

Review Article

Regulatory Acceptance and Use of Serology for Inactivated Veterinary Rabies Vaccines

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Summary

In April 2013 the mouse antibody serum neutralization test (SNT) was formally incorporated into European Pharmacopoeia monograph 0451 for potency testing of inactivated veterinary rabies vaccines. The SNT is designed to replace the highly variable and pain and distress causing NIH mouse rabies challenge assay. The adoption of the SNT meets the European ambition (i.e., EC and CoE) to replace, reduce and/or refine laboratory animal testing. However, regulatory acceptance and use of 3R models, such as the SNT, remains challenging. This paper aims at clarifying the process of acceptance and use of the SNT. For this purpose it reconstructs the process and reveals barriers and drivers that have been observed by involved stakeholders to have played a role. In addition it extracts lessons to stimulate regulatory acceptance in similar future processes. The incorporation of the SNT into the monographs went relatively quickly due to a thorough test development and pre-validation phase, commitment and cooperation of relevant stakeholders and a strong project coordination of the international validation study. The test was developed by the Paul Ehrlich Institut, a leading European OMCL. This facilitated its European regulatory use. The use by industry is in a critical phase. At this stage product specific validation and the question whether the SNT will be accepted outside Europe are important influencing factors.

Keywords: rabies vaccine potency testing, serological assay SNT, regulatory acceptance and use, process reconstruction, drivers and barriers

1 Introduction

"I would wish to remind you all, whether you are sitting on the side of industry or on the side of regulators that, from my personal viewpoint, especially in the field of veterinary medicine, we are suffering from over-conservatism. This is really some-

thing which we all have to overcome." (Jean-Marc Spieser, late Head of Department of Biological Standardization, OMCL Network European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe (CoE), Strasbourg¹)

Rabies – Latin for madness – is one of the oldest diseases known to mankind. It is an acute and deadly disease caused by

¹ Introductory speech at the Workshop of the Paul-Ehrlich-Institut (PEI): Potency testing of veterinary vaccines: the way from *in vivo* to *in vitro*, December 2010, Langen, Germany (Jungbäck, 2012).

Abbreviations

ANSES, French Agency for Food, Environmental and Occupational Health Safety; ARA, actual regulatory acceptance by regulatory authorities; BSP, Biological Standardization Programme; CoE, Council of Europe; EC, European Commission; EDQM, European Directorate for the Quality of Medicines & HealthCare, Council of Europe; EFPIA, European Federation of Pharmaceutical Industries and Associations; EMA, European Medicines Agency; EPAA, European Partnership for Alternative Approaches to Animal Testing; FI, formal incorporation into regulatory requirements; IU, international unit; IVI, The Institute of Virology and Immunoprophylaxis (Swiss OMCL); NCE, National Center for Epidemiology (Hungarian OMCL); NIH, National Institutes of Health; OIE, World Organization for Animal Health; OMCL, Official Medicines Control Laboratory; PEI, Paul Ehrlich Institut (German OMCL); Ph. Eur., European Pharmacopoeia; QC, quality control of vaccine batches; RFFIT, Rapid Fluorescent Focus Inhibition Test; SC, Biological Standardization Steering Committee of the Ph. Eur.; SNT, mouse antibody serum neutralization test; UI, use by industry for regulatory purposes; USDA, US Department of Agriculture; ÚSKVBL, Institute for State Control of Veterinary Biologicals and Medicaments (Czech OMCL)

Received January 26, 2015;
Accepted April 16, 2015;
Epub May 4, 2015;
<http://dx.doi.org/10.14573/altex.1501261>



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a viral infection of the central nervous system. Once a patient develops symptoms of rabies, no treatment is available. More than 60,000 deaths are reported annually from rabies with more human deaths occurring in Asia than anywhere else in the world (30,000 deaths per annum).² Rabies vaccines, both human and veterinary, are crucial in fighting the disease. Every year more than 20 million people are vaccinated against rabies after being bitten. 40% of them are under the age of 15.³

The first rabies vaccine was developed in 1885 by Louis Pasteur and Emile Roux and was originally harvested from infected rabbit nerve tissue and inactivated subsequently. In the 1960's concentrated and purified cell-culture and embryonated egg based inactivated rabies vaccines were developed, which were much more consistent and safer in batch quality compared to the previously produced vaccine batches. Nonetheless, these inactivated rabies vaccines still derive from living organisms which may lead to variations, e.g., in the antigen amount or antigen conformation of the final product. Therefore, each vaccine batch is subjected to mandatory quality control testing. This includes safety testing⁴ and potency testing.⁵

The standard protocol for rabies vaccine potency testing is the NIH mouse rabies challenge assay, which was introduced almost 60 years ago and has remained largely unchanged ever since. This assay raises serious ethical concern in terms of animal welfare. Each final batch needs potency testing (Casey et al., 2011; Krämer et al., 2009), which means infecting a group of mice with the rabies virus and immunizing half of the group with the rabies vaccine. The other 50% of these animals show signs of rabies, which include severe suffering, and die (Bruckner et al., 2003). Although the test was never officially validated the NIH mouse rabies challenge assay – from now on referred to as the NIH test – has been used by regulatory authorities for over 50 years and has thereby gained its status of gold standard. However, it has a number of weaknesses. First, the test parameters differ from the natural situation, e.g., the intracranial challenge route does not reflect the natural route of infection nor does the intraperitoneal vaccination reflect the normal route of immunization (Romberg et al., 2012; Wunderli et al., 2006), and test results show high variability of up to 400% (Bruckner et al., 2003; Krämer et al., 2009). These drawbacks of the NIH test have made rabies vaccine potency testing a high priority in terms of the 3Rs, i.e., models to replace, reduce or refine the conventional animal model (Russell and Burch, 1959).

