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Abstracts

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Toxicology of Colubridae (Rear-Fanged Snakes) Venom from Uzbekistan

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There are about 3000 snake species on the earth. Of these, 400 species are venomous and dangerous for humans. The family Colubridae is the largest family in the suborder Serpentes, which comprises more than 60% of all snake species. In the snake fauna of Uzbekistan , there are more than 10 snake species of the family Colubridae. Of these, the venoms of some snake species are toxic for humans and animals.

Despite a high number of the snakes Colubridae in comparison with other snakes, the biochemical composition and the mechanism of the effect of neurotoxins on biological membranes remain relatively poorly studied.

The goal of our work is to develop the methods of extraction and purification of biologically active compounds, neurotoxins, from the venoms of Colubridae snakes and to study the mechanism of the effect of purified components with the aim of their application in medicine and pharmaceutical industry.

Six snake species, namely, Coluber ravergieri, Natrix tessellata, Psammophis lineolatum Brandt, Coluber tyria (Linneus), Coluber rodorochachis and Elaphes dione Pallas (the family Colubridae) are widespread in Uzbekistan . However, these snakes have not been studied zootoxicologically, as yet.

For the first time, the toxicological properties of the venom of Colubridae snakes were studied in tested preparations (for mice, toxicity of the venom (LD50) using the venom of Psammophis Lineolatum brandt -7,21 mg/kg, Coluber ravergieri - 9,67 mg/kg, Coluber tyria (Linneus) - 9,65 mg/kg, Coluber rodorochachis - 10,64 mg/kg, Elaphe dione Pallas - 9,84 mg/kg and Natrix tessellata- 20,23 mg/kg. The venoms showed the phospholipase and protease activities.

The venoms and fractions of Colubridae snakes were for the first time shown to form single ion channels on bilayer lipid membranes with predominating cation selectivity.

The venoms and fractions of Colubridae snakes uncouple oxidative phosphorylation of isolated mitochondria. The venom of Coluber ravergieri in experiments on frog neuro-muscular synapses acts similarly to cobra neurotoxins, blocking holinergic receptors of postsynaptic membranes

The venoms and preparations made from snake venoms are in wide use in medicine as anesthetics and anti-inflammatory drugs against various kinds of pains. In this respect, the study of neurotoxins of the Colubridae snakes open new avenues for the use of natural biomaterials for the prevention of cardio-vascular diseases, which show a tendency of a wide distribution in various states of the world.

[P02]

The Development of the Quantified Check List for Safety Manegement in the Poisonous and/or Deleterious Substances-handling Manufacturing Industries

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T ABSTRACT: The authors have developed the quantitative risk assessment method of the check list, giving added weight to their check statements individually. In order to build the system of the quantified check list, firstly, the investigation reports of the whole accidents related to the poisonous and deleterious substances in Japan were collected from the manufacturing industries for the limited period of recent five years. Secondary, from the points of view of the organization factors and the technical factors, the accident causes were investigated and analyzed into the detailed factors. According to the statistical analysis for the cause factors, the importance of the cause factors was quantitatively calculated. Thirdly, approximately twenty check statements were extracted from each accident as the safety measures to avoid the causes in principle. The more important a check statement was, the more times the statement was repeatedly extracted. The number of times the statement was extracted was obtained. These deducted check statements were classified into and distributed to the organization factors and the technical factors. Consequently fourthly, based on both the importance of factors and the extracted times, every statement was expressed in numerical value. And, about four hundreds check statements in total were deduced. And finally, the importance on the statements weighed simplistically into four magnitudes.

Keywords: risk, assessment, check list, poison, management, accident, process

Automatic Identification of Color Change Reaction by Chemical Agent Using RGB Value

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When persons are exposed to chemical agents, they are given damage immediately. Therefore, it is necessary to detect chemical agents on the contaminated area immediately, and a portable chemical agent detector which we can operate easily on the contaminated area is very useful. There is a chemical agent detector whose principle is based on color change reaction between a chemical agent and reagents. The detector has some features like low false detection rate and high detection sensitivity. However, the operation of the detector is complicated due to injection of reagents etc. If the color change is quantified optically, it is possible to develop an automatic chemical agent detector with high reliability.

We conducted the color change test to study the feasibility of automatic detection with simulants solution. We used dimethyl sulfate (DMS) as the simulants of sulfur mustard and triethyl phosphate (TEP) as the simulants of nerve agent. In the experiments, we added simulants solution of constant concentrations (DMS: 0.04-2μg/ml, TEP: 10-100mg/ml) to the detection paper for color reaction and gave color to the paper. Then, we took an image of the color change by CCD camera and analyzed RGB value of the image. And we found the simple algorithm of automatic detection for chemical agents by optical analysis.

There are a correlation between the concentrations of simulants solution and the color density for the sulfur mustard detection paper. The correlation between the concentrations and (R+G+2B)/4 values is shown in Figure 1. We propose the algorithm to determine the concentration level by (R+G+2B)/4 values.

For the nerve agents detection paper, we can determine the existence of nerve agents only from R value. We will conduct the color change test with chemical agents and make an advanced algorithm for automatic detection at the next step.

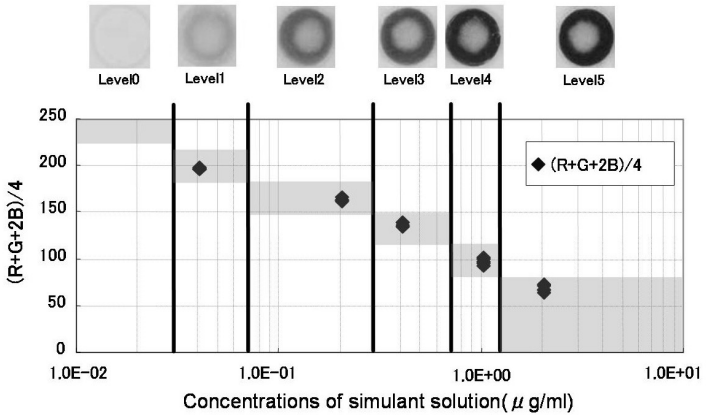

Figure1 Determination of concentration levels on the detector paper for sulfur mustard by RGB values

[P04]

Detection of Microorganisms by TOF-MS

Naohiro MURONOI, Hideyuki HAYASHI, and Shiroh HISAJIMA

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The effectiveness of the microorganism detection technology which used the mass spectrometry was examined. The following two kinds of methods were tested, and both detection performances of the microorganism were verified. In this report, we present the results.

①We aimed at the sensitivity improvement for the mass analysis method to detect and identify the microorganism. We tried the method to use the concentrated plate when analyzing the mass of surface protein of the microorganism directly.

