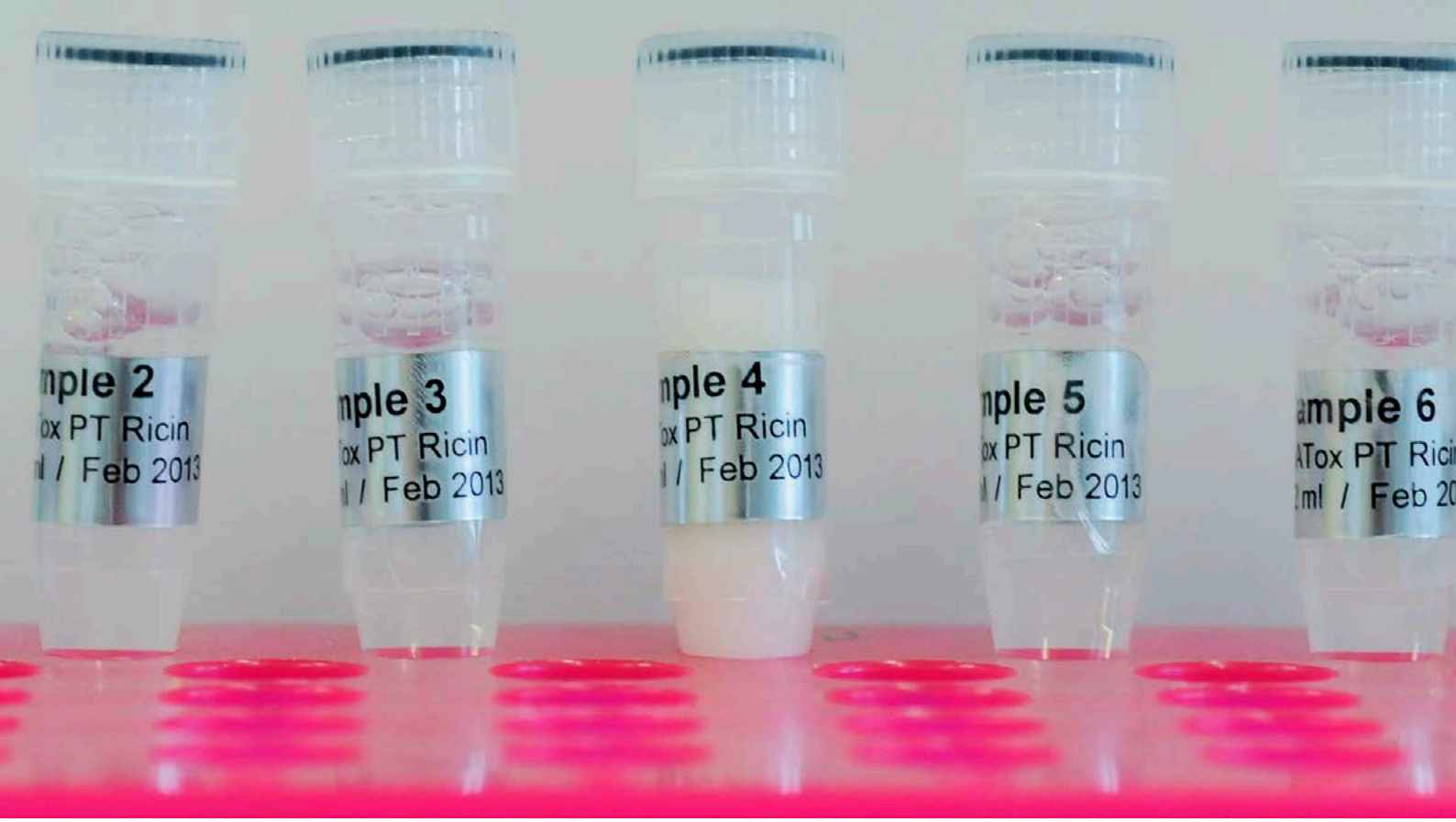


Sample set for the interlaboratory comparison test for ricin

International Round-Robin Test in the Field of Toxinology

Marc-André Avondet

Next to traditional chemicals, radioactive materials and biological agents, toxins have become a growing threat in civilian and military environments. These trends have led, amongst others, to the initiation of several international research projects aiming for the development of countermeasures. Interlaboratory comparison tests in the context of the EU project EQuATox have shown that an appropriate response to this growing threat will require extensive developments in toxin analysis. For many toxins, certified reference standards continue to be lacking and the requirements for quality assurance are not met everywhere.

The Toxinology Branch of Spiez Laboratory is responsible for the support of the army, national institutions and cantonal authorities in the area of toxins. The focus of this work is on the toxins listed in Schedule 1 of the Chemical Weapons Convention (CWC) – ricin and saxitoxin – as well as further 18 toxins listed by the Australia Group. The Toxinology Branch in Spiez provides the necessary scientific competence and is responsible for the expansion of the capacity in laboratory analysis. The latter has priority in the project work and for the response to crisis situations.

Toxins that are produced by plants, animals and microorganisms [1] can be analysed primarily by using the classical methods of biochemical analysis. For the detection of protein-based toxins such as ricin, gel electrophoresis, mass spectrometry, immunological essays (ELISA and LFA) as well as functional essays are standard tests. Lower molecular weight toxins such as saxitoxin are usually analysed using chromatographic and mass spectro-

Figure 1: Consortium partners of EQuATox

Partner No.	Organisation	Country	Work Package(s)
1	Robert Koch Institute Berlin	Germany	1 & 3
2	European Commission – Joint Research Centre	Belgium	5 & 6
3	Institut Scientifque de Santé Publique (ISP/WIV)	Belgium	6
4	University of Helsinki VERIFIN	Finland	4 & 7
5	ANSES – Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail	France	5
6	Toxogen GmbH	Germany	6
7	Swedish Defence Research Agency - FOI	Sweden	2 & 6
8	VBS BABS LABOR SPIEZ	Switzerland	4 & 6
9	ChemStat Bern	Switzerland	3 - 6

Table 1: Consortium partners of the research project EQuATox and distribution of the work packages

Work Package (WP)	Task Description
1	Coordination and Management
2	Information Research within Europe
3	Interlaboratory Comparison Test Ricin
4	Interlaboratory Comparison Test Saxitoxin
5	Interlaboratory Comparison Test Staphylococcus Enterotoxin B
6	Inter-laboratory Comparison Test Botulinum neurotoxin
7	Quality Management
8	Information Dissemination (EQuATox intern & extern)

Table 2: List of the work packages 1–8

metric methods such as GC-MS, HPLC with fluorescence detectors, or LC-MS/MS.

Over the years, three detection methods have been accredited in Spiez (STS 0054 Testing laboratory for the detection of biological agents). In accordance with standard EN/ISO 17025, the Toxinology Branch has to regularly participate in round-robin tests in order to maintain its accreditation. This is not self-evident for laboratories working in this field, because international interlaboratory comparison tests are not offered anywhere as a matter of course. For many years, Spiez Laboratory has maintained good collaborations with the Biological Toxins Group of the Robert Koch Institute (RKI) in Berlin. In 2009, a first interlaboratory comparison test for ricin was conducted together with the RKI and other participants. This first project demonstrated that such round-robin tests require considerable effort, and that there was a need to further advance the entire field of toxin analysis. There was instant agreement that a follow-up programme needed to be devised to cover a broader range of toxins. Given that many potential partners of such a project depend on external funding sources, the choice for funding was the EU's 7th Framework Programme (FP-7) under the topic SEC-2011.5.4-1 *"Towards Standardisation of CBRN Detection and Identification"*.

The Project EQuATox

In early December 2010, coordinated by the RKI, a project proposal was submitted under the acronym EQuATox (Establishment of Quality Assurance for the Detection of Biological Toxins of Potential Bioterrorism Risk). Its financing was approved by the EU in early summer of 2011. The project lasted three years and finished at the end of 2014 [3].

Figure 1 and table 1 show the nine partner organisations that participated in the consortium ("inner circle"), coming from five different EU countries and Switzerland. 36 laboratories from 20 different countries took part in the EQuATox project as so-called participating laboratories (see also http://equatox.eu).

The overall project EQuATox was made up of eight work packages (see table 2). The focus was on the interlaboratory comparison tests for four groups of toxins (work packages 3 to 6).

Interlaboratory Comparison Test Ricin
In February 2013, the participants received nine samples (see figure 2), and they had four weeks to analyse them using their own methods and standards. The sample set consisted of eight liquids and one pulverised solid.

At Spiez Laboratory, sample screening was performed using protein determination, gel electrophoresis (SDS-PAGE) followed by in-gel digest and LC-MS/MS. Quantification was made using immunological methods (ELISA). In order to assess the biological activity, a cytotoxicity essay in cell culture was employed. Four publications on ricin [5,6,7,8] explain in detail the possible approaches and results, as well as the reference data of the reference standards used.

Summary of the results of work package 3:
- Less than half of the 17 participating laboratories were able to quantify all samples correctly
- There was insufficient analytical differentiation between ricin and agglutinin (RCA120)
- The measurement data for the quantification of ricin (samples with high concentration) were of sufficient quality to calculate a consensus reference value

Information concerning the work packages four to six can be found in the scientific journal TOXIN [4, open access].

Findings and Conclusions
From the perspective of the Toxinology Branch of Spiez Laboratory, the findings of EQuATox can be summarised as follows: The project duration of three years was too short, and the administrative effort (internally as well as externally) was considerable; the financing quota was rather modest. Nevertheless, without EQuATox, the state of development of the Toxinology Branch of Spiez Laboratory would be significantly lower today. Due to the collaboration between EQuATox and the OPCW Laboratory a first 'confidence building exercise' involving ricin will be conducted by the OPCW in 2017, in which Spiez Laboratory will participate.

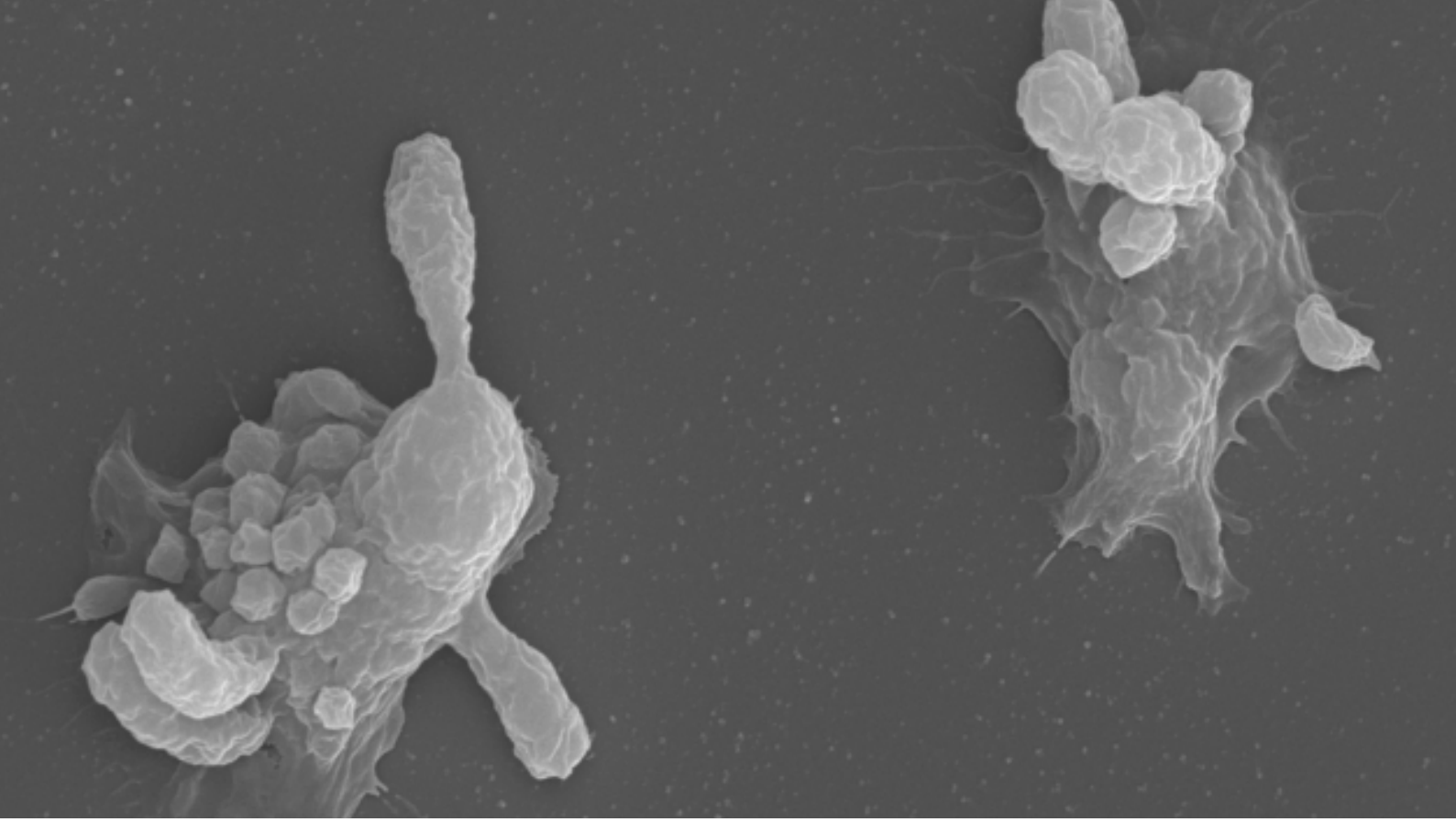
The plenary session of the closing conference of EQuATox in Helsinki in 2014 described the general requirements for further developments, which present themselves based on this interlaboratory comparison, as follows:

- Development and definition of performance criteria for the selected detection methods
- Agreement of standard methods for specified analytical approaches
- Closure of existing gaps in the methods of analysis, with regard to sensitivity, specificity and the analysis of very large numbers of samples
- Development of certified reference materials
- Reagents and methods: systematic exchange of know-how, and supplies of available reagents, tools and strains (European Repository)
- Opportunities for training and instruction
- Regular conduct of round-robin tests, to be increasingly more demanding with regard to the methods chosen
- Expansion of the scientific competence in Europe with regard to toxins
- Sustainable consolidation of the European Toxin Network
- Mutual support in the fields of security and public health

To implement these measures, the EU has issued a call for tender (Horizon 2020 work programme 2016-2017, Call – SECURITY, Topic SEC-03-DRS-2016: Validation of biological toxin measurements after an incident: development of tools and procedures for quality control) [9]. Since the beginning of 2016, Spiez Laboratory is partner of a consortium that, on 25 August 2016, submitted an application under this call. On 16 January 2017, the EU approved this application and the project is expected to start in the middle of this year.

Documents and Publications

[1] Preface Biological Toxins – Ancient Molecules Posing a Current Threat
Toxins 2015, 7(12), 5320-5321; doi: 10.3390/toxins7124888
[2] Proposal FP7 SEC-2011.5.4-1 EQuATox (02.12.2010)
Consortium Agreement (12.09.2011)
Annex I - «Description of Work» (12.01.2012)
Grant agreement for Coordination and Support Action Nr. 285'120 (20.03.2012)
[3] http://cordis.europa.eu/project/rcn/103025_en.html
[4] http://www.mdpi.com/journal/toxins/special_issues/detect-identi-toxins
[5] Characterization of Ricin and R. communis Agglutinin Reference Materials
Toxins 2015, 7(12), 4906-4934; doi:10.3390/toxins7124856
[6] Recommended Immunological Assays to Screen for Ricin-Containing Samples
Toxins 2015, 7(12), 4967-4986; doi:10.3390/toxins7124858
[7] Recommended Mass Spectrometry-Based Strategies to Identify Ricin-Containing Samples , Toxins 2015, 7(12), 4881-4894; doi:10.3390/toxins7124854
[8] An International Proficiency Test to Detect, Identify and Quantify Ricin in Complex Matrices Toxins 2015, 7(12), 4987-5010; doi:10.3390/toxins7124859
[9] https://ec.europa.eu/research/participants/portal/desktop/en/ opportunities/h2020/topics/sec-03-drs-2016.html



REM images of *N. fowleri* trophozoites

Naegleria fowleri – a rare but dangerous amoeba

Dr. Matthias Wittwer,
Nicole Liechti,
Susanne Thomann,
Dr. Nadia Schürch

For several years Spiez Laboratory has been working with *N. fowleri* – a pathogen that causes the usually lethal primary amoebic meningoencephalitis – because of its bioterrorism potential. Since 2009, two PhD candidates at Spiez have been describing the cellular mechanisms that allow the pathogen to enter the tissue of the host organism through the nasal cavity, to spread and produce its destructive effect. The analytical methods of bioinformatics, genomics and proteomics applied in this work can be utilised to address a broader spectrum of research questions in bacteriology, virology and toxinology.

