# ルナパス・ウイルスセミナー

日時；2024 年 10 月 25 日(金)
会場：浜松コングレスセンター4 階 41 会議室

## （第 1 部）ワクチン・ウイルスベクターの最新評価方法

座長　小出　英孝 Southern Research

**13:00 生ワクチン開発の神経毒力試験の実際**
講師　Dr. Joseph Randall, Southern Research
**13:35 ウイルスベクター治療の可能性探求**
講師　Dr. Jennifer Pickens, Southern Research
**14:10 最新の抗ウイルス/ワクチン評価**
Human 3D culture assay
In vivo 試験のアップデート
講師　小出　英孝, Southern Research

## （第 2 部）進化するウイルス安全性評価

座長　安井　文彦　東京都医学総合研究所

**14:40 ICH-Q5A(R2): A new era for biosafety testing**
講師　DI. Jürgen Ebner, ViruSure
**15:10 NGS: Implementation as an alternative to traditional adventitious agent testing**
講師　Dr. Andy Bailey, ViruSure
**15:30 Rapid and Precise: GMP characterisation of viral vectors using Electron Microscopy**
講師　Dr. Ashley Stephen Layland, neotem Bioanalytics
**15:50 Digital PCR testing and biosafety testing**
講師　Dr. Andy Bailey, ViruSure

**（ご案内）第 31 回日本遺伝子細胞治療学会学術集会**
**第 72 回日本ウイルス学会学術集会**

## Session 合同テーマ

座長　岩田　聖　ルナパス毒性病理研究所

**16:20 ワクチン・抗ウイルス薬の安全性評価そして未来**
講師　小野寺　博志　国立医薬品食品衛生研究所

LunaPath
ルナパス毒性病理研究所

# 要旨：

## 第 1 部）ワクチン・ウイルスベクターの最新評価方法

### 13:00 生ワクチン開発の神経毒力試験の実際
### Neurovirulence Testing of Live Viral Vaccines
### 講師 Dr. Joseph Randall, Southern Research


Abstract:

Southern Research has been at the forefront of testing the safety and efficacy of novel vaccines for over two decades. SR has conducted many biodistribution, immunogenicity, and GLP safety studies for SARS-CoV-2, yellow fever, influenza and many other vaccines in support of INDs and BLAs. The development and safety of vaccines for infectious disease has received a lot of public attention in the past few years due to the SARS-Cov2 pandemic. We have a team of experienced virologists and toxicologists to evaluate the safety and efficacy of vaccines. We continue to invest in key capabilities to evaluate vaccine safety, including the use of rodent and an alternative to NHPs. Confirmation of attenuation of pathogenic traits is necessary for the safe use of potentially neurotrophic vaccines.

The monkey neurovirulence test (MNVT) is the gold standard for neurovirulence safety assessment of oral polio vaccines and has been used for 75 years for lot specific release testing. The MVNT bioassay is also required for testing new vaccine seed lots for live attenuated neurotrophic viruses when there is a change to manufacturing. The dosing procedure involves direct injection of a live virus into the central nervous system and to evaluate neurobehavioral effects and histopathology changes in the brain.
We have validated the MVNT neurovirulence model and have demonstrated the safety of live attenuated viral vaccines for yellow fever and dengue fever. In one such study, we evaluated the neurovirulence of master and working seed lots of 17D viruses for yellow fever in comparison to Stamaril™ and the WHO 17D 168-73 reference vaccine (Tyagi et al, 2023). Endpoints for neurovirulence included changes in clinical scores and microscopic evaluation of the brain and spinal cord. Viral replication in blood was detectable on days 2 and 4, but not on day 6. The yellow fever master and working seed viruses and final vaccine were non-neurotrophic and comparable to the live attenuated WHO 17D 168-73 vaccine.

No single species or test can predict the neurovirulence of all live viral vaccines. SR supports the refinement of animal use and to find alternatives to the MVNT. We have conducted a pilot neurovirulence study in 4-day old suckling CD-1 mice and demonstrated neurovirulence 7-8 days after intracranial administration of WHO 17D 168-73. The higher sensitivity of neonatal mice was expected based on a report in the literature (Fulton and Bailey, 2021). There was 100% neurovirulence in suckling mice at a dose of 50 plaque forming units. We recommend including positive controls in rodent neurovirulence studies to demonstrate neurotrophic differences between wild type or approved

vaccines and novel live attenuated viral strains.

