'Considerations on alternative testing for Rabies vaccine'

Dr. T M Chozhavel Rajanathan

DCVMN Regional workshop: Chemistry & Manufacturing Controls

10 to 13 June 2019

Hyderabad, India

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❑Overview

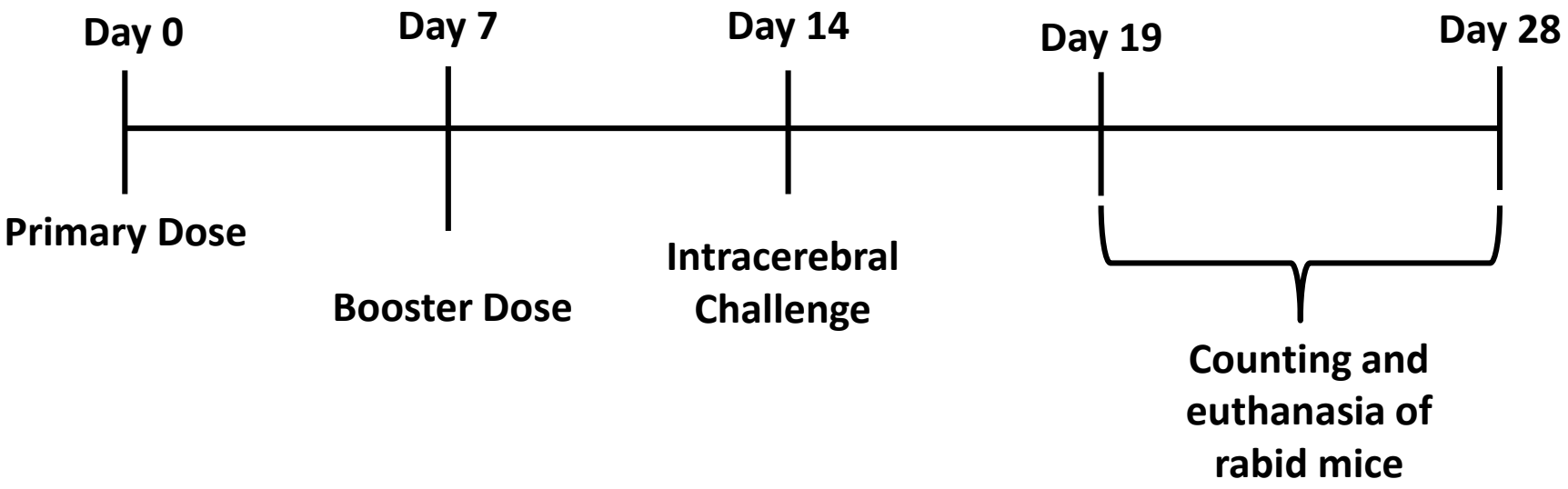
- Summary of 2018 working group discussion on Rabies
- 3R concept for Rabies vaccine
- Development of ELISA method (G-Protein)
- Conclusion
- 3R concepts for other vaccines- Zydus Approach

❑ Working group Discussion summary

Optimization of vaccines' manufacturing, containers and testing for global supply, DCVMN Regional Training Workshop, Hyderabad, 07-10 May 2018

❑ Rabies NIH Potency assay

Sample Dilution	
1/25	1/625
1/125	1/3125



- Worldwide used release test, specification≥2.5 IU per single human dose
- Ph.Eur. 0216, WHO TRS 941

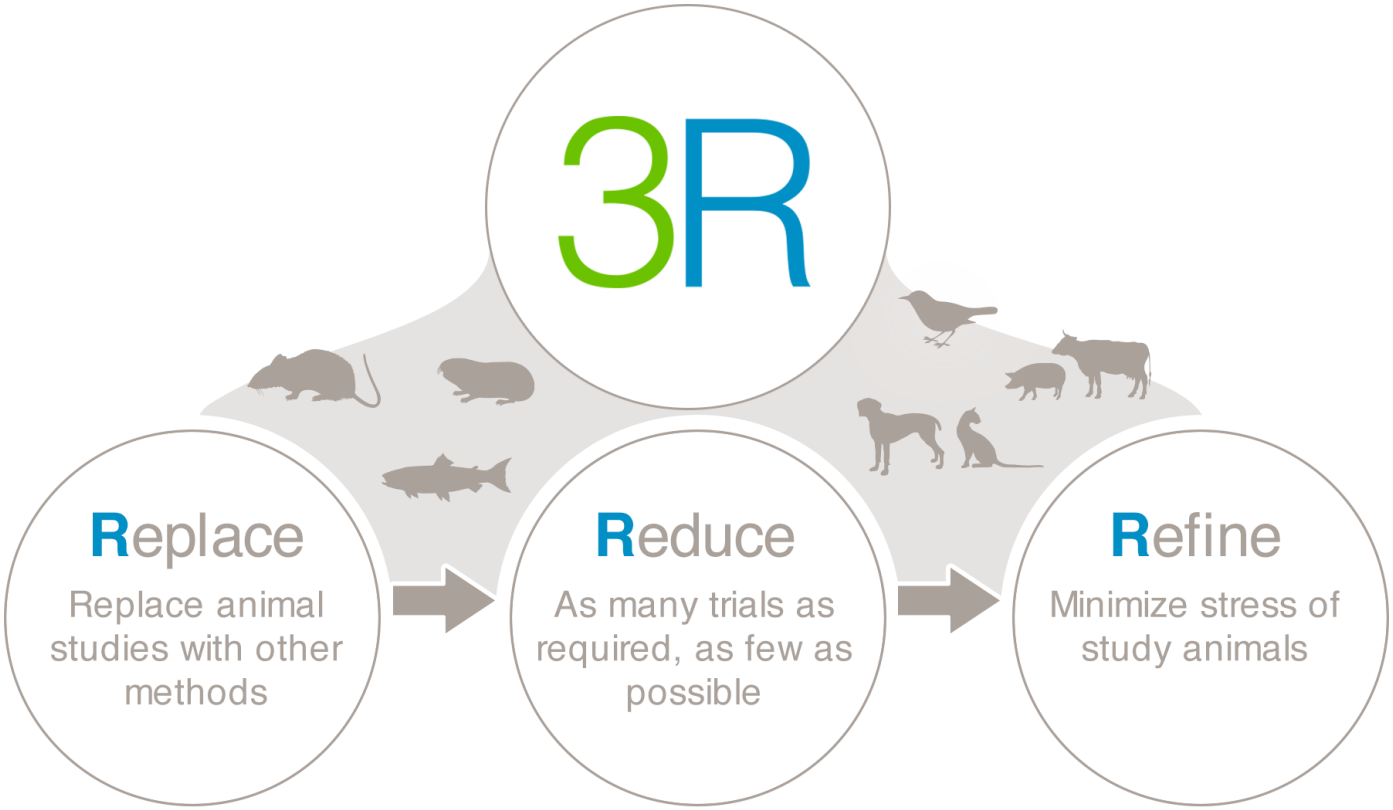
❑ Issues and challenges with the *in vivo* challenge (NIH) test

- Time consuming 28 days to complete the test
- Very high variability : 25-400%
- Hazardous- Safety issues- Need for BSL3 containment due to the use of live rabies virus
- Purity of the animal strain/breed
- Animal usage – Approx. 150 animals per test
- Availability of CVS (challenge virus strain)
- Regulators: in most of the Regulatory Guidelines, NIH is mandatory for final lot release