Naegleria are free living amoebae that occur in soil and bodies of water worldwide. Of the 30 species that have been described, only Naegleria fowleri is dangerous for humans, causing what is called primary amoebic meningoencephalitis (PAM). PAM is an infection of the central nervous system observed predominantly in children and young adults after outdoor activities in lakes, rivers and hot springs. The pathogen enters the nasal cavity when the head is submerged into contaminated water. It then crosses the nasal mucosa. The amoeba then enters the brain along the olfactory nerve, where they can reproduce rapidly given the depressed immune system function inside the central nervous system. As the infection takes effect, brain tissue is progressively dissolved starting at the olfactory bulb. This gave the pathogen its English nickname “brain eating amoeba”. The clinical symptoms of an infection include the sudden occurrence of headaches, a stiff neck, high fever and general discomfort. Because most infected patients are not diagnosed in time and effective therapeutic measures are lacking, death occurs within one or

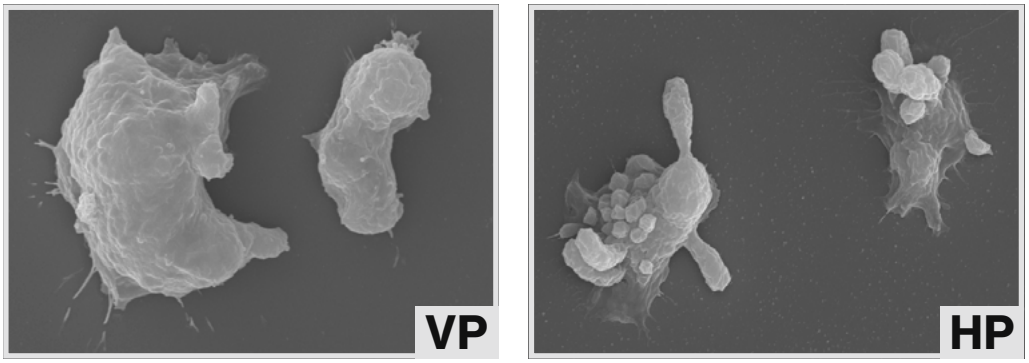


Figure 1: REM images of *N. fowleri* trophozoites

two weeks of the manifestation of the first symptoms. Despite intensive research, the cellular mechanisms of this acute and rapidly progressing disease remained largely unknown up to now. Given its nearly 100 per cent mortality, the rapid disease progression and the lack of therapeutic options for treatment, the Center of Disease Control (CDC, USA) has listed *N. fowleri* as a potential biological weapon.

Since 2009, research on Naegleria has been conducted at Spiez Laboratory in the context of two PhD projects. These projects have focused on the description of the cellular mechanisms (pathogenicity factors) that allow the pathogen to spread in the tissue and to destroy it. There are essentially two strategies that could be applied to identify pathogenicity factors: in the so-called interspecies comparison, the pathogenic organism is compared to a closely related (conspecific), non-pathogenic species. In the case of Naegleria, *N. gruberi* is often used as a closely related apathogenic species.

The second strategy attempts to modulate the pathogenicity of the organism using different cultivation conditions. The establishment of such a model was the objective of the first research project at Spiez Laboratory - this approach is preferred given the uncertain taxonomy of the genus Naegleria. This model is intended to provide the basis for the identification of pathogenicity factors using a comparative proteomic approach in combination with genome sequencing. The sequencing of the hitherto unknown genome of the pathogen is a precondition to describe the pathogenicity mechanism in a whole-cell context.

Modulation of the pathogenicity of N. fowleri

Already since the 1990s, it has been known that the pathogenicity of *N. fowleri* can be increased through propagation in mice. The same effect can be achieved in vitro if the pathogen is cultivated on animal cells. Both methods have the drawback that the Naegleria isolates are heavily contaminated by the genome of the host, or the cell line used, and as a result the genome sequence is distorted. For this reason, an attempt was made to affect the pathogenicity using different amino acid sources (AS) in the growth medium. Experiments at the Institute of Parasitology of the University Bern have shown that the mortality in infected mice can be reduced to 5 per cent when Naegleria are cultivated in a growth medium that uses yeast as AS. On the other hand, the mortality was 100 per cent for Naegleria cultivated in a medium that contained liver hydrolysate as AS.

In order to further characterise the differences between highly pathogenic (HP) and less pathogenic (LP) *N. fowleri*, additional parameters were investigated in vitro. This showed that the generation time of HP *N. fowleri* (2 hours) was half that of LP isolates (approximately 4 hours). Also, the expression of the virulence factors described in the literature could be confirmed by PCR. It was interesting to note that two of the postulated virulence factors were more strongly expressed in the LP isolates than in the HP isolates. These results match the results of cytotoxicity tests, which showed that the LP isolates cause larger damage of the cultivated cell layer than the HP isolates. These results demonstrate that in vitro systems are only suitable to a degree for assessing the pathogenicity of *N. fowleri*. Furthermore, HP and LP isolates can be distinguished morphologically. Raster electron microscopy (REM) images show on average, HP isolates are smaller than LP isolates and that they show a significantly larger number of vesicles on the cell surface (Figure 1).

Genome based analysis of the proteome of N. fowleri

Based on the cultivation system described

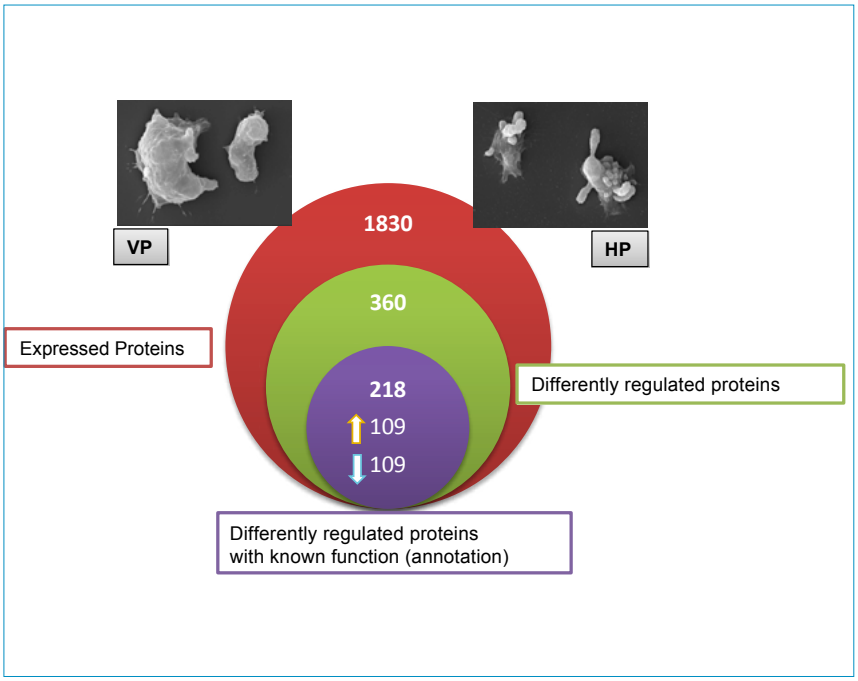


Figure 2: Schematic representation of the protein data. In total, 360 differently regulated proteins were found in the comparison of HP and LP *N. fowleri*. Of these, 218 proteins could be associated with a function.

above and taking the in vitro data into account, in a next step further cellular mechanisms that are associated with pathogenicity will be described at protein level. To identify virulence proteins, one needs to know the genome sequence of the pathogen. The genome describes the amino acid sequence of all proteins that the cell can produce. This data can be compiled in a sequence database. A shotgun proteomics approach is then used to identify the proteins expressed by the pathogen. This method separates the cellular protein extracts in accordance with their size, using gel electrophoresis. Subsequently, the gels are fragmented into some 30 separate size fractions, and the proteins contained in the gel fractions are digested by trypsin. The masses of the proteins so obtained are determined by LC MS/MS (Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer) and matched to those of protein sequences stored in the database, using the MASCOT search algorithm.

The analysis showed that the genome of *N. fowleri* contains 30 million base pairs (20MB) and that it exists in two copies (diploid). Sequencing the transcriptom and the proteome, 17,252 genes were identified. On this basis, the proteomes of LP and HP Naegleria could be compared in a subsequent step. This showed that of the 1830 identified proteins, 360 were expressed differently. 218 of these proteins could be assigned a function, using publically available databases. Compared to the LP isolates, the HP isolates expressed 109 proteins higher (up-regulated) and 109 other proteins lower (down-regulated) (figure 2).

From the assignment of functionality of the regulated proteins it appears that a majority are associated with structures of the cytoskeleton. Also, most of the proteins are located in the plasma membrane. These results correlate with the morphological observations, which show a large number of vesicles on the exterior cell membrane of the HP cells (figure 1).

In order to determine viral factors of *N. fowleri* in an inter-species comparison, the genome of the presumably closest apathogenic relative N. lovaniensis was sequenced using PacBio. The PacBio sequencing technique makes it possible to sequence long DNA fragments and thereby gain a more complete image of the genome.

This sequencing resulted in 1,529,980 DNA fragments with an average length of 6893 base pairs (bp). In a next step, the sequence of the DNA fragments was assembled using so-called genome assemblers. For *N. lovaniensis*, a so-called de novo assembling method was applied because as yet there was no reference genome for it. With this method, the genome of the organism is reconstructed using overlapping sequence segments of the individual sequence fragments. The software “FALCON” was used for the assembly – this software is capable of reconstructing genomes which appear in multiple copies in the organism. Using this de novo assembly, 1,529,980 sequence fragments could be assembled into 112 so-called contigs. The median length of these 112 contigs (N50) was 658,530 bp. Based on these data, the genome of *N. lovaniensis* has a size of 30 million bases (Mb). In addition, the entire circular sequence of the mitochondrial genome of 48553 bp could also be reconstructed. Using so-called ab inito methods, which create gen models using sequencing data of the transcriptome, 15,195 proteins were identified in the genome of *N. lovaniensis*.

The overview of the phylogenetic relationships is important for the subsequent comparative analysis of the pathogenic and apathogenic Naegleria: in a first step, the proteins from *N. fowleri*, *N. lovaniensis* and *N. gruberi* were grouped into protein families using OrthoMCL. The result shows that there are more than 8,000 protein families in all Naegleria species; these therefore form the “core genome” of the Naegleria. To determine the phylogenetic relationships in detail, a phylogenetic tree was constructed from the core genome of the Nae-

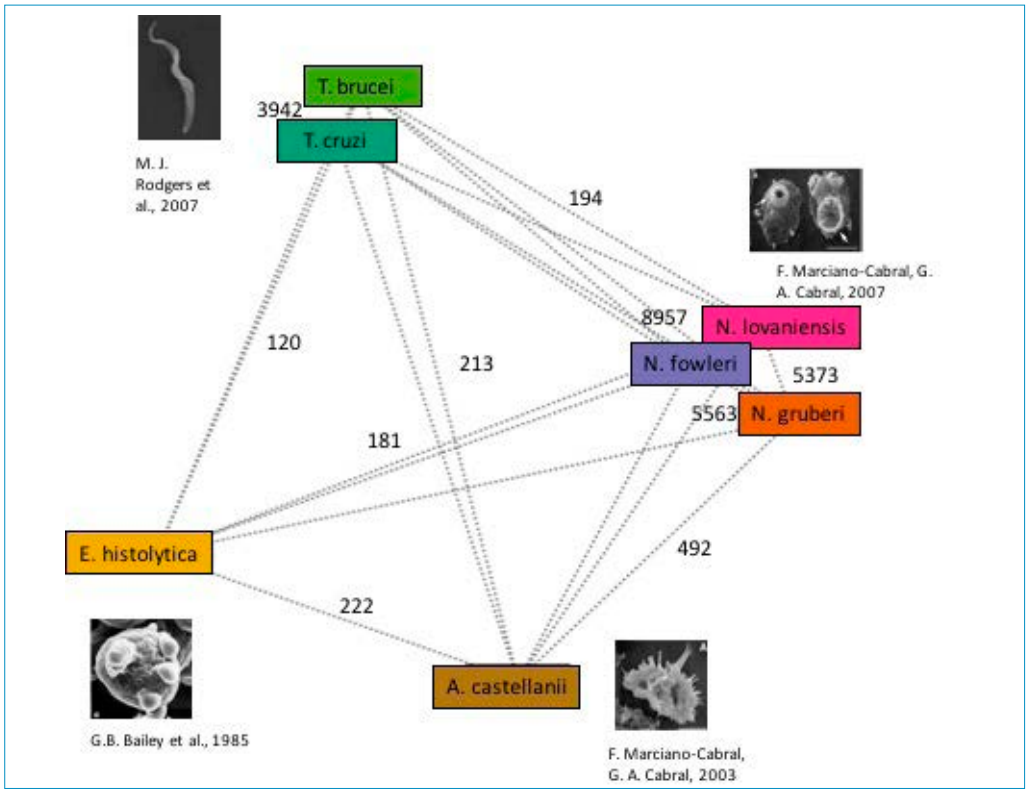


Figure 3: Representation of the degree of kinship of *N. fowleri* with other protozoa. The spacial distance in the representation is inversely proportional to the number of genes that show a high degree of similarity and are present in the respective organisms.

gleria using maximum likelihood methods and bootstrapping. The visualisation of the tree confirms the hypothesis that *N. fowleri* and N. lovaniensis are close relatives. This means that *N. lovaniensis* is the closest non-pathogenic relative of *N. fowleri*, and hence is suitable for interspecies comparison studies to reach conclusions about pathogenicity mechanisms.

A detailed analysis of the proteins that are specific for *N. fowleri* in OrthoMCL clustering shows a connection with the cell membrane. This result coincides with previous findings of the project (Zysset-Burrie et al. [2]). The data show a higher degree of expression of proteins that are associated with the cell membrane. Among the proteins that can be associated with the cell membrane are, amongst others, Rab and Rho GTPases. These GTPases are known to be important regulators in vesicular transport and they play a role in changes of the cytoskeleton. With regard to pathogenicity, the GTPases of *N. fowleri* could be involved in the secretion of proteases for the degradation of the extracellular matrix, or they could play a role in changes of the cytoskeleton related to the adhesion to the host cell and the penetration of the cell matrix.

In order to characterise in detail the differences between *N. fowleri* and *N. lovaniensis* with regard to pathogenicity, the next step will be the improvement of the reference genome for *N. fowleri*. To this end, a protocol for the culture of *N. fowleri* trophocoites is being established at the biosafety laboratory (BSL-3) of Spiez Laboratory. To complement the draft genome, the DNA will be isolated and sequenced using the long read PacBio technique.

Based on the completed genome of *N. fowleri*, pathogenicity factors found by genomic analysis can be studied and further characterised, using proteomic methods. The focus will be on the isolation and identification of membrane proteins of *N. fowleri* and *N. lovaniensis* as well as their suitability as candidates for future therapeutic approaches.

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Ixodes ricinus is the most widespread tick species throughout Europe

Prevalence of tick-borne pathogens in urban areas of Switzerland

Dr. Rahel Ackermann

Throughout Europe, the tick species *Ixodes ricinus* vectors numerous pathogens of bacterial, viral, or protozoic origin. Over the past years, its distribution and therewith the risk of contracting tick-transmitted diseases has significantly expanded in urban and rural areas. So far only limited data have been available on infection rates of ticks in urban areas of Switzerland. In this study, *I. ricinus* ticks collected at 18 urban sites throughout Switzerland were analysed for the presence of various pathogens. 358 out of 1'078 ticks were infected with at least one pathogen, whereof about 20% were carrying two or three different potentially disease-causing agents. Thus, there is a substantial risk of acquiring tick-borne infections, including the possibility of multiple infections as a consequence of a tick bite in urban regions.

