

### 3Rs in a globalised world: EU Manufacturer's point of view

#### Several methods and several approaches required by several countries

- Different methods approved by the NRA
  - Ph. Eur, US, MRBP
  - CHPh
- Several approaches required by Authorities
  - Each commercial lots
    - WHO, MRBP, ChP
  - Significant number of commercial lots + waiving
    - USA
  - Until proof of manufacturing consistency
    - Ph. Eur
  - In the Ph Eur, request for ATT has been deleted in the late 90's for release
    - « the production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity »
  - In 1999, the WHO expert Committee on Biological Standardization stated that the collection of global data on the value of the ATT would be initiated.
    - Ten years later: 25 out of 29 TRS still refers to ATT
      - 9 out of 25 refers to ATT omission once consistency of production established to the satisfaction of the NRA

ATT is still considered as a key safety test!

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### 3Rs in a globalised world: EU Manufacturer's point of view

#### Challenges of multi-regulatory requirements in a global market

- A vaccine may be registered in more than 100 countries with different release requirements
  - The same product lot may be used to supply multiple markets
    - Complexity of the supply chain
      - 2 or more release lines
    - A potential discontinuity of supply (repeat testing for release)
      - Delay in delivery
      - Impact on vaccine access
    - Huge cost
      - Today, *in vivo* = +/- 60% QC
      - Release: *in-vivo* tests or *in vitro/in vivo* in parallel on same lot
      - Validation burden
        - Especially when move from an *in vivo* to another *in vivo* test

Applying several *in vivo* tests does not increase overall vaccines quality!

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### 3Rs in a globalised world: EU Manufacturer's point of view

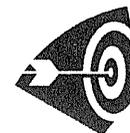
What do Regulators want?  
What do manufacturers want?  
What can we do?

What are the needs for advancing 3R's further?



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### 3Rs in a globalised world: EU Manufacturer's point of view



#### Harmonisation

- One world for *in vivo* tests requirements:
  - Reduction
- Move to alternatives in a harmonized way:
  - Replacement

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### 3Rs in a globalised world: EU Manufacturer's point of view

#### *In vivo* tests established decades ago Justification for animal use

- Variability in starting materials
- Variability in production process
- Limited availability of test methods/controls
- Limited availability of quality systems

#### 50 years of production Huge positive retrospective history

- Better characterisation of the product during development (consistency of starting material)
- Improved optimisation and standardisation of production process (consistency of production process and product)
- Tight in - process control and product monitoring with improved analytical tools (consistency of tests performed on product)
- Use of quality system to guarantee consistency (GMP, QA, pharmacovigilance) (consistency in oversight)

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### 3Rs in a globalised world: EU Manufacturer's point of view

#### • One world for *in vivo* tests requirements

- Mutual recognition of methods between regions  
Release activities
- Need to harmonize Regulations on a worldwide basis  
Moving away from specific local requirements  
Using ICH structure for Vaccine release testing  
Involve WHO in all efforts in order to align WHO Guidelines/Guidances

#### • Move to alternatives in a harmonized way

Under the umbrella of EPAA a Programme Committee and Technical Committee have been installed for replacing *in vivo* tests

- Collaboration between Industry and Authorities
- Sanofi-Patereur, GSK, Novartis, Merck Intervet SP, Pfizer,...
  - EDQM and EMA
  - OMCL's
  - Academia



The European Partnership  
for Alternative Approaches to Animal Testing

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### Conclusion

"It is not sufficient to have validated alternative methods if these are not accepted by authorities in charge of implementation and enforcement. Ways should be sought to increase involvement of authorities at all stages in order to ensure that alternative methods are effectively being used in regulatory compliance testing."\*

Isolated initiative: Poor chance of success  
Regional initiative: Limited impact if not accepted ww

\* EPAA Conference, 2006

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THANK YOU

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FARMACOPEIA  
UFFICIALE  
Farmacopeia Brasileira

USP U.S. PHARMACOPEIA

Edqm  
European Directorate for the Quality of Medicines & HealthCare

ANMAT  
FEDERAL

World Health Organization

Japanese Pharmacopeia

IPG

PHARMACOPEIA

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Consistency testing: the principle

Consistency testing starts from the idea that subsequent lots produced can be compared to an **earlier (reference) lot** (clinical lot, historical lot) which is **thoroughly characterised** with regard to quality, safety and efficacy. This on the **condition** of consistent production, thight in process control and strict application of GMP and Quality Assurance. Lot release is based on equivalence with the reference lot.

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### **S1-3 US-licensed Vaccines, Lot Release Testing, and Animal-based Testing**

**William Egan (PharmaNet Consulting, former FDA CBER)**

This presentation will provide an overview of lot-release testing of vaccines and other biological products at the Center for Biologics Evaluation and Research, FDA. The presentation will also discuss changes to lot-release testing practices, including changes that were implemented for specified biologics and alternatives to lot release for vaccines. The talk will also provide an overview of the use of animal-based testing for vaccines and other biologicals, including changes for the requirement for the General Safety Test. The presentation will present some examples of efforts by FDA in implementing the 3Rs of Russell and Burch.

