



IN VITRO ALTERNATIVES FOR THE IN VIVO RABIES VACCINE POTENCY

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Rabies Vaccine of Human Use





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Rabies vaccines of human use

- Produced in Cell Culture - GMP;
- HDCV, PVCV , PCECV;
- Beta-propiolactone inactivated;
- Purified;
- Highly potent;
- Efficacy and safety has been demonstrated in Clinical Trials.





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Current Good Manufacturing Practices

- Based upon principle that quality must be built in to a product, and not just tested in to a product;
- Requires the validation of all processes and assay methods;





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Guidelines for Production

Minimum quality requirements:

- WHO TRS 941, (2007);
- European Pharmacopoeia (Ph. Eur.) mon. 07/2014 :0216 ;
- Ph. Bras. (2010).





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Guidelines for Production

Minimum potency requirement:

NIH test - ≥ 2.5 IU/dose

- WHO and Ph. Bras. – 16 mice
- Ph. Eur. a suitable n^0
- USA/Canada - duplicated tests





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Drawbacks of NIH test

- Developed in the 1950s;
- Animal welfare and number;
- Inaccurate (low trueness and low precision) so, not validable;
- Takes 4 weeks to conclude;
- **THERE ARE ALTERNATIVES!**





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Alternative in vitro tests:

Final bulk

Ph. Eur. and WHO recommend Ag content assays using:

- SRD;
- ABT;
- ELISA.





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Alternative in vitro tests: Final lot

- Ph. Eur. From 2014 recommends Using, alternatively, validated:
 - **Serological test;**
 - **Immunochemical assay;**





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Which Immunochemical assay?

- **SRD;**
- **ABT;**
- **ELISA.**





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Which Immunochemical assay?

- X ~~SRD~~;
- X ~~ABT~~;
- ✓ **ELISA.**





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Why an ELISA?

- Versatile;
- Simple;
- Sensitive;
- Quantitative;
- Accurate (good trueness and precision) so validable;
- Not indicative of efficacy!





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- 2011 - Paradigm shift – Report of ECVAM/EPAA workshop – Brussels – Belgium - 2010

Biologicals 39 (2011) 59–65



ELSEVIER

Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

Biologicals

journal homepage: www.elsevier.com/locate/biologicals



Meeting report

The consistency approach for quality control of vaccines – A strategy to improve quality control and implement 3Rs

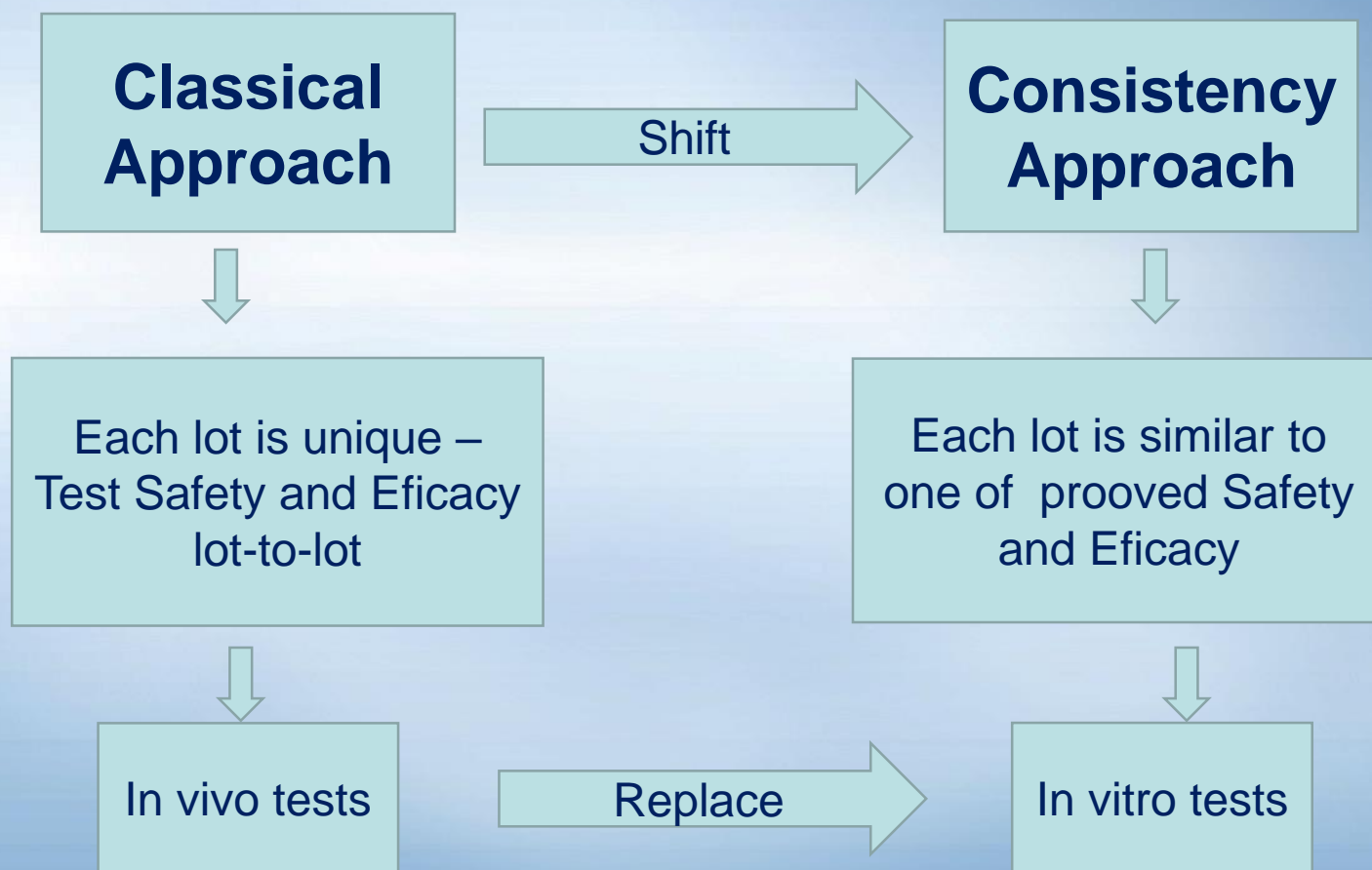
Fabrizio De Mattia^{a,*}, Jean-Michel Chapsal^b, Johan Descamps^c, Marlies Halder^{d,1}, Nicholas Jarrett^{e,2}, Imke Kross^a, Frederic Mortiaux^c, Cecile Ponsar^c, Keith Redhead^f, Jo McKelvie^g, Coenraad Hendriksen^h