Over the last decades several assays have been developed with the goal to replace, reduce or refine the NIH test (see also Schiffelers et al., 2014a) of which the mouse serum neutraliza-

tion test (SNT) of the German Official Medicines Control Laboratory (OMCL), i.e., the Paul Ehrlich Institut (PEI) (Krämer et al., 2009, 2010), is the most recent. This serological assay was designed with the purpose to replace the NIH test for inactivated rabies vaccines for veterinary use. It was validated through an international collaborative study (Krämer et al., 2010) and became part of monograph 0451 for inactivated veterinary rabies vaccines of the European Pharmacopoeia (Ph. Eur.) in March 2012 (European Pharmacopoeia, 2013). Its regulatory acceptance was thereby largely accomplished within the European context. However, its broader international regulatory acceptance and use requires additional steps.

The SNT is in line with the ambition of the European Commission, laid down in Directive 2010/63/EU on the protection of animals used for scientific purposes, to diminish the use of laboratory animals and stimulate the acceptance and use of 3R models to replace, reduce and refine existing animal models. Directive 2010/63/EU states in article 13.2 that “...in choosing between procedures, those which use the minimum number of animals shall be selected” (European Commission, 2010). Furthermore, “The Member States of the Council of Europe have decided that it is their aim to protect live animals used for experimental and other scientific purposes to ensure that any possible pain, suffering, distress or lasting harm inflicted as a consequence of procedures being conducted upon them, shall be kept at a minimum.”⁶ To reach these goals full regulatory acceptance of 3R models is important. This means that they have to pass the following three subsequent sub stages (see also Schiffelers et al., 2012).

Box 1

Sub stages of regulatory acceptance and use of a new test method

FI	Formal incorporation into regulatory requirements
ARA	Actual regulatory acceptance by regulatory authorities
UI	Use by industry for regulatory purposes

This is often an arduous process in which many factors are seen to play a role.

In order to comprehend and augment current processes of regulatory acceptance and use and to facilitate similar imminent processes, it is important to reconstruct and analyze existing trajectories such as the SNT case.

² <http://www.who.int/rabies/about/en/>; last accessed on March 25, 2015

³ http://www.who.int/features/2012/world_rabies_day/en/; last accessed on March 25, 2015

⁴ Safety tests are designed to detect any material or property that may be harmful to the recipient, such as bacterial contamination, infectious virus or toxicity. In the case of viral vaccines in general, and especially of rabies vaccines, the specific problem of residual virulent virus is of the utmost importance. [http://whqlibdoc.who.int/monograph/WHO_MONO_23_\(3ed\)_part5.pdf](http://whqlibdoc.who.int/monograph/WHO_MONO_23_(3ed)_part5.pdf); last accessed on March 25, 2015

⁵ Potency is the capacity of a vaccine to protect the vaccinee against the virus, i.e., rabies. <http://www.oie.int/doc/ged/D8314.PDF>; last accessed on March 25, 2015

⁶ <http://conventions.coe.int/Treaty/en/Treaties/Html/123-A.htm>; last accessed on March 25, 2015

To examine the acceptance process this manuscript addresses the following key questions:

- Which factors influence the acceptance and use of the SNT to replace the NIH test?
- Which additional steps are needed to replace the NIH test by the SNT or other 3R options?
- Which lessons can be drawn from the process of FI, ARA and UI of the SNT to stimulate regulatory acceptance for similar future processes?

The findings derive from literature research, a series of interviews with experts in the field of rabies vaccine testing from standardization bodies, regulatory authorities (e.g., control laboratories) and industry, and international workshops that were attended by the corresponding author (see Appendix 1 in the supplementary data file at <http://dx.doi.org/10.14573/altex.1501261s> for a full description of the methods used). The multilevel perspective on technology transitions (see Section 4) is used to capture the interrelatedness of the factors influencing the regulatory acceptance and use of the SNT.

This manuscript elaborates on earlier work of the authors (Schiffelers et al., 2012, 2014a,b) and offers supplementary in-depth knowledge on the process of regulatory acceptance and use of 3R models through the reconstruction and analysis of the SNT case.

2 Process reconstruction

Below a description is given of the main developments regarding the SNT at the stages of FI, the ARA and the UI. However, we start by describing three pivotal pre-stages that have anticipated the process of FI of the SNT into the European monographs, i.e., the stages of test development, the pre-validation and the international validation (Milne and Buchheit, 2012).

Pre stage I: Test development

The serological assay RFFIT (rapid fluorescent focus inhibition test), which forms the basis for the SNT, has been available for quite a while (Smith et al., 1996) and is described in the World Organization for Animal Health (OIE) Manual of Diagnostic Tests & Vaccines for Terrestrial Animals (OIE, 2012) as a standard approved technique for veterinary rabies vaccines (Krämer et al., 2009). Even though the RFFIT is faster, less painful and uses fewer animals compared to the NIH test, it

has not been widely used and only little data existed concerning the comparability of the RFFIT with the NIH test. In the interest of the 3R principle to replace the NIH test and with the aim to develop an assay with better reproducibility of test results, the PEI developed the SNT serological assay, which is a modification of the RFFIT, as a refinement and reduction model (Krämer et al., 2009).⁷

The advantages of the SNT, when compared to the NIH test, are the reduction in animal use of up to 85% (Krämer et al., 2013), the reduced levels of stress and suffering of the animals involved, the better reproducibility of test results, the reduced amount of time needed to conduct this assay (less than 3 weeks instead of more than 4 weeks), and the fact that the assay is less labor-intensive and therefore less costly than the NIH test (Krämer et al., 2009, 2010). The SNT thereby offers manufacturers a higher speed of release of a vaccine batch. Furthermore, the data, evaluated by the PEI, showed a good correlation between the SNT and the NIH test (Krämer et al., 2009), which in itself was a remarkable outcome considering the variability of the NIH test.⁸

The test results were presented by PEI for the first time at the meeting of the OMCL network in 2007. At that stage several OMCLs were skeptical about the test out of fear of overlooking sub-potent rabies vaccine batches. The PEI promised to provide the stakeholders with additional test results and in May 2008 they presented these results at the annual meeting of the European OMCLs involved in rabies vaccine batch release testing and at the Biological Standardization Steering Committee (SC) meeting in June 2008.⁹ The latter presentation resulted in the initiative to start a collaborative study to confirm the transferability of the assay and its suitability for inactivated veterinary rabies vaccines on the European market.