## US-licensed Vaccines, Lot Release Testing, and Animal-based Testing

William Egan, PhD

Former Deputy Director and Former Acting Director  
Office of Vaccines Research and Review, CBER, FDA

## Lot-release Testing of US-licensed Vaccines

- An amendment (in 1919) to the Biologics Control Act of 1902 provided for lot-release testing by FDA
- “Samples of any lot of any licensed product ... together with the protocols showing results of applicable tests, may at any time be required to be sent to the Director, Center for Biologics Evaluation and Research ... a manufacturer shall not distribute a lot of a product until the lot is released by the Director, Center for Biologics Evaluation and Research.” [21 CFR § 610.2]
- “Licensed product” refers to products licensed under the Public Health Service Act.

## Lot-release Testing of US-licensed Vaccines

- Lot release samples are submitted to the FDA for release testing as well as manufacturer’s lot release testing results.
- Not all submitted samples are tested by CBER; the frequency of testing varies with the product and the product’s history. However, all submitted protocols are reviewed.
- The lot release testing program within CBER is undergoing accreditation to ISO 17025 standards – accreditation by the American Association for Laboratory Accreditation.
- Elimination of lot-by-lot release for well characterized therapeutic recombinant DNA-derived and monoclonal antibody biotechnology products published in the Federal Register, Vol 60, No 236, page 63048, December 8, 1995.

## Lot-release Testing of US-licensed Vaccines

- “Alternatives” to lot-release for vaccines
- Manufacturers meeting certain assurances, as determined by FDA, may submit product license application supplements requesting approval of alternatives to the lot release requirements set forth in their license. Described in the Federal Register, Vol 58, No 137, page 38771, July 20, 1993.
- Allows approved manufacturers to release product directly to market following their own product testing.
- Alternatives to lot release currently exists for several vaccines, for example, Engerix B.

### Animal-based Testing

- General Safety Test (lot release test)
- Potency Testing (lot release test)
- Vaccine Characterization and Safety Testing
  - Neurovirulence Testing
  - Adventitious Agent Testing (cell banks, viral seeds)
  - Pre-clinical Pharmacology/Toxicology Testing
  - Developmental Toxicology Testing
- Other (e.g., tumorigenicity and oncogenicity testing of neoplastic cell lines)

### General Safety Test

- Required by Regulation for licensed vaccines: 21 CFR 610.11
  - “A general safety test for the detection of **extraneous** toxic contaminants shall be performed on biological products intended for administration to humans.”
  - Also performed routinely with investigational products
- The General Safety Test (GST) must be performed on upon a representative sample of the product in the final container from every final filling of each lot of product.
- The GST is performed using guinea pigs and mice.
- The test prescribed in this section need not be performed for Whole Blood, Red Blood Cells, Cryoprecipitated AHF, Platelets, Plasma, or Cellular Therapy Products.  
[21 CFR 610.11(g)(1)]

### General Safety Test: Exception (1)

- To obtain marketing approval for a specified biological product, an “applicant must submit a biologics license application in accordance with paragraph (a) of this section except that the following sections shall not be applicable to such products: ... 610.11 ...”  
[21 CFR 601.2(c)(1)]
- Specified biological products [21 CFR 601.2(a)]
  - Therapeutic DNA plasmid products
  - Therapeutic synthetic peptide products (< 40 amino acids)
  - Monoclonal antibody products for in vivo use
  - Therapeutic recombinant DNA-derived products

### General Safety Test: Exception (2)

“For products other than those identified in paragraph (g)(1) of this section, a manufacturer may request from the Director, Center for Biologics Evaluation and Research or the Director, Center for Drug Evaluation and Research ... an exemption from the general safety test. The manufacturer must submit information as part of a biologics license application submission or supplement to an approved biologics license application establishing that because of the mode of administration, the method of preparation, or the special nature of the product a test of general safety is unnecessary to assure the safety, purity, and potency of the product or cannot be performed. The request must include alternate procedures, if any, to be performed. The Director ... upon finding that the manufacturer’s request justifies an exemption, may exempt the product from the general safety test subject to any condition necessary to assure the safety, purity, and potency of the product.” [21 CFR 610.11(g)(2)]

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### Diseases against which U.S.-Licensed Vaccines Exist