Rodent models of neurovirulence testing are gaining acceptance as an alternative to the MNVT assay. For example, a transgenic knock-in mouse model of neurovirulence that expresses human polio receptors was validated in conjunction with the World Health Organization (Dragunsky et al, 2003). In addition, a neonatal rat model of mumps vaccine neurovirulence has been validated (Rubin et al, 2000). The European Medicines Agency accepts neurovirulence data from mouse influenza models in lieu of the MVNT. Please contact the Departments of Toxicology and Infectious Disease for more information on non-clinical vaccine safety testing.

https://southernresearch.org/services/toxicology/

## ANIMAL MODELS

- Golden Syrian Hamsters
- Rodents and Ferrets
- New Zealand White Rabbits

## *IN VIVO* SERVICES

- Biodistribution and Immunogenicity
- General Toxicology After Repeated Dosing
- Neurovirulence Testing in Mice or NHPs
- Evaluation of Effects on Embryofetal Development

## *IN VITRO* SERVICES

- RT-qPCR
- TCID50
- Analysis of Cytokines (ELISA, Luminex®)
- Histological & Pathology Evaluation
- ELISA for Immunogenicity and Anti-drug Antibodies
- Histopathology & Immunohistochemistry

Stereotaxic injections into brain for neurovirulence and oncolytic viruses

Bilateral Stereotaxic Injections into the Thalamus

## 13:40 ウイルスベクター治療の可能性探求
## Exploring the Therapeutic Potential of Viral Vectors
### 講師 Dr. Jennifer Pickens, Southern Research

Abstract:

There has been a surge in vaccine development research in the wake of recent health threats that include the COVID-19 pandemic, the 2022 Monkey Pox outbreak, and H5N1 highly pathogenic influenza zoonotic transmission this year (2024). The research is aimed at identifying vaccine candidates that exhibit optimal safety profiles across a wide age range; elicit robust and durable cellular and humoral immunity; and are easy, low cost, and scalable to manufacture/distribute. Vaccines come in many varieties from killed, live-attenuated, mRNA, DNA, and viral vectored to name a few. The primary vaccinology approach centers around creating a vaccine that immunologically protects against disease, reduces transmission, and establishes herd immunity. First genetically engineered in 1972, viral vectors remain a promising vaccine platform because they are capable of heterologous expression of antigens without replication, thus inducing a robust and durable humoral and cellular immune response. Various viruses have been used as viral vectors, including but not limited to influenza, lentivirus, retrovirus, measles virus, vesicular stomatitis virus (VSV), and adenovirus. Southern Research (SR) has been instrumental as a preclinical CRO, partnering with government and commercial clients to assess the therapeutic potential of their viral vectored vaccines in small and large animal models. In this seminar, SR will review the preclinical approach to better understand the therapeutic advantage of viral vectored vaccines and review current case studies and data to support advancing these from concept to the clinic.

## 14:20 最新の抗ウイルス/ワクチン評価
## Human 3D culture assay
## In vivo 試験のアップデート
### 講師 小出英孝, Southern Research

Abstract:

SR, chartered in 1941, is one of the oldest independent, not-for-profit research institutes in the United States. For more than 80 years, SR's scientists have addressed many of the most urgent and challenging technological problems of our age. By responding to the needs of private industry and addressing global issues in health, national defense, and the environment, SR has contributed greatly to providing technical solutions in a broad range of areas.

To support its mission of providing sponsors and clients with the highest quality research, SR has disinvested from its Engineering and Energy & Environment divisions, as well as its Fredrick, Maryland,

location, raising more than $150 million to invest in the construction of a 70,000-square-foot flagship building and the renovation of 40,000 square feet of the organization's existing facility, including new biosafety level 3 (BSL-3) space. SR focuses mainly on life science research through **drug discovery and drug development services.**

SR operates in full compliance with federal Good Laboratory Practice (GLP) and animal welfare regulations, and its animal facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

SR has supported US government Operation Warp Speed program under Biomedical Advanced Research and Development Authority (BARDA) funding, entitled "Development of Animal Models for Evaluation of Medical Countermeasures (MCMs) for SARS COV-2" and supported the federal countermeasures acceleration effort. SR has conducted more than $30 million in coronavirus research over the past three years, including support of Phase 1-3 COVID-19 vaccine clinical trials, as well as conducting thousands of HTS screening and refining the underlying chemistry of critical treatments.

Here, we present the update on our latest infectious disease services including Omicron XBB.1.5, Highly Pathogenic Avian Influenza (HPAI) and human metapneumovirus (HPMV) models.

LunaPath
ルナパス毒性病理研究所

# 第 2 部）進化するウイルス安全性評価

## 14:40　ICH-Q5A(R2): A new era for biosafety testing

講師　DI. Jürgen Ebner, BD Manager, ViruSure

Abstract:

The revised ICH Q5A(R2) guideline, a cornerstone for virus safety evaluation of biotechnology products, signals a transformative phase in biosafety testing. This presentation will explore the key updates, including the incorporation of new products, new technologies, innovative approaches in biosafety testing for cell line characterization and bulk harvest testing, and advancements in virus clearance studies.

