Global Harmonization of Vaccine Testing Requirements

Roadmap for elimination of the ATT, TABST & LABST

HUMANE SOCIETY INTERNATIONAL

Preface

Humane Society International and our affiliates are a leading force for animal protection, with offices or programs in more than 50 countries, representing millions of supporters across the globe. We work in cooperation with scientific, governmental, corporate and other stakeholders to effect changes in regulatory science-policy toward replacement of obsolete animal tests with 21st century approaches that are more efficient, predictive and ethical.

HSI, together with leaders in the corporate and philanthropic sectors, established the Animal Free Safety Assessment Collaboration (AFSA) to spearhead a global, cross-sector shift toward modern, species-relevant approaches to safety assessment to better protect people and our planet, and hasten the replacement of animal testing. Within the AFSA Collaboration, a vaccine workstream operates with support from the Bill & Melinda Gates Foundation, Lush Cosmetics, and other stakeholders, to promote global alignment of vaccine regulations, with a view to removing or replacing obsolete animal-based methods, beginning with the Abnormal Toxicity Test, Target Animal Batch Safety Test, and similar variants.

HUMANE SOCIETY INTERNATIONAL

AFSA COLLABORATION

hsi.org

afsacollaboration.org

Humane Society International
1255 23rd St NW, Suite 450, Washington, DC 20037

Glossary of Abbreviations

AFSA	Animal Free Safety Assessment Collaboration
ATT	Abnormal Toxicity Test
AU-PANVAC	Pan African Veterinary Vaccine Center of the African Union
AVAREF	African Vaccine Regulatory Forum
BET	Bacterial Endotoxin Test
BVD	Bovine Viral Diarrhea Virus
CFR	United States Code of Federal Regulation
DCVMN	Developing Countries Vaccine Manufacturers Network
ECBS	Expert Committee on Biological Standardization (WHO)
EDQM	European Directorate for the Quality of Medicines
EFPIA	European Federation of Pharmaceutical Industries and Associations
EPAA	European Partnership for Alternative Approaches to Animal Testing
FDA	Food and Drug Administration, USA
GMP	Good Manufacturing Practice
GST	General Safety Test
HSI	Humane Society International
IBR	Infectious Bovine Rhinotracheitis Vaccine
ICH	International Conference on Harmonization
LABST	Laboratory Animal Batch Safety Test
LAL	Limulus Amebocyte Lysate
MAT	Monocyte Activation Test
NRA	National Regulatory Authority
OIE	World Organization for Animal Health
OMCLs	Official Medicines Control Laboratories
PEI	Paul-Ehrlich-Institute, OMCL, Germany
Ph. Eur.	European Pharmacopoeia
QA	Quality Assurance
QC	Quality Control
TABST	Target Animal Batch Safety Test
TRS	Technical Report Series (WHO)
USDA	United States Department of Agriculture
VICH	International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products
WHO	World Health Organization

Introduction

This roadmap outlines steps that could facilitate deletion of the Abnormal Toxicity Test (ATT),[1] Target Animal Batch Safety Test (TABST), and Laboratory Animal Batch Safety Test (LABST) from global regulations and pharmacopoeias for human and veterinary vaccines batch-release testing.

Building on the work done so far in this field, the roadmap also aims to help foster a more harmonized regulatory environment that both recognizes and encourages alternative methods.

This document has been reviewed and approved by experts and representatives from Argentina, Brazil, China, Europe, India, Russia, South Africa, and the United States (U.S.), who participated in a March 2019 symposium organized by Humane Society International with sponsorship from Lush.

The report from this workshop has been published in the journal *Biologicals*.[2]

[1] In some regions or regulatory frameworks, the test is also called General Safety Test or Innocuity Test.

[2] Viviani et al. 2019, *Biologicals*.