Ixodes ricinus is the most widespread tick species throughout Europe. Its life cycle proceeds through three developmental stages, larvae hatching from eggs, nymphs, and adult males or females (Figure 1). *I. ricinus* serves as a vector ("transmitter") for numerous human and animal pathogens of bacterial, viral, or protozoic origin. These include well-known pathogens such as those causing lyme disease or tick-borne encephalitis, but also several rather unknown agents.

Lyme Borreliosis is the most prevalent tick-borne human disease in the northern hemisphere. It is a multisystemic disease, typically progressing through several stages and affecting joints, the central nervous system, the skin or the heart. Lyme Borreliosis is caused by bacteria belonging to the *B. burgdorferi* s. l. complex, which comprises of 18 species. Small mammals and ground-foraging birds serve as reservoir hosts, maintaining the circulation of the bacteria in the ecosystem. In Switzerland, mean tick infection rates range between 9 and 47%.

Infections with TBEV may cause disease of variable severity in humans, ranging from sub-

Figure 1.:
Developmental stages of *I. ricinus*.
From left to right:
larva, ~0.5 mm,
nymph, ~1.5 mm,
adult male, 2.5–3.5 mm,
adult female, 3.5–4.5 mm.

clinical infections to severe disease with neurological involvement and a potentially fatal outcome. TBEV is maintained within so-called natural foci limited to strict regions, with tick infection rates ranging between <0.1 and 5 %. Rodents, insectivores, and carnivores serve as reservoir hosts of the virus.

Besides these two well-known agents, ticks may transmit numerous other pathogens. For instance, the bacteria *Rickettsia helvetica* and *R. monacensis* may cause a flu-like disease, rash or an eschar. Ticks serve as the vector and main reservoir of *Rickettsia* spp., with a prevalence of 0.5 to 66%. *Babesia* spp. (e.g. B venatorum) are intraerythrocytic protozoan parasites best known to cause animal illness. Human babesiosis is rather rare and usually limited to immunocompromised patients. Between 0.9 and 20% of ticks carry this parasite. *Candidatus* N. mikurensis is found in 0.95 to 23.5% of *I. ricinus* ticks, with rodents serving as reservoir hosts. Human disease is rare and is also usually limited to immunocompromised patients. *A. phagocytophilum* may cause mild self-limiting febrile illness to fatal disease in humans. The epidemiological cycles of A. phagocytophilum involve mammalian hosts and ticks, with infection rates of *I. ricinus* ranging between <1 and 20%. The spirochete *B. miyamotoi* may cause a febrile illness possibly presenting as relapsing fever. Between 0 and 3.2% of ticks carry this bacterium.

Over the past years, the distribution of *I. ricinus* and therewith the risk of contracting tick-transmitted diseases has significantly expanded in urban and rural areas. So far only limited data have been available on infection rates of ticks in urban areas of Switzerland. In this study, we analysed 1'078 questing *I. ricinus* ticks collected at 18 urban sites for the presence of all the above-mentioned pathogens.

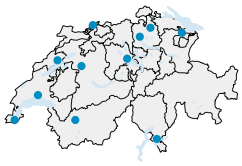
Methods

45 collection sites in urban areas of Switzerland were defined in collaboration with the respective authorities. Ticks were collected by flagging low vegetation and identified based on morphological characteristics. Then, they were homogenised in a buffer solution and nucleic acids were extracted using an automated high-throughput system. The samples were then screened for the presence of *B. burgdorferi* s. l., *B. miyamotoi, Rickettsia* spp., A. phagocytophilum, Babesia spp., and Candidatus N. mikurensis using 9 different real-time (reverse transcription) polymerase chain reaction (real-time [RT-] PCR) assays. Samples positive for Borrelia spp., Babesia spp. and Rickettsia spp. were further examined by Sanger (capillary electrophoresis) sequencing to identify the respective species.

Results and Discussion

A total of 1'079 ticks (66 larvae, 740 nymphs, 138 adult males, 135 adult females) were collected from 18 areas; at 27 sites, no ticks were found (Figure 2). Except for one female *Ixodes hexagonus*, all ticks were identified as *I. ricinus*. Since the number of collected ticks pronouncedly varied between the different areas, calculation of prevalence rates for the different collection sites was not possible. Therefore, overall infection rates were determined.

Four different species belonging to the *B. burgdorferi* s. l. complex were detected, with an



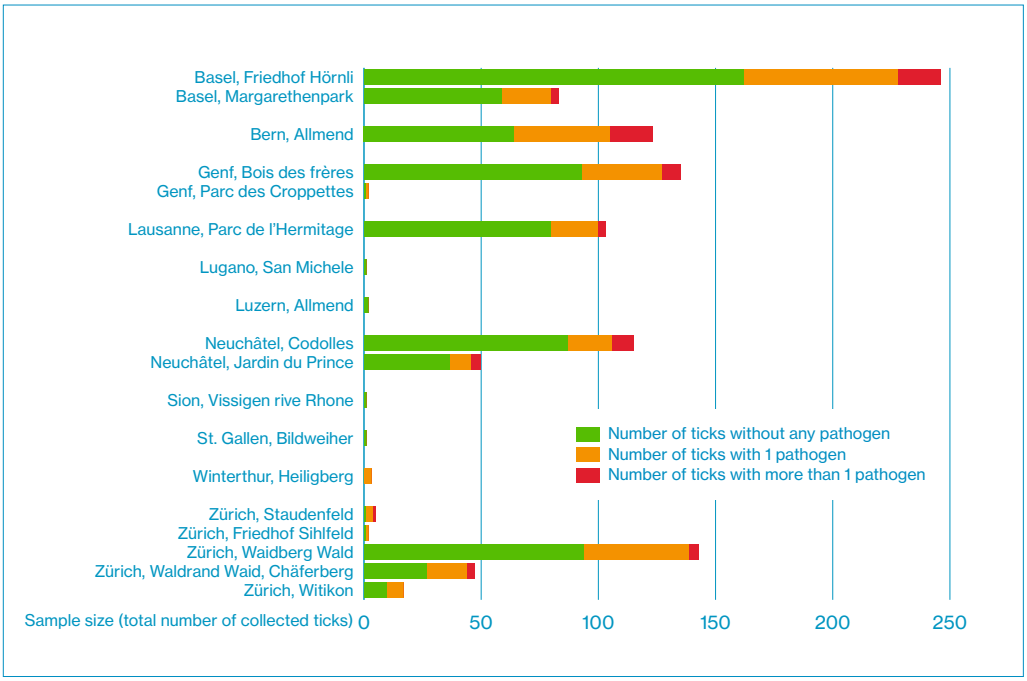


Figure 2. **Urban areas analysed for the presence of pathogens in questing *I. ricinus* ticks.**
The size of the bars indicates the number of ticks collected at the different collection sites. The proportion of ticks with no pathogens is shown in green, the proportion of ticks with one pathogen in orange, and the proportion of ticks with two or three pathogens in red.

overall prevalence of 14.29%: 7.6% *B. afzelii*, 1.11% *B. burgdorferi* sensu stricto, 2.60% *B. garinii*, and 0.84% *B. valaisiana*. 2.13% of the analysed ticks were carrying multiple *Borrelia* spp. All identified species are confirmed agents of Lyme borreliosis and have already been detected in *I. ricinus* ticks in other studies in Switzerland.

Although known to be endemic in natural foci in rural areas of Switzerland, we could not detect any TBEV-positive *I. ricinus* ticks in our present study, supporting the hypothesis that the risk for contracting TBE in urban areas is rather low.

Concordant with previous studies we found *R. helvetica* positive ticks at a prevalence of 13.17% in urban areas of Switzerland. Unlike the repeated detection of *R. helvetica* in ticks, however, human infection with this agent remain rare. In addition to *R. helvetica*, three samples were found to be positive for *R. monacensis*, which was detected for the first time in Switzerland in 2009. 0.83% of urban *I. ricinus* ticks were found to be positive for *B. venatorum*, which agrees with infection rates with this parasite in different European countries. Human babesiosis is a rather rare but a possibly emerging disease in Europe, with about 50 cases reported so far. Although Neoehrlichiosis is a rare human disease, we could confirm the presence of *Candidatus* N. mikurensis in urban ticks, with an overall prevalence of 5.84%. For *A. phagocytophilum*, the causative agent of human granulocytic anaplasmosis (HGA), we found an infection rate of 1.3%. In Switzerland, HGA is so far a poorly known disease. *B. miyamotoi* was found to be present in 2.50% of *I. ricinus* ticks and has also, in previous studies, been detected in forests in our country. Thus, although no disease cases have been reported so far, there is a potential of acquiring such an infection, in urban as well as in rural regions in Switzerland.

In our study, 358 ticks (33.21%) were carrying at least one potentially disease-causing agent: 287 ticks (26.62%) were infected with one, 64 (5.94%) with two, and 7 (0.65%) with three pathogens (Figure 3). Thus, co-infection of ticks and therewith co-transmission of pathogens to humans might have important consequences with respect to disease severity and treatment.

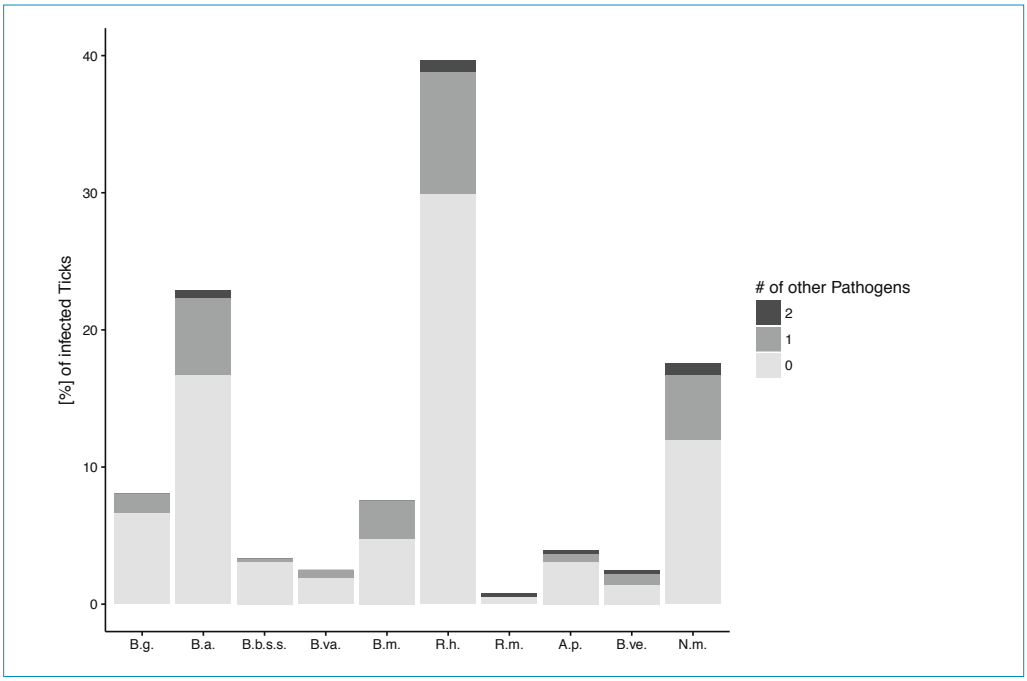


Figure 3. **Number of ticks positive for different tick-borne pathogens.** The overall height of the bars represents the percentage of infected ticks tested positive for the respective pathogen. The proportions at which the pathogens were detected alone or in combination with 1 or 2 others are shown in light gray, dark gray, and black, respectively.

Conclusions

In this study we documented the presence of *B. burdgdorferi* s. l., *B. miyamotoi*, *R. helvetica*, *R. monacensis*, *A. phagocytophilum*, *B. venatorum*, and *Candidatus* N. mikurensis in the urban *I. ricinus* tick population in Switzerland. The pathogen prevalence rates were as high as those in rural regions; thus, there is a serious threat of contracting tick-transmitted diseases in urban areas in our country. Co-infection of ticks with several pathogens was observed in about 20% of infected ticks. Thus, there is a true risk of acquiring multiple infections as a consequence of a tick bite.

This study has been submitted for publication as a research article in Parasites & Vectors.

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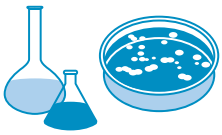
The National Reference Centre for Tick-transmitted Diseases NRZK

Since 2014, Spiez Laboratory in collaboration with two partner laboratories runs the National Reference Centre for Tick-transmitted Diseases (NRZK) on behalf of the Federal Office of Public Health. The NRZK is responsible for the reference (i.e., confirmatory) diagnosis of Lyme borreliosis (ADMED Microbiologie, La Chaux-de-Fonds), tick-borne encephalitis (Spiez Laboratory), and Q fever (CHUV, Lausanne). It develops and validates respective diagnostic methods, provides reference materials, and advises authorities and experts on scientific problems. In the framework of quality assurance, the NRZK annually organises national quality assurance tests and participates in international quality assessments. The NRZK attends national and international scientific events and contributes to further education amongst laypeople as well as experts in the field. Additional information is available online at www.labor-spiez.ch.

Tacit knowledge is essential to make something that is possible into something that actually works. Therefore, tacit knowledge is indispensable for successfully integrating new scientific discoveries into the design of new products and also new weapons.



Spiez CONVERGENCE 2016

Stefan Mogl,
Dr. Cédric Invernizzi

Scientific and technological advances emerging from convergence of biology and chemistry bring enormous benefits but also unknown safety and security threats for society. In 2014, Switzerland[1] started a new workshop series because potential implications for existing arms control regimes need to be discussed between all involved communities. The second edition of Spiez CONVERGENCE held from 5–8 September 2016 provided a unique platform for effective communication between experts from academia, industry and policy making as well as experts involved with the implementation of related arms control requirements.

Ambassador Üzümcü, the OPCW Director-General, opened Spiez CONVERGENCE 2016 by reflecting on chemical weapons use in the Syrian conflict and the third report of the Joint Investigative Mechanism, which had just been released. The very sophisticated technological advances discussed under the concept of convergence stand in stark contrast to the use of crude chemical weapons technology described in these investigations. This chemical weapons use serves as a troubling reminder of how important it is to uphold the provisions of the CWC and BWC, and to ensure they remain effective in view of today's technological advancements.

The program for Spiez CONVERGENCE 2016 was compiled through literature screening looking at developments in basic research, industry applications and issues raised in the arms control community. In sum, the workshop discussed 'hot-science' topics of *possible* relevance to both the CWC and the BWC.

1 Spiez Laboratory, supported by the Center for Security Studies at the Swiss Federal Institute of Technology Zurich (ETHZ), the Division of Security Policy in the Federal Department of Foreign Affairs, and International Relations Defence in the Federal Department of Defence Civil Protection and Sports

Ambassador Ahmet Üzümcü, OPCW Director-General, opens Spiez CONVERGENCE 2016

Chemical synthesis, chemical modification and large molecules were discussed to review developments in the biologically mediated manufacturing of chemicals. The market share of such processes has been negatively affected by a drop in crude oil prices. Nevertheless technologies for converting biomass – sugars, starch, lignocelluloses – into chemical products continue to be developed. While the biochemical conversion of sugars is a well-established industrial process, conversion of starch and celluloses is more challenging. Because sugars and starch are food sources and therefore less desirable as raw materials, lignocelluloses, which are very abundant, offer a valuable alternative. Lignocelluloses are not easy to convert – opening the cell structure to allow access to these polysaccharides requires sophisticated methodologies – but new strategies exist in the form of consolidated bioprocessing. Some progress has been made towards industrial application but for now, sugar and starch remain the dominant feedstock for industrial production of chemical products from biomass. The opposite approach to the breaking down of natural biomaterials is the synthesis of complex carbohydrates from small building blocks. Automated carbohydrate synthesis is becoming commercially available, permitting the synthesis of biomolecules with ever greater complexity; what in the past took months, can be achieved today in hours. The technology offers many possibilities and may make new biomaterials available. This includes new vaccines based on carbohydrate conjugates, for which clinical trials are expected to start soon. A trend in the pharmaceutical industry is to move towards using highly active pharmaceutical ingredients (HAPI) – a third of novel drugs developed fall into this category. HAPI production plants are technologically highly complex and containment standards are similar to high-safety biological facilities. In many aspects they resemble a larger-scale CWC Schedule 1 facility but because of their production profile they remain below the CWC declaration threshold for discrete organic chemicals (DOC)-producing facilities. A further approach for synthesising chemicals is engineering the genome with the aim to convert cells into chemical factories. Recent advances in gene editing enable a shift from reading to writing and editing genomes, and reprogramming cells. The technology is costly and faces many challenges for industrial applications including a time to market of about a decade. Nevertheless, advances in gene editing are revolutionising the field at laboratory scale. A technology on the horizon is the development of a new genetic code using non-standard amino acids, which will expand the chemicals available for protein synthesis. This technology could lead to novel compounds with profound differences in their characteristics compared to the proteins we know from nature.