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Paradigm shift - Biologicals





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- 2011 - Workshop - Ames – USA
- Way forward for Rabies vaccine. Which of the Rs to apply?

Journal of Virological Methods 158 (2009) 84–92



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Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviomet



Potency evaluation of rabies vaccine for human use: The impact of the reduction in the number of animals per dilution

Wlamir Corrêa de Moura^{a,b,*}, Humberto Pinheiro de Araujo^a, Pedro Hernan Cabello^c, Phyllis Catharina Romijn^d, José Paulo Gagliardi Leite^e



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- **2011 - Workshop rabies vaccine testing: -- Ames – USA – Replacement by an ELISA!**

Biologicals 40 (2012) 369–381



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Contents lists available at SciVerse ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals



Meeting report

Report on the international workshop on alternative methods for human and veterinary rabies vaccine testing: State of the science and planning the way forward[☆]

William Stokes^{a,*}, Richard McFarland^b, Jodie Kulpa-Eddy^c, Donna Gatewood^d, Robin Levis^e, Marlies Halder^f, Gayle Pulle^g, Hajime Kojima^h, Warren Casey^a, Alexander Gaydamakaⁱ, Timothy Miller^j, Karen Brown^k, Charles Lewis^d, Jean-Michel Chapsal^l, Lukas Bruckner^m, Sunil Gairolaⁿ, Elisabeth Kamphuis^o, Charles E. Rupprecht^p, Peter Wunderli^q, Lorraine McElhinney^r, Fabrizio De Mattia^s, Koichiro Gamoh^t, Richard Hill^d, David Reed^u, Vivian Doelling^v, Nelson Johnson^v, David Allen^v, Lori Rinckel^v, Brett Jones^v



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2012 – Workshop - ELISA for rabies vaccine testing: -- EPAA Arcachon – France

- International Working Group;
- Two Phase Study Planed.





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2016 - Study ELISA for rabies vaccine testing: -- Phase 1 concluded

Vaccine 35 (2017) 966–971



Contents lists available at [ScienceDirect](#)

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



Replacement of *in vivo* human rabies vaccine potency testing by *in vitro* glycoprotein quantification using ELISA – Results of an international collaborative study



Sylvie Morgeaux^a, Bertrand Poirier^b, C. Ian Ragan^{c,*}, Dianna Wilkinson^d, Ulrich Arabin^e, Françoise Guinet-Morlot^f, Robin Levis^g, Heidi Meyer^h, Patrice Riou^f, Shahjahan Shaid^e, Dmitriy Volokhov^g, Noël Tordo^{i,j}, Jean-Michel Chapsal^k





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Phase 1 Details:

- 5 Labs (3 NCL and 2 Private);
- 3 ELISA formats;
- 7 vaccine samples (4 pot + 3 sub pot);
- 3 virus strains (PM, PV, Flury);
- Agreement, not correlation.
- ✓ EILISA – D1-25 + WI 1112





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2018 - Study ELISA for rabies vaccine testing: - Phase 2

- ELISA proposed to EDQM in 2017;
- Approved BSP 148 project;
- Project started;
- International Collaborative study is being arranged for validation.





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Next step

- ELISA D1-25 + WI 1112 will be proposed to the Ph. Eur. for replacing NIH test;
- Producers will make the mab commercially available, as agreed.





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What else?

- **Serological tests** are in study for human use vaccines in PEI and in INCQS;
- **Veterinary vaccines** – Serological test already validated and described in Ph. Eur. And there are some ELISAs in study.





Thank you!

