Development of 3Rs Alternatives for Determining Potency and Toxicity of Vaccines in Cuba: Current Challenges and Research Projects in Progress

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Summary

For a decade now, Cuba has directed efforts towards the introduction and development of 3Rs alternatives for potency and toxicity testing of vaccines. Significant progress has been achieved for some vaccines, such as the potency of hepatitis B vaccine (neutralization ELISA), the toxicity/potency of diphtheria vaccine (guinea-pig serology-Vero cell), and the potency of tetanus vaccine (mouse serology-ELISA). Finlay Institute is the major manufacturer of vaccines in Cuba and is undoubtedly committed to these activities. The purpose of this lecture is to provide an overview of the state of the science and to show the progress we have made in the introduction and development of 3Rs alternatives for the evaluation of vaccines, as well as in the main research projects now in progress. These include in vitro methods (ELISA, cell culture assays, biochemical, and immunological functional tests), serology, and consistency approaches.

Keywords: vaccines, potency, toxicity, Three Rs

1 Introduction

Finlay Institute is Cuba's biggest manufacturer of vaccines. It is part of a group of around 50 institutions fully devoted to the production of pharmaceutical and biotechnological products and other activities and services related to the protection of human health. Currently, Finlay Institute produces seven bacterial vaccines, including a meningococcal vaccine for serogroup B vaccine that is uniquely produced and is the most clinically effective against this serogroup so far. Moreover, the institution produces and provides antigens that are used in the manufacturing of some other combined vaccines and adjuvants.

Like other Cuban institutions, Finlay Institute is committed to the development, introduction, validation, and implementation of 3Rs (Refinement, Reduction, and Replacement), as first introduced by Russell and Burch in their book, "The Principles of Humane Experimental Technique," published in 1959 (Russell and Burch, 1959). Specifically, there is an ongoing program for developing 3Rs alternatives for a group of animal tests historically used for determining potency and toxicity of vaccines. This program focuses, in part, on the refinement of procedures for the handling of animals during experimentation but is concerned primarily with the reduction of animals in quality control testing and the replacement of classical challenge assays by more ethical and relevant alternative methods that use the current state of the science in this field and the technical and logistic conditions available in our country.

In Cuba we have some experience in the development of 3Rs alternatives. In 2001, a number of Cuban authors presented their work related to the replacement of an immunogenicity test in mice for the recombinant human hepatitis B vaccine at the "Advanced Science and Elimination of the Use of Laboratory Animals for Development and Control of Vaccines and Hormones Congress." They developed and validated an inhibition ELISA using a human anti-hepatitis B immunoglobulin for neutralization, instead of the already commercially available kit for directly determining the content of the *Hepatitis B* surface antigen. The inhibition ELISA demonstrated a good correlation versus the animal potency test ($r=0.995$, $p=0.0008$), and the Abbott commercial *in vitro* method ($r=0.998$, $p=0.0009$), respectively (Chovel and Huerta, 2002). Some years later, the same authors demonstrated the validity of the inhibition ELISA for determining *in vitro* potency of the *Hepatitis B* surface antigen in the presence of other antigens (diphtheria and tetanus toxoids, whole cells of *Bordetella pertussis* and the *Haemophilus influenzae* type b polysaccharide conjugated to protein) for vaccines provided by two different manufacturers. A significant correlation also was obtained regarding the *in vivo* potency test ($r=0.982$, $p<0.05$) and the antigen content *in vitro* test ($r=0.984$, $p<0.05$), respectively (Chovel et al., 2008)

Another interesting experience related to the characterization of the biological activity of the *Haemophilus influenzae* type b polysaccharide (polyribosyl ribitol phosphate, PRP) obtained by chemical synthesis. In the case of PRP, there is no universally accepted animal potency test, and lot release relies on relevant physico-chemical tests (Vérez et al., 2004). Nonetheless, the authors considered it important to develop an immunogenicity test in rabbits to monitor the biological significance of physico-chemical assays and the stability of the new vaccine as part of this characterization. The immunogenicity test showed

acceptable correlations regarding the free PRP content and the molecular distribution by HPLC; both physico-chemical tests associated with the prediction of clinical efficacy of the vaccine when used in the field (Chovel et al., 2007)

At the moment, the ongoing 3Rs methods at Finlay Institute are designed to replace animal challenge assays used for determining potency and toxicity of vaccines with serology tests and other approaches focused on eliminating or drastically reducing the number of animals used during the quality control process of the vaccines.