❑ Global Scenario on alternate strategy to NIH

- ❑ The *in-vitro* ELISA, as an alternative to the NIH test, is :
  - ✓ in accordance with the Ph. Eur. 3Rs strategy: replacement
  - ✓ already used by some manufacturers for blending and monitoring of the consistency of production
- ❑ EPAA (European partnership for alternative approaches in animal testing) has already started a study with industrial collaborative partners to replace Human Rabies potency test (Project Code BSP148) which is been very well supported by all vaccine manufacturers and WHO for Harmonization and make it a release test

❑ Alternatives to Animal Experiments

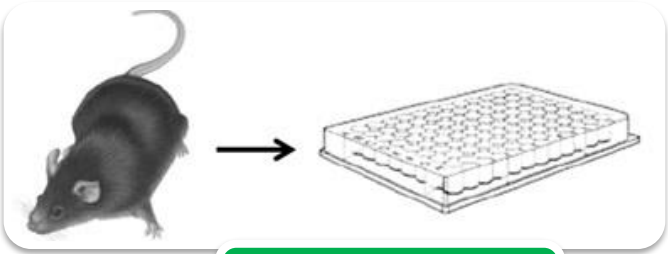


W. M. S. Russell and R. L. Burch in 1959

❑ Immunogenicity Assay/Potency Assays

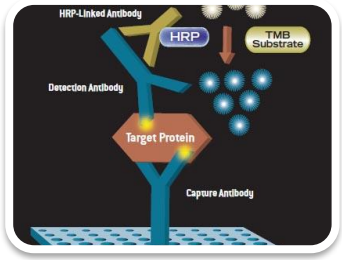
In-vivo

- In-vivo: Early 1900s Lethal Challenge in Animal models (Animal challenge study)
- High cost, time , labour, resources yet high variability.



In-vivo/ In-vitro

- In-Direct ELISA
- Neutralization Assay
- PRNT



In-vitro

- SRID (Influenza)
- ELISA- In-vitro Potency assay to check antigen using specific monoclonal antibody

❑ Development of in-vitro potency assay

- Zydus being one of the vaccine manufacturers of human rabies vaccine has developed an *in-vitro* potency assay based on G-Protein

❑ Publications (1985-2019)

Title	Author	Year
Use of a monoclonal antibody for quantitation of rabies vaccine glycoprotein by enzyme immunoassay.	*Lafon M, et al.,*	J Biol Stand. 1985 Oct;13(4):295-301.
Standardization of an enzyme immunoassay for the in vitro potency assay of inactivated tissue culture rabies vaccines: determination of the rabies virus glycoprotein with polyclonal antisera.	*Thraenhart O, et al.,*	J Biol Stand. 1989 Oct;17(4):291-309.
In vitro rabies vaccine potency appraisal by ELISA: advantages of the immunocapture method with a neutralizing anti-glycoprotein monoclonal antibody.	*Perrin P, et al.,*	Biologicals. 1990 Oct;18(4):321-30.
A relevant in vitro ELISA test in alternative to the in vivo NIH test for human rabies vaccine batch release.	*Gibert R, et al.,*	Vaccine. 2013 Dec 5;31(50):6022-9.
G-protein based ELISA as a potency test for rabies vaccines.	*Chabaud-Riou M, et al.,*	Biologicals. 2017 Mar;46:124-129.
Replacement of in vivo human rabies vaccine potency testing by in vitro glycoprotein quantification using ELISA - Results of an international collaborative study.	*Morgeaux S, et al.,*	Vaccine. 2017 Feb 7;35(6):966-971.
Development of a relative potency test using ELISA for human rabies vaccines.	*Wang Z, et al.,*	Biologicals. 2018 Sep;55:59-62.
Potency test to discriminate between differentially over-inactivated rabies vaccines: Agreement between the NIH assay and a G-protein based ELISA.	*Toinon A, et al.,*	Biologicals. 2019 May 17.

❑ G-Protein ELISA

Biologicals 46 (2017) 124–129

Contents lists available at ScienceDirect

Biologicals

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

G-protein based ELISA as a potency test for rabies vaccines

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ABSTRACT

The NIH test is currently used to assess the potency of rabies vaccine, a key criterion for vaccine release. This test is based on mice immunization followed by intracerebral viral challenge. As part of global efforts to reduce animal experimentation and in the framework of the development of Sanofi Pasteur next generation, highly-purified vaccine, produced without any material of human or animal origin, we developed an ELISA as an alternative to the NIH test. This ELISA is based on monoclonal antibodies recognizing specifically the native form of the viral G-protein, the major antigen that induces neutralizing antibody response to rabies virus. We show here that our ELISA is able to distinguish between potent and different types of sub-potent vaccine lots. Satisfactory agreement was observed between the ELISA and the NIH test in the determination of the vaccine titer and their capacity to discern conform from non-conform batches. Our ELISA meets the criteria for a stability-indicating assay and has been successfully used to develop the new generation of rabies vaccine candidates. After an EPAA international pre-collaborative study, this ELISA was selected as the assay of choice for the EDQM collaborative study aimed at replacing the rabies vaccine NIH *in vivo* potency test.

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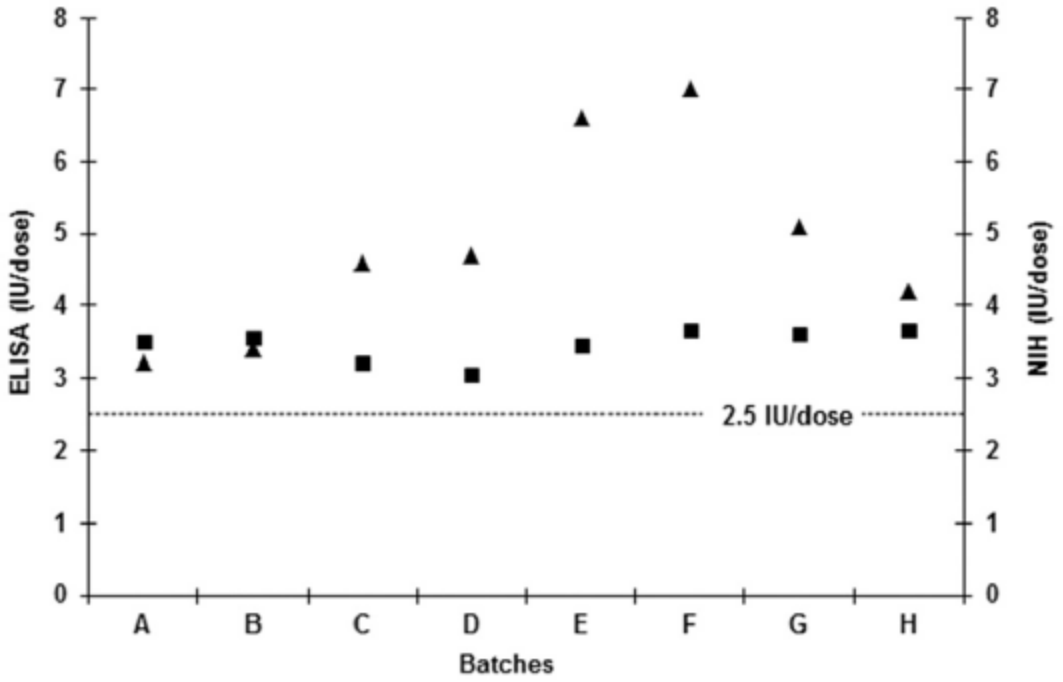
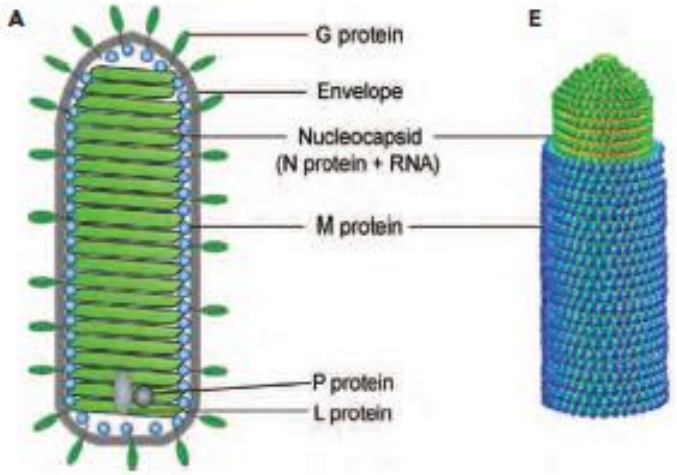