Additive manufacturing or 3D printing of specialised equipment has in recent years and during Spiez CONVERGENCE 2014 been discussed as a development with potential security risks. The technology is maturing and using powder bed (metal, metal alloys, ceramics) melted with electron or laser beam appear to be the most promising approaches for future applications. With the technology maturing, its limitations also become more apparent. As the powder bed process employs continuous welding, it carries an inherent risk of welding defects that could cause material fatigue. Such defects are difficult to detect and cannot be predicted. The melting speed for the layers of powder bed furthermore sets a limit to productivity. This process is an excellent tool for fast prototyping and producing repairs but less

suitable for large scale industrial manufacturing of critical high performance pieces. Promising developments were discussed with regard to 3D printing with biological materials. The technology is based on a layer by layer printing of a bio-ink in a sterile environment using laser printing or inkjet. Future applications include the fabrication of living tissue or the development of tissue models. The goal of reproducing biological function in the concept of 'organ printing' remains a big challenge. 3D printing of biological materials is today a research tool utilised to model tissue functions. It is currently a single use process and reproducibility is a weakness. A key development for future success is that standardised bio-inks of good quality are becoming commercially available.

Genome editing technologies were 2016 included on a US threat list and therefore received much attention from an arms control perspective. Site-directed genome engineering aims at specific modifications of cellular properties – a further step in technological advancement. This became possible after the lowering of cost in sequencing technology has led to large databases and a better understanding of biological systems. CRISPR/Cas9 is a new gene editing technology that is derived from a bacterial defense mechanism against viruses. It permits introducing changes to DNA within cells and it provides the ability to edit genetic code accurately and precisely. Gene editing has been possible for many years using other techniques (i.e. Zinc finger proteins, TALE nucleases) but CRISPR/Cas9 is simpler and more easily accessible. There are two different types of known CRISPR systems and research is shifting today from understanding this technology to applications. The technology is utilised for the development of a broad range of new bio-based products, including therapeutics, antimicrobials, animal health products and crop genetics. Recent developments include in-vivo and ex-vivo drug treatments, but there remain significant challenges in this area. For the delivery of drugs to patients, lipid nanoparticles are promising tools. One particular application of CRISPR/Cas9 based genome editing are gene drives. Gene drives create an inheritance bias for themselves and can force a genetic modification through an entire population, provided, the species has a short generation time and a high population turnover. The technology is expected to work well with insects and is discussed as a form of vector eradication to fight malaria. Current research focuses on spreading genetic sterility. Another approach could be interfering with parasite development in the mosquito. The approach to eradicate an entire population however, poses a whole range of questions in relation to safety and security as well as ethics. With regards to weapons relevance, the implications of gene editing technologies are probably modest. But should a biological weapons program be started today, these technologies would likely become part of it. CRISPR represents a transformational technology that will yield many beneficial applications. And, it demonstrates that risk assessment must not only be based on scientific potential – contextual analysis and effective communications are required to avoid disproportionate reactions.

Omics technologies have moved from genomics to transcriptomics to proteomics and metabolomics. Biologists' focus turned from reading the program of biological systems (DNA) to understanding the systems' functioning. Large sequence databases were accumulated but efforts to better understand biological systems meet unanticipated complexity. Research shows that the proteome organisation is not explainable by the genome organisation alone and that there are further regulatory processes. Another problem is data reliability. For the development of practical applications, industry depends on reliable databases and a significant portion of academic data is not reproducible. It has therefore been suggested that more effort be put into curating databases instead of engaging in further sequencing.

The memory and programming capability of biological systems is researched to look for alternative solutions because of shortcomings in existing technology. For example, conventional data storage media are limited in capacity and deteriorate over a relatively short timeframe. Because of the drop in cost in DNA sequencing and synthesis technology, storing data in DNA could be an interesting option. Long term storage of large amounts of data in DNA encapsulated in glass is today technically feasible, but due to the cost of about USD 1,000 per 1 MB still too expensive. Another interesting application of this technology is to use it for 'barcoding' of materials such as chemicals, intermediates or food products. While recording in living cells is not suitable for long term storage, genomically encoded cells could be used for computing functions. In a layered approach, DNA codes can be programmed and with programmed DNA, devices can be built. Circuits are created from such devices followed by modules based on the circuits etc. This research is increasingly linked to synthetic biology and can be combined with gene editing tools such as CRISPR.

DNA origami is today a technology at the stage of fundamental research with little practical application so far. But nevertheless it offers interesting concepts. DNA Origami uses DNA for building nanostructures. Single, double or bun-

dled DNA strands are used to modulate mechanical stiffness of a structure. By combining such structures with nanoparticles (e.g. gold), researchers succeeded in creating three dimensional behavior of nanoparticles imbedded in the structure, which could be triggered by an external factor (e.g. light). Such dynamic systems could be developed into sensors. The goal of this research is to create molecular structures which interact in a type of 'nanofactory' or to develop molecular robotics.

Tacit knowledge is essential to make something that is possible into something that actually works. Therefore, tacit knowledge is indispensable for successfully integrating new scientific discoveries into the design of new products and also new weapons. This remains true despite the trend of 'deskilling' in the life sciences due to new technologies such as CRISPR. Tacit knowledge is a hindrance for non-state actors but experience from the past shows, it can also be a critical success factor in weapons development programs. However, the lack of tacit knowledge does not prevent the use of improvised or crude weapons systems like chlorine barrel bombs. Such examples show that context is important and that scenario and context must be part of a risk assessment.

The Spiez CONVERGENCE 2016 report ends with a summary of the policy discussion on the last day of the workshop. Convergence may affect the CWC and the BWC at the level of scope as well as with regard to implementation. In terms of scope, assessing how developments like nanomachinery fall under the General Purpose Criterion of the treaties requires a good understanding of the new technology and a continuous review of future developments. When assessing implications of advances in S+T, a general approach is more important than singling out an individual development; doing so carries the risk of missing others. Many of the science trends reviewed under the concept of convergence affect mainly treaty implementation and not treaty scope. A good example are how changes in production technologies affect CWC declarations. Biologically mediated processes have already been addressed by the OPCW SAB, and they affect declaration, as well as implementation – material balance control for verification purposes is not likely to be possible in biotechnology facilities. Another challenge are chemical production plants that are comparable in relevance to Schedule 1 facilities. The problem is not new, but in the past was of little relevance because only few such industry facilities existed. The CWC contains provisions to address this issue but this requires political will. There are several examples of overstating the impact of new scientific discoveries – sometimes by the scientist themselves. Policy responses must be proportional and must consider what is likely to result in applications. 3D printing of process equipment is a good example. The risk of this technology was overestimated in the past. The same may apply to gene editing. New gene editing technologies have added functionality but their limitations as well as practical implications are not yet known. It will be important to follow the development of gene drives. The concept for it was in place but convenient gene editing tools were missing. CRISPR opened the door here for large progress.

The S + T developments described in the workshop report offer promising benefits for society, but their exploitation for harmful purposes by various actors in the context of their dual use potential cannot be disregarded. A point mentioned many times during Spiez CONVERGENCE 2016 was the importance of effective communication – scientists and their work are part of the solution and not part of the problem. A conversation about security threats should be multi-disciplinary, while at the same time recognising that the same thing may be understood differently depending on the community or the context. The results of Spiez CONVERGENCE 2016 are a contribution for developing common understandings in policy discussions in various fora.

This text was adapted from the Executive Summary of the Spiez CONVERGENCE 2016 workshop report. The full report is available under http://bit.ly/2ieaKZh

Workshop report 2016

Liquid Samples for the 39th OPCW Proficiency Test

Sample Preparation for an OPCW Proficiency Test for Chemical Warfare Agent Analysis

Dr. Peter Siegenthaler

Due to its scientific competence and excellent analytical instrumentation Spiez Laboratory has regularly passed OPCW proficiency testing for chemical warfare agent analysis with maximum scores. This testifies that Spiez Laboratory is among the leading institutes in the field of chemical warfare agent analysis. In the spring of 2016, Spiez Laboratory prepared the samples for the 39th OPCW Proficiency Test.

The Organisation for the Prohibition of Chemical Weapons (OPCW) is responsible for the compliance with the Chemical Weapons Convention (CWC). For off-site analysis of suspect samples, the OPCW relies on a worldwide network of Designated Laboratories. Since 1996 the OPCW organises annually two analytical inter-laboratory comparison tests – so-called Proficiency Tests (PT) for the designation of new, as well as the assessment of existing Designated Laboratories. PT participants are required to investigate samples to identify the presence of chemical warfare agents and related chemicals, and to submit a report of the results of their analyses within 15 calendar days.

An internationally accepted accreditation for the analysis of CWC-relevant chemicals under ISO/IEC 17025 as well as regular and success-

ful participation in PT are preconditions for obtaining and maintaining the status of a Designated Laboratory of the OPCW. As of 1996, Spiez Laboratory has participated at least once each year in an OPCW PT, and it completed all 21 tests with success. In 1998, for the first time, the OPCW Director-General pronounced eight laboratories as Designated Laboratories, which included Spiez Laboratory. Of the 26 laboratories that have been designated since then, seven have lost their designation because of false-positive identifications in Proficiency Tests, or because they did not regularly participate in these tests. Today, the OPCW network includes 19 Designated Laboratories; the designation of three laboratories is temporarily suspended because of unsuccessful participation in PT (2016).

For conducting these PT, the OPCW relies on the support of Designated Laboratories with regard to the preparation of the samples as well as the evaluation of the analytical results. Following a request by the OPCW, Spiez Laboratory agreed for the fourth time to prepare the samples for a PT, following previous such undertakings in 1996, 1998 and 2009. A corresponding agreement between the OPCW and Spiez Laboratory was signed in March 2016.

The tasks of the laboratory that prepares the samples for a PT and the stringent quality requirements that the samples must meet are set out in detailed OPCW guidelines. That is to ensure that all PT participants will receive samples with identical composition so that their starting conditions for the analyses are the same. Most importantly, it has to be demonstrated that the samples contain identical amounts of the compounds to be identified, and that they remain stable throughout the duration of the test. To ensure that the criteria with regard to sample homogeneity and the purity and stability of the chemicals are met, considerable efforts are required on the part of the laboratory that prepares the samples. Hence, Spiez Laboratory began in the fall of 2015 to develop different test scenarios, selected potential chemicals, and undertook initial experiments to investigate the behaviour of samples with varying compositions. Based on the results of these studies, the definite composition of the samples was decided in consultation with the OPCW in January 2016.

Dispatching the samples to the participants, the OPCW Laboratory and the Evaluating Laboratory

Following extensive stability and homogeneity tests of multiple sample sets with this definite composition during the period from January to March 2016, the samples for the 39th OPCW Proficiency Test (PT-39) were prepared at Spiez Laboratory at the beginning of April, in the presence of two OPCW representatives. The samples were labelled ‘incineration waste’ and ‘decontamination waste’ according to a fictitious scenario. The scenario was based on an OPCW inspection team conducting a challenge inspection at a suspected chemical warfare agent research facility from which it had collected liquid samples from two respective canisters.

In an inspection with off-site analysis, the OPCW dispatches sample sets that include the sample collected by an inspection team (sample), a positive control sample (control) and a negative control sample (blank) both controls prepared by the OPCW laboratory in order to validate the analysis. This is why for PT-39, two sample sets each containing three liquid samples were prepared for each participant. The sample matrices were mixtures of water and different polar organic solvents. The sample sets were placed in identical containers and labelled in an anonymised form to ensure that the participants could not attribute the samples as ‘sample’, ‘control’ or ‘blank’. Unlike in previous PT, the matrix composition of the two ‘samples’ was different from that of the ‘controls’ and ‘blanks’ which complicated the task of the analytical laboratories. Because the sample matrices were neither pure aqueous nor organic liquids, the participants had to adapt their standard methods for sample preparation. The ‘samples’ and ‘controls’ had been spiked with nerve agent related chemicals at varying concentrations, the number of which was not known to the participants. By definition, the ‘blanks’, on the other hand, did not

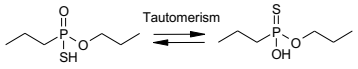
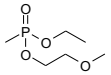
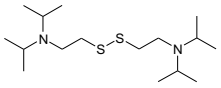
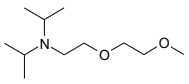
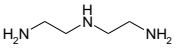
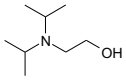
Sample	Spiked Chemicals	Matrices
Sample 1	 Isopropyl methylphosphonate	Water / 2-Propanol Kerosine
	 Methylphosphonic acid	
	 Diisopropyl methylphosphonate	
Control 1	 O-Propyl propylthiophosphonate	Water / Methanol
Sample 2	 Ethyl 2-methoxyethyl methylphosphonate	Water / Acetonitril
	 Bis(2-N,N-diisopropylaminoethyl)disulfide	 2-Methoxyethanol
	 N,N-diisopropylaminoethyl-2-methoxyethyl ether	 Diethylenetriamine
Control 2	 2-(N,N-Diisopropylamino)ethanol	Water / Methanol

Table 1: List of spiked chemicals

contain any relevant chemicals. An additional difficulty for the identification was that the analytical data of two of the eight spiking chemicals were not available from any reference database. To complicate the analysis even further, the two 'samples' were also mixed with kerosene and components of a decontamination agent respectively (see table 1).

The samples were packaged in accordance with the applicable transportation regulations and, on 8 April 2016, dispatched to the participants, the OPCW Laboratory, and the Evaluating Laboratory which is tasked to test and evaluate the sample analysis as well as the reports from the participants. During the weeks following sample dispatch, Spiez Laboratory qualitatively analysed the composition of sample sets that had been randomly selected by OPCW representatives. It monitored the homogeneity of the samples and the stability of the spiked chemicals by quantitative analysis for an extended period of time.

The work conducted in the context of sample preparation and the results achieved by the participants were presented at a PT-39 evaluation meeting in July 2016 in The Hague, in the Netherlands: the majority of the laboratories had successfully identified and correctly reported all eight spiking chemicals. Seven of the nine regular participants had successfully passed PT-39, six with an A score and one with a B score. Two laboratories received a fail score (F) because of false positive identifications.

The feedback received from the OPCW and the Evaluating Laboratory, and also from the participants was positive throughout. The scenario involving different matrix compositions of the samples, as well as the selection of the spiking chemicals was generally considered to have been realistic, challenging and fair. Another positive feedback was that the analytical task had been precisely formulated– an issue which had led to discussions in the past. In PT-39, it had been clearly delineated which chemicals of CWC-relevance that are not specifically listed in the Schedules of the CWC were to be identified and reported. The representative of the Evaluating Laboratory characterised PT-39 as a possible model for future tests with regard to scenario and analytical tasking.

The OPCW thanked Spiez Laboratory for the development of the scenario, its analytical work and the sample preparation including dispatch, which according to the OPCW had been accomplished with a 'Swiss Quality Standard' and thus had contributed significantly to the smooth conduct of PT-39.

