

**3Rs Symposium**

**SESSION III**

**Implementation of the 3Rs:  
Needs and Practical Experiences**



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**3Rs IN VETERINARY  
VACCINE INDUSTRY**

**Dr. René Aerts**  
Director R&D The Netherlands Intervet  
International bv



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**AGENDA**

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- Objectives of vaccine manufacturer
  - Instruments of monitoring vaccine quality
  - Why 3Rs in Industry
  - 3Rs in the company
  - Examples
  - Interaction with Regulators
  - Suggestions for the future



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## OBJECTIVES OF VACCINE MANUFACTURERS

**Develop Safe, Efficacious, Constant quality and Cost-effective products**

**Comply with Regulatory requirements:**

- Environment
- GMP/USDA
- GLP
- Registration




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## INSTRUMENTS TO MONITOR VACCINE QUALITY

**Quality systems**

**GLP:** •Set-up of experiments  
•Documentation  
•Facilities

} **Research phase**

**GMP:** •Documentation materials  
•Documentation batches  
•Consistency  
•QC tests  
•Facilities

} **Production phase**




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## INSTRUMENTS TO MONITOR VACCINE QUALITY

**In-vitro and in-vivo tests**

**During R&D:** Safety / efficacy / quality in target animals,  
including field trials  
Directives / Guidelines / Ph.Eur.

**Production:** QC raw materials  
In-process controls: •Antigenic mass  
•Purity / sterility  
•Inactivation




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## INSTRUMENTS TO MONITOR VACCINE QUALITY

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In-vitro and in-vivo tests

- Final product:
- Sterility
  - Safety
  - Potency



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## WHY 3Rs IN INDUSTRY

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1. Intervet is part of the **society**
  - 5000 staff (families) in 53 countries
  - 800 young educated scientists
  - Strong ethical drive for 3Rs
2. Animal testing is time-consuming and expensive
  - In-vitro potency: 2 days
  - In-vivo potency: 2-6 weeks
  - Containment facilities
  - SPF flocks



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## 3Rs IN THE COMPANY

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During R&D

- Animal Experimental Committee (AEC)
  - Mixed membership
  - Evaluate all animal experiments
  - 3R principle is guideline
  - Sometimes requests authorities are denied
- Project Team + Regulatory Affairs
  - Decide which studies have to be done
  - Regulations plus interpretation
  - Specific requests certain countries



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## 3Rs IN THE COMPANY

### During R&D

#### •Set-up of Batch Release Tests:

**USA:** Preferably in-vitro tests

Safety tests not always in target animals

**Europe:** Mostly in-vivo tests

Evaluate in-vitro possibility

Target animal safety

Modify existing tests



## EXAMPLES IMPLEMENTATION 3Rs

### Reduction

•Increasing batch size:

Investment  
Risk increase  
Equipment  
Increased stock  
Decreased animal use: >30%  
Limits reached

•Combination of studies:

One dose/overdose/repeated  
DO plus efficacy  
Safety & extraneous agents  
Sequential challenge

•Elimination of studies:

Batch safety tests after proven record



## EXAMPLES

### Refinement

Determination of protective

number { No challenge { DOI or field

Batch release on serology instead of

challenge:  
• Cholera  
• Leptospirosis  
• Erysipelas  
• ND

Batch potency test in non-target species



## EXAMPLES

### Replacement

- Extraneous agents viral vaccines
- AE serology to egg-titration
- In vitro potency tests



## INTERACTIONS WITH REGULATORY AUTHORITIES

### Negative for 3Rs

- Interpretation of Regulations
  - Batch tests
  - Ph.Eur. Efficacy
  - ~~studies in general~~ studies in general
- If you have to do it for one country nothing is gained
- Requirements for small variations:
  - Stability data required: potency
  - ~~test~~ ATF discouraged
- Increased role statistics safety/efficacy:
  - Increasing number of animals

} Not one EU



## INTERACTIONS WITH REGULATORY AUTHORITIES

### Negative for 3Rs

- OMCL systems: repetition tests
- Stability guidelines Biotech products
- Difficulty approval in-vitro tests
- Not approved sequential
- ~~in vitro~~ species not acknowledged
- Increasing demands validation potency
- ~~test~~ increased differentiation sub-species
- Increased demands data for compatibility



## INTERACTIONS WITH REGULATORY AUTHORITIES

### Negative for 3Rs

- End result:
- Increased no animal experiments
  - Increased costs / product
  - Increased time to market
  - Increased quality ?