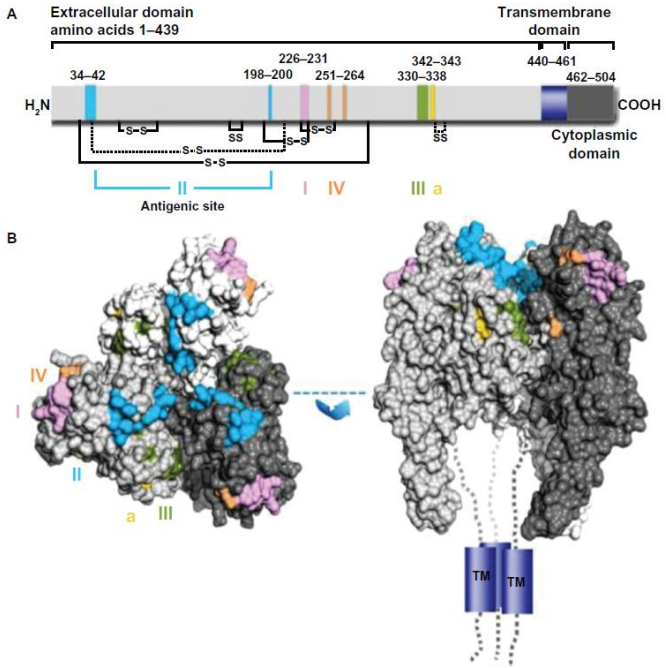
Fig. 2. Comparative analysis of different lots of rabies vaccine at the Filled Product step. The horizontal bar corresponds to the threshold for conformity. Triangle and right-hand y axis: NIH test; Square and left-hand y-axis: ELISA assay.

Riou et al., 2017

❑ G Protein



Fields virology/editors-in-chief, David M. Knipe, Peter M. Howley. – 6th ed.



Nagarajan et al., 2014

- G Protein, which is a trimer of approximately 67kDa, is the major antigen responsible for inducing production of VNAs and for conferring immunity against lethal infection with rabies virus

❑ *In-vitro* Potency assay

- Serum Antibody Assay

  G-Protein ELISA (In-direct ELSIA or c-ELISA)

Challenge can be avoided

- Antigen ELISA
  - ➢ Sandwich ELISA
    - ✓ Polyclonal Sera
    - ✓ Monoclonal ELISA
    - ✓ Polyclonal and Monoclonal ELISA

In-vitro Potency assay

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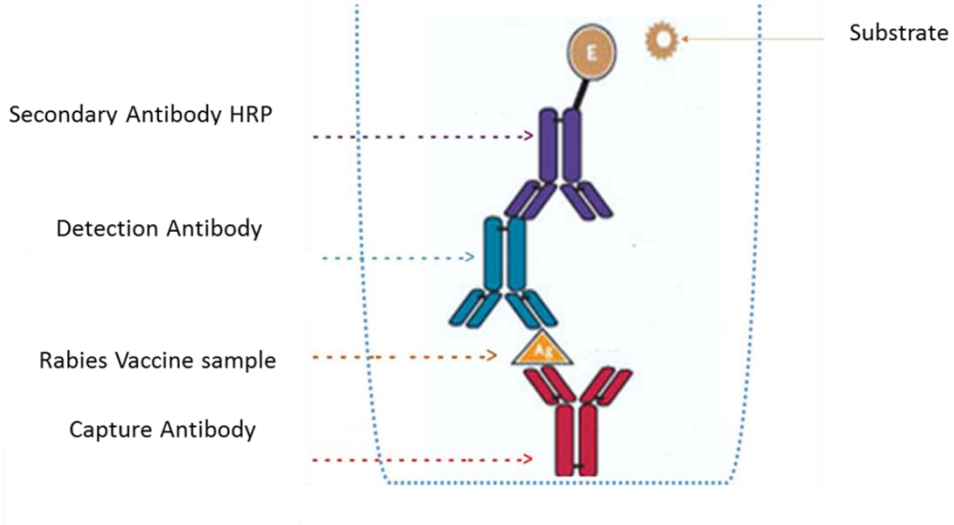
❑ In-vitro Assay Platform

Sandwich ELISA



Critical Reagent:

1. Capture antibody
2. Detection antibody
3. Reference standard

❑ Identification of monoclonal antibody

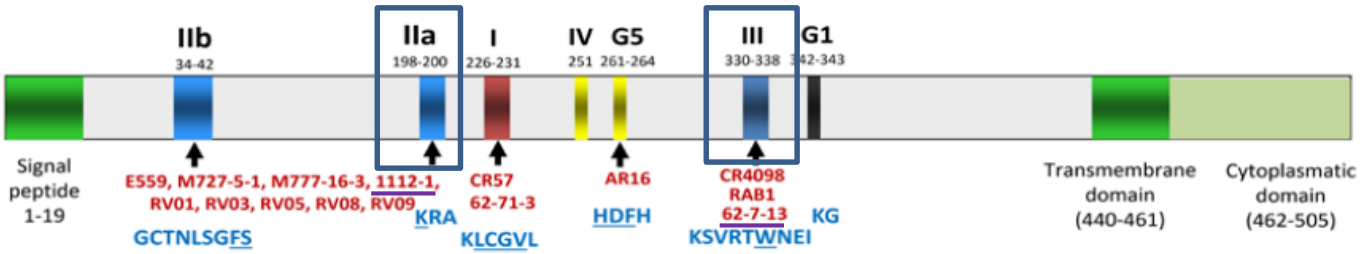


Figure 1: Schematic representation of the RV glycoprotein. Major antigenic sites and their amino acid positions are shown above the bar. Arrows indicate MAb epitopes within antigenic sites. The MAbs addressed in this study are shown in red. Consensus amino acid sequences of the antigenic sites are shown in blue. Underlined residues are invariable.

1.M777-16-3 **(IgG1)** binds to **Site III**

2.62-71-3 **(IgG2b)** binds to **Site II**

❑ Technical Information of the Monoclonal antibody

OPEN ACCESS Freely available online

PLoS NEGLECTED TROPICAL DISEASES

Development of a Mouse Monoclonal Antibody Cocktail for Post-exposure Rabies Prophylaxis in Humans

Thomas Müller[1], Bernhard Dietzschold[2], Hildegund Ertl[3], Anthony R. Fooks[4], Conrad Freuling[1], Christine Fehlner-Gardiner[5], Jeannette Kliemt[1], Francois X. Meslin[6], Charles E. Rupprecht[7], Noël Tordo[8], Alexander I. Wanderler[5], Marie Paule Kieny[9]*

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❑ Technical Information of the Monoclonal antibody

Table 1. Available technical information for candidate MoMAbs.

