Vaccine 37 (2019) 3745–3746

Contents lists available at ScienceDirect

Vaccine

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

Commentary

Towards animal free and science based measures of critical quality attributes for vaccine quality control and release ☆

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ARTICLE INFO

Article history:
Received 11 December 2018
Received in revised form 9 May 2019
Accepted 13 May 2019

Manufacturing of vaccines is a complex and articulated process that includes bulk production, formulation, filling, testing, storage and shipping. Each vaccine lot is monitored during production with standardized and validated methods using hundreds of tests. Eventually, before final release, vaccine lots are tested both by manufacturers and official medicines control laboratories (OMCLs) to demonstrate conformity for critical quality attributes in the release panel. Two critical tests that traditionally require use of animal are pyrogenicity and potency. In this issue of vaccine, two papers report the collaboration between a manufacturer and an OMCL to develop and implement an animal free, *in vitro* assay to measure pyrogenicity of meningococcus B vaccine (1,2). The new assay is science based, more reliable, faster and aligned with the principles of Replacement, Reduction and Refinement (3R) of animal testing. The two papers represent an example of what is scientifically possible and should encourage to prioritize implementation of *in vitro* tests any time it is possible in vaccine and pharmaceutical manufacturing and release.

Today all vaccine lots are subject to release by the relevant regulatory authority; in this context, OMCLs independently from manufacturers, support regulatory authorities in controlling the quality of vaccines for human use by testing all the vaccine lots to be released in the market. Among the different critical quality attributes tested at release, pyrogenicity is of paramount importance in order to ensure that medicinal products intended for the parenteral administration to humans are safe and do not contain unintended pyrogenic compounds.

Animal-based *in vivo* methods such as the Rabbit Pyrogen Test (RPT) have been developed to confirm the absence of pyrogens in parenteral preparations and they played a central role in controlling the safety of vaccines. However, *in vivo* methods have inherent limitations such as high variability, complexity due to the use of animals, the impossibility to properly mimic route of administration and ultimately the human response due to species-specificity response to toll-like receptors. Moreover, the RPT was originally developed to identify potential contaminants in medicinal products that do not intrinsically contain pyrogenic compounds; this represents another limitation for vaccines that contain components with an inherent pyrogenic activity such as the recently developed and approved Bexsero MenB vaccine [3]. Bexsero is a multicomponent vaccine consisting of three recombinant protein antigens and an Outer Membrane Vesicles (OMVs) component from a *Neisseria meningitidis* serogroup B strain; all components are adsorbed onto aluminum hydroxide [4]. The OMVs are inherently pyrogenic because contain both endotoxin (lipopolysaccharide) and non-endotoxin (lipoproteins) pyrogens. For these reasons, application of RPT to Bexsero for measurement of the pyrogenicity attribute required extensive adaptations. However, despite the changes in protocol and sample preparation the RPT demonstrated to be prone to generate highly variable and false positive results reducing the possibility to release and distribute conform vaccine lots to the patients.

The continued challenges faced to properly test Bexsero for a critical quality attribute such as pyrogenicity, created the necessity to develop and apply innovative methods that can substitute RPT both at the manufacturer and the OMCLs.

The MAT (Monocyte Activation Test) is a cell-based *in vitro* test for assessing pyrogenicity, which has been recently introduced in the European Pharmacopeia and also reported in the USP [5,6]. From the scientific and technical point of view, MAT has the following advantages: (i) exhibits a much lower variability with higher sensitivity with respect to the *in vivo* assays; (ii) can be utilized as a fully quantitative test making it more appropriate for

☆ *Commentary for Vaccine to support the two MAT publications from Vipond et al., (NIBSC) and Valentini et al., (GSK).*

https://doi.org/10.1016/j.vaccine.2019.05.039
0264-410X/

vaccines which are inherently pyrogenic; (iii) is physiologically relevant for testing human vaccines since works with human cells. Moreover, from the manufacturer point of view, the use of an *in vitro* assay allows a faster and more reliable testing at release and is aligned with the global commitment for the reduction of animal usage in accordance to the 3Rs principles [7].

