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## Global harmonization of vaccine testing requirements: Making elimination of the ATT and TABST a concrete global achievement

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### ABSTRACT

This one-day symposium organized by Humane Society International (HSI) brought together 18 international experts from Argentina, Brazil, China, Europe, India, Russia, South Africa and the United States to discuss the elimination of the abnormal toxicity test (ATT) from the testing requirements for human vaccines as well as the target animal batch safety test (TABST) and the laboratory animal batch safety test (LABST) for veterinary vaccines. Participants reported on country-specific regulatory requirements and, where present, the perspectives on waiver and elimination of those tests. In addition, the attendees, with HSI in the role of facilitator, moved to define the barriers to the complete elimination or waiving of these tests. This report expounds the outcomes of the symposium, and introduces a proposed roadmap – populated with country specific activities – for the elimination of these tests.

### 1. Introduction

The abnormal toxicity test (ATT), also referred to as General Safety Test (GST) or test for innocuity, is carried out in mice and guinea pigs, and is supposed to detect non-specific contaminants and toxins that may be present in the final product. The laboratory animal batch safety test (LABST) is comparable to ATT, and required for veterinary vaccines. The target animal batch safety test (TABST) is supposed to demonstrate that the vaccine is safe in the target animal, meaning that animals should not show abnormal or systemic reactions.

For nearly 50 years, ATT, TABST and LABST have been required globally for the release of each batch of biologicals for human and veterinary use, despite their questionable scientific relevance and the ethical concerns they raise.

Since the 1980s, the scientific consensus on these tests has shifted, with the most significant turn taking place in 1996 with the publication of several key papers highlighting the inherent limitations of these tests, in particular, their lack of specificity, irreproducibility and concerns regarding their scientific relevance [1–5]. Steady progress has also been made on the operational side of vaccine production, with the introduction of more stringent quality control measures aided by state-of-the-art *in vitro* analytical techniques, stricter control of starting materials, enhancement of Good Manufacturing Practice (GMP) and improved post-marketing pharmacovigilance. All of these measures are aimed at preventing risk of contaminations and ensuring the products' quality and safety.

Together, these advancements have led to calls for the elimination of the ATT, TABST and LABST. Consequently, a number of national agencies, regulatory bodies, and international standard organizations have deleted these tests or are striving towards their elimination from the list of requirements. However, deletion of these tests on a truly global level remains elusive. In this context, the symposium covered in this report sought to define a realistic roadmap for international stakeholders to make the elimination of those tests a concrete global achievement.

### 2. Highlights of presentations on current status of ATT & TABST use

Drs. Klaus Cussler (PEI, Germany) and Lukas Bruckner (Consultant, Switzerland), both nominated experts from the European Directorate for the Quality of Medicines (EDQM), opened the symposium. Dr. Cussler summarized the history of the ATT, which originated in the 19th century, and was used as a test for the quality control of diphtheria sera to quantify phenol-derived preservatives. Similarly, the guinea pig test was developed to detect residual toxin. These tests were later adopted into a host of regulations worldwide, but as general safety tests rather than for their original purpose. Dr. Cussler described the long process leading to the elimination of the ATT in Europe, first for batch release purposes in 1996 (although it was left in the “production” section of the monographs) [3], and then it was deleted completely from all European Pharmacopoeia (Ph. Eur.) monographs in the beginning of 2019 [7]. Dr. Cussler reported that the World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) recently recommended “the discontinuation of the inclusion of the innocuity test in all future WHO Recommendations, Guidelines and manuals for biological products published in the Technical Report Series, and that a clear indication be made in its report that the inclusion of this test in previously published WHO Technical Report Series documents be disregarded.” The ECBS stated that “Good Manufacturing Practices (GMP) and comprehensive quality control measures (including in-process controls), were considered to be more appropriate than the innocuity test in assuring the quality and safety of vaccines and other biological products” [8].