History of hybridomas	E559.9.14	1112-1	62-7-13	M727-5-1	M777-16-3
Mouse strain providing B-cells	BALB/c mice	BALB/c mice	BALB/c mice	BALB/c mice	BALB/c mice
Antigen	ERA G protein	ERA G protein	whole ERA	whole ERA, #167–169	whole ERA, #167–169
Fusion partner (Year of fusion)	P3-X63Ag8 (1979)	653 (1985)	Sp2/0–Ag14 myeloma (1983)	Sp2/0–Ag14 myeloma (1994)	Sp2/0–Ag14 myeloma (1994)
Reference	[35]	[50]	no	no	no
Number of cloning steps	4	Not known	3	4	4
Purity/homogeneity of cell line	Not known	Not known	Sub-cloned 2x, single IgG peak	isotype as pure IgG 2a	isotype as pure IgG 1
Origin of FCS used	New Zealand	USA	USA (GIBCO)	USA (Sigma), Canada (Wisent)	USA (Sigma), Canada (Wisent)
Absence of adventitious agents	Mycoplasma free	n.d.	Per WHO screening request	n.d.	n.d.
Culture conditions					
Medium	Iscove's DMEM 1	DMEM (modified)	Iscove's DMEM 2	HY-HT (10% FCS)	HY-HT (10% FCS)
Cell concentration	10^4–10^6	10^4–10^6	2×10^5	6×10^4–3×10^5	7×10^4–3×10^5
Serum-free culture medium	CD HM or PFHM II protein-free	Not tested	tested but no specification	Ultradoma-PF	Ultradoma-PF
Type of immunoglobulin					
IgG subtytpe	IgG 1 (ELISA)	IgG 1 (ELISA)	IgG 2b (ELISA)	IgG2a (FCA)	IgG 1 (FCA)
Heavy/light chains cDNAs	Yes	Yes	no	no	no
Antigenic site recognized on G	II	II c	III	II	II
Method for determining epitope	sequencing	sequencing	cross-neutralisation	cross-neutralisation	cross-neutralisation
Escape mutants					
derivation	SAD B19	CVS-11	not available	not available	ERA
aa substitutions in G	aa 57 (Leu to Arg)	aa 53 (Gly to Glu)			aa 198 (Lys to Glu)
	aa 217 (Lys to Glu)				aa 286 (Ala to Thr)
Production yield					
Yield in IU/ml (crude hybridoma)	62.5	3	30–60	22–32	11–32

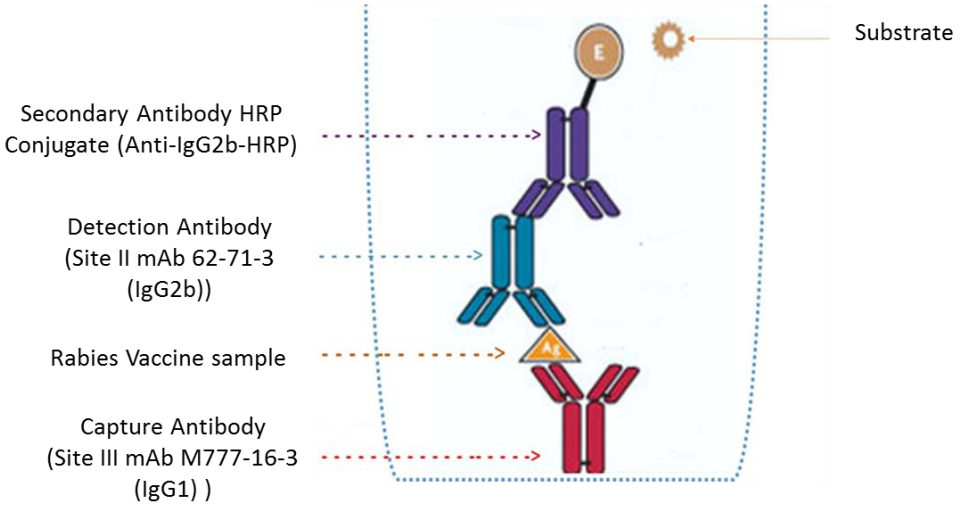
Legend: aa–amino acid, CVS 11–Challenge virus standard 11, DMEM–Dulbeccos' minimum essential medium, ELISA–enzyme linked immunosorbent assay, ERA–Evelyn Rokitniki Abelseth SAD derived RABV strain, FCA–Flouricon-CA Assay, HB–hybridization medium, SAD–Street Alabama Dufferin strain of RABV. Media specification: Iscove's DMEM 1 = Iscove's modified DMEM + HAM F12 (1:1) + 10% FCS; Iscove's DMEM 2 = Iscove's modified DMEM + ITS + antibiotics/antimycotics + L-glutamine + 5% FCS.
doi:10.1371/journal.pntd.0000542.t001

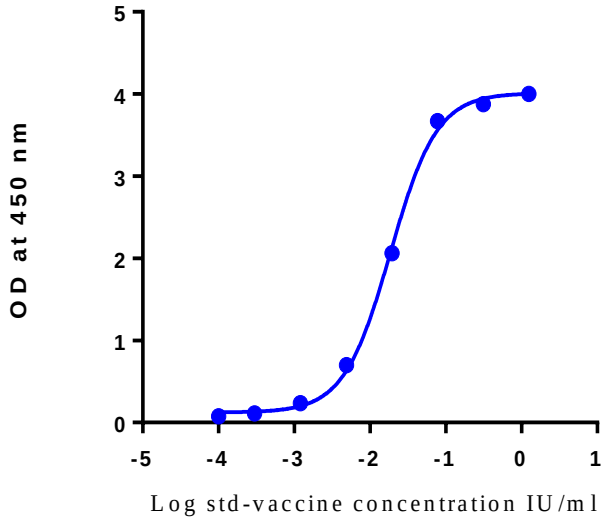
❑Development of G-Protein SW-ELISA method for Rabies vaccine testing

➢In-house highly characterized mAbs binding to the G protein of Rabies antigen

1.M777-16-3 **(IgG1)** binds to **Site III (Capture Antibody)**

2.62-71-3 **(IgG2b)** binds to **Site II (Detection Antibody)**





❑ Sandwich ELISA Procedure

Step 1: Coating of Rabies capture antibody (Site III) Incubate the ELISA plate at 2-8°C for overnight

Day 1

↓

Step 2: 200µL of blocking solution to each well of the ELISA plate and incubate for 1 hrs ± 5 min at 37 ºC± 2° C

↓

Step 3: Add standard and sample in different dilutions

↓

Step 4: Add Detection antibody (Site II)

↓

Step 5: Add anti antibody HRP conjugated

Day 2

↓

Step 6: Substrate , stop reaction and read plate at 450nm

❑ ELISA Data analysis

CombiStats Version 5.0. Monday, 1 April 2019, 17:22:16 [+05:30]. Page 1 of 2

Substance	
Method	Sandwich ELISA
Assay number	1
Technician	Neelam
Date of assay	01/04/19

Remarks:Rabies in-vitro potency assay

Standard		
Id.	INRS-CL107	
Ass. pot.	8.0 IU/mL	
Doses	(1)	(2)
1/40	3.186	3.167
1/80	2.484	2.309
1/160	1.548	1.478
1/320	.840	.853
1/640	.443	.417