In comparison with the normal plate, this method increased the sensitivity about ten times. This test was examined about E.Coli and spores of bacillus subtilis.

②We aimed to simplify the spectrum to facilitate its analysis. Processing the microorganism with the acid before mass analysis, the protein in the microorganism is extracted.

In this method, the spectrum was simplified, so that the peaks can be clarified.

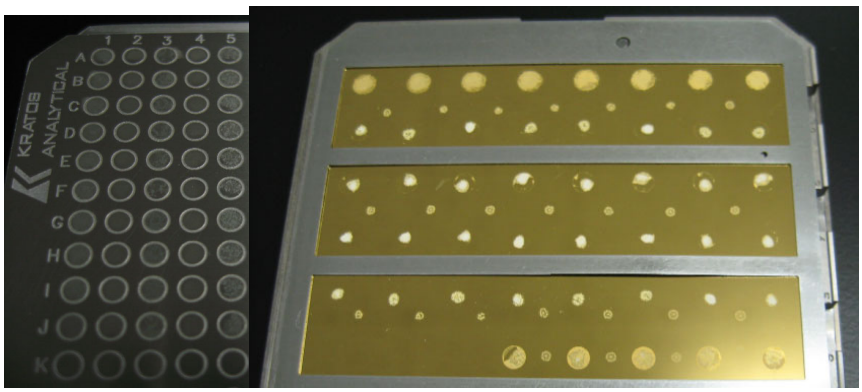

Figure 1　Typical views of the plates prepared for measurements
（Left: Normal plate　　Right: Concentrated plate）

High-Speed Sorting of Microorganism by Photomicrograph

Tadashi OKADA, Kentaroh HAYAKAWA, and Shiroh HISAJIMA

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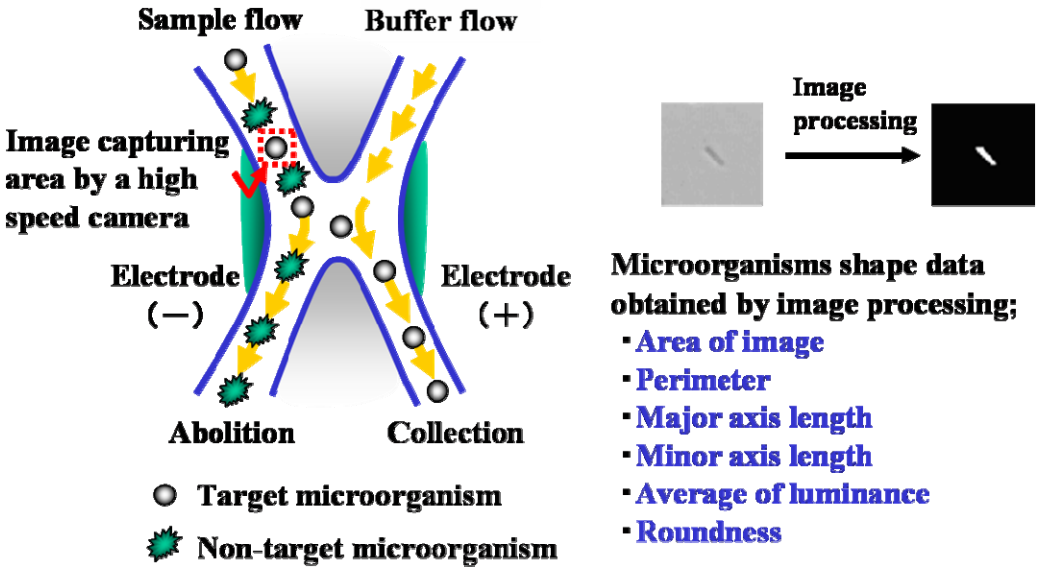
Recently, biological agent's threat becomes an important problem from the viewpoint of the National security. The particles such as dusts and harmless microorganisms exist in the atmosphere. These become "obstructive noise" for the biological agent's identification, and they decrease the accuracy of biological agent's identification and increase the identification time. Therefore, to identify rapidly the biological agent in high accuracy, it is important to separate the biological agent from atmospheric dusts and harmless microorganisms which are contained in samples collected from atmosphere.

We manufactured an on-chip cell sorter which separates target particle from view of the shape. This device obtains microscopic image of the particle which flows flow-channel of microchip with high speed camera, and judge target particle by shape and feature of particle which are calculated from the microscopic image. In that case, the target particles are separated from other particles by the impressed voltage. We confirm separation *Bacillus subtilis* from mixture of *E. coli*, the yeast, and *Bacillus subtilis*. Though the maximum separation rate of this device is 200 cells /second, we will improve this rate to one digit or more in the near future. Moreover, we investigate atmospheric microorganisms, and obtained microscopic image of 24 kinds of atmospheric microorganisms, and make the database concerning those shape and feature. Attention is necessary for the microorganism not included in this database for biological agent detection.

In the future, we plan further investigation, making a reliable database about atmospheric microorganism.



Mechanism of an on-chip cell sorter

[P06]

Discrimination of Biological Agents (Analysis by High-speed Amplification of DNA)

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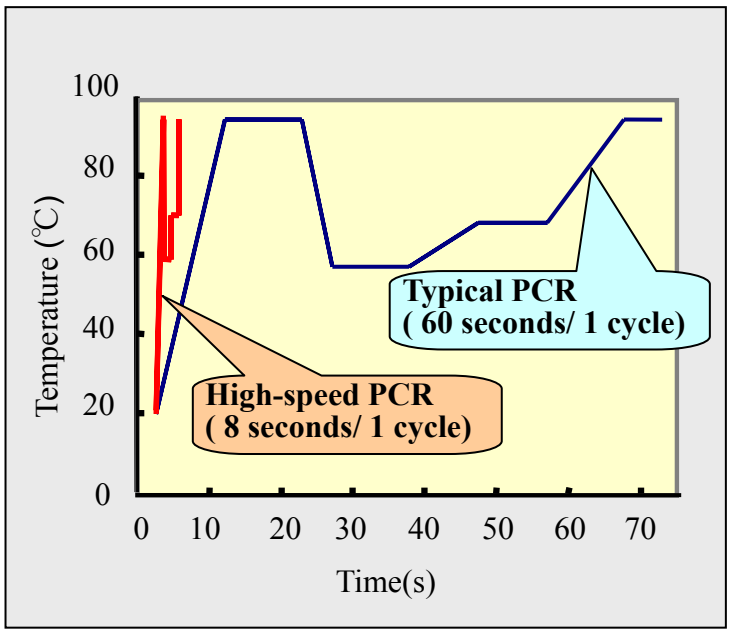
We pay attention to DNA of the microorganism to detect the biological agent, and are developing the system which accurately detects it within 15 minutes using High-speed PCR(Polymerase Chain Reaction).