Pre stage II: Pre-validation

In anticipation of the actual collaborative study, the decision was taken to start a small scale feasibility study to test the transferability of the serological assay to other labs. This transferability study was conducted by the PEI and the Swiss OMCL IVI (The Institute of Virology and Immunoprophylaxis). In this study 4 batches of rabies vaccines were tested using the serological assay and the NIH test in parallel. The conclusion of the pre-validation study was that the serological assay proved to be transferable to other laboratories (Krämer et al., 2010).

⁷ "The serological assay used, involves the immunisation of groups of 6 mice with approximately 1/5th the recommended dose volume of the test vaccine diluted appropriately, or of the reference standard vaccine preparation which is adjusted to the minimum potency allowed in the Ph. Eur. 14 days after immunisation blood samples are taken and the sera are tested individually for rabies antibody using the described virus neutralisation assay. Briefly, sera are titrated on 96 well microtitre plates and incubated for 1h with rabies virus. After adding BHK cells -baby hamster kidney cells- and incubating for 48h the presence of un-neutralised rabies virus is revealed by immunofluorescence. Dilutions of the sera that reduce the number of fluorescent cells by 50 per cent are calculated." (Krämer et al., 2010).

⁸ This conclusion requires some additional wording in the context of the earlier comment that the NIH test in itself is highly variable. Currently experts increasingly criticize the way of thinking in terms of looking for correlation with a poor reference test. Currently there is a tendency to move towards a concordance strategy allowing regulatory approval after a pass/fail correlation using potent and sub-potent batches (Stokes et al., 2012; Schiffelers et al., 2014a).

⁹ The SC consists of the Ph. Eur. group chairs (15, 15V, 6, 6B), the EMA, the WHO and co-opted experts. The SC determines the programme of the BSP and decides on priorities, new collaborative studies and the nomination of project leaders, in consultation with the stakeholders. <http://www.edqm.eu/en/Biological-Standardisation-Programme-Committee-61.html>; last accessed on September 26, 2014

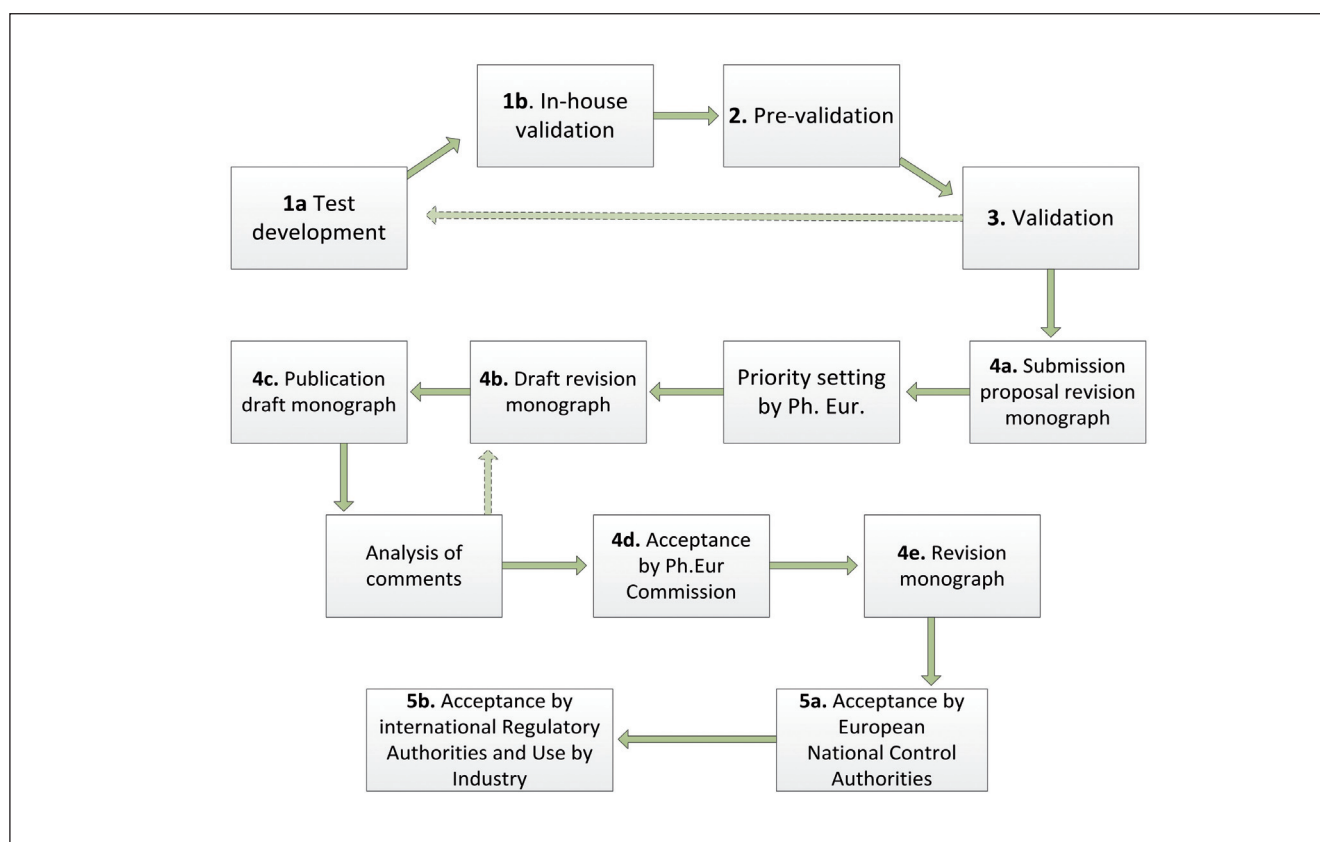


Fig. 1: Ph. Eur. process from test development to test implementation

Based on PPT C. F. M. Hendriksen, AllChemE Seminars. Brussels, 2003.

Pre stage III: International validation

Once shown to be generally applicable, an alternative method can be included into a specific Ph. Eur. monograph or into the general chapters. However, before any candidate assay can be included, the validity of the method, in terms of its robustness and its global applicability, has to be demonstrated in a large scale collaborative study. The aim is to demonstrate the wider transferability of the proposed assay and to confirm its suitability for the potency testing of inactivated rabies vaccines for veterinary use on the European market (Krämer et al., 2010). The less variation is observed in results of a relevant test, the more useable the test is.