The second UNSGM Workshop 2016 in Spiez

2nd UNSGM Designated Laboratories Workshop 2016

Dr. Cédric Invernizzi, Stefan Mogl

The United Nations Secretary-General's Mechanism (SGM) is an important instrument of the Secretary-General to investigate allegations of use of chemical, biological or toxin (CBT) weapons for the international community. Switzerland[1] started in 2015 an initiative aimed at strengthening the roster of designated laboratories that would support allegations of use of biological weapons. The first workshop in this series was conducted in Spiez in November 2015, the second workshop was held from 22–24 June 2016.

The workshop discussed unambiguous identification of a causative agent, the role of a mandate for analysis and the reporting of laboratory results.

The identification of a causative agent is a critical element for an investigation. The respective laboratory data will support the mission's findings and contribute to a final assessment. Notwithstanding that laboratory results are only part of the overall evidence, specific requirements must be met to support an identification. These include multiple orthogonal analytical techniques meeting established acceptance criteria, laboratory accreditation to internationally accepted standards and method specific quality assurance measures. Paramount is an unbroken chain of custody for samples from the start of forensic evidence gathering as well as the subsequent evidence handling. Related training is a must for members of the investiga-

1 Spiez Laboratory supported by the Division of Security Policy in the Federal Department of Foreign Affairs and International Relations Defence in the Federal Department of Defence Civil Protection and Sports

tion team and for off-site laboratories because forensic standards cannot be retrofitted. The accreditation of all the relevant analytical methods at each of the designated laboratories may not be feasible due to limited resources. Therefore a patchwork of laboratories, of which specific capabilities are well known and documented, should be created for the SGM. Such a portfolio will also help a mission to adapt to changing circumstances as any mission must expect the unexpected.

An unambiguous identification through cultivation may in many scenarios no longer be possible. Therefore, it may be appropriate to characterise an identified agent in a given mission context and narrative. The laboratory results are assessed based on probability and relevance of a particular method in such context. This requires developing a scoring system for laboratory methods. The aim would be that the cumulative score for all methods used in characterising an agent would lead to a scientifically satisfactory level of confidence for an identification. Establishing such a scoring system is an iterative process that must be tested with practical examples.

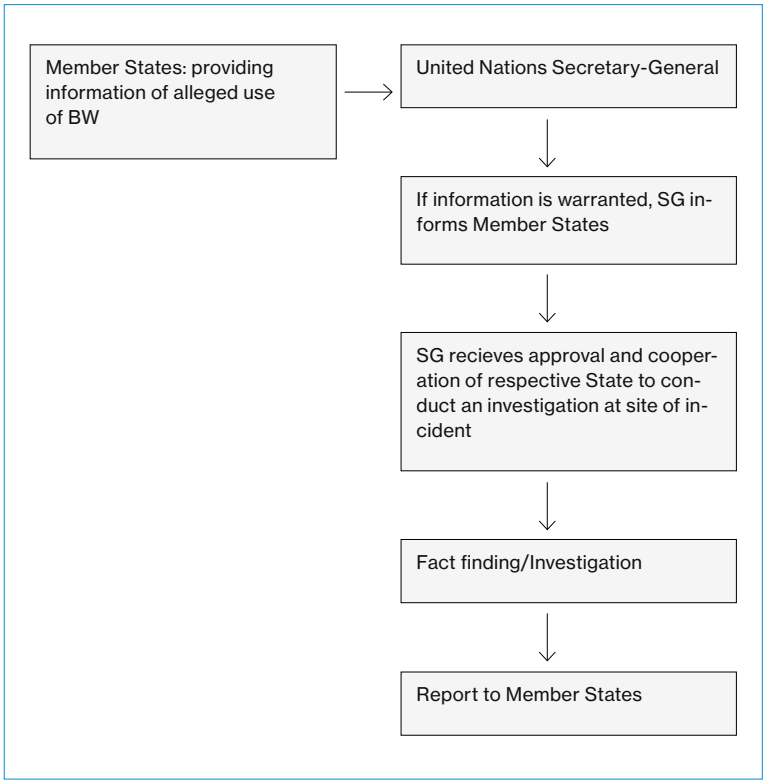
The mandate for analysis is to be understood as a set of instructions and guidelines to off-site laboratories, bearing in mind the need for flexibility depending on the mission context. In particular at the beginning of a mission, the required laboratory experience may not yet be known. The first objective for off-site laboratories is to identify a causative agent. This is followed by a characterisation of the agent which can help assess whether an incident was caused by natural disease outbreak, accidental release or a deliberate biological weapons attack. Should laboratory results alone not permit a final conclusion, they will be essential evidence to support such a conclusion.

If attribution is part of the objective of a mission, a further task for off-site laboratories may be to possibly extract relevant information from the sample. Attribution however may require different types of analysis to link a causative agent to a source or a delivery system to a particular actor. During the conduct of a SGM Mission it is important to manage expectations of stakeholders regarding the information an investigation and its laboratory results can yield, and how much time may be required to establish such information.

OPCW Designated Laboratories perform their off-site analysis independently and without interaction with the inspection team. Contrary to this, laboratories in a biological investigation may have to play a more interactive role with the team, which should be practiced in exercises. Embedding an expert laboratory capability in every investigation team is thus critical to ensure the team's independence in its decision making. The OPCW also has a central laboratory hub managing sample dispatch to off-site laboratories. For an investigation of alleged use of biological weapons this gap may have to be filled. An on-site capability has clear limitations, not only but also in relation to biosafety. An alternative solution would be to task a designated off-site laboratory with the functions of a central laboratory hub.

The reporting of laboratory results as part of the report of an investigation is a critical element to support the findings presented by the investigation team. The correct interpretation of all data collected and an explanation of what can be concluded from this evidence for a non-technical, political audience is crucial for mission success. The report must be robust to withstand technical scrutiny in a wider political and legal context. Obtaining legal guidance during drafting may therefore be necessary. The content of the report must strike a balance between being understandable for a lay audience as well as providing sufficient technical detail to demonstrate that laboratory methods used were appropriate and validated, leading to quality controlled and coherent results. Furthermore, the reporting of laboratory results should include any unusual or unexpected findings.

In conclusion, the gold standard in analysis remains isolation and cultivation of a pathogen to confirm the agent alive. This however is only



Secretary-General's Mechanism (UNSGM) for investigation of alleged use of chemical and biological weapons

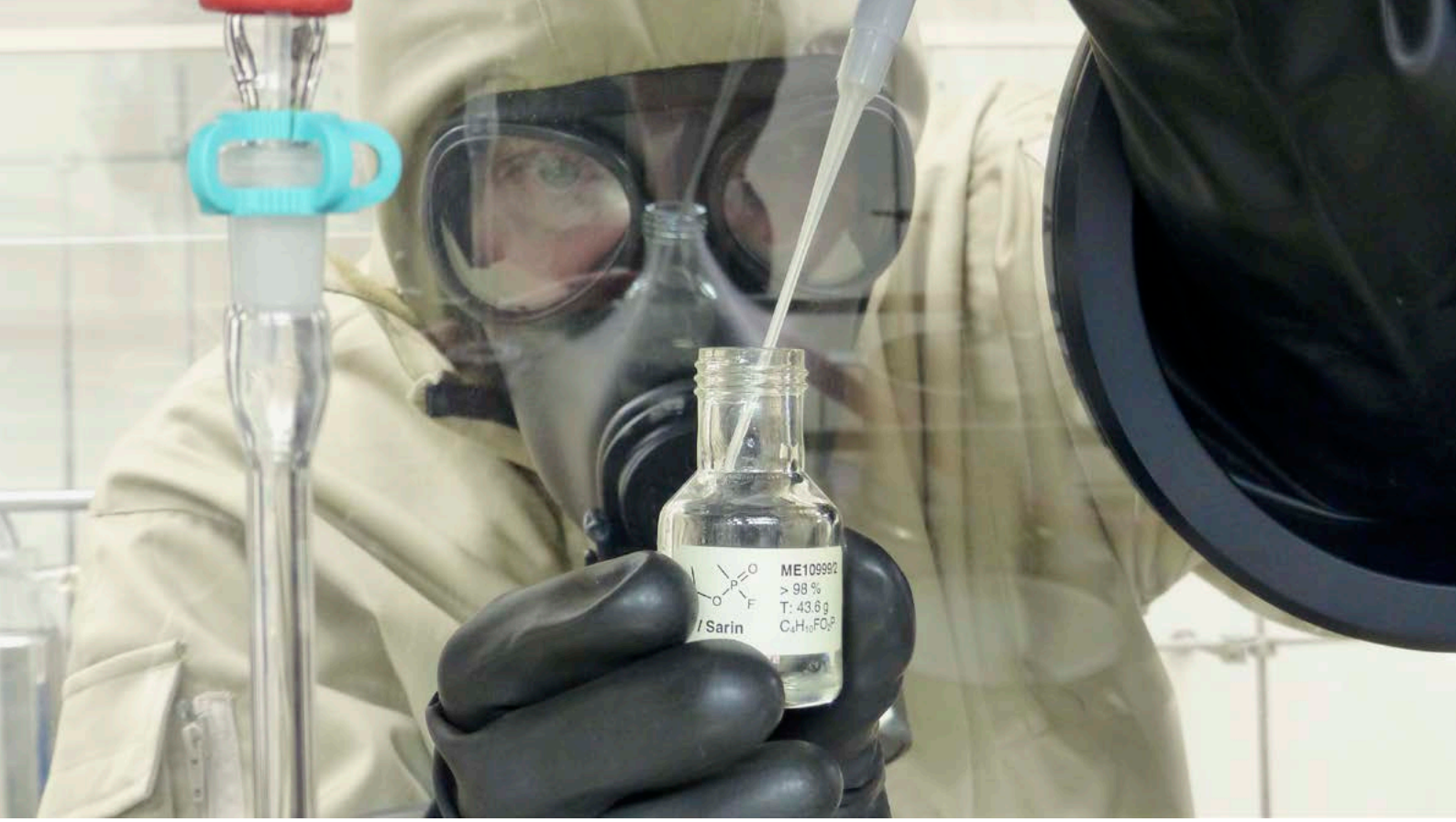
United Nations Office for Disarmament Affairs UNODA

possible if respective samples can be collected in due time. Working with such samples affects sampling and sample handling procedures as well as shipping conditions.

Looking ahead, the absence of a capability portfolio for laboratories makes it difficult for the UNSG to select laboratories with the required capabilities in case of a request for an investigation. This should be addressed with some urgency.

Switzerland will hold a third workshop in June 2017 based on strong support voiced by workshop participants to continue this process and following indications for individual engagement by several workshop participants. In preparation for the next workshop practical steps must be initiated. These include developing a scoring system for laboratory methods and criteria for reporting the results of such methods, both to be tested in a table-top exercise. In parallel, to build confidence among laboratories preparations must commence by parties willing to contribute to inter-laboratory testing.

This text was adapted from the Executive Summary of the 2nd UNSGM Designated Laboratories workshop report. The full report is available under http://bit.ly/2ndZWgb



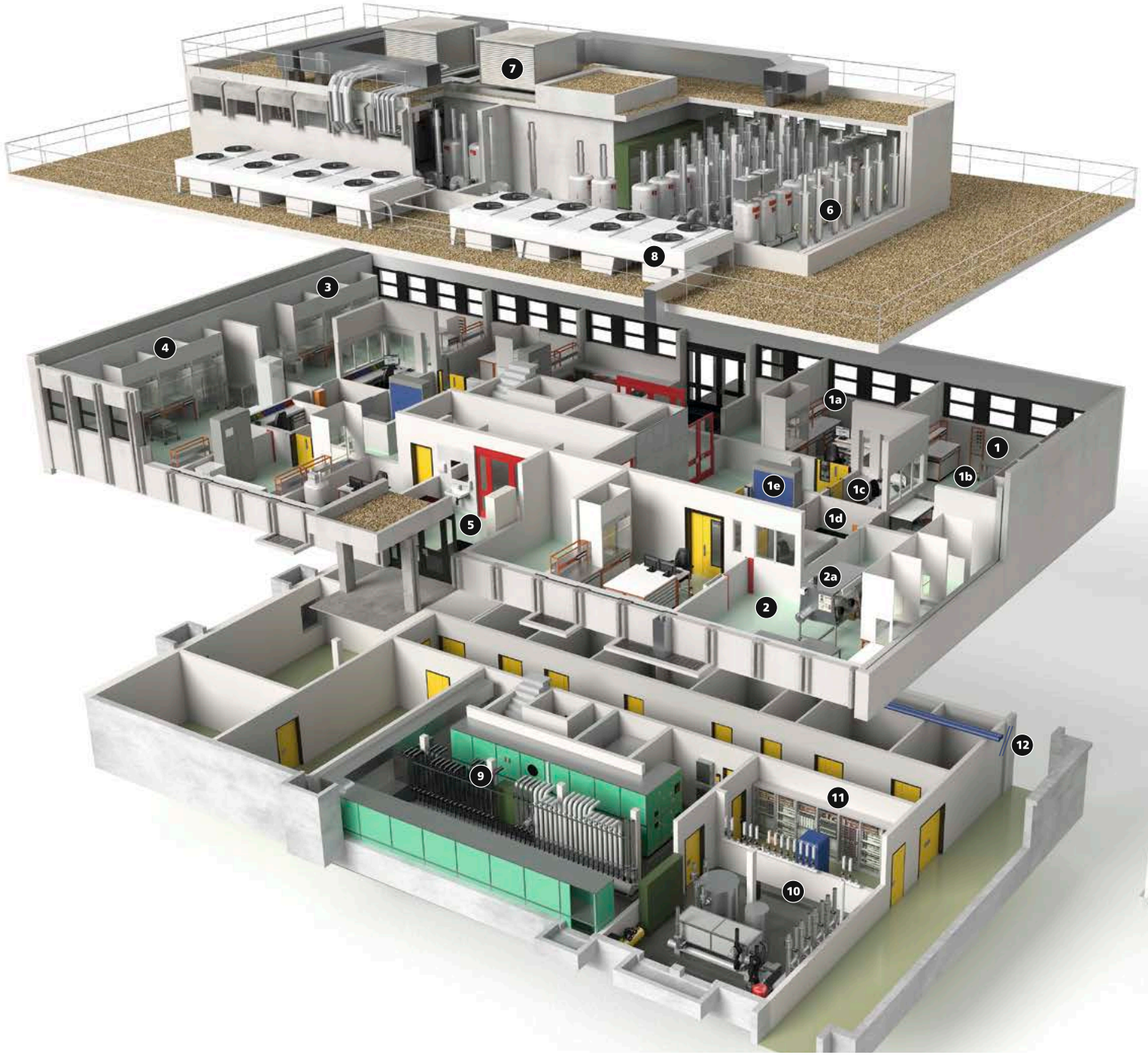
Operator in full protective clothing, handling a toxic substance

Chemical Safety Laboratory

Dr. Christophe Curty,
Benjamin Menzi

The Chemical Safety Laboratory has been designed specifically for the synthesis and handling of highly toxic chemicals. It is the only facility in Switzerland where chemical warfare agents are produced in small amounts for reference purposes and studied with the goal of gaining new insight into the effects of chemical warfare agents and the means of protection against them. These activities help to develop protection measures against the threat of chemical weapons for military personnel as well as the civilian population. Since the commissioning of the Chemical Safety Laboratory in 1981, the facility has been continuously updated to newest safety standards. From June 2015 to June 2016, the lab was completely refurbished.

The Chemical Safety Laboratory provides for the protection of personnel, the environment and neighbouring communities. It includes four independent laboratory units, each assuring the complete containment of toxic agents (HT 1 to 4). Each of these units comprises of a main laboratory (1b), a preparation room (1a), a supervision room (1c) and an air lock system equipped with decontamination showers for use in the case of an unforeseen incident (1d). A specialised air ventilation system (6 and 9) enables the circulation of external air into the interior of the building whilst ensuring a pressure gradient between the different rooms. The entire exhaust air is cleaned using aerosol as well as activated carbon filters (6). An emergency tank automatically collects potentially contaminated wastewater in case of an unforeseen event (13). Liquid and solid wastes are disposed of in a professional and environmentally sound manner after decontamination treatment and subsequent analytical control. Other safety elements include highly qualified and well-trained staff, detailed work instructions and standardised procedures, modern protective equipment, regular medical check-ups for staff and an emergency standby team.