In case that air sample is collected by biological detection system, the number of biological agent which is included in the collecting sample is very small amount. So it is necessary to increase the number of DNA for identifying the microorganism. There are DNA chip etc. as the method for identifying the microorganism by analysis of the DNA. It is necessary to increase the number of DNA before we identify the microorganism by the DNA chip etc. On the other hand, even if a little amount of DNA is included in the sample, the microorganism can be identified by PCR.

It takes 30 minutes to amplify DNA by typical PCR, but by High-speed PCR it is possible to amplify DNA within 5 minutes. As a result, even if the small amount of DNA of an atmospheric microorganism are included in the sample, it was possible to amplify the target DNA peculiarly and identify the microorganism. We show that it is possible to detect the biological agent rapidly by amplifying DNA of the biological agent using high speed PCR.



Temperature Cycles of High-speed PCR and typical PCR

Environmental Monitoring and Ecological Analysis Controlling the Running of the Facilities for Chemical Weapons Storing and Destruction

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4) Regional Center for Ecological Monitoring, Izhevsk, Russia

The experience gained through the activity for environmental safety surrounding the facilities for chemical weapons storing and destruction located in Udmurt Republic of Russian Federation has been reported. Based on the sampling of background and current level of hazardous chemical components the systematic approach for the evaluation of environmental safety has been performed.

Safety is the crucial factor of the Program of chemical weapons destruction in Russia. Thus, the spending for safety amounts from sixty up to seventy per cent of the overall operational cost of the factory. Following such a policy, the comprehensive system for State ecological control and monitoring of the facilities for chemical weapons destruction has been established according to the Russian Federal law assigning the process of chemical disarmament.

As for the specific factory (#1203, located in Kambarka, Udmurt Republic), the activity for ecological control and monitoring is carried out by the Ministry of Natural Resources of Udmurt Republic (Izhevsk) collaborated with the Research Institute of Industrial Ecology (Saratov). Actual work for environmental analysis has been started several months before the launching of facility. Background level in natural conditions surrounding factory's protected area has been previously analyzed. With the facility putting into operation (March 1, 2006), regulating State control of the emission source is performed.

By the two years period of the implementation of State ecological control and monitoring of the factory #1203, the following basic operations have been outlined:

- The result of analysis of facility's emission source reported quarterly for the specific poisonous pollutants, which are here products of lewisite decomposition (arsenic compounds). As shown the requirement of Maximum Permissible Concentration has been satisfied.
- The sampling of ambient air in the factory's protected area is carried out biannually. Along with poisonous substances and its decomposition products, the level of general pollutants is analyzed. Unlike the proper concentration of poisonous gases, unsupervised daily observations have shown critical atmospheric air pollution by general industrial compounds from December till April due to seasonal activity of civil establishments (heating installations mainly).
- The water resources quality is controlled by sampling of wastewater in spots located 500 meters from the spillway both upstream and downstream along Kama river.

- The quality of underground water resources is controlled by the comparison of sampling the well located within the factory territory and the model one located in similar surroundings but far enough to vouch for the lack of facility running effect.

As a result of described activity, a substantial amount of information has been collected on the quantitative characteristics of spatial and timing dynamic distribution of poisonous and hazardous chemical components and general industrial pollutants. Such a database is used for the implementation of the systematic approach for the evaluation of environmental and human safety surrounding the operation of the facility for chemical weapon destruction. Generally, this direction of investigation is performed in the form of expert system using the GIS (Geographic Informational System) technology with corresponding linking with attributive field parameters for natural resources inventory data and ambient conditions.

Pesticides and Nerve Agents Assay Based On Electrochemical Biosensor

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Electrochemical biosensors based on recognition capability of enzyme acetylcholinesterase were found suitable for organophosphates pesticides and nerve agent detection. Capability of developed method was tested using selected insecticies (methamidophos, paraoxon methyl, paraoxon ethyl) and nerve agents (sarin, cyclosarin, soman, tabun, VX). Screen printed sensors with platinum working and auxiliary, and Ag/AgCl reference electrode were used throughout experiments. Concept of assay was established on following of acetylcholinesterase inhibition by analyte. Acetylcholinesterase digested acetylthiocholine and created thiocholine as cholinesterase reaction product was oxidized on working electrode. Mentioned assay was able to detect as low as 10 nA organophosphate within a quarter hour.

[P09]

Salmonellosis due to *Salmonella Enterica* Serotype Typhimurium DT40 in Sparrows (*Passer montanus*) in Japan.

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2) aboratory of Public Health, Azabu University, Kanagawa, Japan
3) Department of Bacteriology, National Institute of Infectious Diseases, Tokyo, Japan.

This paper reports the first known outbreak of *Salmonella enterica* serotype Typhimurium DT40 infection in Eurasian tree sparrows in Japan.

[History] From December 2005, dead sparrows were observed frequently in a wide area of Hokkaido. The number reached 1,517 by July 28, 2006 (Hokkaido Government Office publication). Several organizations conducted investigations into these specimens, but the cause of death was not elucidated.

[Materials and Methods] We carried out pathological and microbiological examination on 15 sparrows collected in the area where the mass deaths occurred, to elucidate the cause. All samples were examined according to regular pathological methods, and the crop, liver, spleen and intestine were offered for general bacterial and *Salmonella* examination. Isolated *Salmonella* strains were subjected to serotype and phage typing. We also performed drug sensitivity tests and genetic screening by PFGE examination and RAPD analysis. To further assess *Salmonella* derived from the sparrows, we inoculated it into paddybirds, using *Salmonella* derived from a food poisoning patient as control.

[Results] Ingluviitis, splenomegaly and hepatomegaly with white nodules were constantly observed. Bacterial and necrotizing ingluviitis was especially characteristic. Histological findings showed colonization by gram-negative, rod shaped bacteria, and necrotizing lesions were observed in many visceral organs. *Salmonella* were isolated from many organs in all sparrows and were identified as serotype Typhimurium. The isolated bacteria did not show citric acid resolution, and catalase testing revealed only a very weak reaction. The isolates showed an indistinguishable band pattern on pulse-field gel electrophoresis and random amplified polymorphic deoxyribonucleic acid testing, and the same antibiotic resistance profile, and were phage typed as phage type 40(DT40). The control birds did not die, but the birds that were inoculated with DT40 from the dead sparrows suffered disease onset or died.