In 2008 the Biological Standardization Program (BSP) of the EDQM initiated and later on coordinated collaborative study BSP105 to broadly validate the SNT test as developed by the PEI. The Standard Operating Procedure and the reporting sheets were provided by the EDQM and vaccines were provided by several participants. The study involved 13 laboratories from 10 different countries – including Canada, the US and EU Member States. It included 8 official control laboratories of regulatory

authorities and 5 manufacturer laboratories. All the laboratories were asked to test the potency of 4 different inactivated veterinary rabies vaccines – representing a range of products available on the EU market and produced by different manufacturers – using the SNT assay developed by the PEI.¹⁰ The results were published in 2010 (Krämer et al., 2010) and were disseminated through presentations at various international congresses.

The collaborative study showed very comparable inter-laboratory results and a good comparison between the results of the serological assay and the NIH test. The sub-potent vaccine failed in both the NIH and serological test. It was therefore concluded that the SNT, as developed by the PEI, is not only a relevant assay but is also a reliable, i.e., reproducible, assay for potency testing of inactivated veterinary rabies vaccines (Krämer et al., 2010). This, combined with the advantages of saving a substantial number of mice, test time and costs, has made the SNT a serious alternative for rabies vaccine potency testing purposes.

It must be noted that the SNT is a single-dose serological assay and a semi-quantitative test that serves as a biomarker for vac-

¹⁰ To anonymize the process, the samples were blinded and the results coded. One laboratory carried out the challenge test in mice to confirm the expected potencies. A sub-potent vaccine was included in the test to check if it would fail. The control sera of different levels of activity were centrally provided and the test results of the different labs were gathered and centrally evaluated through statistical evaluation using the software Combistats at the EDQM.

cine potency. The result of the test is a pass/fail outcome, answering the core question: Is the batch tested significantly better than the minimum level of 1 international unit (IU) as specified in the monograph? This means that the test discriminates between high and low potency/quality rabies vaccine batches, but is not suitable for quantifying potency (Krämer, 2013) (also see Section 3).

Sub stage 1: Formal incorporation into regulatory requirements (FI)

Monograph 0451 for inactivated rabies vaccines for veterinary use already allowed the replacement of the NIH test for batch potency by a validated alternative method, but did not refer to a specific assay. After the completion of the BSP105 the report was submitted to Ph. Eur. Expert Group 15V¹¹ and the assay was recommended for inclusion as an alternative batch potency assay in the Ph. Eur. monograph 0451 (Krämer et al., 2010). The formal incorporation of the SNT into the Ph. Eur. requires the steps as outlined in Figure 1. After the revision was put on the agenda a draft revision was prepared and published in 2011 in *Pharmeuropa*¹² for public consultation. This did not lead to any fundamental opposition and subsequently the European Pharmacopoeia Commission adopted the revised draft of monograph 0451 at its 142nd session in April 2012. It came into force on April 1, 2013. The formal incorporation of the SNT into the European monographs was thereby effectuated.

Sub stage 2 and 3: Actual regulatory acceptance and use by industry (ARA and UI)

Since its revision, monograph 0451 offers the possibility to conduct the SNT instead of the NIH test. In addition the monograph states that, “*In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes such an alternative validated method should preferably be used for routine testing.*” (European Pharmacopoeia, 2013).

Nonetheless, the incorporation of the SNT serological assay does not directly imply the replacement of the NIH test by the SNT, let alone the deletion of the NIH test from the monograph. Both assays are specified and it is up to the OMCLs and the manufacturers to choose the most suitable method. Rabies vaccine batch release is done by several OMCLs in Europe. These results are accepted within the EU by mutual recognition of data within the OMCL network for veterinary vaccine batch release. For veterinary rabies vaccine batch control, the PEI is the leading OMCL and they encourage the SNT for batch release testing. This means that manufacturers are stimulated to use the SNT to demonstrate their vaccine's potency. Other European OMCLs involved in veterinary rabies vaccine batch release are the French ANSES, the Swiss IVI, the Hungarian NCE and the Czech ÚSKVBL. ANSES and IVI have already

adopted the SNT for the quality control (QC) of inactivated veterinary rabies vaccines. The Hungarian and Czech OMCL's are in the process of implementing it.

Manufacturers however carry the final responsibility to prove the validity of the method for their specific product. At the time of writing, one manufacturer had successfully validated the SNT for their product (personal communication with employee) and declared that the NIH test will no longer be conducted for batch release purposes of veterinary rabies vaccines for the European market. Another manufacturer was in the process of validating the SNT for the respective specific product, but ran into some difficulties in meeting the criteria set by the PEI for the product specific validation of the serological assay (personal communication with employee).¹³

3 Factors influencing the FI, ARA and UI of the SNT

This section defines the drivers and barriers that are seen to have influenced the process of regulatory acceptance and use of the SNT. The process of formal incorporation of the SNT into Ph. Eur. Monograph 0451 was accomplished in about three years' time when counting from the first publication of Krämer et al. in 2009. This is very fast when compared to similar processes which often take ten years or more (Cooper and Jennings, 2008). As such the process of the SNT can be viewed as best practice in terms of the FI of 3R models for regulatory purposes. This swift FI was accomplished through a mix of factors.

First of all, rabies vaccine batch release testing has been regularly defined as a hot topic and a priority in terms of the 3Rs. This was fed by the broad agreement on the weaknesses of the NIH test (Stokes et al., 2011, 2012). The PEI, as one of the leading OMCLs in Europe when it comes to rabies vaccines, harnessed the momentum to start developing the SNT and to initiate a pre-validation study which showed good scientific results in terms of reproducibility and correlation with the NIH test. Subsequently, the collaborative study was a well-organized and coordinated process. In BSP collaborative studies project leaders are assigned by the BSP Steering Committee to coordinate the projects in cooperation with the EDQM secretariat, and the subsequent steps are reviewed regularly and approved by the steering committee. The collaborative study included an international group of participants from regulatory authorities (Europe, US and Canada) and from industry. While the primary focus was Europe, non-European partners were encouraged to participate. In addition, the BSP works hand in hand with the European Pharmacopoeia Commission and the European Pharmacopoeia Groups of Experts dealing with biologicals, of which group 15V (Vaccines and sera for veterinary use) is im-

¹¹ Group 15V veterinary sera and vaccines is responsible for the evaluation and approval of veterinary vaccine monographs.