- |  |  |
|--|--|
| ┆ Diphtheria                                     | ┆ Rotavirus                                |
| ┆ Tetanus  | ┆ Poliomyelitis                            |
| ┆ Pertussis                                      | ┆ Pneumococcal disease                     |
| ┆ Mumps  | ┆ Meningococcal disease (A, C,<br>Y,W-135) |
| ┆ Measles  | ┆ Human Papilloma Virus                    |
| ┆ Rubella  | ┆ Rabies                                   |
| ┆ Varicella-zoster (chicken pox<br>and shingles) | ┆ Tuberculosis                             |
| ┆ Hepatitis B                                    | ┆ Yellow Fever                             |
| ┆ Hepatitis A                                    | ┆ <i>Salmonella typhi</i>                  |
| ┆ <i>Haemophilus influenzae</i> , b              | ┆ JEV encephalitis                         |
| ┆ Influenza A and B                              | ┆ Anthrax                                  |
| ┆ Adenovirus                                     | ┆ Smallpox (variola)                       |

### Potency Tests

- Potency and Potency Tests are defined in the US Code of Federal Regulations
  - Tests for potency shall consist of either *in vitro* or *in vivo* tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in § 600.3 (s) of this chapter [21 CFR § 610.10]
  - Specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result [21 CFR § 600.3 (s)]
- The majority of potency tests for vaccines are not animal-based, especially among the more-recently licensed vaccines. The majority of animal-based potency tests reside with older vaccines.

### Potency Test Examples: *in vitro* Tests

- Acellular pertussis vaccines: content of the constituent pertussis antigens by ELISA
- Hepatitis A and B vaccines: protein content by ELISA
- *Haemophilus influenzae* type b, meningococcal, and pneumococcal conjugate vaccines: physical chemical measures (protein content, saccharide content)
- Influenza vaccines: Simple Radial Immunodiffusion for hemagglutinin content
- Measles, mumps, and rubella vaccines: the concentration of live virus, measured as a TCID<sub>50</sub>.
- *Salmonella typhi* (TY21a), BCG vaccine: Number of colony forming units.
- Human Papilloma Virus vaccine (Gardasil): ELISA assay (IVRP).

### Potency Test Examples: *in vivo* Tests

- Anthrax vaccine: guinea pig immunization followed by challenge with anthrax bacteria
- Rabies vaccine: immunization (mice) and challenge with wild type rabies
- Diphtheria and tetanus toxoids: immunization and evaluation of toxin neutralization
- Japanese Encephalitis Virus vaccine (JE-VAX): Murine antibodies measured in a plaque neutralization assay relative to a reference vaccine.

## Haemophilus influenzae type b Conjugate Vaccine

- During studies on the interchangeability of licensed Hib vaccines, it was found that one lot of vaccine that had met all lot-release criteria, including immunogenicity testing in mice, was sub-immunogenic in infants.
- The lot-release criteria, which were, and still are, chemical and physical chemical in nature were augmented with the addition of a new chemical test. Also, immunogenicity testing in mice was deleted.
- No problems observed since the additional chemical test was included.

W. Egan *et al.*, *J Amer Med Assn*, 1995; **273**: 888-9.

## Neurovirulence Testing

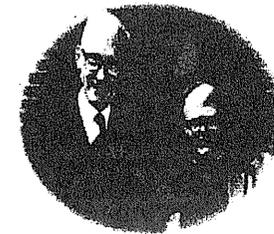
- Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications [February, 2010]
  - "Assessment of neurovirulence might be appropriate for MVSs derived from wild type viruses known or suspected to possess neurotropic or neurovirulent properties, and we recommend that you consult with CBER on appropriate animal models, methods, and scoring systems for the assessment before you initiate such studies. Animal models other than non-human primates may be acceptable. For live-attenuated viruses that are neurovirulent or might revert to neurovirulence (e.g., polio viruses), it might be necessary to assess not only neurovirulence on the MVS or an end-of-production passage level virus stock, but also on the product lot-by-lot."

## Neurovirulence Testing

- Characterization of master virus seed lots
  - Mumps
  - Measles
  - Others
- Lot release testing
  - Polio
- These neurovirulence tests were traditionally carried out with monkeys.

## Russell And Burch: The 3 R's (1958)

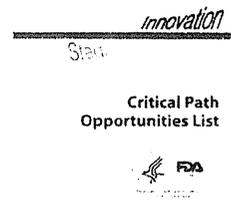
- Replacement: use of non-sentient material instead of conscious living vertebrates
- Reduction: lowering the number of animals required to obtain enough information of adequate precision
- Refinement: any development leading to a decrease in the incidence or severity of inhumane procedures



### The 3 R's and CBER

- Various activities during the past several decades to develop alternatives or refinements to animal-based testing. Examples include,
  - ELISA based assay for rabies potency
  - Transgenic mouse assay for OPV neurovirulence testing
  - Rat-based model for neurovirulence testing of mumps virus vaccine and, subsequently, additional vaccines
  - Toxin neutralization assay for anthrax potency
  - Vero cell based toxin neutralization for diphtheria

### FDA Critical Path Initiative



60. Improved, more quantitative and reliable non-animal based tests of vaccine potency would assist in development of vaccines for conditions such as rabies and smallpox.

