

Dr Jean-Michel Chapsal

Human Rabies vaccine glycoprotein G ELISA as an alternative to the challenge test: selection of a candidate method and future strategies

Jean-Michel CHAPSAL¹, Sylvie MORGEAUX², Eriko TERAO³

(1) EPAA - (2) Control Laboratory Department, ANSM, France - (3) EDQM, DBO/BSP, Council of Europe

Immunization with the native trimeric form of the rabies glycoprotein G induces the production of neutralising antibodies and protection against lethal challenge. In human rabies vaccines manufacturing, antigen quantification is used for the formulation of the final vaccine lot. Official release control of human rabies vaccines relies upon an *in vivo* potency test in mice, the NIH test (1). The NIH assay is a significantly variable test based on immunisation followed by an intracranial virus challenge and is using a large number of mice of which many suffer tetany symptoms. The replacement of the NIH test is thus a high priority for the implementation of the 3R concept.

Following the International workshop on Alternative Methods for Human and Veterinary Rabies Vaccine Testing : State of the Science and Planning the Way Forward organised by NICEATM and ICCVAM in Ames in 2011, an EPAA meeting in 2012 focused on gaps in technical knowledge and validation of *in vitro* G antigen quantification methods for potency testing, with the view to propose a strategy for the replacement of the NIH test. Participants stressed out that the current *in vivo* assay should not be used for correlation with the *in vitro* methods since it is highly variable, and that an agreement strategy should therefore follow. It was also agreed that the alternative glycoprotein G ELISA method should be able to discriminate between potent and sub-potent batches.

An International Working Group including regulators, rabies science specialists and vaccine manufacturers was formed to coordinate an inter-laboratory study aiming at identifying the most suitable replacement assay. A protocol was established to compare several ELISA methods, using potent and sub potent human rabies vaccine lots. The data from this study indicated a good agreement between the ELISA and the NIH test. One of the tested ELISAs was selected for its ability to discriminate potent from sub-potent lots but also to detect the main virus strains used in vaccine manufacturing (2, 3). The results of this study were presented in 2015 at an EPAA group workshop and were published (3).

Based on these results, an international collaborative study (coded BSP148) was launched by the Biological Standardisation Programme of the Council of Europe and the EU Commission to further validate the transferability and robustness of the selected ELISA. This BSP study should support to the global replacement of the *in vivo* NIH test by an *in vitro* method for the official release potency test of human rabies vaccines.

References

1. Laboratory techniques in rabies: the NIH test for potency. Seligmann EB. Monogr Ser World Health Organ., 1973,(23):279-86.
2. G-protein based ELISA as a potency test for rabies vaccines. Chabaud-Riou M, Moreno N, Guinard F, Nicolai MC, Niogret-Siohan E, Sève N, Manin C, Guinet-Morlot F, Riou F. *Biologicals*, 2017,46:124-29.
3. Replacement of *in vivo* human rabies vaccine potency testing by *in vitro* glycoprotein quantification using ELISA - Results of an international collaborative study. Morgeaux S, Poirier B, Ragan I, Wilkinson D, Arabin U, Guinet-Morlot F, Levis R, Meyer H, Riou P, Shaid S, Volokhov D, Tordo N, Chapsal JM. *Vaccine*, 2017, 40(5):369-81