Sample 1		
	RV90004	
Ass. pot.	? IU/mL	
Doses	(1)	(2)
1/40	2.953	2.957
1/80	2.050	2.030
1/160	1.231	1.294
1/320	0.635	0.651
1/640	0.339	0.359

Sample 2		
Id.	BS016	
Ass. pot.	? IU/mL	
Doses	(1)	(2)
1/40	3.093	3.098
1/80	2.241	2.241
1/160	1.42	1.535
1/320	0.726	0.743
1/640	.386	.383

Sample 3		
Id.	RV70004	
Ass. pot.	? IU/mL	
Doses	(1)	(2)
1/40	3.080	3.156
1/80	2.316	2.437
1/160	1.408	1.406
1/320	.741	.832
1/640	.380	0.40

Model: Sigmoid curves (5-parameters)
Design: Completely randomised
Transformation: y' = logit(y)
Variance: Observed residuals

Common slope(factor) = 1.17133 (1.15095 to 1.19171)
Correlation | r |: 0.998111 (Weighted), 0.999090 (Unweighted)
Asymptotes: 3.89034 and 0.0334428
Asymmetry factor: g = 1.77342

…. Continued

Source of variation	Degrees of freedom	Sum of squares	Mean square	Chi-square	Probability
Preparations	3	0.0228899	0.00762996	10.8455	0.013 (*)
Regression	1	18.8601	18.8601	8936.10	0.000 (***)
Non-parallelism	3	0.00195204	0.000650681	0.924897	0.819
Non-linearity	12	0.0273969	0.00228307	12.9809	0.370
Standard	3	0.000243894	8.12981E-05	0.115560	0.990
Sample 1	3	0.00568989	0.00189663	2.69593	0.441
Sample 2	3	0.0135054	0.00450181	6.39901	0.094
Sample 3	3	0.00795764	0.00265255	3.77041	0.287
Treatments	19	18.9123	0.995385	8960.85	0.000 (***)
Residual error	20	0.0422110	0.00211055		
Total	39	18.9545	0.486014		

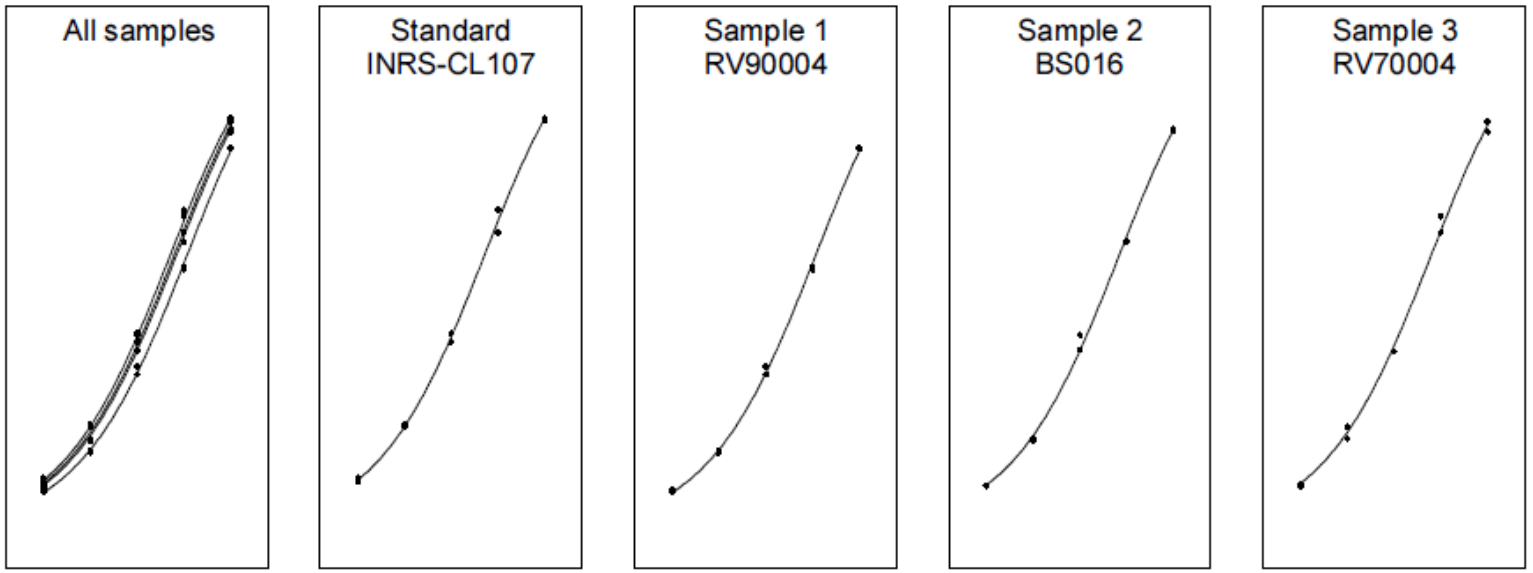
Sample 1			
	RV90004		
(IU/mL)	Lower limit	Estimate	Upper limit
Potency	5.97767	6.23526	6.50357
Rel. to Ass.	?	?	?
Rel. to Est.	95.9%	100.0%	104.3%

Sample 2			
Id.	BS016		
(IU/mL)	Lower limit	Estimate	Upper limit
Potency	7.00256	7.30203	7.61415
Rel. to Ass.	?	?	?
Rel. to Est.	95.9%	100.0%	104.3%

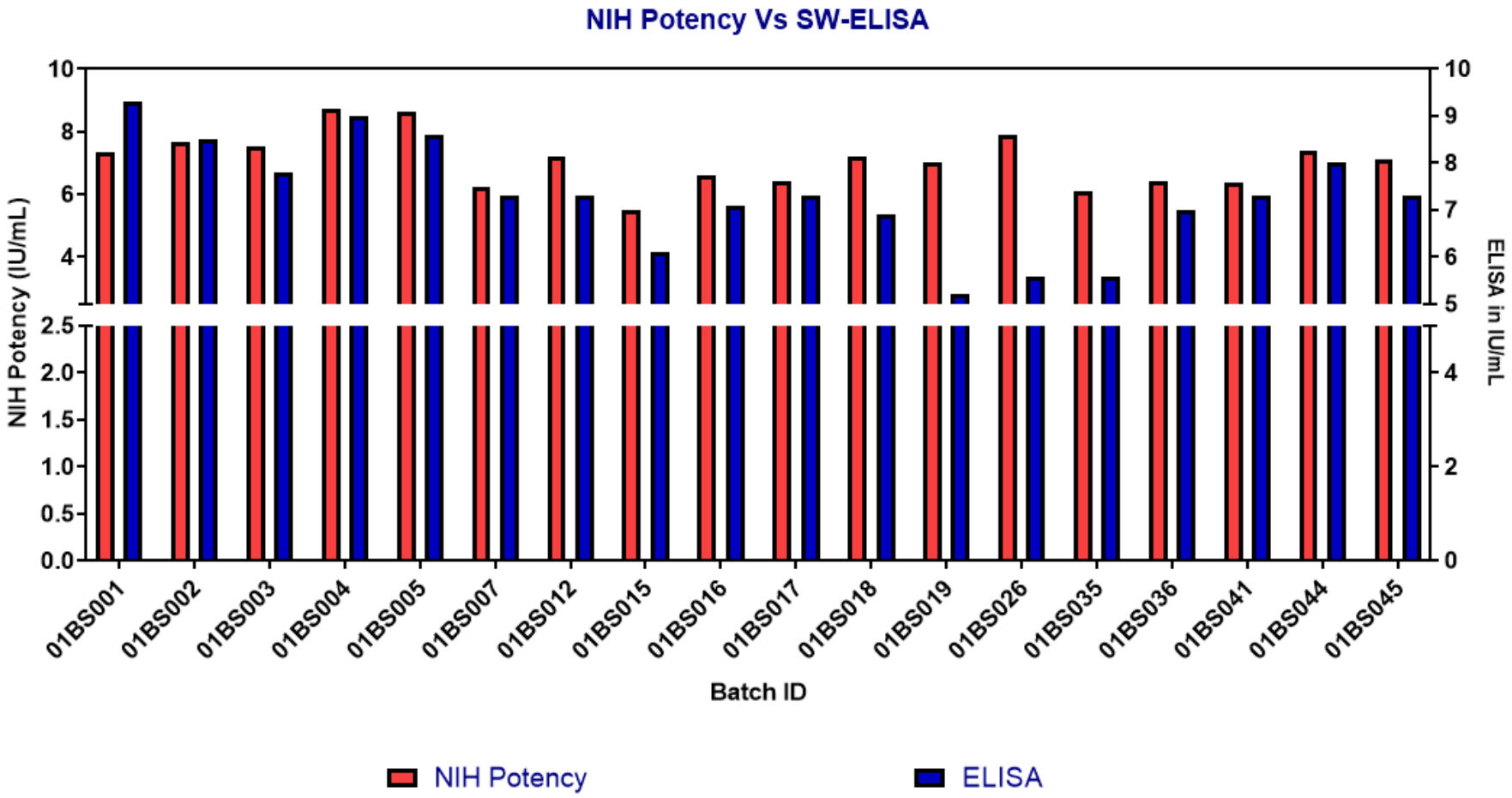
Sample 3			
Id.	RV70004		
(IU/mL)	Lower limit	Estimate	Upper limit
Potency	7.24547	7.55495	7.87756
Rel. to Ass.	?	?	?
Rel. to Est.	95.9%	100.0%	104.3%