¹² *Pharmeuropa* contains draft pharmacopoeial texts for which the European Pharmacopoeia Commission is seeking comment. <https://www.edqm.eu/en/Pharmeuropa-Texts-for-comments-1587.html?mbID=56>; last accessed on March 25, 2015

¹³ In light of this example it is interesting to note that the Expert Group on the Application of the 3Rs in Regulatory Testing of Medicinal Products of the European Medicines Agency (EMA) started a discussion on the possibilities for a lighter product specific validation for 3R models (communication with involved expert).



portant in this context.¹⁴ This group of experts played a critical role in encouraging communication between the stakeholders and promoting regulatory acceptance.

Nonetheless, these policy entrepreneurs also had to face several challenges at the stage of FI. The preparation of the critical reagents as a renewable resource (e.g., specific antibodies or reference antigens) is important in such collaborative studies, as is the availability of a range of products from the market, including different formulations and the use of sub-potent or borderline samples to test the system (Milne and Buchheit, 2012). The availability of reagents and of appropriate samples proved challenging. In addition, the BSP has to adhere to many regulations when sending biological samples to countries outside Europe and these biological materials run the risk of being impounded at the borders (communication with BSP employee). Another challenge was convincing participants to cooperate in the collaborative study. Collaborative studies are complicated and energy consuming processes and several participants were uncertain about the benefits of taking part. Some stakeholders were anxious that participation could in the end lead to a forced use of the SNT. As a result, engaging these participants in this process took a lot of persuasion and reassurance from the side of the initiators of the collaborative study.

When it comes to the use of the SNT for regulatory purposes the monograph is clear about the preferred method, namely an alternative validated method (see Section 2 sub stage 2: ARA). However, the monograph does not fully elucidate under what exact circumstances the 3R option will be accepted. From the side of European Regulatory Authorities the actual regulatory acceptance (ARA) of the SNT is imminent. As mentioned, the central OMCLs PEI, ANSES and the IVI already use the SNT and the other two European OMCLs involved in rabies batch release testing are in the process of implementing it. However, with the NIH test remaining part of the monograph, the Ph. Eur. leaves a certain amount of discretionary space to the regulatory authorities and manufacturers to choose the method they consider most suitable. This discretionary space is a source of uncertainty for manufacturers in terms of whether and under what conditions the 3R model will in the end be accepted. Moreover, regulatory acceptance of test results from industry, based on the SNT, requires product specific validation, which is perceived to be a significant hurdle (Casey et al., 2011). It costs manufacturers time and money and requires parallel testing, which temporarily increases the use of animals. It thus de-

pends on the cost benefit analyses of the manufacturer whether this final critical step will actually be taken. This is even more the case for veterinary rabies vaccines due to the fact that the price margins for veterinary vaccines are usually smaller than for human vaccines. This can influence the cost-benefit analyses of whether or not to use a 3R model to the disadvantage of the new model.

Besides, manufacturers have expressed their concern about the fact that the SNT assay is a pass/fail test that offers no information on the amount of antibodies induced by the vaccine. Additionally, several respondents mentioned that the SNT, despite the good results of the collaborative study, is observed to cause some difficulties in terms of non-responders. This is connected with the immune response of mice, which tends to be somewhat unpredictable. One of the respondents remarked: *"With a lot of good will you can use it. However, it is the question whether there is enough good will within industry to overcome these hurdles."*¹⁵

To circumvent the problems of non-responders and of not getting information on the amount of antibodies induced by the vaccine, several stakeholders have expressed a preference to move straight over to *in vitro* methods (i.e., antigen quantification models). Manufacturers for example have expressed a preference for glycoprotein tests like ELISA's, which they often already use for in-process control purposes. Glycoprotein tests allow quantification of the amount of antigen in the vaccine and to keep a more accurate control on the quality of the vaccine.¹⁶ This point of view is shared by the US Department of Agriculture (USDA) responsible for the licensing and batch release of veterinary rabies vaccines onto the US market. The USDA is not completely at ease with the SNT. Despite the conclusions of the collaborative study, they feel the SNT is not informative and sensitive enough. They are therefore investigating the possibilities of a direct transition towards antigen quantification tests (see also Section 4). In response to this discussion, the PEI recently developed a multi-dose serological assay to quantify the potency of inactivated rabies vaccines for veterinary use (Krämer et al., 2013). At this stage it is still unclear to what extent this development will influence the current discussion.

For now, some manufacturers have indicated that they will work towards combining the SNT with an antigen quantification assay with the future perspective to replace the SNT altogether by antigen quantification assays, if subsequent batches of a vaccine prove to be potent (see also Section 5: steps ahead).¹⁷

¹⁴ The European Pharmacopoeia Commission refers requests for revision to group 15V.

¹⁵ It should be noted that the NIH test faces comparable problems, but this did not become a major hurdle due to the fact that the test was developed in a period where the acceptance criteria for a test were much lower.

¹⁶ Antigen quantification is mainly seen to be of relevance for non-adjuvanted vaccines, since the adjuvant generally interferes with the antigenicity test. For this reason manufacturers might prefer to use antigen quantification for in-process testing and serology for final batch testing to demonstrate consistency.

¹⁷ In the European context the use of antigen quantification for QC purposes could lead to difficulties for the OMCLs in terms of retesting. Article 82 of Directive 2001/82/EC enables European Member States to have specific vaccine batches – among which rabies vaccines – retested at an Official Medicines Control Laboratory (OMCL). In this process, known as "Official Control Authority Batch Release" (OCABR), tests conducted by the manufacturer are repeated (Cooper and Jennings, 2008). Repeating the antigen quantification tests however is a challenge due to the fact that these tests are custom made for a specific product. As a result the tests differ slightly per manufacturer. This means that the OMCL responsible for the retesting of a rabies vaccine must be able to conduct a large variety of specific antigen quantification tests.