2 Serological approaches for potency of DPT vaccines

Traditionally, challenge methods have been used for determining potency of some vaccines. Nevertheless, these methods are quite variable, cumbersome, and time-consuming, and they require large numbers of animals and induce substantial levels of suffering. Besides, the new developments and strategies in vaccine production make extensive quality control of the final product less relevant, and even superfluous (Hendriksen, 2006). That is why many efforts have been made in the search for relevant methods for the replacement of the animal challenge assays used for the potency of vaccines. One of the most feasible alternatives is serology. This means the estimation of the protective capacity of a new batch of vaccine by antibody titration in immunized animals. In fact, for diphtheria and tetanus toxoids the multi-dilution challenge methods have been replaced by *in vitro* toxin neutralization in cultures of Vero cells (Miyamura et al., 1974) and by an ELISA procedure (Winsnes et al., 1999), respectively. An additional advantage of serology compared to challenge is the use of quantitative endpoints (antibody titers) instead of qualitative endpoints (death or survival), allowing a significant reduction in the number of animals used (Cussler et al., 1999).

WHO/EP included in the respective monographs the serological tests as valid 3Rs alternatives to animal challenge tests. But even the serological approaches imply the use of a great number of animals, mainly in multi-dilution assays. The possibility of using a single-dose test as a reduction approach requires an extensive and rigorous comparison regarding the multi-dilution assay. That is why in Latin America the estimation of potency of the diphtheria and tetanus components in vaccines has been based on a different approach, a test known as the NIH/FDA method. This method is more economical because it uses far fewer animals than the WHO/EP method. The assay consists of the subcutaneous immunization of at least 4 guinea-pigs with half the total human dose of vaccine. The animals are bled for no more than 6 weeks, and the titers of antibodies against diphtheria and tetanus are estimated in pooled sera by *in vivo* seroneutralization tests using corresponding toxin standards. It has been demonstrated that a titer higher than (or equal to) 2 IU of antitoxin/ml is a measurement of protection in children. Moreover, the epidemiological experience in the United States and Latin America over the past 35 years using adsorbed toxoids passing the test requirements has been excellent. Nevertheless, in terms of standardization, the method has been criticized due to its low discriminating power, the lack of a reference vaccine, and a problem in the application of statistics (Kreeftenberg, 2002). In addition, challenge methods are ethically incorrect and should be substituted.

For that reason, and in line with the progress of 3Rs in the field of diphtheria and tetanus potency, Finlay Institute implemented a program to replace, within a moderate period of time, the *in vivo* seroneutralization assays used for determining the antitoxin titers in the sera of immunized guinea-pigs. In principle, an indirect ELISA for determining anti-tetanus antibodies was developed and validated (unpublished results). The method was able to determine in a reliable way anti-tetanus antibodies in vaccines of different origin and in the presence of other antigens, and the correlation against the Toxin Neutralization Test was acceptable. Likewise, in collaboration with Finlay Institute, Lara et al. developed a cell culture test for determining anti-diphtheria antibodies in the same guinea pigs' sera. The method is an *in vitro* protection test because it is based on the sensitivity of Vero cells to diphtheria toxin and the protection against it induced by neutralizing antibodies. As for the Tetanus ELISA, the Vero cell showed a good correlation in regard to the *in vivo* seroneutralization test (unpublished results).