## INTERACTIONS WITH REGULATORY AUTHORITIES

### Positive for 3Rs

- Collaboration on development of improved potency tests:
  - > Ery
  - > Lepto
  - > ND
- Support for relieving requirements batch safety tests (for some countries)
- Support for replacing in-vivo extraneous agents tests



## WHAT DOES INDUSTRY NEED FOR FURTHER IMPLEMENTATION 3Rs

1. Realistic, harmonized approach regarding need for animal testing in Europe: stability, variations, etc.....
2. Guidance from CVMP on requirements for in-vitro potency tests
3. Interaction (discussion) between Regulators and Industry
4. Accept QA/QC system for batch release



**3Rs Symposium**  
**SESSION III**  
**Implementation of the 3Rs:**  
**Needs and Practical Experiences**



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# 3Rs Symposium

## SESSION III

Implementation of the 3Rs:  
Needs and Practical Experiences

### **Experience in Validation of Alternative Methods: Case Studies (Tetanus and Diphtheria Vaccines)**

**Dr. Marie-Emmanuelle BEHR-GROSS**  
Division IV  
European Directorate for the Quality of Medicines  
(EDQM)  
Council of Europe

ME. Behr-Gross, Strasbourg, 7-8/11/2002

2

### **Implication of Ph. Eur. in animal welfare Policy for application of 3R in monographs**

#### **ACTION PLAN**

- 1) Phasing out superfluous animal testing
- 2) Replacing in-vivo by in-vitro tests
- 3) Reducing animal numbers by modifying design
- 4) Refining animal tests

#### **REGULATORY FRAME**

**EC Council Directive 86/609/EEC**  
**European Convention for Protection of Vertebrate  
Animals for Experimental and Other Scientific Purposes**

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3





**BSP019-BSP035**

Publication: Pharmeuropa Bio 2000-2, 2001-2, Symposium: 6/02  
Consequences: Revision proposal 2.7.8 Assay of tetanus vaccine adsorbed, (Pharmeuropa 14.3)

Methods A & B: challenge test in guinea pigs and mice:  
for development, major change in manufacturing process,  
validation & monitoring of Method C

Method C: determination antibodies in guinea pigs for batch  
potency testing

Design of tests: Multiple dilution -> single-dilution

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10

**Method C: 2Rs in 3 steps**

1) Immunisation with reference vaccine (shown effective in  
clinical studies or representative thereof) and test vaccine  
Test consistency to be demonstrated

2) Immunisation with test vaccine: multiple dilution assay;  
reference serum from GPs immunised with reference vaccine  
Sufficient data multiple dilution assay available

3) Immunisation with test vaccine: single dilution assay

--> Refinement & Reduction

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11

**Successful validation of alternative methods based on  
BSP projects**

- 1. Involvement scientists (PL/SA), pilot labs,  
statisticians
- 2. Evaluation potential alternative method(s):  
costs, time, performance, availability reagents
- 3. Evaluation data on target biological by alternative(s)
- 4. Pre-selection of methods/model
- 5. Elaboration study plan (sequential phases)
- 6. Identification participants
- 7. Selection samples: marketed + borderline quality  
product(s)

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12

**Successful validation of alternative methods based on BSP projects**

- 8. Sourcing non commercial reagents & test samples
- 9. Preliminary assays using SOPs of pilot labs
- 10. Determination parameters influencing results for level of needed standardisation
- 11. Careful drafting protocols & documents
- 12. Provision data reporting sheets
- 13. Independent centralised statistical evaluation (each step)
- 14. Retrospective statistical evaluation if needed
- 15. Critical analysis of outcome (each step)
- 16. Additional experiments if needed
- 17. Adaptation study plan based on completed steps