Another barrier is the fact that rabies vaccine manufacturers produce their vaccines for a global market. Due to a lack of harmonization of regulatory requirements, they have to take many different regulatory requirements into account. This withholds the adoption of an alternative model for regulatory purposes. At a global level the NIH test still is the leading procedure and all regulatory frameworks entail – a variation of – this assay (Schiffelers et al., 2014a). Sticking to the NIH test therefore often is the most secure option for manufacturers in terms of international regulatory acceptance. In this context, a regulator remarked: *“The lack of harmonization is the main argument industry plays. But if manufacturers inform regulatory authorities about the possibilities of serology and its acceptance in Europe they have the potential to convince these authorities.”* In practice this effort will normally only be made if profitable in one way or the other to the manufacturer or if required by law. Nonetheless, some manufacturers are seen to have a strong company policy on the topic of the 3Rs and they have already taken substantial steps to phase out the use of the NIH test and to adapt to the changed European requirements. As such they function as frontrunners in the field.

To conclude, both the pre-validation study and the collaborative study were very important in proving that the alternative method works in the hands of all the participants. The process from test development to FI was facilitated by strong policy entrepreneurs and a clear problem ownership and process management – first the PEI, then the BSP, then the European Pharmacopoeia Commission and group 15V. Additionally, the intense collaboration within the OMCL network, the early involvement of a statistician to design the study and analyze the data during the validation process and the wide dissemination of the study results have all added to the swift incorporation of the SNT into monograph 0451. However, the swiftness also came with a price. The persuasiveness of the initiators/project-coordinators was a big driver for the adoption of the SNT into the Ph. Eur. monographs, but may have partly led to restrained attention to the drawbacks of the SNT as perceived by manufacturers and regulators such as the USDA.

4 Analyses

To analyze the influence of the different drivers and barriers on the regulatory acceptance and use of the SNT, they are positioned in the multilevel perspective on technology transitions (Schot and Rip, 1996), which was developed to better understand complex technology transition processes such as the acceptance and use of 3R models (Schiffelers et al., 2012). Such system innovations are almost always *“the result of the interplay between many factors and actors”* (Geels, 2006). Consequently an integrative approach is needed to comprehend such processes, and this is offered by this multilevel perspective. It addresses three levels of factors that influence technology transitions (Geels, 2006; Kemp, 2010; Schiffelers et al., 2012):

- the micro- or niche level where innovations are developed and validated;

- the meso- or sociotechnical regime level which includes the existing rules and regulations, expertise, dominant practices and the standing institutions;
- and the macro- or sociotechnical landscape level which comprises the material infrastructure, the existing political culture and coalitions, social values, the macro-economy, demography and the natural environment.

Successful technology transitions require alignment of the developments at these three levels. An aggregation of the developments at these levels can only occur if an innovation (e.g., a 3R model) meets the needs of the meso- and the macro-level (Schiffelers et al., 2012). If a new technology does not comply with these needs, it will be incapable of escaping from the niche where it was developed (Kemp, 2010). On the other hand, the meso- and macro-level have to open up to alternative ways of thinking in order to give a new technology a serious chance to break through.

Apart from playing a role in the analysis of the influences on regulatory acceptance and use, a distinction between these three levels is also helpful in defining those influences that offer better opportunities in terms of improving the acceptance and use of an innovation. The factors at the micro- and partly the meso-level for example tend to offer more possibilities for change than the broader societal developments at the macro level.

The process of FI of the SNT can be defined as a success in terms of getting a 3R model incorporated into regulatory requirements. The SNT was able to escape from the niche in which it was developed and validated and to become part of the European regulatory regime, i.e., the monographs of the Ph. Eur. This is mainly the result of the solid basis that was created scientifically, in terms of the process during the pre-stages that anticipated the FI and by the legislative context that stimulates the acceptance and use of 3R models. Additionally, its actual regulatory acceptance within Europe is largely accomplished and its use by industry in the European context is slowly progressing. However, its regulatory acceptance and use by regulators and industry at a global scale still is a big challenge.

Figure 2 summarizes the different forces (as described in Section 3) that are observed to have played a role in the process of regulatory acceptance and use of the SNT, using the multilevel perspective on technology transitions. It shows the opposing forces at hand.

In the European situation the drivers have been able to outweigh the barriers in the sub stages of FI and ARA. For the broader ARA – i.e., outside Europe – and for the UI it is still highly uncertain whether the drivers will be able to outweigh the barriers. Here the previously mentioned discussion about the drawbacks of the SNT plays an important role. In the US these drawbacks are observed to outweigh the benefits of the SNT and as a result the USDA does not opt for the use of the SNT. Instead they are investing in a direct transition towards full *in vitro* methods, i.e., antigen quantification. Several manufacturers have also expressed a preference for this approach. They question the added value of the SNT and instead would like to invest in *in vitro* methods, which most of them already use for production and in-process control purposes. The SNT for them