Nevertheless, in spite of those promising results, the use of both methods in Cuba is limited because of the low supply of guinea pigs for potency and toxicity of vaccines. That is why an alternative serological approach in mice has been followed. Briefly, mice were immunized with a third part of the vaccine human dose, bled after 28 days, and the pooled sera were tested for anti-diphtheria and anti-tetanus antibodies by ELISA. Hyper immune sera against diphtheria and tetanus were produced to be used for titration. Despite some previous reports, the same dose-response range was obtained for both sera, making the evaluation of the response against both antigens on the same sera feasible. In the case of tetanus, previous experiments had demonstrated a strong relationship between the titers of anti-tetanus antibodies in vaccine-immunized guinea pigs and mice when analyzed by an *in vivo* Toxin Neutralization Test. Hence, it was not surprising to see a similar performance between the mouse serological method and the guinea pig Toxin Neutralization Test, especially when used with adsorbed tetanus monovalent vaccines or combined with diphtheria.

A similar behavior was seen for diphtheria. In Figure 1, the good correlation obtained for the anti-diphtheria antibodies in murine sera can be observed when compared with the titers reached by the *in vivo* seroneutralization test in guinea pigs after the analysis of the same vaccine samples. Both ELISAs not only showed a good agreement regarding the Toxin Neutralization Test in guinea pigs, but they also were able to discriminate between vaccine batches with different biological activity. Nevertheless, the concordance was affected in the presence of inactivated whole cells from *Bordetella pertussis*, because this antigen increased in a significant way the response of anti-tetanus and anti-diphtheria, as broadly reported for the mouse model. The overestimation in the presence of pertussis components needs further evaluation; perhaps this could be solved with

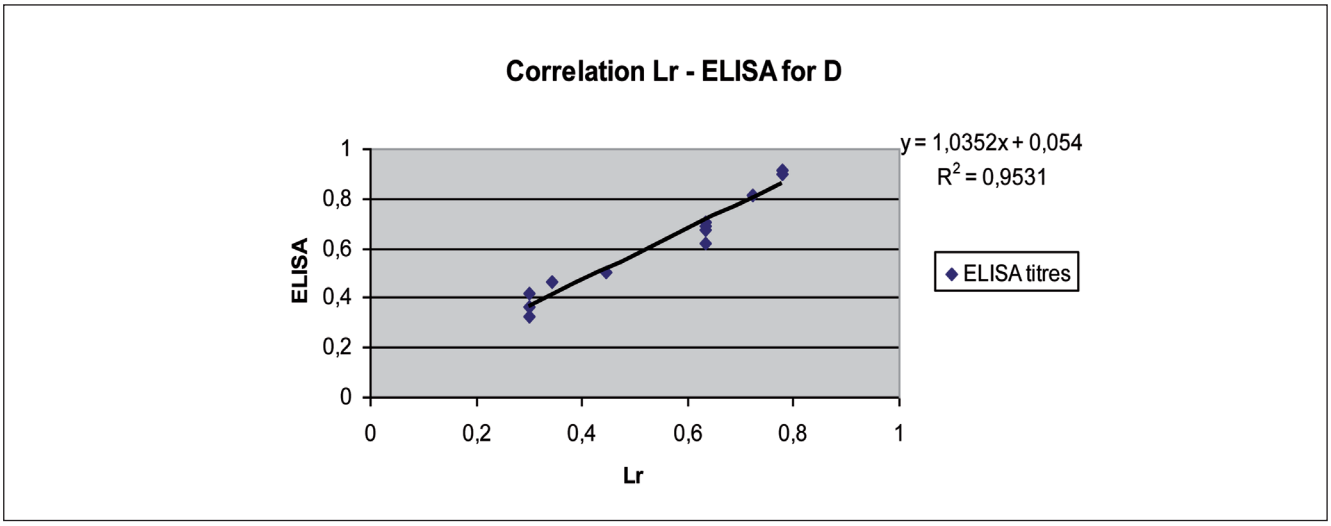

Fig. 1: Correlation between an ELISA and the *in vivo* Toxin Neutralization Test for diphtheria

an adjustment of the dose used to immunize the animals. The clinical relevance of the mouse model should be demonstrated because the correlate of protection is based on the guinea pig model. The mouse serological approach has shown the potential for use, after proper validation, as a replacement for the *in vivo* seroneutralization tests.