Chemical Safety Laboratory

1 **Unit HT-1 Synthesis**
1a Preparation (-20 Pa)
1b Laboratory (-40 Pa)
1c Supervision (+10 Pa)
1d Personnel decontamination
1e Airlock
2 **Unit HT-2 Synthesis**
2a Glovebox
3 **Unit HT-3 Sample preparation**
4 **Unit HT-4 Detection and decontamination**
5 Emergency station
6 Exhaust air centre HT-1 / HT-2 (active carbon filters)
7 Exhaust air heat recovery system
8 Heat exchanger for cooling unit
9 Air supply centre
10 Heating, climatisation, sanitary installations
11 Electrical centre
12 Laboratory wastewater
13 Decontamination tank

Chemical Weapons Convention
Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction, Organisation for the Prohibition of Chemical Weapons, The Hague, 1994
The Convention entered into force on 29 April 1997. Switzerland signed it on 14 January 1993, and ratified it on 10 March 1995. The Organisation for the Prohibition of Chemical Weapons (OPCW, https://www.opcw.org/, 2017) has the mandate to oversee the implementation of the Convention at the international level.
The Convention is the first multilateral disarmament treaty that aims at the elimination of an entire category of weapons of mass destruction within a defined time frame and under a global verification mechanism. It prohibits the development, production, acquisition, retention, stockpiling and use of any kind of chemical weapon. Only activities for so-called purposes not prohibited are permissible, such as medical, pharmaceutical, or protective purposes.
The Convention separates toxic chemicals and their precursors into three groups, depending on their toxicity and possible applications. Schedule 1 contains the actual and potential chemical warfare agents as well as their precursors that have no civilian application. Schedule 2 contains potential chemical warfare agents and precursors which have civilian application. Schedule 3 contains industrial chemicals which, however, may be used in the composition of chemical warfare agents.

The ventilation system of the Chemical Safety Laboratory had been in operation for over 30 years, and it has now been fully replaced. Although the safe operation of the facility was guaranteed throughout, it had become increasingly difficult and at time impossible to find the necessary spare parts. The refurbishment work took place from June 2015 to June 2016, and was carried out in two phases. Two laboratory units were closed down and refurbished at the same time, whilst the other two units continued to operate in order to support our activities. armasuisse (Federal Office for Defence Procurement) was responsible for the project management. The installations for incoming air (9) and exhaust air (6) were either replaced or completely overhauled. As a result of the new air-conditioning system, the temperature and humidity in the laboratory units can now be precisely regulated, the control of parameters is no longer based on a pneumatic but an electronic system and the redundancy capability for the exhaust air treatment has been increased. An air lock system has been installed for entering the laboratory units and because of this, system air pressure fluctuations inside the building could be reduced to a minimum. The air pressure within the different laboratory units is now visually displayed. Furthermore, a system for heat recovery from the exhaust air has been installed (7). Entry to the building and the access control system were modernised or replaced. As a result of these renovations we have now a modern facility at our disposal that offers optimum usability and maximum safety for our activities.

The Chemical Safety Laboratory is a unique and indispensable platform for the synthesis of highly toxic chemicals, chemical warfare agents and their related compounds. Following the principle of double containment, syntheses are being conducted in a glovebox specifically developed for this purpose. From the initial mixing of the reagents to the final purification of the toxic agents formed, all steps of a synthesis are conducted semi-automatically in a safe environment. All reaction parameters are being directed, regulated and monitored from the supervision room using a process control system. In order to further enhance safety standards, a two-person-rule is in force: Whilst one operator is working in the laboratory wearing full protective gear, all tasks are being supported and monitored by a second operator from the control room. As a Designated Laboratory of the Organisation for the Prohibition of Chemical Weapons (OPCW), Spiez Laboratory must be capable of providing reference standards for analytical purposes on short notice. The Chemical Safety Laboratory supports different divisions and branches of Spiez Laboratory by providing chemical substances that are not commercially available for a range of activities. These include: method development for the analysis of chemical warfare agents; devel-

A specialised air ventilation system enables the circulation of external air into the interior of the building whilst ensuring a pressure gradient between the different rooms.

Glovebox for the synthesis of toxic chemicals

opment of analytical databases; unambiguous identification and quantitation of respective substances; testing of protection equipment such as protective clothing and filters; training of military NBC specialists in detection and analysis procedures. Furthermore, the facility is used for the assessment of new synthetic methods developed in the academic domain, which today are finding their way into industrial chemical manufacturing and which could be used for the production of chemical warfare agents, and it provides room for evaluation and testing of chemical detection and decontamination systems with live agents. Spiez Laboratory applies the knowledge and expertise gained: to arms control and non-proliferation initiatives, to its support of the implementation of the Chemical Weapons Convention (CWC) and to the Swiss chemical emergency response team.

Our activities fully comply with all relevant national regulations. Internationally and in accordance with the Chemical Weapons Convention, even the smallest amounts of Schedule 1 chemicals produced, processed or consumed in whichever form must be declared to the OPCW. The Chemical Safety Laboratory, which has been declared as an 'other schedule 1 facility for protective purposes' (under Article VI of the CWC), is inspected regularly by OPCW inspection teams. This is carried out to ensure that no illicit activities are being conducted at the laboratory. The last OPCW inspection took place from 5 - 9 December 2016 and it was confirmed that our facilities and activities are in full compliance with the applicable regulations.

Entry to the sample receipt unit

Safe Sample Triage in Spiez

Dr. Beat Aebi,
Daniel Jordi

The sample receipt unit (SRU) is a safety facility for the entire Spiez NBC Centre. It enables the safe and technically correct assessment, receipt, acquisition, and temporary storage of a large number of NBC samples as well as their forwarding to the analytical laboratories (Cold Triage). The SRU can also process individual samples that pose a potential, as yet unknown NBC hazard (Hot Triage). The SRU of the Spiez NBC Centre is a unique facility in Switzerland.

"For the first time, a radioactively contaminated rice sample was discovered in Japan close to the wrecked Fukushima NPS. The exposure exceeded the permitted limit – despite the fact that thousands of random samples previously collected had remained well below this level."
(Spiegel Online, 17 November 2011)

"Unpaid bills, annoying advertisements or bank statements in the red: anyone who opens his/her letterbox has to expect unpleasant news. When Sabtir Khokhra (37) picks up his mail, however, he is genuinely frightened. For two months already, the tailor receives threatening letters in his shop at the Bahnhofstrasse in Zurich. The content is always the same: a white powder and a business card from his shop. No sender, no message, no postage stamp."
(Blick, 21 November 2015)

Whether we are talking about hundreds of individual samples after a nuclear incident or about a few threatening letters: In Switzerland, Spiez Laboratory can analyse any and all of them. The SRU, which started operation in the

spring of 2016, was constructed in order to make the receipt of samples with potential NBC risks more efficient and safe. Through two acquisition channels, thousands of known samples (e.g. after an accident at a nuclear power station) and also single suspect samples can be processed and investigated.

In case of an accident at a nuclear power station, it is of critical importance that the authorities as well as the population rapidly receive information about the spread of radioactive radiation. In such a scenario, hundreds of samples will have to be managed over a few days, but the substances to be measured are known. The analytical results form the basis for information to be released to the population about areas which are safe to stay in, and about areas where precautionary measures such as evacuation will have to be ordered. They also provide information concerning the situation with regards to environmental pollution (soil, grass and other samples). Finally, the analytical results form the basis for decisions about the lifting of protective measures.

The SRU offers the ideal facility to ensure the logistics of processing up to a thousand samples per day and to forward these samples to the analytical laboratories of Spiez Laboratory. In this so-called Cold Triage, the samples are registered and barcoded. All recorded data are transferred automatically to Spiez Laboratory. In such a situation, Spiez Laboratory collaborates with the NBC Defence Laboratory of the Swiss Armed Forces, and can thereby increase its human resource capacity in a very short time.

The roofed-over forecourt is used for the receipt and initial inspection of the samples. The rooms to the right of the entrance are dedicated for the acquisition and storage of large numbers of samples (Cold Triage/Orange Arrow), the rooms to the left of the entrance are dedicated for the assessment of individual, potentially dangerous samples (Hot Triage/Red Arrow). They contain a luggage screening facility and two gloveboxes with air locks, negative pressure and filtration systems.

Receipt of large numbers of samples (Cold Triage)
In the case of an NBC mega event, the specialised detachment SRU of the NBC Defence Laboratory of the Swiss Armed Forces can activate the SRU without delay, and can assess, receive, and register hundreds of samples per day in the computer system and forward them to the appropriate laboratories.

Before entering the SRU, the delivered material will be assessed for a first time. Once classified as safe, it will be passed on for further assessments and registration within the SRU. After registration in the computer system, the samples will be sorted and forwarded to the respective laboratories of Spiez Laboratory.

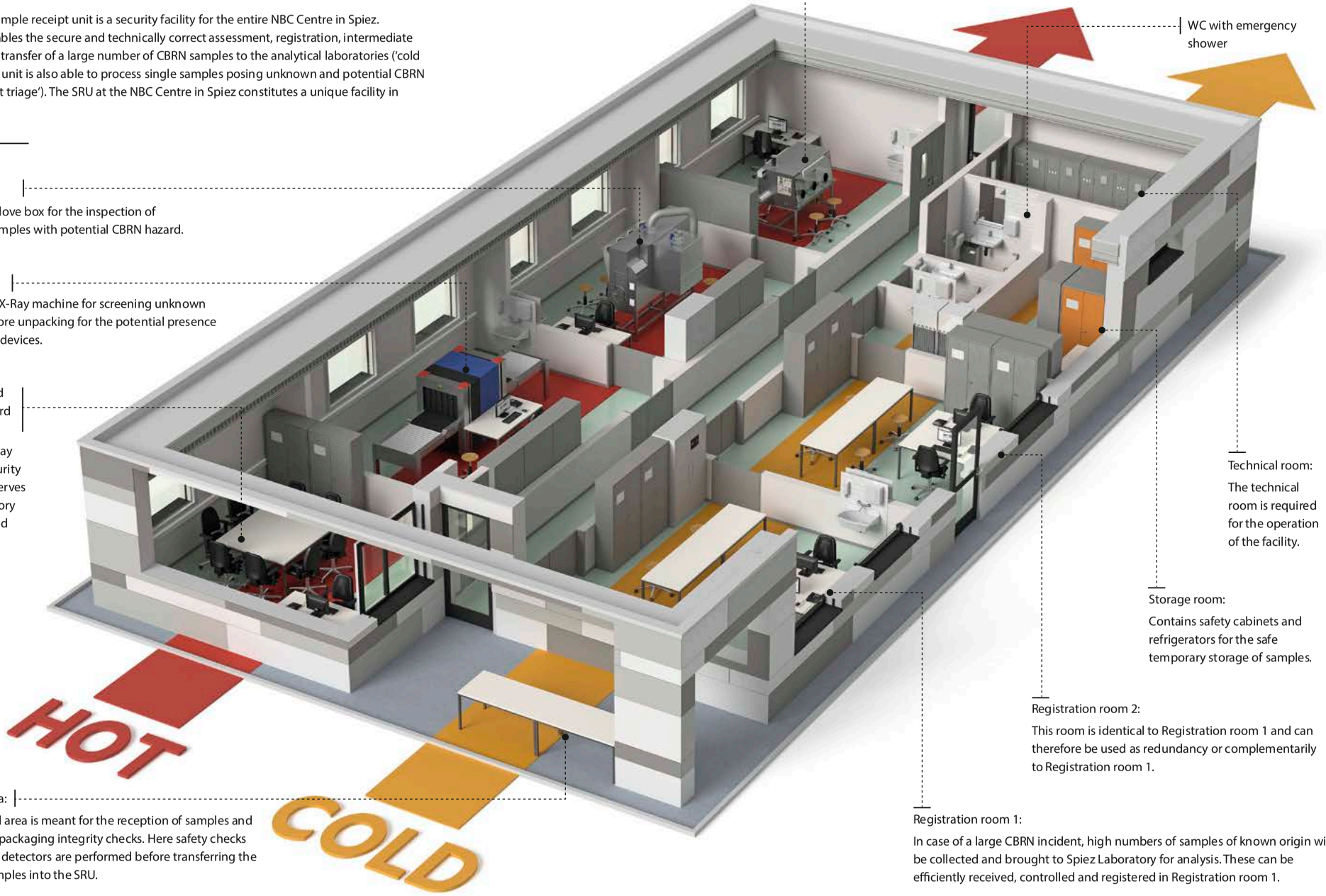
Receipt of individual samples (Hot Triage)
Individual samples with an uncertain background (e.g. a suspect letter or suspicious package), or samples with a elevated hazard potential, must be processed by Hot Triage specialists of Spiez Laboratory. If explosive materials are suspected, the EOD specialists of the NBC-EOD Centre of Competence of the Swiss Armed Forces, will be brought in. After a first inspection the material can be investigated further inside two gloveboxes. Samples with increased hazard potential will be opened exclusively within these gloveboxes.

Safety
The main purpose of the SRU is to ensure the safety of the staff and the facilities of the NBC Centre Spiez. It functions as a locking system and prevents the contamination of personnel, the premises and materials/equipment. This can only be achieved thanks to well-designed procedures, experienced and safety conscious specialists, and high quality equipment. These factors together minimise the danger of unintentional releases of NBC substances into the building. On one hand, samples are inspected already upon arrival; on the other hand, sample containers will be opened exclusively in one of the two gloveboxes of the Hot Triage. A release of NBC material from the SRU into the outside environment, for example as a result of a fire despite smoke detectors and the fire alarm, is unlikely. The small amounts of solvents and acids required within the SRU are stored in separate safety containments. The SRU (including the forecourt) are positioned on a firewater basin that can hold up to 30,000 litres of water.

NBC Centre Spiez

THE CBRN SAMPLE RECEIPT UNIT SRU

The CBRN sample receipt unit is a security facility for the entire NBC Centre in Spiez. The SRU enables the secure and technically correct assessment, registration, intermediate storage and transfer of a large number of CBRN samples to the analytical laboratories ('cold triage'). This unit is also able to process single samples posing unknown and potential CBRN hazards ('hot triage'). The SRU at the NBC Centre in Spiez constitutes a unique facility in Switzerland.



Hot Triage 1:

Contains a glove box for the inspection of unknown samples with potential CBRN hazard.

X-Ray room:

Contains an X-Ray machine for screening unknown samples before unpacking for the potential presence of explosive devices.

Logistics and security guard office:

The office may house a security guard and serves for preparatory meetings and logistical purposes.

Delivery area:

This covered area is meant for the reception of samples and performing packaging integrity checks. Here safety checks with mobile detectors are performed before transferring the received samples into the SRU.

Hot Triage 2:

Contains a glove box for the preparation (e.g. dilution) of chemical samples before their transport to the analytical laboratory.

WC with emergency shower

Technical room:

The technical room is required for the operation of the facility.

Storage room:

Contains safety cabinets and refrigerators for the safe temporary storage of samples.

Registration room 2:

This room is identical to Registration room 1 and can therefore be used as redundancy or complementarily to Registration room 1.

Registration room 1:

In case of a large CBRN incident, high numbers of samples of known origin will be collected and brought to Spiez Laboratory for analysis. These can be efficiently received, controlled and registered in Registration room 1.

The research shock tube of the Collective Protection Branch at Spiez Laboratory

Shock Tube Study of Pencil Probes

André Zahnd (Spiez Laboratory), Dr. Ronny Lorenzo (armasuisse Science and Technology)

Due to its specialised know how in NBC Protection Spiez Laboratory provides a wide range of services for the Swiss Federal Office of Procurement (armasuisse) as well as many collaborative projects, such as the Shock Tube Study of Pencil Probes. Pencil Probes are pressure sensors for free field measurements of shock-waves. To determine the performance and quality of two different types of pencil probes, a comprehensive qualification test series was carried out in the research shock tube of Spiez Laboratory, which has proved to be an ideal facility for such tests.