## 15:10　NGS: Implementation as an alternative to traditional adventitious agent testing

講師　Dr. Andy Bailey, CEO, ViruSure

Abstract:

Revision 2 of the ICH Q5A guideline on Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin discusses in depth the expectations for meeting virus safety requirements in the modern era. This presentation will explore some of the practical implications and strategies that can be applied when endeavouring to meet these virus safety requirements. Using case studies, it will explore various aspects, including how to best control risk in raw materials and biological materials as well as introduce how next generation sequencing (NGS) can be effectively integrated into these control strategies.

## 15:30　Rapid and Precise: GMP characterisation of viral vectors using Electron Microscopy

講師　Dr. Ashley Stephen Layland, Project Director, neotem Bioanalytics-IIT GmbH

Abstract:

The advent of transmission electron microscopy (TEM) marked a pivotal moment in the history of microbiology, as it became the first technique capable of visualising viruses in their natural state. This

groundbreaking discovery has had a profound impact on our understanding of these enigmatic particles. In the present era, TEM continues to be a cornerstone of the biopharmaceutical industry, playing a pivotal role in ensuring the safety of biologics.
This presentation will highlight the critical role of TEM in GMP-quality assessment, where its unparalleled resolution allows for a rapid and detailed investigation of vaccine products and viral vectors, including Adeno-Associated Viruses (AAV) ensuring product safety and efficacy. By facilitating the ultrastructural investigation of viral vectors and enabling the determination of key parameters such as particle integrity, morphology, distribution and filling grade (full/empty ratio), TEM continues to play a crucial role in the comprehensive characterisation and quality assurance of viral vectors.

## 15:50　Digital PCR testing and biosafety testing

講師　Dr. Andy Bailey, CEO, ViruSure

Abstract:

Digital PCR (dPCR) is an advanced molecular biology technique used for the precise and absolute quantification of nucleic acids (DNA or RNA) by end-point analysis and Poisson statistics. Unlike qPCR assays, which provide relative quantification in relation to a standard curve, dPCR divides the sample into thousands of individual partitions. Each partition is analyzed for presence (positive) or absence (negative) of a fluorescent signal, allowing for the detection and counting of single DNA or RNA molecules. With its high sensitivity and precision, coupled with reduced susceptibility to inhibitors, it can be an essential tool in biosafety testing: viral load quantification, residual host cell DNA quantification, copy number variation, genetic stability and titre determination in cell and gene therapy products. Digital PCR provides precise and absolute quantification of nucleic acids. Therefore, its usage in biosafety testing ensures consistently higher qualities in final products and this presentation will highlight how and where this testing can used effectively to support product safety.

## 演者紹介：

### Joseph Chase Randall, PhD.
### Southern Research

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|  | Joseph has more than 25 years of drug development experience in the design, conduct and reporting of regulated toxicology studies in support of IND and NDA applications for pharmaceutical and biotechnology companies. Areas of therapeutic expertise include the safety assessment of antivirals, vaccines, and drugs to treat cancer and neurodegenerative diseases. He has developed liposomal nanoparticle formulations and vascular targeting agents in oncology. His current focus is on central nervous safety of live viral vaccines and oncolytic viruses and the reproductive safety testing of novel vaccines and genetic therapies for rare diseases. |

### Jennifer Pickens, PhD
### Director of Infectious Disease Research, Southern Research

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|  | Dr. Pickens has over 15 years scientific leadership experience, where she is an infectious disease and preclinical drug development subject matter expert (SME) with a focus on the advancement of cutting-edge and first-in-class vaccine and therapeutic medical countermeasures in the nonclinical and clinical setting. Before joining Southern Research in 2019 and again in 2022, she earned her Ph.D. in Infectious Diseases from the College of Veterinary Medicine at the University of Georgia in 2011 under the mentorship of S. Mark Tompkins and later completed postdoctoral fellowships (2012-2017) within the laboratories of James E. Crowe, Jr at the Vanderbilt Vaccine Center and Ralph A. Tripp at the University Georgia. Her career has focused on managing preclinical research programs aimed at identifying and assessing the efficacy of novel antiviral therapeutics against potentially lethal respiratory viruses (i.e. respiratory syncytial virus (RSV), metapneumoviruses (hMPV), SARS-CoV-2 and influenza). During the COVID-19 pandemic, her work focused predominately on developing the nonclinical commercial and government A/BSL-3 SAR-CoV-2 program at Southern Research before joining the Biomedical Advanced Research & Development Authority (BARDA) in 2021 as |