In the recent years MAT has been used primarily for detecting the presence on non-endotoxin pyrogens during product development and several studies demonstrated that MAT provides reliable and reproducible results for different products [8–10].

The two publications presented in this issue of Vaccine [1,2] describe for the first time the development of MAT applied to test and release a vaccine product. Vipond et al., report the experience of the National Institute for Biological Standards and Control (NIBSC) in the development and validation of the MAT as a replacement for the RPT, for the consistency/safety testing of Bexsero vaccine. Beside the assay development, the authors also describe the work done to set a specification value for testing Bexsero batches that had been demonstrated to be safe in clinical trials in different age groups and report also about the decisional procedure used to evaluate the MAT results. Valentini et al., describes the perspective of the manufacturer (i.e. GSK vaccines) reporting the development and validation of a similar MAT for Bexsero vaccine. In this article, the authors describe all the key experiments performed for assay development and the correlation of the MAT with RPT with a significant dataset of Bexsero vaccine lots tested with both methods at release.

The development, validation and implementation of the MAT for Bexsero testing and release at GSK Vaccines and at the OMCLs can be considered an outstanding example of a private–public collaboration based on scientific and technical exchanges between the two teams. While the two published articles reflect the separate and specific experiences at the NIBSC and at GSK Vaccines, behind that there are many years of close scientific collaborations between the two institutes. This close collaboration has allowed the development of a robust and sensitive assay for measuring the safety of the Bexsero vaccine increasing the quality of the controls both at the manufacturer and at the OMCLs. The MAT for Bexsero is now accepted by different regulatory authorities and is included in the release panel of the vaccine.

The results and conclusions reported by the two articles bring also more general and important considerations on the use of animal-based *in vivo* methods for characterization and release of vaccine products. While the *in vivo* methods have historically played a central role in safeguarding the quality of vaccines, the inherent variability of animal-based assays can make them less suitable than appropriately designed *in vitro* assays for monitoring consistency of production and for assessing the potential impact of manufacturing changes.

The use of appropriate *in vitro* methods will reduce and ultimately eliminate the use of animals in line with 3Rs principles [7], EU Directive 2010/63/EU about the protection of animals [11] and FDA efforts related to ICCVAM [12,13]. At the same time the use of properly scientifically developed *in vitro* methods at release and during process changes will improved evaluation of critical quality attributes while substantially reduces the time and cost of releasing vaccine lots.

The example described with the MAT for Bexsero to measure the pyrogenicity attribute will be definitively applied also to other attributes for the same vaccine (e.g. Potency attribute) and to other vaccines. In the context of MAT applied to Bexsero or other vaccines I foresee future developments and improvements on three areas: (1) human cells from individual donors may be substituted with specific cell lines in order to improve robustness and reproducibility and avoid complications in finding proper donors; (2) proper reference material of vaccine drug products should be prepared and used by manufacturers to set specifications in order to take into consideration experiences in clinical/technical development and during production; (3) efforts should be also considered to prepare proper standards to be distributed to any laboratory in order to allow a proper standardization of the assay with interested partners worldwide.

The progresses made by the scientific community in the mechanisms of action of antigens and adjuvants and in the development of novel technologies will pave the way to develop new *in vitro* methods. In the near future, I expect the manufacturer of vaccine products to significantly invest their efforts in the development and implementation of innovative *in vitro* methods in order to increase the performance of the assays used to measure the critical quality attributes at release and ultimately to distribute effective and safe vaccines to patients. A close collaboration with health and regulatory authorities, OMCLs and other key stakeholders will further increase the success of this effort as documented by the Bexsero MAT experience.

Declaration of Competing Interest

RR is a full-time employee of GSK group of companies.

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