[Discussion] In Europe, North America and New Zealand, mass death of wild birds such as finches and especially sparrows due to *S. enterica* serotype Typhimurium has been reported and this infectious disease is attracting attention as a cause of sharp decline in populations of these birds. The pathological findings concerning the dead birds in these countries correspond with those of the present case. We considered the situation in Europe and America where there was a mass outbreak in the winter season, and our results indicated that Salmonella Typhimurium was also the cause of the mass death of

sparrows reported throughout Hokkaido. In New Zealand, an increase in human infection by *S. enterica* serotype Typhimurium was seen at the same time as the epizootic causing high mortality in sparrows occurred, suggesting that sparrows were the source of infection in humans. Therefore caution must be taken to guard against *S. enterica* serotype Typhimurium infection in public health, animal health and for conservation of species in wild birds. Phage type DT40, which we detected in the present study, had not previously been isolated from humans and animals in Japan, despite its importance as a cause of mass death of sparrows in Norway, North America and the U.K. We confirmed the high pathogenicity of DT40 based on an infection experiment. The route or mechanism by which this agent entered Japan is not known.

[P10]

A Comparison of Neuroprotective Efficacy of Newly Developed Oximes (K203, K206) and Commonly Used Oximes (Obidoxime, HI-6) in Tabun-Poisoned Rats

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2) Center of Advanced Studies, Faculty of Military Health Sciences, Hradec Kralove, Czech Republic

The neuroprotective effects of newly developed oximes (K203, K206) and commonly used oximes (obidoxime, HI-6) in combination with atropine in rats poisoned with organophosphorus nerve agent tabun at a sublethal dose (180 μg/kg i.m.; 80% LD_{50}) were studied. The tabun-induced neurotoxicity was monitored using a Functional observational battery and an automatic measurement of motor activity. The neurotoxicity of tabun was monitored at 24 hours and 7 days following tabun challenge. The results indicate that only K203 and obidoxime in combination with atropine allow all tabun-poisoned rats to survive within 7 days following tabun challenge while two non-treated tabun-poisoned rats and one tabun-poisoned rat treated with K206 or HI-6 in combination with atropine died within 7 days. Only one of newly developed oximes (K203) combined with atropine seems to be effective for a decrease in tabun-induced neurotoxicity within 24 hours after tabun sublethal poisoning although it is not able to eliminate tabun-induced neurotoxicity completely. On the other hand, the neuroprotective efficacy of commonly used oximes (obidoxime and HI-6) as well as one of newly synthesized oxime (K206) is significantly lower in comparison with K203 according to the number of eliminated tabun-induced neurotoxic signs at 24 hours after tabun challenge. Due to its neuroprotective effects, K203 appears to be suitable oxime for the antidotal treatment of acute tabun poisonings.

The study was supported by the grant of the Ministry of Defense, No MO0FVZ0000501.

Structure-Activity Relationship as a Tool for the Development of New Promising Oximes

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Acetylcholinesterase (AChE; EC 3.1.1.7) reactivators are important group of drugs used in the case of intoxications with highly toxic organophosphorus compounds such as pesticides (paraoxon, chlorpyrifos etc.) and nerve agents (sarin, tabun etc.). After the sarin terroristic attack in Tokyo subway, their development employed many scientists from both - military and civilian sectors. Due to the rapid synthesis and evaluation of the biological activity of many new chemically different structures of new potential antidotes in our laboratories, we would like to discuss relationship between structure of AChE reactivators and their biological activity. Because of wide range of organophosphorus nerve agents and pesticides, we would like to focus only on reactivators of cyclosarin-inhibited AChE. Presented results are based on our in vitro studies with more than one hundred structurally different AChE reactivators, which were conducted during last five years. In this presentation, the main structural requirements influencing reactivation potency of currently available compounds are discussed.

[P12]

Novel Reactivators of Acetylcholinesterase against Paraoxon Intoxication

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3) Centre of Advanced Studies, Faculty of Military Health Sciences, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic

The reactivators of acetylcholinesterase (AChE, EC 3.1.1.7) are very important components in the treatment of intoxications caused by organophosphate inhibitors such as nerve agents and pesticides [1]. These inhibitors covalently bind to active site of mentioned enzyme and irreversibly inhibit its activity. The reactivator breaks the inhibitor-enzyme covalent bond and restores its activity. Unfortunately, there is no reactivator applicable for every type of inhibitor; it means that every structural change in the molecule of inhibitor needs a specific structure of the reactivator [2].

Several series of AChE reactivators have been prepared in the Czech Republic since 2003. Their design was primarily focused on nerve agents; however, they were also excellent reactivators of paraoxon-inhibited AChE [3]. The SAR study of reactivators for paraoxon was developed and will be presented.

[1] Bajgar, J. *Adv. Clin. Chem.* **2004**, *38*, 151.
[2] Marrs, T.C.. *Pharmacol. Therapeut*. **1993**, *58*, 51.
[3] Musilek, K.; Holas, O.; Jun, D. Dohnal, V.; Gunn-Moore, F.; Opletalova, V.; Dolezal, M.; Kuca, K. *Bioorg. Med. Chem.* **2007**, *15*, 6733.

The work was supported by the Ministry of Defence of Czech Republic No. FVZ0000501.

Aflatoxin Assay Using an Acetylcholinesterase Based Biosensors

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The presented study approach acetylcholinesterase (AChE) as a biorecognition element useful for construction of low cost; however, low detection limit analytical devices for aflatoxin (AF) assay. AChE activity can be easily followed by measuring one of the enzyme reaction products: thiocholine that could be either chronoamperometrically oxidized or led to react with Ellman´s reagent resulting in strong shift of absorbance at 412 nm. Microplate photometric assay and amperometric biosensors were chosen as convenient transducers. We have approved feasibility of AChE for analytical application during the first phase of experiments. In the further round, we tested analytical properties of photometric microplates and amperometric biosensors during measuring protocols. Finally, we can proclaimed that obtained limit of detection was under 10 ppb for the both of described methods when solution of AFB1 was assayed. Another way of AChE based biosensor performance was investigation of complexes stability when biosensor was placed into reaction cell. Dissociation of complex AFB – AChE had dissociation rate constant k_{dis} = 0.0047±0.0005 s^{-1}. The half time ($t_{1/2}$) of complex dissociation was 146 s.