A serological approach has been also introduced for the pertussis component in DPT vaccines produced at Finlay Institute. Traditionally, the potency of whole-cell *Bordetella pertussis* vaccines has been determined by the Kendrick test, an intracerebral challenge test in mice in which prediction of the vaccine efficacy in children has been demonstrated (Kendrick et al., 1947; Medical Research Council, 1956). Although the test has shown an acceptable reproducibility in official collaborative studies with correct identification of potent and sub-potent samples in most laboratories experienced in its use, it has been deeply criticized due to the great variability, the poor reproducibility and robustness, the unacceptable pain and suffering incurred by the animals, the use of a challenge route unrelated to the natural mode of infection, among other technical, logistic and ethical issues (Xing et al., 2002; Corbel and Xing, 2004). Considering the problems inherent in the Kendrick test, attempts have been made to develop more acceptable tests. Serology seems to be one of the most suitable alternatives to the Kendrick test, considering it is a fast, highly reproducible and specific, easy-to-perform, and relatively cheap method. Besides, its application would drastically reduce the animal stress produced during the challenge test. The assay, known as Pertussis Serological Potency Test (PSPT), involves immunization of mice with serial dilutions of reference and test vaccine. After 4 weeks, mice are terminally bled and the total antibody titer to pertussis antigens is determined by ELISA using whole-cell bacterial suspension as coating antigen (Ark et al., 1994).

We implemented the PSPT by using the same strain used for the challenge test (strain 18323). This approach looks for a relationship between the protection and the total amount of antibodies against the broad range of antigens present in whole-cell vaccines. At the same time, our laboratory designed an in-house serological method using an inactivated bacterial suspension from a local strain. Table 1 shows the results of the potency of pertussis

Tab. 1: Comparison among MPT and serological tests for Pertussis

Sample	MPT	PSPT-18323 ELISA	In house-ELISA
DPT 1	4 UI/ml	4.3 UI/ml	5.2 UA/ml
DPT 2	4 UI/ml	5.1 UI/ml	6.3 UA/ml
DPT 3	4.9 UI/ml	6.6 UI/ml	7.6 UA/ml
DPT 4	14.8 UI/ml	14.2 UI/ml	21 UA/ml
DPT 5	17.4 UI/ml	23.6 UI/ml	30.6 UA/ml
DPT 6	9.7 UI/ml	17.7 UI/ml	16.2 UA/ml
DPT 7	17.8 UI/ml	30 UI/ml	39 UA/ml

component in DPT vaccines by Kendrick test compared with the respective antibody titers by using the serological assays. Though preliminarily, the results revealed a strong relationship between the protection by Kendrick and the total antibodies against the antigens from *Bordetella pertussis*. Nonetheless, beyond the need of further evaluation for increasing the amount of vaccine samples, including samples from different origin and activity, some complementary testing should be done to gain information on the functionality of the antibodies as part of the characterization of the humoral response and its role in protection.

Thus, we have demonstrated that, for the vaccines produced at Finlay Institute, serological methods are a very good alternative to be used as potency tests instead of the currently used challenge assays.

3 Ongoing 3Rs alternatives for toxicity of vaccines

Vaccine monographs normally include as specifications assays addressed to determine the potential toxicity of the antigens and other components of vaccines. These assays constitute the safety profile of vaccines, and the methods currently used to monitor it are based on animals. Generally, they are ethically controversial because they imply the use of toxins and painful procedures for the management of animals during the testing. The state-of-the science today allows the use of *in vitro* and other approaches more suitable and less aggressive to animals.