Dr. Bruckner focused his presentation on the TABST, highlighting the factors that make it unsuitable as a tool for the demonstration of safety of veterinary vaccines. One of the key factors is the test's inherent risk of generating false-positive or false negative results when used to test products manufactured through already well-controlled processes (seed-lot system, extensive testing of starting materials, and GMP and/or quality assurance) and that as such have extremely low probabilities

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**Abbreviations**

ATT	Abnormal Toxicity Test	ICH	International Conference on Harmonization
AU-PANVAC	Pan African Veterinary Vaccine Center of African Union	IPC	Indian Pharmacopoeia Commission
BSP	Biological Standardization Program	LABST	Laboratory Animal Batch Safety Test
CFR	United States Code of Federal Regulation	NCL	National Control Laboratory
CVB	Center for Veterinary Biologics	NRA	National Regulatory Authority
ECBS	Expert Committee on Biological Standardization (WHO)	OCABR	Official Control Authority Batch Release
EDQM	European Directorate for the Quality of Medicines	OIE	World Organization for Animal Health
EPAA	European Partnership for Alternative Approaches to Animal Testing	PCR	Polymerase Chain Reaction
EURL ECVAM	European Union Reference Laboratory for alternatives to animal testing, European Commission Joint Research Centre	PEI	Paul-Ehrlich-Institut, OMCL, Germany
FDA	Federal Drug Administration, USA	Ph. Eur	European Pharmacopoeia
FSBI “SCEEMP”	Federal State Budgetary Institution “Scientific Centre for Expert Evaluation of Medicinal Products” of the Ministry of Health of the Russian Federation	QS	Quality Standards
GMP	Good Manufacturing Practice	TABST	Target Animal Batch Safety Test
GST	General Safety Test	TRS	Technical Report Series (WHO)
IABS	International Alliance for Biological Standardization	USDA	United States Department of Agriculture
		VAC2VAC	Vaccine Lot to Vaccine Lot Comparison by Consistency Testing
		VICH	International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products
		VSM	Veterinary Services Memorandum
		WHO	World Health Organization

of being out of specification. These products are usually highly purified and may only contain extremely low concentrations of potential contaminants that fall well below the detection limit of TABST. Another factor is the potential impact of health and immune status of the test animals on the reliability of test results.

Dr. Bruckner highlighted that TABST was eliminated from the Ph. Eur. after intensive discussions within the expert group for veterinary vaccines. It was deleted from all monographs for veterinary vaccines with 3 exceptions: Porcine actinobacillosis vaccine, Porcine progressive atrophic rhinitis vaccine, and Tetanus vaccine for veterinary use, where it was renamed as a specific residual toxicity test [9]. It was noted that prior to its deletion in 2012, TABST could be waived provided that at least 10 consecutive batches from separate final bulks had been tested and found compliant with the test. The possibility to request a waiver was introduced based on the outcome of a retrospective analysis of TABST data performed by the Advisory Group on Alternatives to Animal Testing in Immunobiologicals [4].

Dr. Bruckner and Dr. Halder acknowledged the work initiated by the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) in 2009. Two recently developed guidelines (VICH GL50 and 55) address criteria for waiving the TABST for live and inactivated veterinary vaccines [10,11]. VICH GL50 was implemented in 2014, and the revised GL50 and the new GL55 were implemented in 2018. These guidelines, which are also referred to by OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2018 [12], are instruments that National Regulatory Authorities (NRA) can use to offer waivers to the test.

The situation is not as encouraging for the elimination of LABST. While a Paul Ehrlich Institute retrospective analysis of LABST data revealed that the test is no longer relevant and not effective in detecting problematic batches [1], only the Ph. Eur. has proceeded to its removal for veterinary vaccines (1997) [3]. It is still a requirement in the vast majority of countries. It should be noted that VICH is currently developing a set of harmonizing criteria for waiving LABST, and that this is expected to serve as an official guideline for VICH aligned countries (and, hopefully, also for those not aligned) to grant waivers. This guideline is undergoing public consultation since October 2019 and hopefully it will be published in 2020.

Some concrete examples of the challenges related to securing waivers for TABST, and how this impacts manufacturers, have been

reported by MSD Animal Health, whose products are registered in more than 100 different markets. In 2015, 84 non-European countries accepted the company's requests for waiver of TABST, yet to this day, the company (and, for that matter, other manufacturers) faces a complex situation, as still some countries require the TABST to be performed. These countries can be divided in three categories as follows: 1) some countries are ready to accept a vaccine batch without TABST results from the manufacturer, but continue to perform the test locally; 2) others will grant a waiver for TABST, but only when presented with data on a large number of batches submitted with a variation application; and 3) some countries will grant a waiver for TABST for new products but not for legacy products.

Meeting participants were then presented with specific regulations, details and rationale for the use, waiver, or deletion of ATT and TABST from the testing requirements in the respective countries.