[P14]

Molecular Genetical Analysis of Nosocomial Infections Caused by *bla*CTX-M-3-harboring Strains of *Enterobacteriaceae*

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β-lactam resistance profiles of 3,975 nosocomial isolates of *Enterobacteriacea*e, i.e., *Citrobacter freundii* (n=163), *C.koseri* (n=125), *Enterobacter aerogenes* (n=233), *E.cloacae* (n=423), *Escherichia coli* (n=1,100), *Klebsiella oxytoca* (n=288), *K.pneumoniae* (n=789), *Morganella morganii* (n=104), *Proteus mirabilis* (n=66), *Providencia rettgeri* (n=34), *P.stuartii* (n=58), and *Serratia marcescens* (n=496), from inpatients were investigated. Three hundred and twenty isolates (8.2%) showed resistance to third generation cephalosporins (3GC). Of the 226 monobactam-resistant isolates in above 3GC-resistant strains, 35, i.e., *C.freundii* (n=3), *C.koseri* (n=2), *E.aerogenes* (n=2), *E.cloacae* (n=12), *E. coli* (n=2), *K.oxytoca* (n=2), *K.pneumoniae* (n=10), and *S.marcescens* (n=2), expressed a typical ESBL-cefotaximase profile (cefotaxime MIC>ceftazidime MIC) with clavulanic acid synergy. Molecular methods identified 35 ESBL-producing strains harboring a plasmid–mediated CTX-M-3 type ESBL gene *(bla*CTX-M3).

CTX-M-3 型βラクタマーゼ遺伝子保有腸内細菌による院内感染の分子遺伝学的解析

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入院患者から分離された腸内細菌科の細菌 3,975 株、即ち、*Citrobacter freundii* (163 株), *C.koseri* (125 株), *Enterobacter aerogenes* (233 株), *E.cloacae* (423 株), *Escherichia coli* (1,100 株), *Klebsiella oxytoca* (288 株), *K.pneumoniae* 789 株), *Morganella morganii* (104 株), *Proteus mirabilis* (66 株), *Providencia rettgeri* (3 株 4), *P.stuartii* (58 株), *Serratia marcescens* (496 株)のβラクタム薬耐性傾向を調べた。320 株(8.2%)が第 3 世代のセファロスポリン（3GC）に耐性であった。3GC 耐性でモノバクタムに耐性を示す 226 株の内 35 株、即ち、*C.freundii* (3 株), *C.koseri* (2 株), *E.aerogenes* (2 株), *E.cloacae* (12 株), *E. coli* (2 株), *K.oxytoca* (2 株), *K.pneumoniae* (10 株), *S.marcescens* (2 株)がクラブラン酸に阻害される典型的な cefotaximase タイプの ESBL (cefotaxime の MIC>ceftazidime の MIC)を産生することが確認された。これらの株が保有するラクタマーゼ遺伝子について分子遺伝学的解析を行った結果、35 株全てがプラスミド性に CTX-M-3 型 ESBL 遺伝子(*bla*CTX-M3)を保有することが明らかになった。

Medical Measurement against Flue Pandemic-including the social measurement

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Different consideration is necessary against the so-called NBC(Nuclear, Biological and Chemical) Hazard compared with measures against natural disaster.

Nowadays, outbreak by new type (new strain) influenza is feared, in which the casualty number is suspected to increase with parabolic curve.

Measures for maintaining social activity is also thought to be important as well as effective medical system. Our trial is presented.

RESULTS: Measures should be prepared from macroscopic viewpoint in addition to microscopic methods.

Macroscopically, the following points are characteristic: The hardware/mechanical damage is minimal. However, the negative effects for people's activity or workforces are prominent. The area involved will become worldwide. Repeated or recurrent attack will be caused. Therefore, inhibition of social activity will last for long-term, as well as the severe influence in mental/psychological aspects in the public. Early recognition and information system are essential.

Microscopically, it is essential to prepare the necessary sanitary goods as well as to educate hygiene and the preventive medicine for public widely, in order to inhibit/minimize the outbreak.

DISCUSSION and CONCLUSIONS: For measuring outbreak, (1) medical/emergency system, including hospital, police, rescue/fire department, self-defense forces, (2) governmental supporting system should be prepared. Moreover, if it is considered to minimize the long-term influence of outbreak, coordination between (3) the lifeline maintaining organization/company, (4) other enterprises, schools, and systems of local residents' association system should be added. Manuals or guidelines against the outbreak, and drill should be repeated from various different viewpoints.

鳥インフルエンザ・新型インフルエンザに対する医療対応―社会的視点を踏まえて

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NBC(Nuclear, Biological and Chemical Hazard)災害では、地震・風水害等の自然災害における医療とは異なった対応が必要である。

特に、大規模感染症、中でも発生が危惧される新型インフルエンザでは、被害が経時的に放物線状に増大する可能性がある。災害医療対応を考える上でも社会的な視点がより重要と考えられる。我々の取り組みの現状を提示し、問題点、これからのあり方を検討した。

検討結果：自然災害と比較した違いとして、新型インフルエンザによる大規模感染症発生時の大きな特徴としては、巨視的・微視的観点から指摘できる。

被害の形としては、前者として、ハード面での直接被害は極僅かであるが、その規模が世界的なこと、被害の波が繰り返す可能性があること、大規模社会活動休止期間が長期にわたることによる社会の疲弊が想定されること、広範囲の多数の住民に精神／心理学的影響が大きいこと、後者としては、個々の感染を通して速やかな拡大が想定されることから、緊急医療に加え、予防・衛生的な対応が普及するする必要があることがあげられる。

考察・まとめ：新型インフルエンザによる大規模感染症発生時は、(1)医療施設・警察／消防／自衛隊等の医療対応にあたる機関、(2)地方自治体・政府等の公的施設・機関対応、に加え、長期対応を想定した(3)ライフライン関係の機関・企業の準備態勢、(4)その他の企業・学校・地域住民体制等の全ての組織を含んだ協力体制が整備される必要がある。そのための多面的な視点からのマニュアル・ガイドライン作成、訓練の施行が必要である。

Characterization of Highly Pathogenic Avian Influenza Viruses Isolated During 2006-2007 in Myanmar