Initial Situation and Project Goals

Reliable pressure data from shock-waves, be it side-on or reflected, represent important pieces of information, not just for the characterisation of explosives and shock-waves but because they also form part of the framework from within which, e.g. armored vehicles and protective structures, are assessed and qualified. Therefore, being able to determine the reliability, quality and weaknesses of the sensors used to obtain these measurements is a prerequisite for planning reliable experiments.

Commonly applied methods to calibrate pressure gauges are typically (semi-) static, due to their high degree of reproducibility. However, these procedures are hardly comparable to shock-waves, either from the absence of thermal and electromagnetic immissions on the sensors or the lack of dynamics. Thus, calibration charts provided by the manufacturers, given that they rely on more than one data point, fully characterise the sensors performance in ideal conditions but not in field measurements.

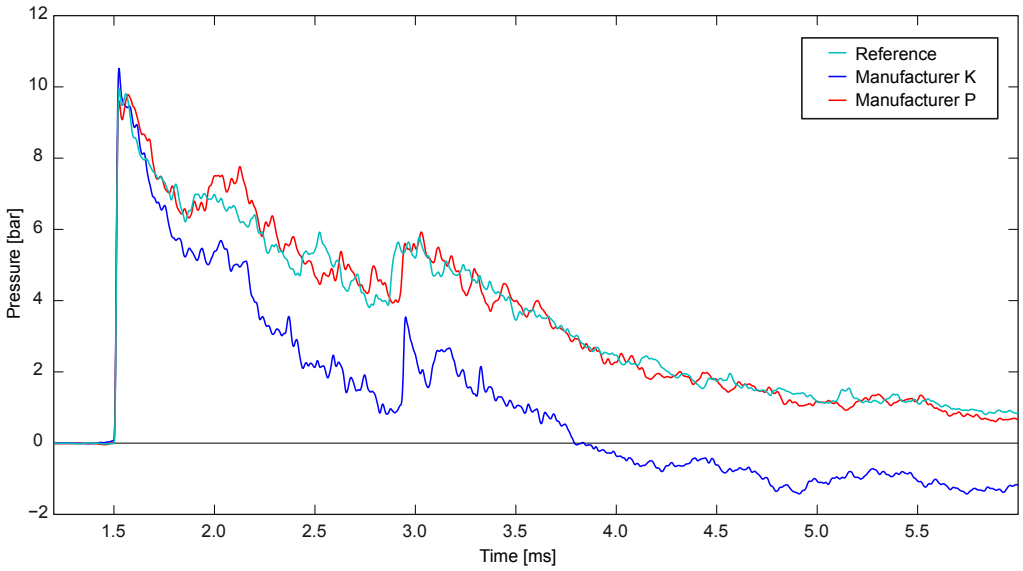


Figure 1: Comparison of the pressure profiles as measured by the two types of pencil probes

Towards the end of 2015 another major manufacturer (K) started to provide laboratories with pencil probes. Naturally, the question arises how this new product compares to the de-facto standard of the industry, the well-established pencil probes of the competing manufacturer (P). Interestingly, the two providers apply different methods of calibration, albeit both rely on (semi-) static ones.

The conducted test series in the shock tube had to answer three questions:

- How do these pencil probes compare to a reference sensor?
- Are their cross sensitivities to acceleration and thermal influences similar?
- And finally: Do the differing methods of calibration have an effect on the performance of the sensors?

Experimental Setup

The shock tube allowed the excluding of environmental influences such as composition of the ground, anchoring of the tripods or atmospheric influences while preserving the characteristics of life fire shock waves, albeit without the electromagnetic radiation typically generated by explosions.

In order to simulate a shock-wave span representing the operating pressure range of the pencil probes the correct shock tube configuration had to be found by performing numerous calibration shots, accompanied by numerical simulations which were provided by the project partner armasuisse Science and Technology. To reduce interferences from the individual deflections and the turbulences in the wake of the passing shock wave, only three pencil probes, as well as two reference sensors were fitted inside the shock tube for each shot. Five pencil probes with identical pressure ranges (17 bar) from each of the two major suppliers were fitted with a custom designed mount. While nine of the probes were new (P2-P5, K1-K5), one of the established supplier's devices (P1) had already been in service with armasuisse Science and Technology for several years and is of different build than its newer colleagues. All of the pencil probes were freshly calibrated by the manufacturers prior to the experiments.

For each shot pencil probes of both suppliers without any protective layer on the sensors were installed inside the shock tube. To ensure reproducibility, the pencil probes were rotated through all possible positions. With each setup three shots at four different pressure levels (2 bar, 6 bar, 10 bar, 15 bar) were performed, resulting in 120 configurations measured. Additionally, to determine the thermal sensitivity of the two different types of pencil probes, three sensors of the same manufacturer with different protective layers (none, Al tape, Si paste) were analysed.

Results

All pencil probes performed persistently well. While reproducibility was exceptional, accordance between the two types was not. Figure 1 compares the pressure profile measured by the reference sensor and one of each suppliers' pencil probes during the same shot. The reference sensor and P's pencil probe are quite in tune. However, while the rise time and peak

pressure measured by K's pencil probe is in accordance to the other two sensors, the pressure inside the shock tube seems to fall much faster according to K's sensor and it even plots a negative pressure phase, which cannot occur inside a closed shock tube.

In figure 2 the average peak pressure for each pencil probe (with 1 sigma margin) is plotted against the same value normalised with the average peak pressure measured by the reference sensor (with the 1 sigma margin as well). The graph demonstrates that manufacturer P has greatly improved its sensor design from one generation (P1) to the latest generation (P2-P5). Even though the reproducibility of the measurements of pencil probe P1 is on par with its successors, it overestimates the peak pressure of the shock wave on average by 15 % and its sensitivity seems to be less constant over its pressure range. However, the latest generation of P's pencil probes underestimates the peak pressure on average by 3 % while otherwise delivering an almost flawless performance.

With respect to peak pressure, the pencil probes of manufacturer K show a similar behavior over the pressure range. Reproducibility is certainly on par with its competitor's sensors but in contrast, K's pencil probes tend to overestimate the peak pressure on average by 6 %. As sensitivity to electromagnetic radiation (especially to the infrared spectrum) is to be expected both manufacturers suggest that some form of protective layer has to be applied. As virtually no such radiation is emitted in shock tubes, all pencil probes were initially used without insulation.

Figure 3 confirms that the pencil probes made by K are influenced by the temperature gradient across the shock wave and shows which insulation provides the best results. K's sensors showed a pronounced reaction to the temperature gradient across the shock wave, which explains the performance observed in figure 1, while P's sensors coped comparatively well.

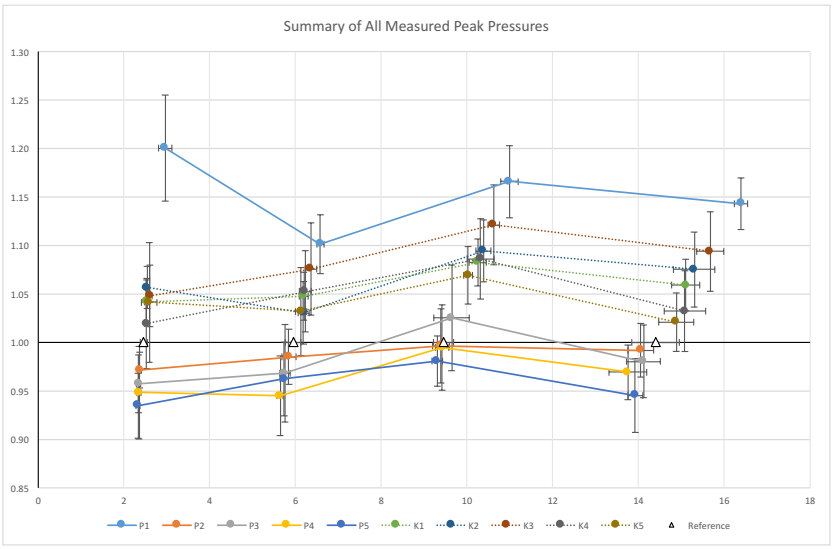


Figure 2: Summary of the results (peak pressures)

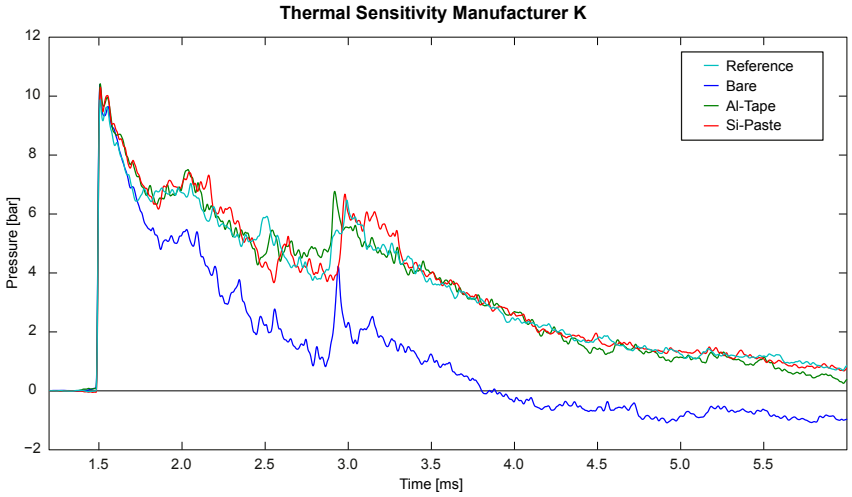


Figure 3: Influence of different insulation methods on sensor accuracy, manufacturer K

Conclusions and Outlook

The shock tube has proven to be ideal to verify the factory calibration of the pencil probes and to compare the performance and quality of the two competing products. The production of reproducible shock waves to characterise the

Spiez Laboratory's services for the Swiss Federal Office of Procurement (armasuisse):

- Technical assessments and experimental qualification tests on NBC Collective Protection Components
  → Service Level Agreement with armasuisse real estate, section UNS
- Certification of Conformity of NBC Collective Protection Components and NBC Filters
  → Service Level Agreement with armasuisse real estate, section UNS
- Technical assessments and experimental qualification tests of NBC Personal Protective Equipment
  → Service Level Agreement with armasuisse, section KBLA FBLAL
- Qualification tests on NBC filters for Personal Protective Masks
  → Service Level Agreement with armasuisse, section KBLA FBLAL
- Technical assessments and experimental qualification tests of NBC Protective Fabrics and on Polymers
  → Service Level Agreements with armasuisse, section KBLA FBLAL and sections UNS
- Onsite NBC inspections in infrastructure objects of the Swiss Armed Forces
  → Service Level Agreement with armasuisse real estate, section UNS
- Qualification tests on NBC protection systems in vehicles and temporary installations
  → Service Level Agreement with armasuisse real estate, section LAS

measuring equipment, greatly enhances the confidence in the results obtained in live fire tests by armasuisse Science and Technology.

The results show that both manufacturers produce pencil probes which are capable of accurately measuring the pressure profiles of shock waves with an exceptional level of reproducibility. However, the factory specific calibration methods may result in over- (K), respectively underestimating (P) the shock wave's peak pressure. Furthermore, the sensitivity of K's pencil probes to the temperature gradient across the shock wave and possible methods of mitigating these effects have been investigated. The project "Shock tube study of Pencil Probes" is a typical win-win project not only for both partners involved but also for the manufacturers.

The whole endeavour has been published in a scientific paper which was presented by Spiez Laboratory at the international symposium MABS 24 (Military Aspects of Blast and Shock) in Halifax, Canada.
Further investigations on the sensor's behavior with regards to varying mounting angles are planned.

Project Partners and Funding
This project was conducted in collaboration between Spiez Laboratory and armasuisse Science and Technology and was funded by the project partners.

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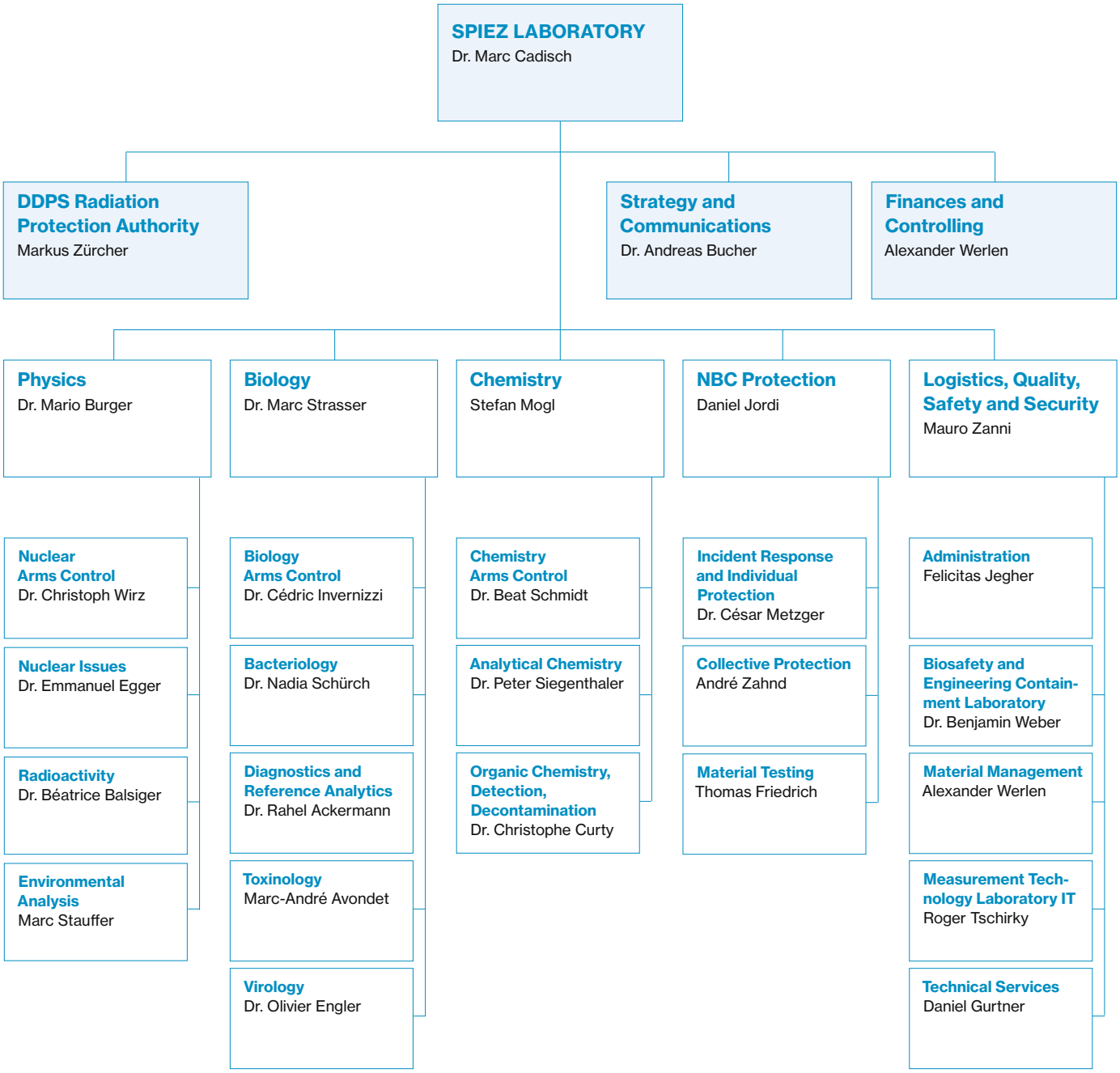
Organisation



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 2015–September 2016

Accredited laboratory		number	Type and partner
STS 0019	Chemical analysis/verification	1	Sample preparation for the 39th OPCW Proficiency Test
STS 0022	Adsorbents and Respiratory	1	Aerosol Retention Rate of HEPA Filters, WIS Munster
STS 0028	Radionuclides	5	– Round Robin: Gamma emitting radionuclides in a reference sample (epoxy resin) - (IRA/FOPH, Dec. 15) – Radiochemistry: Water analysis using alpha spectrometry, ICP-MS and LSC (German Federal Office for Radiation Protection, Oct. 15) – Uranium isotopes in drinking water (IAEA, Oct. 15) – Characterising of reference material (sediment) using gamma spectrometry and ICP-MS (IAEA, May 16) – PT ALMERA 16: Analysis of water, spruce needles, sediment and dried clover using gamma spectrometry, ICP-MS and LSC. LLC-measurement of Sr-isotopes after radiochemical preparation (IAEA, Sept.16)
STS 0036	Polymers and Rubber	8	– Identification of plastic granulates by FTIR – Tensile test at - 30 °C – Flexural test (modulus) – Flexural test (strength and elongation) – Tear resistance - Elmendorf method – Charpy notched impact properties – Izod impact properties – Melt flow/volume rate MFR/MVR
STS 0054	Biological toxins	1	Protein determination by dyebinding andphotometry.
	Medical biochemistry	0	
	Diagnostics of bacteria – drinking water	6	Public Health England
	Diagnostics of bacteria – molecular biology	2	– INSTAND anthrax, tularemiea, Q-fever (Nov. 15 (2015-B-12), May 16 (2016-B-04) – EMERGE May 16 (2015-B-03)
	Diagnostics of viruses – molecular biology	2	– INSTAND Dengue Virus PCR Sept. 16 – INSTAND West Nile Virus PCR Sept. 16
	Diagnostics of viruses serology	1	INSTAND FSME-Serology June 16
STS 0055	Ventilation	0	
	Air blast effects	0	
	Ground shock effects	0	
STS 0101	Main and trace elements	3	– International Soil Exchange 2015-04 – Ielab potable water Round I & II & III – IAEA TEL 2015-01 (Wasser)
	Air pollutants	0	

Presentations

Our scientists attend and actively contribute to conferences and offer their input to training courses dealing with NBC protection issues. Below is a selection of the presentations, given by our specialists during 2016.