In **India**, the Indian Pharmacopoeia Commission (IPC) traditionally required ATT for product testing, but after the introduction of GMP and Good Laboratory Practices principles, and in view of efforts for global harmonization of testing requirements, the IPC's recommendations now state that, for certain products including vaccines, ATT may be omitted for routine lot release once the consistency in production has been well established to the satisfaction of the national regulatory authority and when good manufacturing practices are in place. Each lot, if tested by the National Control Laboratory, should pass the test for abnormal toxicity [13]. The test is still required for newly developed products until the consistency in production is established to the satisfaction of NRA. In case of established products, the vaccine manufacturers need to apply for the waiver as a post-approval change for omission of ATT in compliance to country's regulatory guidelines. Some companies have requested waivers, and are currently engaged in a dialogue with the regulatory authorities and with the IPC on the possibility of a deletion of the test. Despite the progress achieved, manufacturers would prefer a more streamlined process and better guidance from the NRA for post-approval changes. Post-approval changes require filing an application to the regulatory authority. Review can take between 2 and 4 months and manufacturers must be in compliance with updated IPC requirements. No information was presented on the status of TABST by India.

In the **United States**, in 2015, the Federal Drug Administration (FDA) stated that the GST was no longer required for ensuring the safety of licensed biological products. Consequently, GST was revoked from applicable regulations [14]. For already licensed vaccines for

which GST is part of the license, manufacturers can request to discontinue its use from product release testing through an official request, i.e. submission of a supplement to the biologic license. These requests are usually granted by FDA, provided appropriate quality assurance safeguards and controls are in place for the particular vaccine [15]. In the case of veterinary vaccines, the United States Department of Agriculture (USDA) still requires TABST, but has implemented VICH GL50 and GL55 and allows exemptions based on supportive data (the same is true in Japan).

In **Brazil**, ATT is no longer stipulated in the requirements for vaccines (the full deletion of the test from the Brazilian Pharmacopoeia is currently being assessed). Manufacturers have the possibility to stop performing the test for already registered products, under the condition that they send proper communication (through an annual report) to NRA in Brazil, in which they state their compliance with the regulations. However, the removal of TABST is not imminent even though interest has been expressed by representatives of the Ministry of Agriculture. No specific activity has yet been initiated in this regard.

A similar situation was reported by the representative from **Argentina**, where ATT has been removed from the Pharmacopoeia (except for the acellular pertussis vaccine, where it should be revoked in the next edition of the Pharmacopoeia), and is not required for marketing authorizations for the new products. No information was presented on the status of TABST.

In **South Africa**, regulatory requirements align with WHO requirements and Ph. Eur. Monographs. The country's National Control Laboratory (NCL) has taken the lead in elimination of ATT and informed the South African regulatory authority that it has halted all *in vivo* testing. This implies that ATT is no longer performed for lot release of human vaccines. The NRA in South Africa does not perform any batch release testing for veterinary vaccines, nor does it perform any post-import testing, so TABST and LABST (or any other *in vivo* batch release testing for veterinary vaccines) are not a concern. A report was made on the interest expressed by the Pan-African Veterinary Vaccine Center of African Union (AU-PANVAC) to work on the application of VICH GL50 and GL55 guidelines in Africa.

Both **China** and **Russia** stipulate use of ATT in their pharmacopeias. Russia reported its positive experience in using the test to detect various impurities. The ATT performed in Russia is similar to the one that was used in the US [16] and to the method outlined in the International Pharmacopoeia [17] versions, which differ from the former European version which contained less stringent requirements. Currently, as part of a global trend towards reduction of *in vivo* testing as well as harmonization of requirements for medicine quality control, the Federal State Budgetary Institution, "Scientific Centre for Expert Evaluation of Medicinal Products" (FSBI "SCEEMP"), of the Ministry of Health of the Russian Federation is developing approaches to the creation of a step-wise program for the implementation of the 3R principles. Groups of drugs are being identified for which ATT can be omitted in the first place (for the time being, they are considering orphan drugs, local anesthetics, etc., but not yet vaccines). In the future, the Centre aims to conduct systematic comparative *in vitro* and *in vivo* tests not only for groups of biologicals, but also for each individual biological product, taking into account the composition of the drug (the presence of excipients of different origin), production technology, and other factors, in order to obtain reliable information for decision making.

### 3. Discussion highlights

The second part of the symposium consisted of a roundtable to discuss evidence supporting the deletion or waiver of ATT and TABST (/LABST), the barriers preventing some stakeholders from pursuing the transition, and to begin laying out a roadmap for further actions.