### Thank you

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#### S1-4 生物学的製剤基準とワクチンの品質確保にどこまで動物実験は有用か

小島 肇(国立医薬品食品衛生研究所)

動物実験の3Rs (Reduction : 削減、Refinement : 苦痛の軽減、Replacement : 置き換え) の普及が世界的に叫ばれる昨今、すべての試験法において動物実験の見直しを求める国際的な動向が続いている。動物実験は今日まで我々の安全・安心を支えてきたが、生体は未だブラックボックスである一方、種差、系統差などの問題も抱えている。従来までの動物実験という経験に基づく試験法のみならず、作用機構 (Mode of Action: MoA) や証拠の価値 (Weight of Evidence: WoE) を考慮し、最先端の科学を導入した方法の利用が必須となってきている。

日本における3Rsは、欧州の化粧品規制を念頭に置いた化粧品企業が主流であったこと、replacementに当たるin vitro試験が中心であったことから、その他の分野への普及は進んでいない。特に欧米に比べ、生物学的製剤基準とワクチンの品質確保への対応は遅れがちである。しかし、ICH (日米EU医薬品規制調和国際会議) やISO (国際標準化機構) などで試験法の国際協調が迫られ、医薬品や医療機器のグローバル化が進む今日、いつまでも欧米追随ではすまされないと感じている。

そのような状況の中、欧米ではワクチンの品質確保に動物実験の3Rsを配慮するワークショップなどが開催されるなど活発に活動がなされている。生物学的製剤基準とワクチンの品質確保に果たしてきた動物実験の役割は大きく、その存在を否定するわけではない。しかし、今後、本分野においてもどのような動物実験の3Rsの国際対応を進めるべきかを日本でも検討する時期に来ていると考えている。

### 生物学的製剤基準とワクチンの品質確保に どこまで動物実験は有用か



Hajime Kojima,  
JaCVAM, NIHS

### Outline

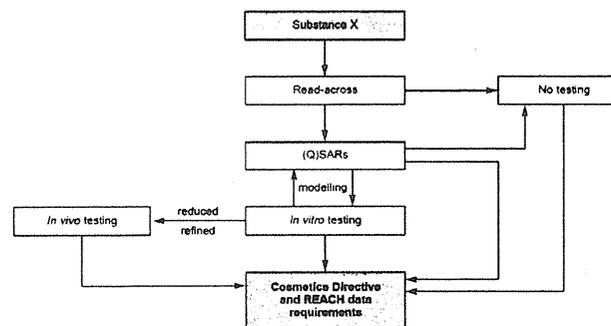
- Three Rs
- International Validation Guidance
- International Centre for Alternatives to Animal experiments and JaCVAM
- Interenational update on Vaccine potency testing

### DECLARATION OF BOLOGNA

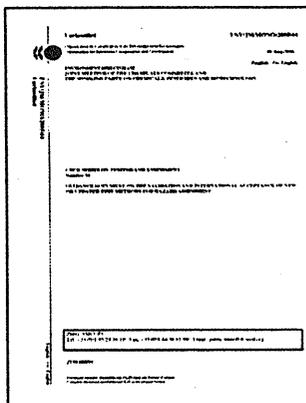
The "Three Rs" of Russell and Burch, reduction, refinement and replacement, had their origin in a project initiated in 1954 by the Universities Federation for Animal Welfare (UFAW), which led to the publication in 1959 of *The Principles of Humane Experimental Technique*, by W.M.S. Russell and R.L. Burch. In 1978, David Smyth used the word alternatives to define the Three Rs. In their book, Russell and Burch stated that "The greatest scientific achievements have always been the most humane and the most aesthetically attractive, conveying that sense of beauty and elegance which is the essence of science at its most successful". They defined: Reduction alternatives as methods for obtaining comparable from the use of fewer animals in scientific procedures, or information from the same number of animals. Refinement alternatives as methods which alleviate suffering and distress, and which enhance animal Replacement alternatives as methods which permit a given purpose to be achieved without conducting experiments or other scientific procedures on animals.