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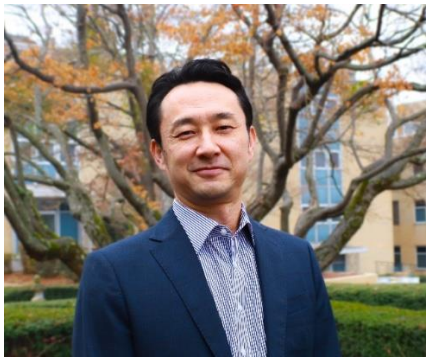
| | a nonclinical and infectious disease SME/Biologist. She returned to Southern Research in 2022 and is the current Director of Infectious Disease Research. She works alongside commercial and government clients providing scientific and operational guidance through the lifecycle of their pre-IND pipelines. |

## Fusataka Koide
## Senior Director, Global Client Engagement, Southern Research



Fusataka Koide, M.S., is the Director of Global Client Engagement at SR and managing the groups of Proposal writes, Business Development and Marketing team. His responsibilities include tracking and oversight of proposal activity and progress, market assessment, development of the strategic business plan, incorporating marketing and sales strategy for each capability area, and building robust processes that allow the division to meet its financial goals.

Mr. Koide also serving as the vaccine and animal model subject matter expert for SR. Mr. Koide has over 20 years of experience in vaccine animal model development including 14 years of direct experience with nonhuman primate (NHP) infection and vaccine/antiviral testing. His experience in NHPs and small animal models for evaluation of vaccine covers a wide range of infectious diseases including SARS-CoV-2, Monkeypox (MPXV), Rabbitpox (RPXV), HIV/SIV, Chikungunya, MRSA, plague, HPAI, Dengue, Zika, and Yellow Fever. Specific examples of the vaccine studies include efficacy evaluation of live attenuated tetravalent dengue vaccine in Cynomolgus and Rhesus Macaques (GLP and non-GLP), Dengue vaccine neurovirulence and vaccine viscerotropism studies in Cynomolgus Macaques (WHO TRS protocols) and efficacy evaluation of protein-based Smallpox Vaccine in Cynomolgus monkeys challenged with Monkeypox Virus (FDA Animal Rule). His work has included analysis of humoral and cell-mediated immune responses in vaccinated and/or infected animals with an emphasis on attempting to identify correlates of immune protection against disease. Many of these efforts have been supported by the NIH's NIAID/DMID. Mr. Koide was a PI for

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| | supporting NIAID COVID-19 Task Order B08 (Contract No. HHSN272201000022I) entitled “Development and Use of a Non-Human Primate Model of SARS-CoV-2 Infection”. He has also served as PI for another COVID-19 Task Order for BARDA BIO-AMT 1021 (Contract No. HHSO100201700018I) entitled “Development of Animal Models for Evaluation of Medical Countermeasures for SARS-CoV-2”.<br>As the leader of Southern Research’s vaccine development program, Mr. Koide provides support to many clients in the form of preclinical efficacy testing and reports that support IND applications submitted to the U.S. FDA. |

**Jürgen Ebner**
**BD Manager, ViruSure**

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|  | Jürgen Ebner studied food- and biotechnology at the University of Natural Resources and Life Sciences. He joined ViruSure in 2009 and gained a lot of experience in biosafety testing of biopharmaceuticals. His area of expertise comprises the design and execution of Viral- and Prion Clearance Studies. |

## Andy Bailey, PhD
## CEO, ViruSure

Originally a chemist/biochemist, Andy later specialised in Virology serving for 9 years at the MRC Virology Unit in Glasgow, Scotland, studying Herpesviruses and Adenoviruses. In 1995, he moved to the industry sector, initially as Director of Virus Validation services with Q-One Biotech Ltd, and later at the Pathogen Safety group of Baxter Healthcare in Vienna, Austria.

Over the last 25 years Andy has been actively involved in the virus and prion safety field, presenting at numerous regulatory agencies and conferences either in support of product registration or as an invited speaker at expert workshops and conferences. This has included presentations at the UK MHRA, German PEI, French AFFSAPS, US FDA, EMEA and PMDA (Japan) supporting regulatory submissions for various products. He has extensive experience in regulatory affairs and virus safety issues. He currently serves as an external expert for the EU SCENIHR committee on emerging human health risks, and continues to be a frequent presenter at international biopharmaceutical conferences.

In 2005 he founded ViruSure with the goal of providing a high quality testing service to the biopharmaceutical industry, a philosophy which remains a key focal point of the company.

## Ashley Stephen Layland, PhD
## Project Director, neotem Bioanalytics-IIT GmbH

Graduating as a research assistant in 2011, Ashley Stephen Layland began his career in cell culture technology at Xell AG (which is now part of Sartorius) where he acquired eight years of experience in upstream processing and cultivation. From 2012, Ashley then gained further in-depth experience in quality assurance management, virology and electron microscopy from Charles River Laboratories and the Centre for Ultrastructural Diagnostics located at the University of Bielefeld. Fast forward to 2022, Ashley assumed the position of project director at neotem Bioanalytics and now leads a GMP-certified company, applying electron microscopy for

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| | the viral safety assessment of biologics and the characterisation of virus products. |