…. Continued

Substance	
Method	Sandwich ELISA
Assay number	1
Technician	Neelam
Date of assay	01/04/19



❑ 18 Batch Data of NIH Potency Vs SW-ELISA



❑ Cost of Animal Potency

Rabies Vaccine Testing								
	Method	No of Animals used per Batch	Cost per animal (Approx. on the lower side) in Rs.	Husbandry cost for 28 Days per animal in RS.	Total cost for a 28 day batch release study in Rs.	No of Rabies batches taken in 2017-18	Total Animal Cost for releasing the batches in Rs.	Remarks
Current Method	NIH Animal Potency	136	200	300	68,000	60	40,80,000	Handling of challenge virus, Facility to do challenge study

❑ Cost of Sandwich ELISA

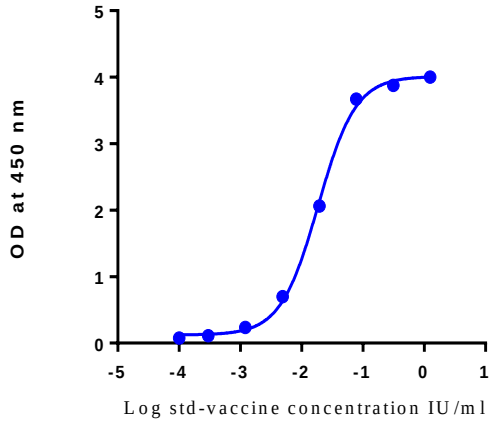
	Method	Time for Test	Cost of the Assay per plate (Approx. for testing 5 batches) in Rs.	No of Rabies batches taken in 2017-18	Total Cost for testing 60 batches in Rs.
Alternate Method	In-house Developed Sandwich ELISA (EDQM harmonizing to approve)	1 Day	300	60	18,000

❑ Zydus approach to Development of *in-vitro* potency assay G-Protein ELISA method for Rabies vaccine testing

- In-house highly characterized mAbs binding to the G protein of Rabies antigen

1. M777-16-3 **(IgG1)** binds to **Site III**
2. 62-71-3 **(IgG2b)** binds to **Site II**

- Developed sandwich ELISA and made the standard curve
- Assay range is from **1.25IU/mL to 0.01IU/mL**
- Screened 18 batches of Rabies vaccine by ELISA method
- Correlation between NIH and ELISA was studies
- More validation under QC is under progress



❑ G-Protein ELISA

Contents lists available at ScienceDirect

Biologicals

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

Potency test to discriminate between differentially over-inactivated rabies vaccines: Agreement between the NIH assay and a G-protein based ELISA

Audrey Toinon[1], Nadege Moreno[1], Heloise Chausse, Emilie Mas, Marie Claire Nicolai, Fabien Guinchard, Isabelle Jaudinaud, Françoise Guinet-Morlot, Patrice Riou, Catherine Manin*

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Keywords:
Rabies vaccine
ELISA
Potency test
NIH test
BPL inactivation

A B S T R A C T

The NIH assay is used to assess the potency of rabies vaccine and is currently a key measure required for vaccine release. As this test involves immunization of mice and subsequent viral challenge, efforts are being made to develop alternative analytical methods that do not rely on animal testing. Sanofi Pasteur has reported the development of a G-protein specific ELISA assay that has shown agreement with the NIH test. In this study we have generated several non-conform vaccine lots by an excessive inactivation with β-propiolactone (BPL) and assessed the capacity of both tests to detect the corresponding consequences. Excessive BPL inactivation causes G-protein unfolding, altering in turn viral morphology and the continuity of the G-protein layer in the viral particle. Both the NIH and the ELISA tests were able to monitor the consequences of excessive inactivation in a similar manner. Of note, the experimental error of the ELISA test was well below that of the NIH test. These results increase the prospect that the ELISA test could be considered a suitable candidate for the replacement of the NIH test.

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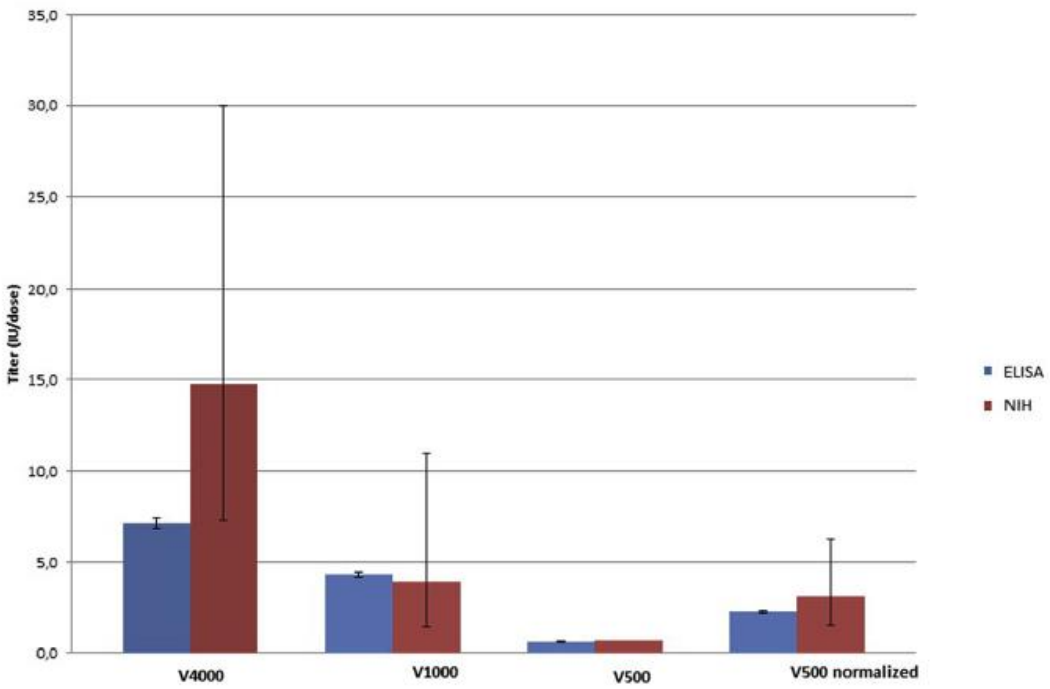

Fig. 5. Agreement between in vivo NIH and in vitro ELISA for each experimental vaccine (V4000; V1000; V500; V500 normalized).