Reduction, Refinement and Replacement Alternatives and Laboratory Animal Procedures Adopted by the 3rd World Congress on Alternatives and Animal Use in the Life Sciences, Bologna, Italy, 31 August 1999

Figure 1: The linking of non-animal tests and approaches into integrated testing strategies

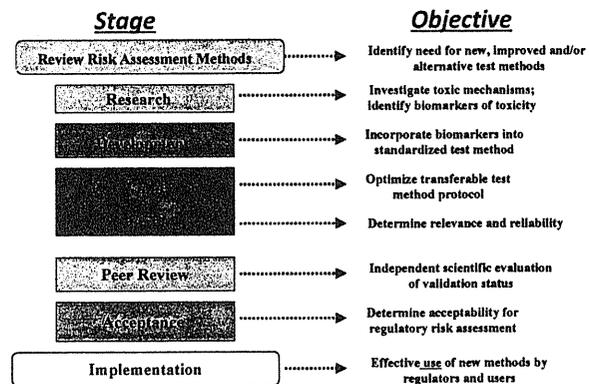


## International Validation Guidance: OECD Guidance Document 34



- ◆ **Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment, 2005**
- ◆ Developed by experts from international community
- ◆ ICCVAM contributions/participation
  - ◆ 1995 ICCVAM Draft/Workshop
  - ◆ 1996 Solna Workshop
  - ◆ 2003 Stockholm Workshop
  - ◆ 2005 Expert Consultation, U.S.A.
- ◆ Provides:
  - ◆ Test Method Validation and Acceptance Criteria that should be addressed for new proposed test methods
  - ◆ Information that should be provided to support the validity of new and revised test methods

## Test Method Evolution and Translation Process: Concept to Implementation



## A general connectional framework

- Module 1: Test Definition
- Module 2: Within-laboratory repeatability and reproducibility
- Module 3: Between-laboratory transferability
- Module 4: Between-laboratory reproducibility
- Module 5: Predictive capacity
- Module 6: Applicability domain
- Module 7: Performance standards

## VALIDATION =

- confirming the validity of a process or method =
- agreeing that it is reliably delivering relevant information

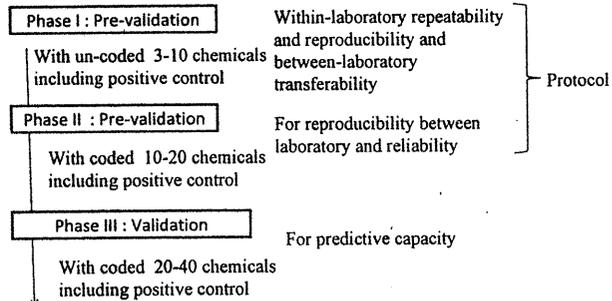
## Reliability =

- Within & between laboratory reproducibility = same input >> same output

## RELEVANCE=

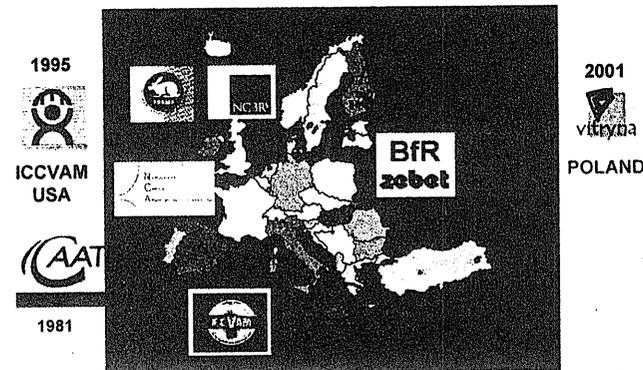
- Information generated is useful for the purpose, i.e. risk/safety assessment, quality control,

### Phases for a validation study



Validation reports, statistical report, chemical selection report, final protocol, Back ground Review Document and performance standard (if necessary)

### INTERNATIONAL CENTRES FOR ALTERNATIVES TO ANIMAL EXPERIMENTS



### JaCVAM: Japanese Center for the Validation of Alternative Methods

This ad hoc organization created at National Institute of Health Sciences (NIHS) in Japan, 2005. by the Ministry of Health, Labour and Welfare (MHLW).

#### JaCVAM's Goal

- To promote the 3Rs in animal experiments for the evaluation of chemical substance safety in Japan
- To establish guidelines for new alternative experimental methods through international collaboration.

### JaCVAM mission

- 1) coordination of peer review and regulatory acceptance of new and revised test methods
- 2) support of validation work for new and revised test methods
- 3) promotion of the 3Rs
- 4) working to promote international partnership surrounding the issue of alternative methods.

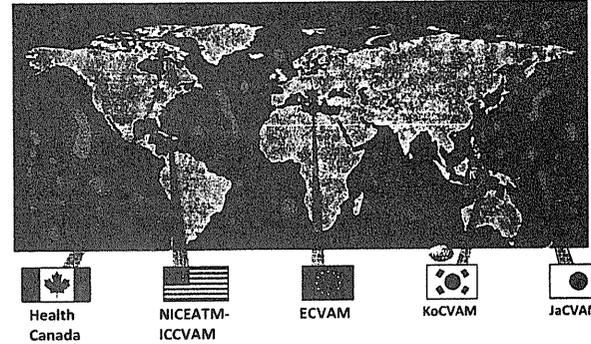
### Regulatory acceptance

- The Bovine Corneal Opacity and Permeability (BCOP) Test Method for Identifying Ocular Corrosives and Severe Irritants
- The Isolated Chicken Eye (ICE) for Identifying Ocular Corrosives and Severe Irritants
- Skin sensitization assay, LLNA: DA
- Skin sensitization assay, LLNA: BrdU-ELISA
- *In vitro* skin irritation testing: EPISKIN
- *In vitro* skin corrosion testing: Vitrolife-Skin, EpiDerm