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In Myanmar, its first highly pathogenic avian influenza (HPAI) outbreak occurred in March 2006, followed by two outbreaks in 2007. Here, we analyzed HPAI viruses of H5N1 subtype isolated in 1st and 2nd outbreaks in Myanmar. Firstly, we performed phylogenetic analysis of the hemagglutinin (HA) gene of four HPAI isolates (A/chicken/Pyigyitagon/204/2006 from 1st outbreak in March 2006, A/chicken/Hmawbi/517/2007, A/guinea fowl/North Okkalarpa/834/2007, and A/quail/Mingalardone/866/2007 from 2nd outbreak in March 2007). It showed that the isolate from 1st outbreak belongs to clade 7 of the classification system created by WHO/OIE/FAO H5N1 Evolution Working Group, whereas all isolates examined from 2nd outbreak belong to clade 2.3.4 (Fujian-like strain). This result showed that genetic origins of HPAI viruses differ between two outbreaks, suggesting that at least two introductions of the HPAI viruses have occurred in Myanmar. Thus, result here suggested the importance of the monitor of cross-border movements of poultry or poultry-related materials to minimize the expansion of HPAI virus exposures. In addition, we examined the pathogenicity of A/chicken/Hmawbi/517/2007 for chickens and domestic ducks. All chickens died by 48 hours after intranasal inoculation of the virus (mortality; 100%), whereas only two ducks died by day 6 and 8 after inoculation (mortality; 25%). Moreover, HA inhibition assay of post-infection serum from surviving ducks revealed that all of the surviving ducks seroconverted by day 14 after inoculation. These results showed that A/chicken/Hmawbi/517/2007 causes lethal infection for not only chickens, but also domestic ducks, suggesting that the control measures on domestic ducks are important in Myanmar to eradicate the HPAI virus from the country.

(Acknowledgements) Authors thank Drs. Win Win Myint, Wai Zin Thein, Ah Mar, Cho Cho Su Mon, L. Tu Ming Gawng, Tin Aye Kyi, Kyaw Sunn (Myanmer LBVD) for providing the isolates used in the study.

[P17]

Characterization and Application of Glycopolymers Carrying Sialyl Lactosamine Repeats for Inhibition against Influenza Virus Infection

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Influenza viruses infect cells through binding of virus hemagglutinins to sialic acid-containing carbohydrates as receptors on the host cell surface. Influenza viruses isolated in humans and other animals seem to have originated in wild birds. Sialic acid-containing carbohydrate molecules expressed on the host cell surface are essential determinants for both cross-species transmission and epidemics in a specific host. Influenza viruses recognize sialic acid linkages in the receptors, such as sialyl lactosamine (sialyl LacNAc) structures, sialylα2-3/6Galα1-3GlcNAcβ1-R and sialylα2-3/6Galβ1-4GlcNAcβ1-R.

To control cross-species transmission of influenza viruses, it is necessary to elucidate the mechanisms of the interaction between influenza viruses and sialo-glycoconjugate receptors expressed on different hosts. Inhibitors containing receptor carbohydrate determinants that prevent virus entry may be useful tools to address such issues. We synthesized and characterized sialyl glycopolymers carrying lactosamine repeats as influenza virus inhibitors. We also applied such glycopolymers carrying multivalent sialyl LacNAc oligosaccharides for further investigation of the molecular mechanisms underlying the interaction of influenza viruses with different species through specific carbohydrate structures.

Small Exotic Animals as Potential Reservoirs of Zoonotic *Bartonella* Species

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[Objectives] Last decade, it has been reported that zoonotic *Bartonella* species were isolated from various small mammals in a number of countries. With globalization, many wild and captured animals have been traded as pets throughout the world. Therefore, we investigated the prevalence of *Bartonella* species among imported small animals whether these animals may serve as the reservoirs of the organisms or not in Japan.

[Materials and Methods] The prevalence of *Bartonellae* was bacteriologically investigated in 546 mammals (6 families including 23 genera, and 28 species) imported as pets from Asia (China, Thailand, and Indonesia), North America (U.S.A), Europe (Netherland and Czech), and the Middle and Near East (Egypt and Pakistan) to Japan. A phylogenetic analysis was performed by DNA sequences of citrate synthase gene (*gltA*) of the isolates.

[Results] Of the 546 mammals examined, 367 animals were wild and captured and 179 animals were bred and maintained in breeding facilities. In total, 137 (37.3%) wild animals were infected with *Bartonellae*, while only 5 (2.8%) domesticated animals harbored the organisms. A total of 407 isolates obtained was classified into 53 sequencing types (STs) based on *gltA*, and classified into 11 groups, including 4 related groups with the type strain of *B. washoensis*, *B. grahamii*, *B. elizabethae*, and *B. clarridgeiae* by phylogenetic analysis. The sequence homologies between the isolates and each related type strain of *Bartonella* species showed 94.6% to 97.4% for *B. washoensis*, 98.4 to 98.7% for *B. grahamii*, 95.5% to 100% for *B. elizabethae*, and 96.2% for *B. clarridgeiae*, respectively. The other 7 groups were distantly related to any existing *Bartonella* species. *B. washoensis*-, *B. grahamii*-, and *B. clarridgeiae*-like bacteria were obtained from only family *Sciuridae*, while *B. elizabethae*-like bacteria were isolated from different three families such as *Sciuridae*, *Muridae*, and *Dipodidae*. Of the 142 *Bartonella* positive animals, 25 animals were found to be co-infected with different STs of *Bartonella* species.

[Conclusions] We revealed that imported pet animals carried several *Bartonella* species with a high rate including probably 7 novel species and may serve potential new reservoirs of zoonotic *Bartonella* species in Japan.

[P19]

Detection of the Borrelia Burgdorferi Sensu Lato and Anaplasma Phagocytophilum in Host-seeking Adult Lxodes Ricinus Ticks Collected in Serbia

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Lyme borreliosis and human granulocytic anaplasmosis (HGA) are tick-borne infections. To evaluate prevalence rate of these pathogens, a total of 287 unfed adult Ixodes ricinus ticks were collected from vegetation in 2001, 2003, and 2004 at 18 localities throughout Serbia. Using PCR technique, we detected species-specific sequences, rrf-rrl rDNA intergenic spacer for Borrelia burgdorferi sensu lato and p44/msp2 paralogs for Anaplasma phagocytophilum, respectively, in total DNA extracted from the ticks. These prevalence rates were that for B. burgdorferi sensu lato (42.5%) and A. phagocytophilum (13.9%). The presence of five B. burgdorferi sensu lato genospecies, namely, B. burgdorferi sensu stricto, B. afzelii, B. garinii, B. lusitaniae, and B. valaisiana was identified by restriction fragment length polymorphism (RFLP) analysis. The most frequent B. burgdorferi sensu lato genospecies was B. lusitaniae, followed by B. burgdorferi sensu stricto. Coinfection by B. burgdorferi sensu stricto and B. lusitaniae was the frequently observed among ticks infected with B. burgdorferi sensu lato genospecies. Coinfection by B. burgdorferi sensu lato and A. phagocytophilum appeared in 24 ticks. Sequencing of p44/msp2 paralogs of Serbian A. phagocytophilum showed that they were unique and distinct from those of A. phagocytophilum in other countries. These findings indicate a public health threat in Serbia of tick-borne diseases caused by the B. burgdorferi sensu lato and A. phagocytophilum.