Date	Subject
12.01.2016	Dr. Christoph Wirz: Kontrolle des Comprehensive Test Ban Treaty CTBT, Nationale Alarmzentrale, Zürich
20.01.2016	Dr. Marc Cadisch: Schweizer Engagement bei der Beseitigung von Chemiewaffen, Forum Gesellschaft und Politik, Thun
29.01.2016	Dr. Cédric Invernizzi: Epidemics and Pandemics: The Role of Research Centers of the Swiss Federal Department of Defence, Civil Protection and Sports, DCAF GCSP, Geneva
03.02.2016	Dr. Marc Cadisch: National Laboratory Systems, GCSP National Laboratory Systems, Geneva
11.02.2016	Dr. Matthias Wittwer: OMICS: Zeige mir deine Gene und ich sage dir woher du kommst, wer du bist, und wohin du gehst, NGS GV GINT, Thun
01.03.2016	Dr. Olivier Engler, Analyzing the virus-neutralizing capacity of sera from VSV-ZEBOV vaccine recipients: Current state and outlook; EBOVAC Meeting, Vienna, AUT
31.03.2016	Dr. Christoph Wirz: Der Iran-Deal, abcsuisse, Zürich
11.04.2016	Dr. Matthias Wittwer: Whole genome phylogeny of Swiss *F.t. holarctica isolates*, ECCMID, Amsterdam, NL
19.04.2016	Dr. Peter Siegenthaler: Preparation of the Samples for the 39th Official OPCW Proficiency Test, Evaluation Meeting 39. OPCW Proficiency Test, The Hague, NL
19.05.2016	Dr. Gilles Richner: Field Study of the Climate Impact on Activated Carbon Filters. 7th International Symposium on CBRN Protection and Decontamination, WIS Munster, DEU
14.06.2016	Dr. Marc Cadisch: Schweizer Engagement bei der Beseitigung von Chemiewaffen am Beispiel von Syrien, GV AVAG, Spiez
14.06.2016	Dr. Cédric Invernizzi: Dual Use Research of Concern (DURC), Annual Meeting of the Swiss Society for Microbiology, Bern
15.06.2016	Stefan Mogl: Technologies as Challenge for Arms Control, International Security Forum, Geneva
29.06.2016	Dr. Peter Siegenthaler: Investigation of Chlorine Exposure in Environmental and Material Samples at the Spiez Laboratory, OPCW SAB-23 Meeting, The Hague, NL
02.07.2016	Dr. Marc Cadisch: Logistische Herausforderungen im Umfeld von Probenentnahmen und Analysen, QV Feier Logistiker, Thun
06.08.2016	Dr. Beat Schmidt: Best Practices and Capacity Building, OPCW Mentorship/Partnership Programme, Windhoek, NAM
16.08.2016	Dr. Peter Siegenthaler, Dr. Martin Schär, Andreas Schorer: BioMedical Sample Analysis, Trace Analysis and LC-MS Screening Strategies, Technical Exchange Meeting with DSO National Laboratories, Singapore
25.08.2016	Dr. Beat Schmidt: Swiss Day Fellowships on Disarmament, UNODA, Spiez
21.09.2016	Dr. Martin Schär: Application of High-Resolution Mass Spectrometry to Verification Analysis in the Context of the Chemical Weapons Convention, Bruker Life Science Mass Spectrometry Seminar, Basel
22.09.2016	André Zahnd: Shock Tube Verification of the Factory Calibration of Pencil Probes, MABS Halifax, CDN
23.09.2016	Dr. Marc Cadisch: ABC-Risiken im vernetzten Denken: Herausforderung des Unbekannten, Nationale ABC-Schutz Konferenz, Bern
05.10.2016	Stefan Mogl: Swiss Workshops on UNSMG Designated Laboratories, FOI Workshop, Umea, SWE
06.10.2016	Andreas Schorer: Einsatz des Agilent 7200 GC/Q-TOF in der Analyse von chemischen Kampfstoffen, Agilent MS User Meeting, Kassel, DEU
12.10.2016	Dr. Christophe Curty: Chemical Weapons Sample Stability and Storage, Science for Diplomats at EC-83, OPCW, The Hague, NL
21.10.2016	Dr. Mario Burger: Nuklearer/radiologischer Terrorismus, Bevölkerungsschutzkonferenz BSK, Neuenburg
27.10.2016	Stefan Mogl: Chemische/Biologische Waffen – Herausforderungen für die Rüstungskontrolle, Vorlesung CSS ETHZ *Aktuelle sicherheitspolitische Fragen*, Zürich
19.11.2016	Dr. Marc Strasser: Natürliche oder beabsichtigte biologische Gefahren, Berner Tagungen, Konferenz der LabMed Schweiz, Bern
21.11.2016	Dr. Marc Strasser: Diagnostischer Nachweis und biological preparedness in der Schweiz, Blockkurs Katastrophenmedizin, Universität Zürich, Zürich
30.11.2016	Stefan Mogl, Dr. Beat Schmidt: Report from Spiez Convergence 2, Side Events, CSP-21, OPCW, The Hague, NL
22.12.2016	Dr. Nina Mosimann: Gamma Spectrometry at Spiez Laboratory, Seminar on Ultrafast Science and Technology, Institut für Angewandte Physik, Universität Bern, Bern

Publications

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

Media Review

22.01.2016	Thuner Tagblatt	«Im Einsatz gegen chemische Waffen»
20.02.2016	20 Minuten	«Radiologischer Terror: Es würde auf jeden Fall Angst und Panik geben»
29.02.2016	Jungfrau Zeitung	«Innovative Spezialanalytik in Spiez»
04.03.2016	Beobachter	«Atomunfall – bedingt notfallbereit»
11.03.2016	Neue Luzerner Zeitung	«Syrienkrieg – drastischer Einblick für Schüler»
30.03.2016	Watson	«Islamischer Staat, Chemie- und Nuklear-Waffen: Wie real ist die Gefahr?»
30.03.2016	Spiegel Online	«IS-Terror mit ABC-Waffen: Sehr reales Risiko»
31.03.2016	Basler Zeitung	«Anschläge mit C-Waffen: Die psychologische Wirkung wäre gross»
22.04.2016	ats	«Tschernobyl: le drame nucléaire a laissé des traces en Suisse»
13.06.2016	Horizonte – Das Schweizer Forschungsmagazin	«Der Dual-Use-Joker»
03.08.2016	Der Bund	«Milzbrandfall in Sibirien: Ein gängiges Antibiotikum reicht»
03.08.2016	ARCINFO.ch	«Après la radioactivité, l'air pur de la Suisse»
05.08.2016	Jungfrau Zeitung	«Deutlich mehr Zeckenstiche»
09.08.2016	Le Courrier	«Le joker du double usage»
19.08.2016	Berner Zeitung	«Sperrzone – Besuch im Labor Spiez»
07.09.2016	Neue Zürcher Zeitung	«Giftgasangriffe in Syrien: Gegen jede Kultur der Straflosigkeit»
08.09.2016	Radio SRF	«Assad liess offenbar erneut Chemiewaffen einsetzen»
08.09.2016	Neue Zürcher Zeitung	«Wächter über das C-Waffen-Verbot»
25.09.2016	Sonntagszeitung	«Ein Kinderspiel für Techno-Terroristen»
25.10.2016	Der Bund	«Biotechnologe: Die neue Gefahr der Biowaffen»
04.11.2016	ETH Zürich	«Bioweapons and Scientific Advances» Center for Security Studies
18.11.2016	Neue Zürcher Zeitung	«Schweiz wappnet sich gegen Pocken»
13.12.2016	The Diplomat	«Soviet Uranium Mines Still Have Deadly Impact in Kyrgyzstan»
28.02.2017	Neue Zürcher Zeitung (NZZ)	«Neuer Botox-Test soll Mäuse verschonen»
28.02.2017	Universität Bern	«Maus-Stammzellen auf Chip könnten Tierversuche ersetzten»
01.03.2017	Radio SRF	«Universität Bern will Tierversuche überflüssig machen»
13.03.2017	Puls, Schweizer Radio und Fernsehen	«Botox-Test am Chip statt an Labormäusen»

Physics Division

M. Díaz-Asencio, J. A. Corcho-Alvarado, J. A. Sánchez-Cabeza, A. C. Ruiz-Fernández, M. Eriksson
Reconstruction of Recent Sedimentary Processes in a Carbonate Platform (Gulf of Batabano, Cuba) Using Environmental Radiotracers
Estuaries and Coasts (2016) 39: 1020

Carnero-Bravo V, Sanchez-Cabeza JA, Ruiz-Fernández AC, Merino-Ibarra M, Hillaire-Marcel C, Corcho-Alvarado JA, Röllin S, Diaz-Asencio M, Cardoso-Mohedano JG, Zavala-Hidalgo J
Sedimentary records of recent sea level rise and acceleration in the Yucatan Peninsula
Science of the Total Environment 573 (2016), 1063-1069

José Corcho
Kontrolle Abwasser aus Isotopenlabors
Labornotiz LN 2016-01 CORJ

José Corcho
Bestimmung von Strontium-Isotopen in der Prüfstelle STS 0028
Labornotiz LN 2016-02 CORJ

José Corcho
Stilllegung kerntechnischer Anlagen: Rückbauanalytik
Labornotiz LN 2016-03 CORJ

Emmanuel Egger, Béatrice Balsiger, Markus Aster
Erfahrungsbericht zum Einsatz des Mobilen Messsystems Radioaktivität MMR der A-EEVBS an der Übung «WEILAR»
Labornotiz LN 2016-01 EGM BALB AST

Cédric von Gunten
Bestimmung von Spurenelementen in Bleiproben mittels offenem Aufschluss und ICP-Optischer Emissions-Spektrometrie
Labornotiz LN 2016-02 VGC

Jasmin Ossola
Validierung der Selenbestimmung mit dem optischen Emissionsspektrometer - 5100 ICP-OES Dual View
Labornotiz LN 2016-01 OSJA

Nina Mosimann
Berechnung der gewichteten mittleren Aktivität in Genie2k
Labornotiz LN 2016-01 SNIN

André Pignolet
Laborabwasser-Neutralisationsanlage, Jahresbericht 2015
Labornotiz LN 2016-01 PAN

Hans Sahli, Christoph Wirz
Nukleare Forensik – Zusammenfassung Kurs in Karlsruhe und Stand Analytik im LS
Labornotiz LN 2016-01 SAHH-WIC

Stefan Röllin
Auftrag-und Probenverwaltung in der Gruppe Radioaktivität und Abw Lab 1A
Labornotiz LN 2016-01 ROF

M. Staufer, A. Pignolet, J. A. Corcho Alvarado
Persistent Mercury Contamination in Shooting Range Soils: The Legacy from Former Primers
Bulletin of Environmental Contamination and Toxicology, Nov. 2016

Marc Stauffer
Validierung der Wolframbestimmung mit dem ICP-Massenspektrometer «NexION 300D»
Labornotiz LN 2016-02 STM

Marc Stauffer
Validierung des elektrischen Schmelzaufschlusssystems «LeNeo»
Labornotiz LN 2016-03 STM

Marc Stauffer
Ringversuchsergebnisse 2015 der Prüfstelle für die Bestimmung von Haupt- und Spurenelementen sowie ausgewählten Luftschadstoffen STS 0101
Labornotiz LN 2016-04 STM

Marc Stauffer, Jasmin Ossola
Entwicklung und erste Versuche zur Spezifikation von Quecksilber in wässerigen Lösungen mittels LPLC-ICP-MS
Labornotiz LN 2016-01 STM

Yuheng Wang, Konstantin von Gunten, Barbora Bartova, Nicolas Meisser, Markus Astner, Mario Burger, Rizlan Bernier-Latmani
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Entwicklungen im Bereich nukleare Rüstungskontrolle - Technische Sicht aus Spiez
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Biology Division

Rahel Ackermann
Validierung des real-time PCR Nachweises von Anaplasma plasma phagocytophilum
Laborbericht LS 2016-04

Rahel Ackermann
Validierung des real-time PCR Nachweises von Rickettsia spp.
Laborbericht LS 2016-09

Chinmay Dwibedi, Dawn Birdsell, Adrian Lärkeryd, Kerstin Myrtennäs, Caroline Öhrman, Elin Nilsson, Edvin Karlsson, Christian Hochhalter, Andrew Rivera, Sara Maltinsky, Brittany Bayer, Paul Keim, Holger C. Scholz, Herbert Tomaso, Matthias Wittwer, Christian Beuret, Nadia Schuerch, Paola Pilo, Marta Hernández Pérez, David Rodriguez-Lazaro, Raquel Escudero, Pedro Anda, Mats Forsman, David M. Wagner, Pär Larsson, Anders Johansson
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Denise Siegrist, Christian Beuret
Evaluation des FilmArray (Biofire Diagnostics)
Laborbericht LS 2016-02

Susanne Thomann
Evaluierung der Empfindlichkeit von Bacillus cereus ssp. auf Copsin mittels der Microdilution Methode
Laborbericht LS 2016-06

Matthias Wittwer, Fritz Wüthrich, Nadia Schürch
Implementierung der neuen molekularen Technologien Next Generation Sequencing und digtal PCR im Rahmen eines europäischen Proficiency Tests
Laborbericht LS 2016-08

Matthias Wittwer, Fritz Wüthrich
Validierung des real-time PCR Nachweises von Coxiella burnetii
Laborbericht LS 2016-13

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Chemistry Division

Michael Arnold
Prüfung von Nachweispapieren für flüssige Kampfstoffe: KNP der Schweizer Armee und PDF1 aus KDTC-Kit von NBC-Sys
Labornotiz LN 2016-03 ARND

Michael Arnold
Prüfung von DETINDIV-Nachweissensoren für gasförmige Nervengifte des Herstellers NBC-Sys
Labornotiz LN 2016-02 ARND

Michael Arnold
Labor Test FLIR-Nachweiskits FIDO C2
Laborbericht LS 2016-12

Andreas Bielmann
Schlussbericht Evaluation Peptide Synthesizer
Labornotiz LN 2016-01 BIAN

Elena Consoli
Albumin – sulfur mustard bioadducts. Investigation of the synthesis of the relevant bi- and tri-peptides, [S-HETE]-Cys-Pro and [S-HETE]-Cys-Pro-Phe, and crosslinked bioadduct Cys2[ETE]
Laborbericht LS 2016-07

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