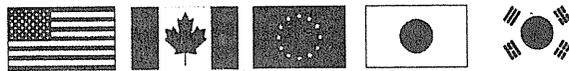
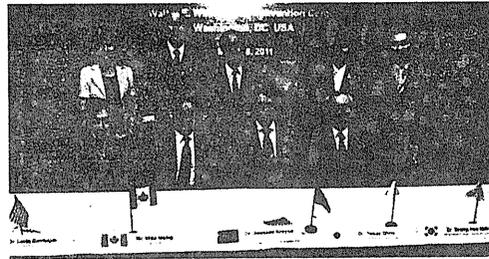
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### ICATM Organizations

ICATM is a voluntary international cooperation of national organizations: Canada, the European Union, Japan, South Korea, and the United States.



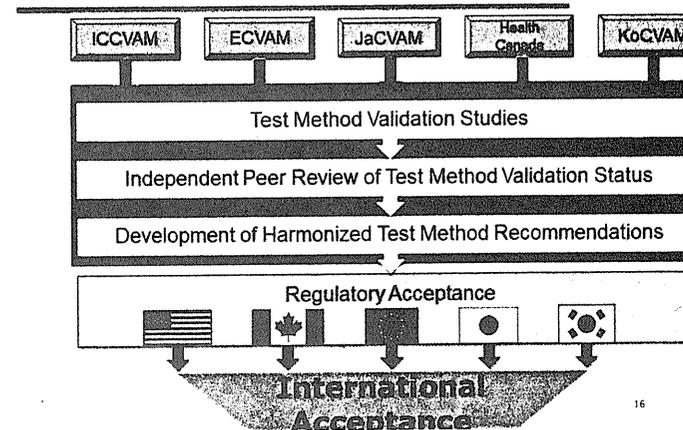
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International Cooperation on Alternative Test Methods (ICATM)

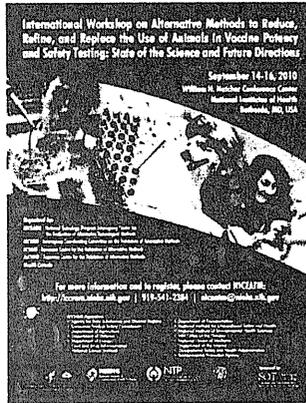
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### ICATM Cooperation



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## Vaccine Potency and Safety Testing Workshop



- NIH, Bethesda, Maryland
- September 14-16, 2010
- Organized by NICEATM-ICC<sup>1</sup> with ECVAM, JaCVAM, and IACVAM
- SOT co-sponsorship
- Attended by nearly 200 scientists from 13 countries
- Presentations by:
  - Regulatory authorities
  - Experts from industry
  - Experts from academia
- Poster session

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## Workshop Goals

1. Review the state of the science of available 3Rs alternative methods for vaccine potency and safety testing and discuss ways to promote their implementation
2. Identify knowledge and data gaps that need to be addressed to further advance alternative methods for vaccine potency and safety testing
3. Identify and prioritize research, development, and validation efforts needed to address these gaps

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## Human Vaccine Potency and Safety Testing: Recommended Priorities for Future 3Rs Efforts

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| <ul style="list-style-type: none"> <li>■ Highest Priorities: Potency Testing                             <ul style="list-style-type: none"> <li>- Diphtheria and Tetanus toxoids</li> <li>- Pertussis (whole cell and acellular)</li> <li>- Rabies</li> <li>- Anthrax</li> <li>- DTP pentavalent vaccines</li> <li>- Inactivated Polio vaccine</li> <li>- Combined vaccines</li> </ul> </li> </ul> | <ul style="list-style-type: none"> <li>■ Highest Priorities: Safety Testing                             <ul style="list-style-type: none"> <li>- Vero cell assay for Diphtheria</li> <li>- Transgenic mouse test for oral polio vaccine (OPV)</li> <li>- <i>In vitro</i> assay for Tetanus toxoid</li> <li>- Histamine (HSA) assay for Pertussis</li> <li>- Development of Massive Parallel Sequencing (MP) and other emerging technologies</li> </ul> </li> </ul> |
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## Veterinary Vaccine Potency and Safety Testing: Recommended Priorities for Future 3Rs Efforts