For diphtheria and tetanus toxoids a specific toxicity test must be performed for detecting the presence of residual active toxin after the detoxification process. Under physiological conditions, a reversion from toxoid to toxin could happen, and so a test for this specific purpose also is carried out. Our laboratory designed and is validating a Vero cell test for determining toxicity of diphtheria toxoids. The method has been demonstrated to be very sensitive, robust, and specific. Some unspecific toxicity to the cells produced by some components of the sample (thiomersal used as preservative and formaldehyde used for detoxification) has been studied, and the results have demonstrated that the toxicity induced by thiomersal and formaldehyde are completely unrelated to the presence of residual toxin. Nonetheless, some actions have been taken to minimize this interference. Likewise, the potential interference of aluminium hydroxide present as adjuvant in vaccine has been reported, but the evaluation is performed in non-adsorbed samples.

In the case of whole-cell *B. pertussis* toxicity, the mouse weight gain test (MWGT) is the only test currently specified by the WHO, EP, and US requirements. It can be considered as a general, non-specific test measuring overall toxicity, since a number of pertussis toxins may affect the weight gain of mice. Correlation of the results of the MWGT with adverse reactions in children has been reported (Hilton and Burland, 1970; Perkins et al., 1970). Despite that, MWGT has disadvantages in terms of sensitivity, because samples with levels of Pertussis Toxin (PT) and levels of endotoxins higher than present in vaccines pass the test. That is why some alternatives based on improved knowledge of the mechanisms of pathogenicity of *B. pertussis* have been evaluated in order to replace or to complement the MWGT for whole-cell vaccines.

We have focused on the monitoring of endotoxins and the residual active PT content, both regarded as the major contributors to the reactogenicity of whole-cell vaccines. The endotoxin content has started to be evaluated by LAL using the WHO International Standard for Bacterial Endotoxin, independent of the minor differences between the LPS from *Escherichia coli* and *B. pertussis*. The specification of the endotoxin content is based on our own results because whole-cell vaccines vary widely in this parameter, and so far there is no agreement as to what constitutes an acceptable level. In regard to PT, the introduction of a cell culture (CHO cells) test is being considered. As it stands, the CHO test is based on the clustering of cells in the presence of PT as observed and scored under an inverted microscope (Gillenius et al., 1985). The method must be implemented in non-adsorbed samples because the adjuvant can cause CHO cell death. The major concern for its implementation could be the aggregation, which may cause the test to fail to detect residual toxin in toxoid after chemical detoxification (Kataoka et al., 2002). That is why Finlay Institute also is thinking about the possibility of introducing a newly developed method based on cAMP production in A10 cells induced by residual PT. This method has shown to be able to detect PT in the presence of the other components of acellular DPT vaccines, and it is a very promising candidate to replace the Histamine Test (Hoonakker et al., 2010). This method might be useful for determining residually active PT in whole-cell vaccines too.

Regarding pyrogenicity of our vaccines, despite use of the LAL, a research project is under development for the introduction of an *in vitro* pyrogen test based on pro-inflammatory cytokine release from cryopreserved whole blood and a human monocytic cell line, respectively (Spreitzer et al. 2007; Nakagawa et al., 2002). Its application, compared to the pyrogen test in rabbits, will allow the more sensitive detection of pyrogens from Gram negative bacteria and also from Gram positive bacteria, fungi, *inter alia*.

4 Other advanced 3Rs approaches to be implemented

Together with serology, some other *in vitro* approaches are planned to be introduced as part of the 3Rs program to complement or partially replace the animal potency tests in the routine quality control of vaccines. Antigen quantification is one such test. This approach has been known to be successfully used for potency testing of live attenuated vaccines, either by determining the number of live particles in bacterial vaccines (BCG, typhoid and cholera) or by virus titration in cell cultures using endpoints in viral vaccines (Oral Polio, MMR, yellow fever and influenza vaccines). In the case of viral inactivated vaccines, this method has been based on the binding of key protective

antigens to specific antibodies in an *in vitro* immunoassay (Hendriksen, 2008). That's the case for rabies vaccines, where ELISAs were developed using monoclonal antibodies directed to glycoproteins and nucleoproteins of the rabies virus as an alternative to the NIH potency challenge test in mice (Rooijakkers et al., 1996; Bruckner et al., 2003). A similar approach has been followed for the veterinary inactivated leptospira hardjo vaccine. Therefore a monoclonal antibody ELISA for antigen quantification is planned (in conjunction with serology) for some of our inactivated bacterial vaccines such as the meningococcal BC vaccine and the trivalent human leptospirae vaccine. Nonetheless, it should be taken into account that adjuvant must be removed and thus, it has to be demonstrated that removal is successful in terms of antigen recovery, as well as in retaining antigen integrity.