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13

**• 1. Involvement scientists, pilot labs, statisticians (BSP019)**

- Project leaders: Dr. R. Winsnes, Dr. C. Hendriksen
- Pilot labs: RIVM, NMA, NIBSC
- Statisticians: A. Daas, A. Akkermans

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14

**2. Evaluation potential alternative method(s): costs, time, performance, availability reagents (BSP019)**

- Candidate methods based on
- Limes flocculationis (Lf), ELISA, Haemagglutination (HA), ToBI tests, RIA, double antigen time resolved fluorescence immunoassay (DELFI), etc...
- Methods validated for potency testing
- ELISA (Layton, 1980; Simonson et al, 1986, Gupta and Siber, 1994, Hendriksen et al, 1994, Aggerbeck et al, 1996, ..)
- ToBI (Hendriksen et al, 1991, 1994)
- HA (Pitzura et al, 1983)

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15



**7. Selection samples: marketed + border line (BSP019)**

**Table 1. Specifications of tetanus toxoid vaccines used in the collaborative study**

Vaccine	Tested in Phase	Composition	Adjuvant	If content*
Reference ERTA	I, IIa & IIb	T	Al(OH) <sub>3</sub>	54 Lf/ampoule
TET C	I, IIa	DTP Hib	AlPO <sub>4</sub>	ca.10 Lf/ml
TET D	I, IIa	D'	AlPO <sub>4</sub>	ca.10 Lf/ml
TET E	I, IIa	D'	AlPO <sub>4</sub>	ca.15 Lf/ml
TET F	I, IIa & IIb	DTP	AlPO <sub>4</sub>	ca.15 Lf/ml
TET H	I, IIa	DTP	Al(OH) <sub>3</sub>	ca. 5 Lf/ml
TET I	IIb	T	Al(OH) <sub>3</sub>	ca. 10 Lf/ml
TET K	IIb	DTPaP	Al(OH) <sub>3</sub>	ca. 10 Lf/ml

D = Diphtheria, T = Tetanus, P = Pertussis, Hib = *Haemophilus influenzae* type b, aP = Acellular pertussis.

\*Data established in a preliminary study at one of the co-ordinating laboratories

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19

**8. Sourcing non commercial reagents & test samples**

**9. Preliminary assays using SOPs of pilot labs**

**10. Determination parameters influencing results for level of needed standardisation (BSP019-BSP035)**

**Table 6. Materials provided by the organising laboratories**

Test system	Materials	Supplier
ELISA	ELISA plate GPTA-6 Rabbit-anti-guinea pig HRP conjugate Tetanus toxoid, lot MWC S208/A/F-6	Maxisorp, Cat.no. 442404 (from RIVM) EDQM (produced by RIVM) Sigma A5545 NIBSC
ToBI test	PolyStyrene roundbottom microtitre plate ELISA plate, flat bottom GPTA-6 Tetanus toxin, lot T417, 300 Lf/ml Equine-anti-tetanus IgG, lot GTL34 Equine-anti-tetanus IgG (HATPO), lot 32-33, peroxidase conjugated	Greiner 650101 (from RIVM) Greiner 655092 (from RIVM) EDQM (produced by RIVM) RIVM RIVM RIVM

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20

**11. Careful drafting protocols & documents**



BIOLOGICAL STANDARDISATION PROGRAMME  
**Human Tetanus Vaccine Alternative In Vitro Assays**  
 Preliminary Report of results of Phase I Study  
 Project Leaders: Dr. C. Henschel & Dr. R. Wittman  
 Participants: RIVM, NIBSC, SLA

Strasbourg

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21

## 12. Provision data reporting sheets (BSP019)

Laboratory: \_\_\_\_\_  
 Technician: \_\_\_\_\_ Date: \_\_\_\_\_ Assay No 1  
 SOP: ELISA Ph Eur