Toinon et al., 2019

❑ Checklist for Replacing the in-vivo NIH with in-vitro assay

Scenario	Yes/No
Are all manufacturers interested in moving from in-vivo NIH potency to in-vitro (ELISA) method	Yes
Are the critical reagent available to implement the assay	Yes
Method validation/correlation for the assay performed	Yes
Are enough lots tested or Is it possible to get more manufactures share the final lot for validating the Assay	Yes
Can a harmonized training provided to all manufacturers	Yes (DCVMN/NIBSC)

❑ Conclusion

- Zydus Cadila as one of the leading vaccine manufacturers of Human Rabies vaccine is interested to collaborate and validate the assay platform through DCVMN network program
- It is time for considering this method as an alternate method for batch releasing and to replace the NIH potency

❑ Sandwich ELISA for HAV (IVRP)

European Directorate for the Quality of Medicines & HealthCare
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INFORMATION LEAFLET Ph. Eur. BIOLOGICAL REFERENCE REAGENT

Hepatitis A vaccine ELISA detection antibodies set BRR batch 5 composed of:

Anti-hepatitis A virus primary detection antibody BRR batch 5

Conjugated secondary detection antibody BRR batch 5

1. **Identification**

Catalogue code: Y0001623 Unit Quantity: ca 295 µL

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❑ Sandwich ELISA for HAV (IVRP)

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde (where applicable) and antimicrobial preservative content (where applicable) and the assay have been carried out on the final bulk vaccine with satisfactory results, these tests may be omitted on the final lot. If the assay is carried out using mice or other animals, then provided it has been carried with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Identification

The vaccine is shown to contain hepatitis A virus antigen by a suitable immunochemical method using specific antibodies or by the mouse immunogenicity test described under Assay.

the aluminium adjuvant used for the vaccine, prepare at least three dilutions of the vaccine under examination and matching dilutions of the reference preparation. Allocate the dilutions one to each of the groups of animals and inject subcutaneously not more than 0.5 ml of each dilution into each animal in the group to which that dilution is allocated. Maintain a group of unvaccinated controls, injected subcutaneously with the same volume of diluent. After 28 to 32 days, anaesthetise and bleed all animals, keeping the individual sera separate. Assay the individual sera for specific antibodies against hepatitis A virus by a suitable immunochemical method (2.2.14).

Calculations. Carry out the calculations by the usual statistical methods (5.7) for an assay with a quantal response.

From the distribution of reaction levels measured on all the

In vitro assay

Carry out an immunochemical determination of antigen content (2.2.14) with acceptance criteria validated against the *in vivo* test. The acceptance criteria are approved for a given reference preparation by the National Regulatory Authority in the light of the validation data.

Labelling. The label states (1) the biological origin of the cells and; (2) the adjuvant used for the preparation of the vaccine.

❑ Sandwich ELISA for HPV (IVRP)

World Health Organization

Post ECBS version
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 12 to 16 October 2015

Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines

Replacement of: TRS 962, Annex 1

A.9.11 Potency

An appropriate quantitative test for potency by an *in vivo* or *in vitro* method should be performed on samples that are representative of each final vaccine lot. The method and the analysis of data from potency tests should be approved by the NRA. The vaccine potency should be compared with that of a reference preparation, and the limits of potency should be agreed with the NRA. The NRA should approve the reference preparation used. If an *in vivo* potency test is used, this test may be omitted on the final bulk. The method of testing for antigen potency in an in vitro test could be quantitative with respect to the antigen content or relative to a reference preparation.

Because of the diversity in the reactivity of vaccines containing HPV VLPs produced by different manufacturing techniques and differences in the adjuvants used for the vaccine formulation, it is unlikely that International Standards will be suitable for the standardization of assays of vaccines from all manufacturers. Consequently, International Standards will not be developed for the potency of each HPV type. Manufacturers should establish a product-specific reference preparation that is traceable to a lot of vaccine, or bulks used in the production of such a lot, which has been shown to be efficacious in clinical trials. The performance of this reference vaccine should be monitored by trend analysis using relevant test parameters and the reference vaccine should be replaced when necessary. An acceptable procedure for replacing reference vaccines should be in place *(45, 46)*.

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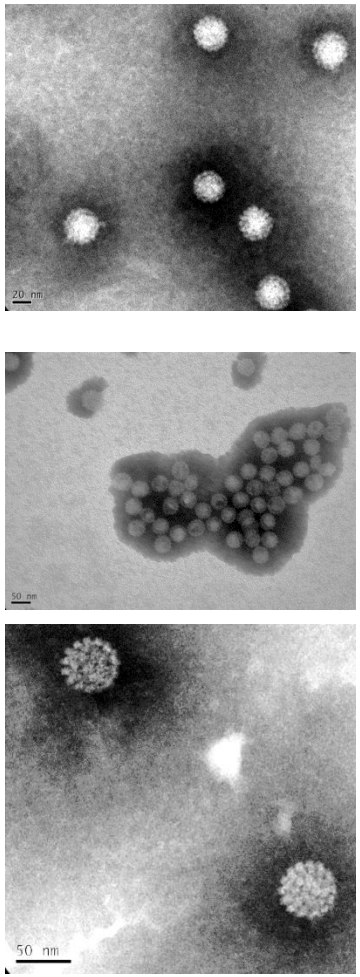
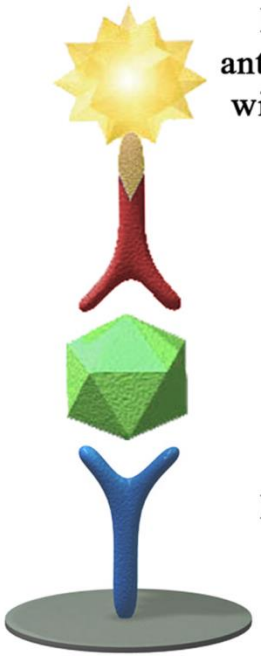
❑ Sandwich ELISA for HPV (IVRP)



Sandwich ELISA
(In-vitro)