The other 3Rs approach is called the consistency approach. Its principle emerges from the new generation of vaccines. These vaccines are based on new technologies and are produced in a consistent way. Here, the stress of quality control is on in-process monitoring rather than final batch testing (Metz et al., 2002). This means that, if consistency in production is demonstrated, then testing could rely on a battery of easy-to-use *in vitro* assays to characterize (fingerprint) the vaccine and confirm consistency. Hence, the number of animals needed for quality control of conventional vaccines will be reduced to an absolute minimum. However, the implementation of the consistency approach implies the use of very expensive equipment for the physico-chemical characterization of the antigen structure. For us, the use of a combination of immunological and biochemical methods such as ELISA using monoclonal and polyclonal antibodies, SDS-PAGE, 2D-electrophoresis, among others seems to be more accessible. In our opinion, this approach has a great potential for DPT and meningococcal BC vaccines in the near future.

5 Conclusions

As shown, serology has been the best and the most frequently implemented strategy to replace animal challenge methods for determining potency of vaccines produced by Finlay Institute in Cuba, so far. At the moment, serological methods for diphtheria, tetanus and whole-cell pertussis vaccines are under development/validation (including correlation versus clinical data, when necessary) in order to be presented as official testing procedures for lot release to the Cuban National Control Authority for their approval in a relatively short period of time. Likewise, cell culture assays have been selected as the most suitable and relevant 3Rs alternatives to monitor the toxicity and safety of vaccines. Finally, the implementation of new strategies in vaccine quality control such as the consistency approach is being considered for an almost total elimination of animal use while increasing the relevance of quality control.

References

Ark, A., Kapelle, I., Akkermans, A., et al. (1994). Development of pertussis serological potency test. *Biologicals 22*, 233-242.

Bruckner, L., Cussler, K., Halder, M., et al. (2003). Three Rs approaches in the quality control of inactivated rabies vaccines. The report and recommendations of ECVAM workshop 48. *ATLA 31*, 429-454.

Chovel, M. L. and Huerta, N. (2002). Validation of an in vitro potency test for the Cuban Hepatitis B vaccine. In F. Brown, C. Hendriksen, D. Sesardic, and K. Cussler (eds.), *Advancing science and elimination of the use of laboratory animals for development and control of vaccines and hormones, Dev. Biol. 111*, 305-312.

Chovel, M. L., Pérez, R. L., Oviedo, M., et al. (2007). Relationships among physico-chemical and biological tests for a synthetic Hib-TT conjugate vaccine. *Vaccine 25*, 194-200.

Chovel, M. L., Lara, A., Abreu, N., et al. (2008). Validation of an inhibition ELISA for determining in vitro potency of recombinant Hepatitis B surface antigen in vaccines combinations from two manufacturers. *Biologicals 36*, 375-382.

Corbel, M. J. and Xing, D. K. L. (2004). Toxicity and potency evaluation of pertussis vaccines. *Expert Rev. Vaccines 3*, 89-101.

Cussler, K., Morton, D. B., and Hendriksen, C. F. M. (1999). Humane endpoints in vaccine research and quality control. In C. F. M. Hendriksen and D. B. Morton (eds.), *Humane endpoints in animal experiments for biomedical research*, 95-101. The Royal Society Medicine Press, London (UK)

Gillenius, P., Jaatmaa, E., Askelof, P., et al. (1985). The standardization of an assay for pertussis toxin and antitoxin in microplate culture of Chinese hamster ovary cells. *J. Biol. Stand. 13*, 61-66.