Put the absorbance data in the coloured cells of the plate-scheme

R	A	Column numbers													
		1	2	3	4	5	6	7	8	9	10	11	12		
Sample A	B														
Sample B	C														
Sample C	D														
Sample D	E														
Sample E	F														
Sample F	G														
Sample G	H														
Nab															
Nag															

remarks: \_\_\_\_\_

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22

## 13. Independent centralised statistical evaluation (BSP035)

Lab	Rep	Time	Plate 1						
			A	B	C	D	E	F	G
1	1	-	0.183	0.090	0.035	0.013	0.033	0.027	0.122
	2	-	0.189	0.081	0.038	0.015	0.041	0.030	0.119
	3	-	0.179	0.077	0.035	0.013	0.034	0.029	0.118
2	1	-	0.223	0.094	0.040	0.015	0.042	0.029	0.120
	2	-	0.188	0.086	0.036	0.013	0.033	0.028	0.117
3	1	30	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
	2	30	0.148	0.089	0.053	0.044	0.061	0.051	0.111
4	1	-	0.400	0.080	0.038	0.027	0.061	0.025	0.188
	2	-	0.230	0.059	0.044	0.018	0.039	0.036	0.139
5	1	-	0.166	0.077	0.034	0.013	0.034	0.026	0.117
	2	-	0.169	0.081	0.037	0.015	0.034	0.029	0.110
6	1	-	0.178	0.077	0.034	0.013	0.030	0.024	0.098
	2	-	0.173	0.077	0.039	0.013	0.040	0.027	0.120
7	1	-	0.152	0.063	0.039	0.014	0.033	0.026	0.102
	2	-	0.205	0.099	0.035	0.017	0.044	0.065	0.162
8	1	-	0.259	0.089	0.047	0.023	0.048	0.038	0.131
	2	-	0.134	0.067	0.016	0.007	0.024	0.017	0.054
9	1	15	0.124	0.083	0.022	0.008	0.027	0.021	0.098
	2	15	0.162	0.065	0.029	0.010	0.028	0.018	0.089
	1	30	0.160	0.095	0.035	0.014	0.044	0.028	0.106
	2	10	0.162	0.065	0.029	0.010	0.028	0.018	0.089
	2	15	0.172	0.070	0.030	0.010	0.029	0.021	0.107
10	1	-	0.201	0.079	0.034	0.012	0.035	0.025	0.112
	2	-							

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23

Table 4a – Titres of the samples (ELISA)

Lab	Rep	Time	A	B	C	D	E	F	G
1	1	-							
1	2	-							
1	3	-							
2	1	-							
2	2	-							
3	1	30							
3	2	30							
4	1	-							
4	2	-							
5	1	-							
5	2	-							
6	1	-							
6	2	-							
7	1	-							
7	2	-							
8	1	-							
8	2	-							
9	1	15							
9	2	15							
9	1	30							
9	2	10							
9	2	15							
9	2	30							
10	1	-							
10	2	-							

**14. Retrospective statistical analysis (BSP019)**

**Exploring the perspectives for single dose assay using data from the collaborative study**

- A. Daas
- A. Akkermans

**Proceedings of symposium Tetanus vaccine for human use**

**Pharmeuropa Special Issue October 2000**

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25

**15. Critical analysis of outcome (each step)**

**16. Additional experiments if needed ( BSP019)**

**Phase IIa: unexpected failures in challenge in 2 labs**

**Phase II therefore extended (2 additional labs)**

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26

**17. Adaptation study plan based on completed steps (BSP019-035)**

**Establishment of Guinea Pig antiserum Clostridium tetani guinea pig antiserum (vaccines - human use) BRP**

**First candidate: potency not optimal  
-> second developed**

**BRP calibrated by challenge to be used as positive run control, not as reference serum for potency determination for in-vitro part of serological potency assays**

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**Experience from BSP019-BSP035**  
-> start BSP034: comparable study plan

**AIMS**  
**Validation serological methods for batch potency testing of diphtheria vaccines (human use)**  
+  
**Assessing tetanus potency test vaccines by ELISA/ToBI (same samples)**

**Production & calibration of reference GP diphtheria antiserum BRP (BSP056)**

**Successful completion**  
-> significant progress for animal welfare

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28

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**Acknowledgments to BSP contributors / 3R**



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**Experience in Validation of Alternative Methods: Case Studies (Tetanus and Diphtheria Vaccines)**

**Dr. Marie-Emmanuelle BEHR-GROSS**  
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