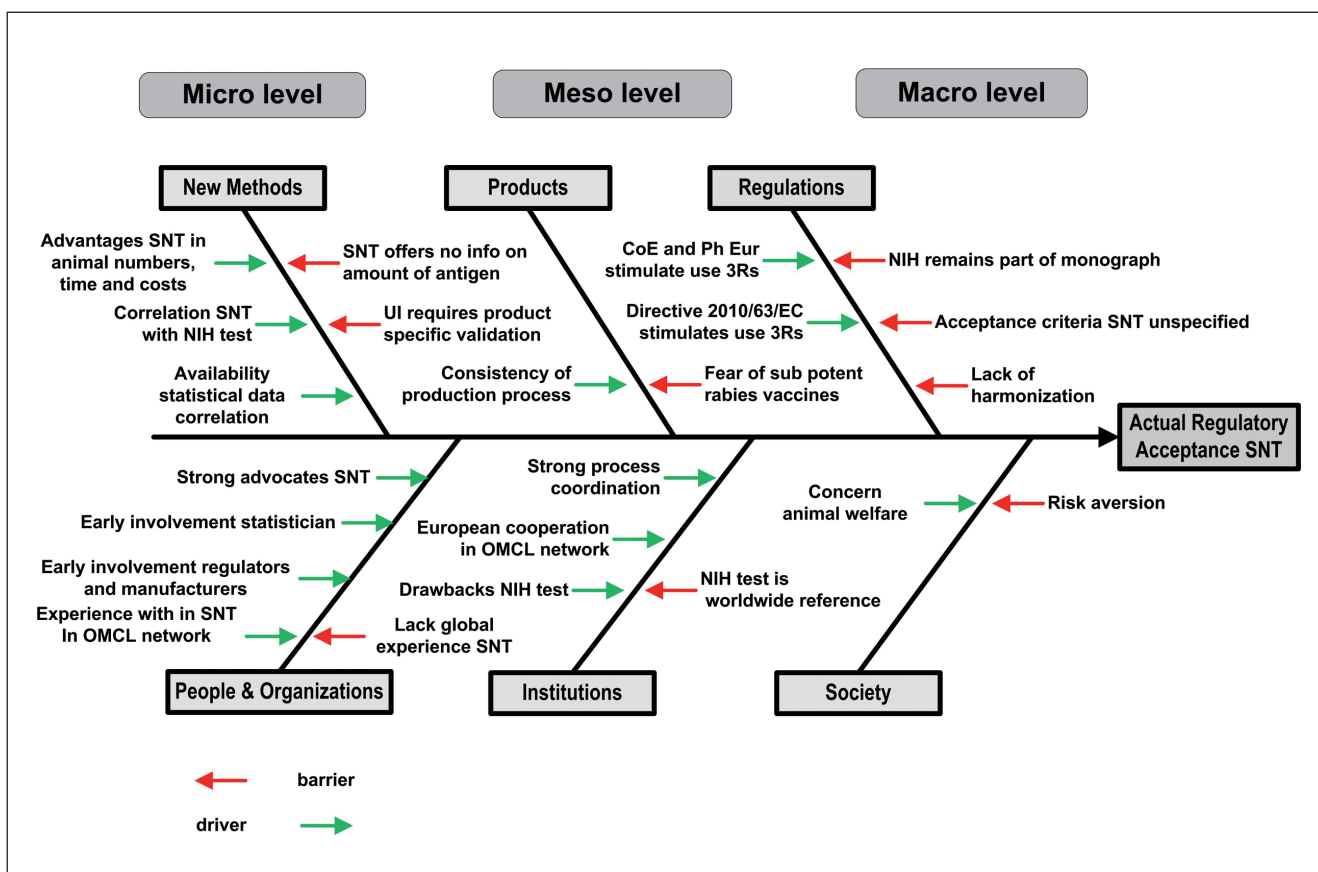


Fig. 2: Multilevel perspective on drivers and barriers influencing regulatory acceptance and use of the SNT

Based on Schiffelers et al. 2012.

is merely an intermediate step or even an unnecessary step from the NIH test to the use of *in vitro* methods as part of a consistency approach (see Section 5). In this context, several respondents from industry suggested that representatives from industry should have been involved earlier on in the process of the development and pre-validation of the SNT. According to them this could have prevented that the discussion on the drawbacks of the SNT test surfaced at the phase of UI.

5 Lessons learned and steps ahead

From the perspective of the European ambition to diminish the use of laboratory animals and to stimulate the use of 3R models (see Section 1), it is important to not only clarify the process of regulatory acceptance and use of the SNT, but also to answer the following questions:

– Which lessons can be learned from the process of FI, ARA

and UI of the SNT to stimulate regulatory acceptance for similar processes in the future?

– Which additional steps are needed to replace the NIH test by the SNT or other 3R options?

The case of the SNT teaches us several important lessons regarding the regulatory acceptance and use of 3R models.

First of all it shows that there must be a firm commitment of the key stakeholders to allocate time and money to take part in such a project, to exchange method details, reagents, test samples and adhere to the specific rules of the collaborative study. The strong commitment from European stakeholders such as the PEI, the EDQM and the BSP was fed by legal texts of both the CoE¹⁸ and the EC¹⁹ calling for minimizing the suffering, pain and distress caused to animals used for scientific purposes.

Secondly, effective interaction between central stakeholders within industry and regulatory authorities on an international level is essential. Interaction is necessary to exchange and transfer available scientific data with regard to the 3R model

¹⁸ The European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (1986, ETS 123)

¹⁹ Directive 2010/63/EU on the protection of animals used for scientific purposes

and to discuss criteria that have to be met for regulatory acceptance and use. It is pivotal that this is done within the regulatory framework the 3R model is destined for, which was, in this case, the EDQM and the OMCL network. In this way support from the regulators is stimulated from the very beginning. Early involvement is also needed from the side of industry. IFAH²⁰ can fulfill the network role for manufacturers – comparable to the OMCL network at the side of regulators – but it is also important to involve manufacturers individually owing to the fact that the manufacturers are competitors. Furthermore, it is important to firmly involve regulatory authorities from other parts of the world to anticipate what is needed for broader regulatory acceptance.

Thirdly, the SNT process shows the importance of a well-designed and coordinated validation process which starts with a small scale feasibility study and moves to a large scale method transfer and validation, subsequently involving more labs and more products. Whether a 3R method will be fit for regulatory acceptance depends on the availability of reproducible test data. Regulators need proof that an assay does what it is intended to do, i.e., to make a distinction between a potent and a sub-potent vaccine batch. To be able to compare and interpret the data of the different stakeholders in a meaningful manner the early involvement of statistical knowledge to ensure a well-designed study is of great importance.

Fourthly, the validation process requires a strict process management with predefined steps to be taken and questions to be answered. This needs to be supervised by well informed and committed coordinators with the legitimacy to get the parties together.