#### 3.1. Identification of barriers and proposals to overcome them

It emerged from the discussion that the global deletion of ATT and TABST is hindered by a variety of factors, including legislative, organizational and technical shortcomings. Additionally, in some instances, a long-held tradition of relying on an older, established method prevents acceptance of a new rationale even if the latter is scientifically sound. The participants listed some of the key barriers, such as lack of global coordination, inadequate communication between manufacturers and regulators, the requirement for testing with slightly different methods of local preference, and regional unfamiliarity with new approaches or technologies. Also discussed was a major bias influencing many countries and stakeholders: the general reluctance to shift to non-animal based methods because of the perception of technical difficulties regarding their implementation, concerns regarding costs to be incurred in the transition, and their long-term sustainability.

Participants discussed how specific safety tests (other than ATT) are used to evaluate safety risks inherent to some vaccines. For example, toxoid vaccines are tested for residual toxicity to check the complete inactivation of the toxin; acellular pertussis vaccines are tested using CHO cells; diphtheria vaccines are tested using Vero cells, and tetanus vaccines may in future be tested with the BINACLE assay currently validated by EDQM [9]. These tests have a specific purpose, and have already either replaced animal tests or will do so in the near future.

During the discussion, one country offered a differing view on the removal of ATT, indicating that it relies on it not only as a test for toxicity, but also as an instrument for the identification of contaminants and counterfeit products or medicines, clearly stating that due to this expanded use, the respective regulatory agency is not convinced of the appropriateness of its deletion. Other participants voiced concerns regarding this approach, underlining that ATT is not an appropriate tool to identify counterfeit batches, and that potential contaminants would be better identified using a number of validated and more specific, non-animal tests that aim at detecting specific contaminants (e.g., microbiological, or residual contaminants [5,6]). These methods include, for example, mass spectrometry, sterility test, bioburden, pyrogens (e.g. Monocyte Activation Test), and endotoxins (Bacterial Endotoxins Test or Limulus Amebocyte lysate test; cell-based tests, Polymerase Chain Reactions (PCRs), and functional assays. Compared to those validated tests, ATT does not fulfill International Conference on Harmonization (ICH) validation criteria (i.e. specificity, reproducibility and detection limit) of a quality control test [18]. Notably, validation of ATT to modern standards would be not possible, as it lacks explicit acceptance criteria and specific endpoints in relation to its objective of detecting contaminants [6].

After exchanging perspectives on the scientific and practical shortcomings of ATT and TABST, the discussion focused on key factors preventing a global consensus on their removal. These factors include the lack of harmonization across different NRAs, different regulatory requirements, guidance and country specific decision-making. Participants agreed that such lack of harmonization presents significant burden in terms of costs, time, resources on those manufacturers that produce vaccines for global market and, therefore, are required to perform these animal-based tests to satisfy different country-specific requirements. Differences in requirements exist, in part, due to the fact that respective countries have varying expertise in evaluating and requiring implementation of GMP and quality systems, and in oversight and surveillance activities that are needed to effectively monitor manufacturing processes and enforce GMP. In these contexts, tests like the ATT and TABST are regarded by some countries as more practical than any other methods, and are therefore relied upon for identification of potentially unsafe batches. However, a possible solution would be to use more specific validated safety tests instead.

It was also noted that the burden imposed by lack of global harmonization does not merely rest with manufacturers; it also affects both people and animals in need for vaccination because product availability

may be impacted (e.g., number of batches released to the markets, shortened product shelf-life due to the time required for the release testing and cost of a single dose).

The last barrier the participants discussed was of socio-ethical character and relevance; namely, the ethical dimension of using animals for testing when non-animal based methods exist, particularly when the latter are also safer, more reliable, and cheaper. Participants agreed that different cultures have different perceptions of animal welfare, and although not directly impacting the focus of the symposium, it was agreed that each participant could champion non-animal based methods in conversations or discussions with relevant stakeholders by emphasizing the benefits provided by those methods and citing existing data and experience. Sparing countless animals currently being used in meaningless box-checking exercises would be a positive outcome.