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|---|--|
| <ul style="list-style-type: none"> <li>■ Highest Priorities: Potency Testing                             <ul style="list-style-type: none"> <li>- Rabies</li> <li>- <i>Leptospira sp.</i> serovars</li> <li>- <i>Clostridium sp.</i> serovars</li> <li>- Erysipelas</li> <li>- Fish vaccines</li> <li>- Poultry vaccines</li> <li>- Foreign animal diseases</li> <li>- Non-adjuvanted vaccines</li> <li>- Those for which the functional antigen identified/characterized</li> <li>- Human &amp; veterinary products for same organism</li> <li>- New vaccines</li> </ul> </li> </ul> | <ul style="list-style-type: none"> <li>■ Highest Priorities: Safety Testing                             <ul style="list-style-type: none"> <li>- Extraneous agent testing with animals are still used—esp avian vaccines</li> <li>- Inactivation testing for killed vaccines, e.g. rabies</li> <li>- Residual toxicity testing</li> <li>- Subunit protein or DNA vaccines</li> <li>- New vaccines</li> </ul> </li> </ul> |
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**International Regulatory Requirements for Vaccine Safety and Potency**

**Testing: Roundtable Discussion**

Canada *Richard Isbrucker, Ph.D., Health Canada, Canada.*

Europe *Ralph Woodland, Ph.D., Veterinary Medicines Directorate, United Kingdom.*

Japan *Yoshinobu Honuchi, Ph.D., Pharmaceuticals and Medical Devices Agency, Japan.*

WHO *JinHo Shin, D.V.M., Ph.D., World Health Organization, Switzerland.*

U.S. FDA *Theresa Finn, Ph.D., Center for Biologics Evaluation and Research, U.S. FDA.*

USDA *Richard E. Hill Jr., D.V.M., Center for*

*Procedia in Vaccinology 5 (2011) 156 – 163*

**Strategic approaches for developing alternative tests for safety and potency of vaccines**

Yoshinobu Horiuchi, Masaki Ochiai, Michiyo Kataoka, Akihiko Yamamoto, Chun-Ting Yuen, Cathy Asokanathan, Michael Corbel, Takeshi Kurata and Dorothy Xing

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## Conclusion of workshop

The workshop participants recommended enhanced international harmonization and cooperation efforts and closer collaborations between human and veterinary researchers to expedite progress. Implementation of the workshop recommendations is expected to advance new methods for vaccine testing that will reduce animal use, benefit animal welfare, and ensure continued and improved protection of human and animal health.

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## Upcoming Vaccine Alternatives Workshops

- International Workshop on Alternatives to the Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines Workshop
  - Follow-up to the August 2011 Montreal Workshop
  - Proposed date: September 2012
- International Workshop on Alternative Methods for *Leptospira* vaccine potency testing
  - Tentative dates: October 1-4, 2012, National Centers for Animal Health, Ames, IA
- International Workshop on Alternative Methods for Human and Veterinary Clostridial Vaccine Potency Testing
  - Proposed date: Fall 2013

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## AGAATI/ECVAM Workshop on the Validation of Alternative Methods for the Potency Testing of Vaccines.

The workshop on validation of alternative methods for the potency testing of vaccines was organised by ECVAM (European Centre for the Validation of Alternative Methods) and AGAATI (Advisory Group on Alternatives to Animal Testing in Immunobiologicals), and it was held in Angera, Italy, on 14-16 November 1997 under the co-chairmanship of Coenraad Hendriksen (RIVM, Bilthoven, The Netherlands) and Jean-Marc Spieser (EDQM, Strasbourg, France). The 17 participants, all experts in vaccine quality control and/or validation procedures, came from international regulatory bodies and organisations, national control laboratories and vaccine manufacturers. The aim of the workshop was to discuss, in an informal atmosphere, the complex issue of guidelines for the validation of alternative methods to potency testing of vaccines in laboratory animals, in particular: a) to review existing guidelines for validation; b) to discuss specific guidelines for the validation of alternative methods to the potency testing of vaccines and to agree on strategies for the preparation of guidelines; and c) to discuss and make recommendations for the implementation of validated alternative methods for regulatory purposes.

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### ECVAM's contributions to the implementation of the Three Rs in the production and quality control of biologicals.

A summary is presented of the activities initiated, and the progress achieved, between April 1993 and December 2001 in implementing the Three Rs in one of the main priority areas of the European Centre for the Validation of Alternative Methods (ECVAM) - the production and quality control of biologicals. These have included organising eight key workshops, and financial contributions to, and sponsorship of, relevant international workshops, symposia and conferences. Noteworthy activities include financial support and/or participation in a number of prevalidation and validation studies. These involved alternative methods for the batch potency testing of: human tetanus vaccines; human and veterinary tetanus antisera and immunoglobulin; rabies vaccines; Leptospira hardjo vaccines; Clostridium perfringens vaccines; and erysipelas vaccines. They also involved a cell culture test for specific toxicity testing of diphtheria toxoid vaccines. In addition, ECVAM funded a study on the use of humane endpoints for vaccine quality control tests involving severe suffering, such as the potency testing of erysipelas, rabies and pertussis vaccines. ECVAM has also contributed financially to the compilation of manuals and expert reports, and to training in test methods. Following the report of an ECVAM Task Force, ECVAM financially supported the prevalidation of some in vitro methods for the potency testing of a recombinant hormone. A proposal is presented for promotion of regulatory acceptance, and suggestions are made for possible future activities.