Characterization of Rickettsial DNA (Spotted Fever Group) From Ticks Collected In Kagoshima Prefecture, Japan

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In 2007, 251 ticks were collected by flagging method in Kagoshima prefecture, Japan. These ticks were dissected and the DNA was extracted from salivary glands of the individual ticks. PCR was performed using *gltA* primers specific for spotted fever rickettsiae in each of tick specimens. Of 251 ticks, 16 were PCR-positive (6.3%): 7 of 21 *Haemaphysalis hystricis* (positive rate 33%), 2 of 140 *H. formosensis* (1.4%), one of 9 *H. flava* (11%), and 6 of 12 *Amblyomma testudianruim* (50%). None of *H. longicornis* were positive (0/69 ticks). Sequence and phylogenetic analyses based on *gltA* revealed that (i) rickettsial DNAs from *H. hystricis* were closely related, but not identical, to *Rickettsia montanensis* (similarity 99%), (ii) DNA from *H. formosensis* was far from any other rickettsia species (similarities 93 to 96%), and (iii) DNAs from *A. testudinarium* were identical to that of *R. tamurae*. The rickettsial *rompA* and 16S rDNA amplified from *H. hystricis* were further analyzed. The results showed that the amplified DNA sequences from *H. hystricis* were located distantly from any other rickettsia species sequences (similarities 87 to 91 %) in the phylogram of *rompA*, and were closely related to *R. rickettsii* (similarity 99%), *R. massilliae* (99%), and followed by *R. japonica* (98%) in the tree of 16S rDNA.
In Kagoshima prefecture, it is one of endemic areas for Japanese spotted fever. However, in this study, *R. japonica* which is causative agent of Japanese spotted fever was not detected. Taken together, this study suggests that rickttsiae from *H. hystricis* and *H. formosensis* seems to be new spotted fever group rickettsiae in Japan and may be a new causative agent of Japanese spotted fever.

[P21]

Leptospirosis in Squirrels Imported from United States to Japan

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Leptospirosis is a worldwide zoonosis caused by infection with Leptospira interrogans sensu lato species. Leptospira is mostly transmitted to humans through contaminated water or soil and by direct contact with a variety of infected animals. To date, a variety of wild animals have been imported from foreign countries to Japan.

In this study, 2 men working at an animal trading company were infected with Leptospira spp. Leptospira isolates from 1 patient and 5 of 10 squirrels at the company were genetically (flagellin gene and DNA gyrase B subunit gene sequences) (Fig 1) and serologically identical and were identified as Leptospira kirschneri.

These findings show that exotic pets represent a substantial hazard. The outbreak demonstrated how new infectious diseases could be emerging because of importation from overseas. If, during shipping and housing of the animals, the infection were to have expanded among southern flying squirrels, the infection rates and risk for humans would have increased. The leptospirosis cases reported here warn against importing exotic animals.

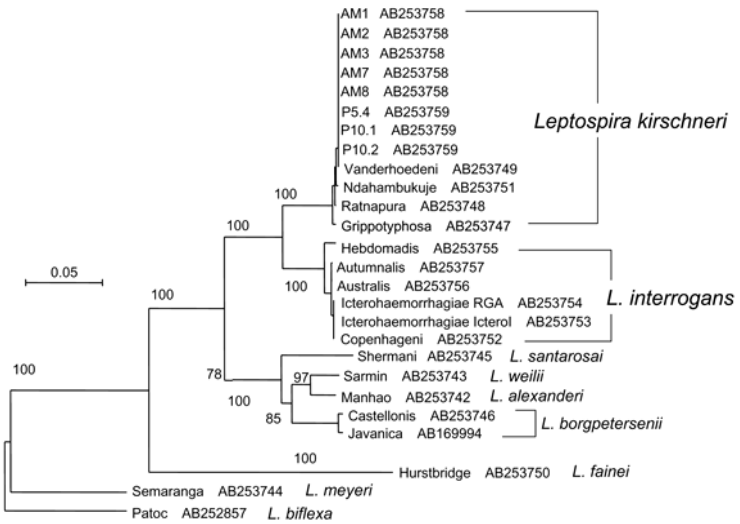

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Figure 1. Phylogenetic tree based on the *Leptospira* DNA gyrase B subunit gene (*gyrB*) sequence. The sequences obtained have been deposited in DDBJ/GenBank/EMBL with accession numbers indicated.

Mutation mechanism of the highly pathogenic avian influenza virus into humans

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The highly pathogenic avian H5N1 influenza A viruses have spread to numerous countries in Asia, Europe and Africa, infecting not only large numbers of chickens and related poultry, but also an increasing number of humans, with lethal effects. In this context, infection and transmission mechanism of the virus into the avian, and other animals including humans must be elucidated in order to construct the safety for poultry food. We confirmed that 1) Chicken and Quail intestine express human type influenza virus receptors *(N*-linked Neu5Ac2-6Gal-sequences). Therefore, chickens and quails in the market have molecular character as an intermediate host for H5N1 transmission to humans, and could generate new influenza viruses with pandemic potential. 2) Amino acid substitution in H5N1 avian virus Hemagglutinin, Ser227Asn; Glu75Lys, Leu123Pro, Asn193Lys; Leu129Val, Ala134Val; Gln192Arg, Asn182Lys are responsible to bind human type receptor (Neu5Ac2-6Gal-sugar chains). We have characterized several native highly pathogenic H5N1 strains which bind to human receptor with pandemic potential. 3) Global surveillance of the mutation of receptor binding specificity of highly pathogenic avian influenza viruses (H5N1, H9N2 and H7N7) is necessary.

[P23]

A Study on the Preventive Law for Influenza (H5N1) In Japan

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Establishment of effective control measures against influenza (H5N1) pandemic is of urgent need all over the world. Damage and influence by infectious disease are different among nations and law functions as a control system only for domestic risks in each nation. Therefore, we need a global standard to decrease risks of pandemic diseases. In Japan, risks of influenza (H5N1) is estimated to be "class 2" in the preventive law, so-called '*Kansensho-Ho*', in accordance with the WHO's regulation. Influenza (H5N1) pandemic is estimated to affect at least 4 million citizens (30% of the population) in Tokyo. However, we are facing the absolute lack of the preparedness. For example, there are 34,000 medical doctors, including all fields, but only 12 hospitals (92 beds) are authorized for quarantine of patients in the class 2 category. Thus, from practical views of public health, the quarantine and prevention systems of Japan appears apparently insufficient. In terms of well-coordinated preparedness for pandemic in Japan, a variety of risks should be properly recognized and classified. We present ideas to decrease risks of the pandemic, including improvement of the corresponding laws.