Consensus also emerged regarding the need to improve the communication on the benefit of implementing improved quality systems and GMPs in product manufacturing, making the release phase faster and cheaper. However, justification needs to be provided in specific regions for the increased costs related to improving GMP standards, qualification of instruments, validation of new procedures and personnel training. Furthermore, it will need to be made more evident how these expenses will end up benefitting regulatory authorities, manufacturers and the public. Also, more effort could be made in prioritizing these themes and issues in the political, regulatory and industrial agendas of many countries and regions that currently find themselves between a rock and a hard place with regard to providing their populations with safe and effective products for prevention and care with very limited financial and technical resources at their disposal. It was noted that the initial cost of establishing, validating and implementing a new *in vitro* method to replace an *in vivo* test will be more than offset in the long run by savings in cost associated with animal based studies and batch failures resulting from large variabilities of such tests.

### 3.2. Proposed strategy

Participants were encouraged by the recent suggestion of the WHO Expert Committee on Biological Standardization to eliminate the test for innocuity from all WHO recommendations for vaccines [7], with the hope that this would trigger similar decisions among the regulatory authorities of the countries that follow the WHO recommendations and the International Pharmacopoeia. The International Pharmacopoeia, published by the WHO, currently includes the general chapter “3.7 Undue toxicity”, which requires for certain antibiotics an animal test in mice. Following the decision taken by the WHO ECBS at its 69th meeting to discontinue the inclusion of the innocuity test in future WHO documents on vaccines and other biologicals, the Secretariat of the International Pharmacopoeia will submit the corresponding proposal to the 54th meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations to omit the general chapter on “Undue toxicity” and its reference in monographs on antibiotics in the next edition of the compendium.

A general consensus emerged among the participants on the opportunity to encourage the WHO in implementing the ECBS recommendation in all biological products Technical Report Series (TRS). This would facilitate the decision making process for deletion of the test from country specific regulations, as well as from release testing in manufacturers’ and national control laboratories. Similarly, participants considered the work done within the VICH with the implementation of GL50 and GL55 and the acknowledgement by the OIE in its Terrestrial Manual [12] to be very important and a fundamental step to reach out to additional stakeholders and influence their decisions.

Symposium participants agreed on the crucial need for systematic and continued engagement of a large variety of stakeholders, and their commitment to do further work towards global deletion of ATT and

TABST/LABST. This could be done, for example, by this symposium’s participants becoming champions of the cause and speaking up for it in as many venues as possible, by sharing their network of contacts, by extending support to those who are willing to engage, and to stakeholders in countries outside of their own. They also agreed on the need to 1) improve the dissemination/ease of accessibility/retrievability of available key messages and case studies, 2) improve the strength of the existing networks, 3) help engaging new stakeholders, 4) facilitate the creation of new connections, 5) maintain ongoing engagement with stakeholders in their outreach activities, and 6) maintain a constant communication flow on the topic.

### 4. Symposium outcomes and the roadmap

Participants came to a shared vision on the cornerstones upon which to build an actionable strategy, specifically that dialogue, mutual comprehension and information exchange are key instruments. These will need to be strengthened and fostered globally to make progress toward, and eventually achieve, the elimination of ATT and TABST/LABST.

There was consensus that HSI should develop and disseminate a roadmap, directly collaborating with each stakeholder, as a concrete guiding tool to be employed globally. The roadmap contents are as follows:

- A stakeholders’ outreach kit, including key messages clearly stating the reasons for the elimination (or if deemed necessary, for a given period, the waiver) of those tests, together with a list of key publications on sets of data or case studies that could be shared and used to substantiate the key messages.
- A country-specific strategy, including an analysis of the current state of regulatory environment with regards to the use of ATT and TABST/LABST in vaccines batch release testing requirements; a general description of the stakeholders and their roles in this matter; and a general description of agreed actions.

HSI accepted responsibility to work on such a document and its distribution, to follow up on specific action items with the symposium’s participants, and to foster international communication and collaborations.

Overall, the feedback on the symposium from the participants was very positive. It was received as an opportunity to lay the foundations of enhanced cooperation between interested stakeholders, both from regions that already achieved the deletion or waiving of said tests, and those that are considering it or that will be confronted with the challenge in the future. It has also been an opportunity to outline the current status of the removal and waiver of ATT, TABST and LABST, and as a means to begin securing additional necessary concrete actions to make their elimination a concrete global achievement.

### Disclaimer

This manuscript and the views expressed herein are those of the authors and do not necessarily reflect the views or policies of the various regulatory authorities and organizations.

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LV wrote the manuscript. Then MH, MG, LB, KC, GS were involved in the composition, reviewing, and editing of the manuscript. All the other authors contributed equally to the review of the manuscript. No author received funding for their involvement.

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