### On going Validation Study of Alternatives in Korea

- *In vitro* method for specific toxicity of pertussis vaccine using ELISA and enzyme-HPLC
  - Report submitted to WHO (2011.6)
- *In vitro* assay for Japanese Encephalitis vaccine with ELISA

**JaCVAM**  
 Japanese Center for the Validation of Alternative Methods  
 Office: New Testing Method Assessment, Division of Pharmacology,  
 National Biological Safety Research Center (NBSRC),  
 National Institute of Health Sciences (NIHS)

日本語 English Google Search WWW ja-cvam.jp

About JaCVAM Update on JaCVAM Academic activities Submission of Alternative Methods to JaCVAM International Cooperation

**Thank you for your attention**

**Policy and Mission** JaCVAM's policy and mission is to promote the 3Rs in animal experiments for the evaluation of chemical substance safety in Japan and establish guidelines for new alternative experimental methods through international collaboration

the 3Rs in animal experiments—Reduction (of animal use)  
 Refinement (to lessen pain or distress and to enhance animal well-being)  
 Replacement (of an animal test with one that uses non-animal systems or phylo-genetically lower species)  
 (OECD GD34)

**News** Contents

Ⓜ [NEW] news texts dummy texts news texts dummy texts news texts dummy texts(2009.7.15)  
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 Ⓜ news texts dummy texts news texts dummy texts news texts dummy texts (2009.7.3)

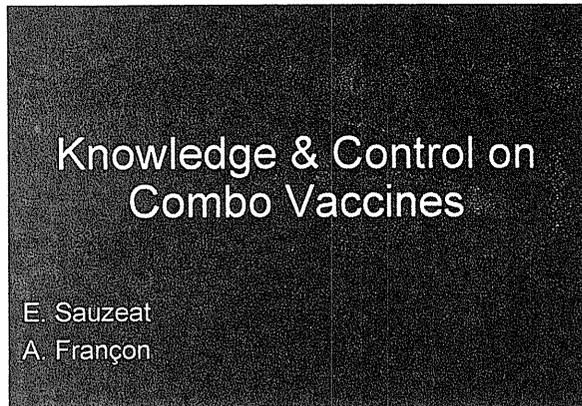
Ⓜ About JaCVAM  
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 Ⓜ JaCVAM Activities

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 インターネット | 保護モード: 有効

## **S2-1 Knowledge & Control on Combo Vaccines**

**Elisabeth Sauzeat (Sanofi Pasteur)**

- To get robust and efficient product, formulation development have to take into account aluminum adjuvant and antigen characterization and interaction
- Aluminum adjuvant characterization is key to understand adsorption and non adsorption of antigen.
- The need of interaction characterization between adjuvant and antigen is key for efficiency and robustness of the multivalent vaccine.
- This knowledge allows us to get a stable and complex balance between antigens & adjuvant.
- Finally, Combo Vaccine formulation is a case by case development depending on the adjuvant and antigens characteristic



## Abstract

- To get robust and efficient product, formulation development have to take into account aluminum adjuvant and antigen characterization and interaction
- Aluminum adjuvant characterization is key to understand adsorption and non adsorption of antigen.
- The need of interaction characterization between adjuvant and antigen is key for efficiency and robustness of the multivalent vaccine.
- This knowledge allow us to get a stable and complex balance between antigens & adjuvant.
- Finally, Combo Vaccine formulation is a case by case development depending of the adjuvant and antigens characteristic

## Agenda & Objectives

### Agenda :

- Characterization : Aluminum Adjuvant
- Formulation development
  - Characterization of Interface between adjuvant and Antigens
  - Process Development

### Objectives of this presentation

- Present the knowledge we have on aluminum adjuvant and interaction with antigens
- Present our Hexavalent development

## History of Aluminum Adjuvants

- Aluminum-based adjuvants are by far the most commonly used.
- In 1926, Glenny demonstrated the adjuvant activity of aluminum compounds with the use of an alum-precipitated diphtheria toxoid vaccine. These adjuvants, commonly referred to as 'alum adjuvants,' are presently used in vaccines licensed in the United States and are the most widely used vaccine adjuvants in licensed vaccines worldwide.
- Aluminum adjuvants have an extensive record of safety. Billions of doses of aluminum adsorbed vaccines have been used to inoculate children and infants for more than 50 years.