Detection of *Leptospira* Antigen by ELISA and Immunochromatographic Assay

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[Introduction] Laboratory test of leptospiral infection is based on either isolation of the pathogen from the specimen or the detection of specific serum antibodies. The pathogen isolation is laborious and expensive and may not be successful. And the antibody detection is not effective during the early period of infection, but once the antibody is produced, its presence continues for a long time. The aim of our study is to develop an assay method for detecting the *Leptospira* antigen in urine for early diagnosis of leptospiral infection. This time, we report the preliminary study on the *Leptospira* antigen detection by the anti *Leptospira* antibody-based ELISA and immunochromatographic assay (ICA).
[Materials and Methods] The whole organism sonicates of *Leptospira* spp. and urine samples collected from the mice that were inoculated whole organism of *L. interrogans* serovar Autumnalis were prepared. ELISA was carried out by conventional method using HRP-labeled antibody and 3, 3', 5, 5' -tetramethylbenzidine as a substrate. ICA device was constructed by manual procedure. ELISA and ICA were used for detecting *Leptospira* antigen, using anti-*L. biflexa* antibody. Real-time PCR amplification of the flagellin gene (*flaB)* was performed to determine the cell number in urine sample.

[Results and Discussion] The minimal concentration of *Leptospira* antigen in PBS which could be detected by ELISA and ICA was 40 ng/mL and 1,000 ng/mL, respectively. 25 of 50 (50%) urine samples could be determined the cell number in a range from 10^3 to 10^7 cells per mL by real-time PCR. However ELISA was positive for 46 of 50 (92%) urine samples, and there observed discrepancy between results of real-time PCR and ELISA. It was suggested that some soluble antigens came out in the urine collected from infected mice, and then ELISA was more sensitive than real-time PCR as a result. ICA was positive for 19 of 50 (38%), and its sensitivity was low. It was indicated that ICA, without washing process, was susceptible to the effect of interference components in urine.

[Conclusion] Both ELISA and ICA are able to detect *Leptospira* antigen in urine. But further improvement is needed to establish a rapid and simple assay method for the testing of leptospiral infection.

[P25]

Treatment of Implants in the Oral Surgery Which Prevents Medical Malpractice

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Recently, treatment of an inn plant in the oral surgery is popularized remarkably, and generally popularized. A Wide is in the adaptation disease worldwide due to the material and the progress of the technique. However, the report of the medical malpractice along with this is on the increase, too. Report a method in consideration of the operating plan that safety was pursued to this.

医療過誤を防ぐ口腔外科におけるインプラント治療

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Ⅰ目的: 近年, 口腔外科におけるインプラント治療は目覚しく普及し, 一般的に普及している. 世界的にも適応症は材料とテクニックの進歩によって広がっている. しかしながら, これに伴う医療過誤の報告も増加している. これに対して, より安全性を追求した手術プランを考慮した方法を報告する.
Ⅱ概要: インプラントの手術において、その埋入位置、方向、角度や深さは重要な因子となる. 我々は, 迅速かつ効果的により高精度なインプラントの埋入を行うために, 誤差補正機能を有する 3D - CT ナビゲーションシステムを用いたオリジナルサージカルガイド(Kis-System)を作製した.
Ⅲ結果: ガイドシステムを使用したインプラント手術は良好であり, 開発後 10 年を経過するが医療過誤に発展するような問題もなく経過している.
Ⅳ考察と結論:インプラント関連メーカーから様々なサージカルガイドが販売されている. 中には大きな誤差によって手術中にトラブルが生じる報告も耳にする. 我々の CT データの誤差補正機能を用いたオリジナルサージカルガイド(Kis-System)はインプラント手術を安全に行えた.

Measures to Prevent Medical Malpractice Related to Radiopharmaceuticals

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Radiopharmaceuticals are defined as “radiation emitting drugs” in the ministerial ordinance Regulations for Manufacturing and Handling of Radiopharmaceuticals of the Ministry of Welfare (current Ministry of Health, Labour and Welfare). They are offered in radiation shielding containers to prevent exposure to radiation. Differently from ordinary pharmaceuticals, it is difficult to directly read the labels on the vials and syringes. Therefore, we offer the following measures to prevent mix-ups while preventing exposure to radiation in hospital settings.

The representative forms of supply of radiopharmaceuticals are vials, syringes, and freeze-dried products that are prepared in hospital.

Vial products are becoming less common in order to prevent exposure to radiation during the transfer to disposal syringes in the hospital. When transferring radiopharmaceuticals into disposal syringes, we use peel-off labels taken from the product's lead container and attach the labels to the surface of dedicated tungsten containers to accommodate and shield the disposal syringes containing the drug. In addition, we offer dedicated product name stickers for the disposal syringe rods.

Syringe products are most commonly used as pre-filled syringes. Compared to vial products, syringe products are effective in preventing exposure to radiation because the preparation does not involve the transfer of drug solutions. The drugs can be easily administered simply by attaching a syringe rod ans a needle to the tip of the syringe. Products of this type are often taken out of the lead container beforehand for well-timed administration. Therefore, in order to prevent mix-ups of the syringes (with tungsten shielding) after taken out of the containers, the product name and radiant quantity are shown on the plastic parts that fix the tungsten shield and the syringe. We also provide dedicated product name stickers to attach to the syringe rods.

Finally, freeze-dried products for in-hospital preparation are prepared (radiolabeling) in hospital settings using separately supplied ^{99}Mo-^{99}mTc generators. The preparation is done basically by mixing. If the products are heated or mixed in a fixed order, we provide dedicated heaters or marketing equipment to ensure correct preparation. Also, we provide dedicated product name stickers to be attached to the rod of the disposal syringes to which prepared drug solution is transferred.

[P27]

Statistical Consideration of Incident Report Case in Dental Medic

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I considered dangerous frequent occurrence time and other things in dental medical treatment from the result of the analysis based on incident reports of about 200 people

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歯科医療従事者におけるインシデントレポート事例の統計的考察

松元 長泰

まつもと歯科クリニック，東京，日本

約 200 名のインシデントレポートをもとに分析した結果を踏まえて、歯科医療において危険な多発時間などを考察した。

It Fries Medical Care Cooperation and A Second Opinion About The Oral And Maxillofacial Surgery

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In the oral and maxillofacial surgery treatment importance of the medical care cooperation and the second opinion is pointed out from the difference of the treatment with the primary care organization (the dental office)

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