Despite the positive experience of the SNT collaborative study, the validation process of 3R models is mostly very challenging due to the required correlation with the existing *in vivo* assay, which is very difficult if not impossible to achieve. *In vivo* assays habitually are a poor reference owing to the often highly variable test results (Schiffelers et al., 2014a). Therefore, a change in the way of thinking is needed to help 3R models out of their niches and into the existing regulatory regime. Such a different way of thinking in terms of validation is the concordance strategy in which regulatory approval and implementation of an alternative method can be obtained after a pass/fail correlation using sub-potent batches instead of by seeking a full correlation with the conventional animal model (Stokes, 2012).

The next challenge, as mentioned, is regulatory acceptance of the SNT outside Europe and its use by manufacturers for batch release purposes. For this, several actions are needed, i.e., ongoing international communication among regulatory authorities and between regulatory authorities and manufacturers and continuing harmonization efforts. A first step in this direction, although non-binding, was taken in 2013 by the World Organization for Animal Health (OIE) through the adoption of the SNT

for potency testing of inactivated veterinary rabies vaccines in their Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.²¹

The acceptance and use of the SNT for human inactivated rabies vaccines is an additional step to be taken. Human rabies vaccines are generally less complex in composition as they are non-adjuvanted. Moreover, the RFFIT (the predecessor of the SNT) is already recognized by the WHO as a valid alternative method (WHO, 2007). Nonetheless, it was agreed that further research and validation is needed for serological assays such as the RFFIT and the SNT to gain broader acceptance for human rabies vaccine potency testing (Casey et al., 2011).

In terms of QC testing of vaccines the consistency approach is an important alteration in the way of thinking. This approach, which has already gained its merits in the area of well characterized vaccines, is winning terrain in the discussions about batch release testing of classical vaccines, like rabies vaccines. The consistency approach is based upon the principle that the quality of a vaccine is the result of the strict application of a quality system and consistent production (Hendriksen et al., 2008; Kulpa-Eddy and Dusek, 2011). With this approach, the focus is changed from batch release testing to in-process control. It implies that a consistent production process is key to the quality of a vaccine. The approach allows replacing animal bioassays like the NIH test on the final batch by a battery of meaningful non-animal tests with enhanced capacity to compare new batches with batches of proven quality (Hendriksen et al., 2008). This approach requires a combination of immune-chemical and physico-chemical tests performed in-process and on the final product. Such a combination of tests, together with adherence to the guidelines of Good Manufacturing Practice (GMP) (De Mattia et al., 2011), shall ensure that all produced batches are of the same quality as the batches that have proven to be safe and efficacious during licensing (Kulpa-Eddy and Dusek, 2011). For conventional products – like rabies vaccines – it is believed that the consistency approach will lead to a substantial reduction in animal use for potency testing purposes in the final batch (Hendriksen et al., 2008), even though some animal testing may still be required during prelicensing or validating manufacturing changes (Kulpa-Eddy and Dusek, 2011).

The Ph. Eur. and EMA underscore the potential of the consistency approach and are taking gradual steps in this direction. The general notices of the *Pharmacopoeia* (Supplement 8.2) for example were revised in June 2013 in order to address the consistency of production approach in the context of reduction of animal testing. This change entered into force on July 1, 2014. The monograph on veterinary rabies vaccines is currently adapted to these general notices.

For the process of actual regulatory acceptance, regulators depend on the data on the production process of a specific vaccine

²⁰ The International Federation for Animal Health (IFAH) is the global representative body of companies engaged in research, development, manufacturing and commercialization of veterinary medicines, vaccines and other animal health products in both developed and developing countries across the five continents.

²¹ http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.13_RABIES.pdf



from manufacturers. This means that industries need to share their *in vivo* and *in vitro* data with regulators. Industries however tend to be very cautious in sharing test information with regulators as long as they are uncertain how this might influence the regulatory decision regarding their product. Manufacturers therefore ask for more clarity on the acceptance criteria when it comes to the evaluation of their product. Regulators in turn are very cautious with regard to specifying precise acceptance criteria as long as they do not know what kind of data they can expect. This leads to a “catch 22” in which both parties are waiting for the other to take the first step (Schiffelers et al., 2014a). “To enable the submission of results of screening tests outside the drug specific regulatory decision processes, the EMA is now working on the so called safe harbor concept. In this way we can compare these data with the results of the conventional tests as described in the current requirements within a neutral context.” (personal communication with involved expert). Within such safe harbors manufacturers can discuss their test results with regulators without this having direct consequences on the evaluation of their product.

The process of regulatory acceptance and use of 3R models to replace the NIH test for rabies vaccine potency testing has long been characterized by inertia. This inertia is fed by the dreadfulness of the disease and the fear of missing sub-potent rabies vaccine batches. Moving away from the well-known NIH test entails taking a risk in this highly risk averse context. The biggest chance for successful change in such high risk areas is created by thinking in terms of evolutions rather than in terms of revolutions (Schiffelers et al., 2012). For this reason the acceptance and use of 3R methods in this area requires an incremental process, i.e., no change in terms of radical developments but new test regimes that gradually grow out of old ones (Geels, 2002). The regulatory acceptance and use of the SNT has to be placed in this context. Even though *in vitro* methods might be preferable in several respects, the use of antigen quantification for potency testing purposes of adjuvanted vaccines still needs to overcome significant technical problems. Optimization/validation of these *in vitro* models is therefore likely to require a considerable additional amount of time. For the purpose of moving away from the NIH test within a limited timeframe, the use of the SNT is a recommendable intermediate step in the transition towards in-process control using *in vitro* methods. So, even though there is a preference among several stakeholders to make a direct move to *in vitro* methods, a more gradual approach which starts with combining serology with *in vitro* methods, is in our view a very sensible approach.

To conclude, the process that led to the formal incorporation of the SNT into Ph. Eur. monographs demonstrates the importance of the four C's of Commitment, Communication, Collaboration and Coordination (Schiffelers et al., 2014b). However, the case of rabies vaccine potency testing reveals a fifth important C, namely the C of Continuity. To gradually replace the NIH test, continuity is very important. Many stakeholders have worked for decades to replace the NIH test. Their enduring effort can be seen as one of the central drivers in phasing out the NIH test and to ultimately change the collective mindset in favor of the 3Rs.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgment

We thank the Doerenkamp-Zbinden Foundation for funding this project. We thank the respondents for sharing their expertise and perspectives with us.

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