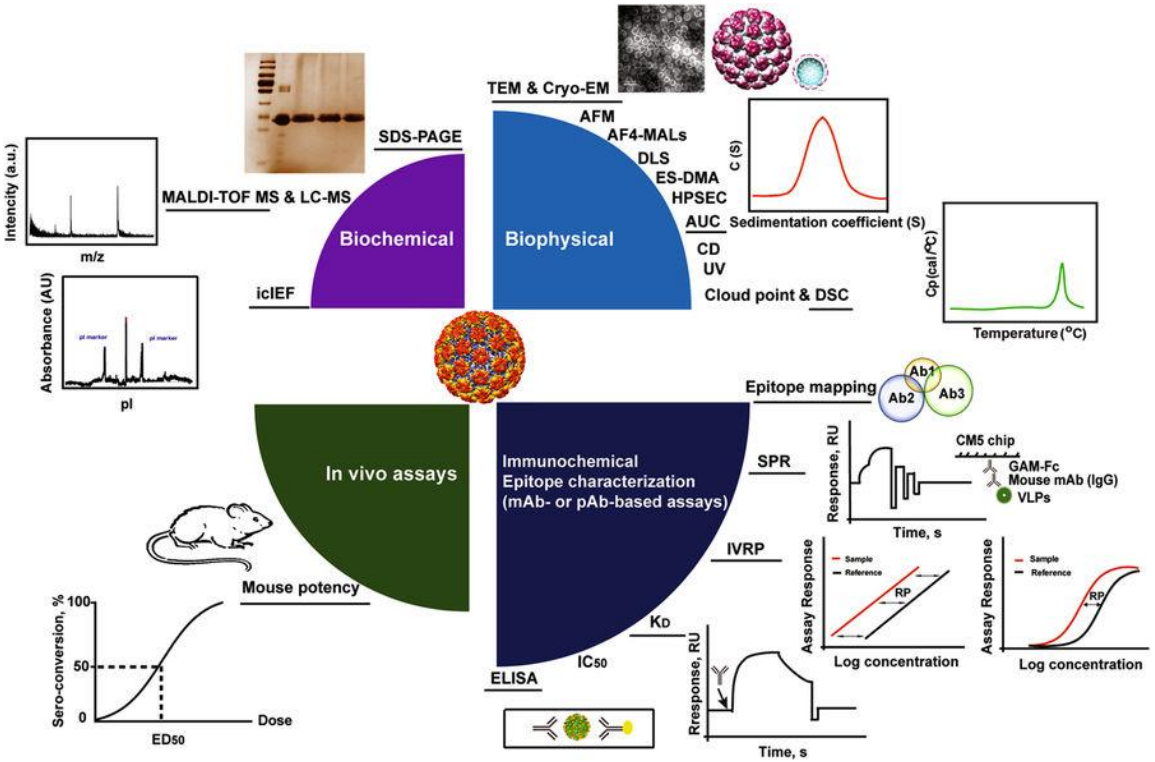
Monoclonal antibody labelled with peroxidase

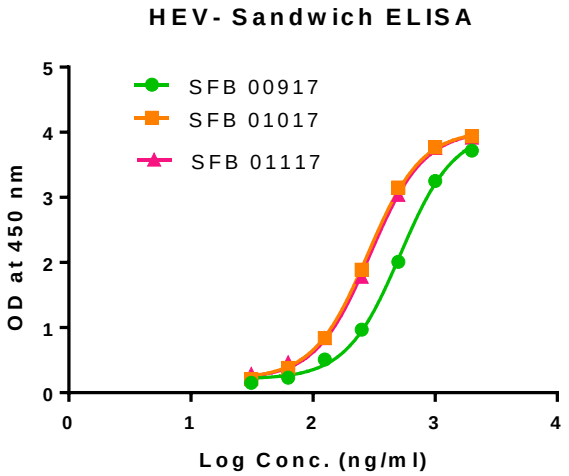
→ Recognizes only VLPs

Virus

Monoclonal antibody → Recognizes only VLPs

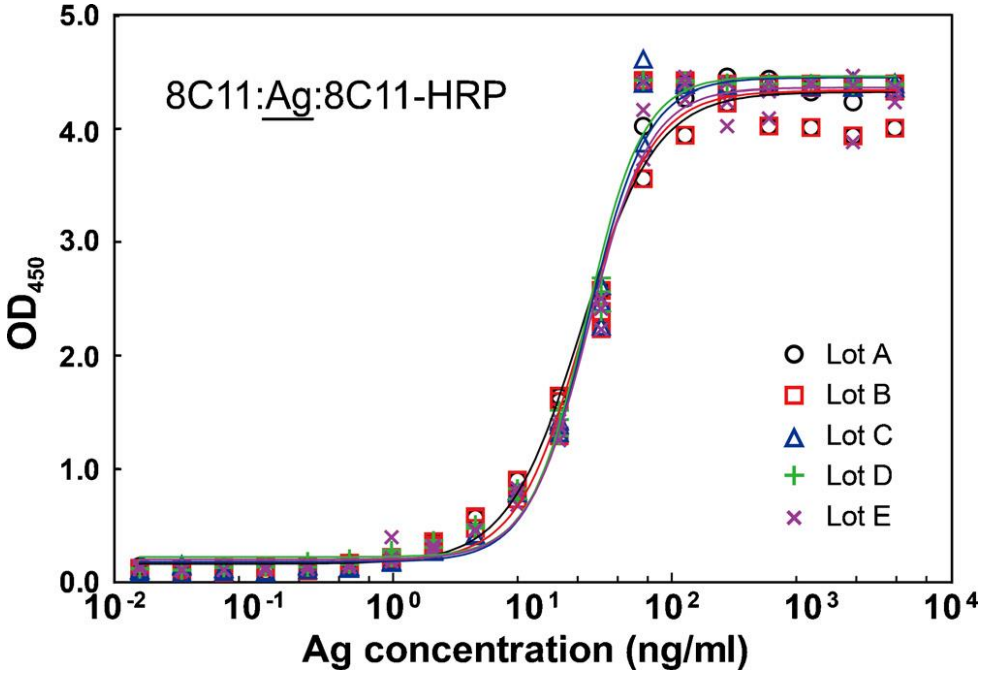
❑ Sandwich ELISA for HEV (IVRP)





X Huaung et al., 2017, npj Vaccines

❑ Sandwich ELISA for HEV (IVRP)



Antigenicity analysis using 8C11 (or 1B7) as capture Ab and detection Ab in a sandwich format for multiple lots of final aqueous product of Hecolin®. Highly consistent EC50 values for multiple p239 final aqueous product lots were listed .

Table 2
Antigenicity determination of multiple lots of p239 final aqueous product with two different ELISAs with 8C11 or 1B7 as capture and detection in a sandwich ELISA.

Lot no.	P239 antigenicity[a]	
	EC_{50} (ng/ml) (n – 3)	Relative antigenicity[b]
Sandwich ELISA (8C11: Ag: 8C11-HRP)		
Lot A	24.3 ± 0.2	**1.00**
Lot B	22.7 ± 0.4	1.07
Lot C	25.3 ± 1.5	0.96
Lot D	24.6 ± 1.2	0.99
Lot E	25.7 ± 1.0	0.94
Sandwich ELISA (1B7: Ag: 1B7-HRP)		
Lot A	90.7 ± 3.7	**1.00**
Lot B	85.5 ± 4.3	1.06
Lot C	90.2 ± 4.5	1.01
Lot D	91.7 ± 2.8	0.99
Lot E	91.9 ± 3.0	0.99

Based on these data, product consistency and process reproducibility were demonstrated with five commercial scale production lots.

[a] p239 antigenicity was assessed in a sandwich ELISA with mAb 8C11 or 1B7 as capture and detection Ab. Data from five p239 production lots (with a p239 concentration range of 2.8 to 3.5 mg/ml) are presented. Each reported relative EC_{50} value is an averaged value from three independent measurements.

[b] Relative antigenicity was calculated by normalizing EC_{50} value of a given lot to that of Lot A.

M. Wei et al. / Vaccine 32 (2014) 2859–2865

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Thank You