Hendriksen, C. F. M. (2006). Replacement, reduction and refinement in the production and quality control of immunobiologicals. *AATEX 11*, 155-161.

Hendriksen, C. F. M. (2008). Replacement, reduction and refinement alternatives to animal use in vaccine potency measurement. *Expert Rev. Vaccines 8*, 313-322.

Hilton, M. L. and Burland, W. L. (1970). Pertussis-containing vaccines: The relationship between laboratory toxicity tests and reactions in children. *Sym. Series Immunobiol. Stand. 13*, 150-156.

Hoonaker, M., Ruiterkamp, N., and Hendriksen, C. F. M. (2010). The cAMP assay: A functional in vitro alternative to the in vivo Histamine Sensitization test. *Vaccine 28*, 1347-1352.

Kataoka, M., Toyoizumi, H., Yamamoto, A., et al. (2002). Chinese Hamster Ovary (CHO) cell clustering does not correlate with in vivo histamine-sensitisation when measuring residual activity of aldehyde-treated pertussis toxin (PT). *Biologicals 30*, 297-302.

Kendrick, P. L., Eldering, G., Dixon, M. K., and MIsner, J. (1947). Mouse protection tests in the study of pertussis vac-

cines: a comparative series using the intracerebral route of challenge. *Am. J. Public Health 37*, 803-810.

Kreeftenberg, J. G. (2002). Consistency testing of diphtheria and tetanus to replace potency testing for lot release. In F. Brown, C. Hendriksen, D. Sesardic, and K. Cussler (eds.), *Advancing science and elimination of the use of laboratory animals for development and control of vaccines and hormones, Dev. Biol. 111*, 291-298.

Medical Research Council (1956). Vaccination against whooping cough: Relation between protection in children and results of laboratory tests. *Br. Med. J. 2*, 454-462.

Metz, B., Hendriksen, C. F. M., Jiskoot, W., and Kersten, G. F. (2002). Reduction of animal use in human vaccine quality control. *Vaccine 20*, 2411-2430.

Miyamura, K., Nishio, S., Ito, A., et al. (1974). Micro cell culture method for determination of diphtheria toxin and antitoxin titres using VERO cells. *J. Biol. Stand. 2*, 189-201.

Nakagawa, Y., Maeda, H., and Murai, T. (2002). Evaluation of the in vitro pyrogen test system based on pro-inflammatory cytokine release from human monocytes: Comparison with a human whole blood culture system and with the rabbit pyrogen test. *Clin. Diag. Lab. Immun. 9*, 588-597.

Perkins, F. T., Sheffield, F., Miller, C. L., and Skegg, J. L. (1970). The comparison of toxicity of pertussis vaccines in children and mice. *Sym. Series Immunobiol. Stand. 13*, 41-49.

Rooijakkers, E. J., Uittenboogaard, J. P., Groen, J., and Osterhaus, A. D. (1996). Rabies vaccine potency control: Comparison of ELISA systems for antigenicity testing. *J. Virol. Methods 58*, 111-119.

Russell, W. M. S. and Burch, R. L. (1959). *The principles of humane experimental technique*. London, UK: Methuen & Co LTD.

Spreitzer, I., Loeschner, B., Schneider, C. K., et al. (2007). 10 years of experience with alternative pyrogen tests (monocyte activation tests). AATEX 14, Spec. Issue, 587-589.

Vérez, V., Fernández, V., Hardy, E., et al. (2004). *A synthetic conjugate polysaccharide vaccine against Haemophilus influenzae type b. Science 305*, 522-525.

Winsnes, R., Hendriksen, C., Sesardic, D., et al. (1999). Serological assays as alternatives to the Ph.Eur. challenge test for batch release of tetanus vaccines for human use. *Dev. Biol. Stand. 101*, 277-288.

Xing, D., Das, R. G., O'Neill, T., et al. (2002). Laboratory testing of whole-cell pertussis vaccine: a WHO proficiency study using the Kendrick test. *Vaccine 20*, 345-351.

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