Motivation and Approach

For more than 50 years, the ATT, TABST, and LABST have been requisite tests worldwide for the release of human and veterinary vaccines and other biologicals. However, since 1995, a series of papers have highlighted the inherent limits of those tests, including their lack of specificity and the difficulties related to reproducibility, calling into question their scientific validity and regulatory value.[3]

At the same time, vaccine production has made significant progress through introduction of strict controls over starting materials and development of Good Manufacturing Practice (GMP), Quality Assurance and Control (QA/QC) and pharmacovigilance systems. Together, these systems have contributed to the creation of an environment in which these safety tests can be considered obsolete.

Multi-stakeholder discussions that arose during a 2015 workshop on harmonization of 3Rs in biologicals organized by the European Partnership on Alternative Approaches to Animal Testing (EPAA) further highlighted the opportunity for change towards allowing waivers and deletion of these tests by some regulatory bodies and international standards organizations.

However, several factors still hinder a full transition away from the tests, including:

- Lack of global coordination;
- Lack of regulatory harmonization (which can lead to local retesting, usually with slightly different locally preferred methods;
- Lack of familiarity with new approaches or technologies in some regions;
- Inadequate communication between manufacturers and regulators; and
- An imbalanced system of incentives/ protections from risk for manufacturers.

Mitigation of some of these factors would represent a fundamental step toward global elimination of these tests, an outcome that would spare the lives of countless thousands of animals each year, while ensuring more prompt and potentially more reliable availability of vaccines worldwide.

This was the perspective informing the March 2019 HSI symposium, which resulted in a consensus around a two-pronged approach that leverages both pragmatism and inclusion:

1. Pragmatism because the bases for elimination of ATT, TABST, and LABST testing are clear and compelling, so the question becomes one of reinforcing these conclusions, making decisions regarding next steps, and taking charge of further actions to secure elimination of those tests.

2. Inclusion because, to secure elimination of these tests in countries other than Europe and the U.S., stakeholders from those countries need to be involved so that their perspectives, experiences and safety-related worries can openly surface and be constructively addressed.

The roadmap we present in this document was agreed during that symposium.

[3] Duchow et al, 1995; Kramer, et al. 1996; Schwanig et al., 1997; AGAATI, 2002; Garbe et al., 2014

Status of the ATT

The process of deleting the ATT from the European Pharmacopoeia (Ph. Eur.) began in 1997 with the decision to end its mandatory use for batch-release testing of human and veterinary vaccines. However, reference to the test remained in some monographs in the "production section" until 2017, when the European Directorate for Quality of Medicines (EDQM) took steps to ensure its full deletion, with all reference to the test removed effective 2019.

In the U.S., regulations prescribing the ATT-equivalent General Safety Test (GST) were revoked in 2015, at which time the Food and Drug Administration (FDA) formally stipulated that the "GST requirements are no longer necessary or appropriate to help ensure the safety, purity, and potency of licensed biological products."[4] For licensed products, if the requirement to perform the GST is part of the biologic license, a manufacturer who desires to discontinue the GST must submit a supplement to their licensing agreement reporting the change under 21 CFR 610.12, and FDA would need to approve this change.

In November 2018, the World Health Organization (WHO) Expert Committee on Biologicals Standardization (ECBS)[5] took the historical decision to discontinue "*the inclusion of the innocuity test in all future WHO Recommendations, Guidelines and manuals for biological products published in the Technical Report Series (TRS), and that a clear indication be made in its report that the inclusion of this test in previously published WHO TRS documents be disregarded.*"[6]

Despite this promising momentum, the ATT remains a routine requirement in a number of major markets, including China, Japan, Mexico, Russia. In others, e.g. India, the possibility of waivers upon approval by a national regulatory authority is being assessed.

[4] https://www.federalregister.gov/documents/2015/07/02/2015-16366/revocation-of-general-safety-test-regulations-that-are-duplicative-of-requirements-in-biologics

[5] https://www.who.int/biologicals/expert_committee/POST_ECBS_2018_Polio_Web_9_Nov_2018.pdf?ua=1

[6] Main outcomes of the meeting of the WHO Expert Committee on Biological Standardization held from 29 October to 2 November 2018: http://www.who.int/biologicals/expert_committee/ECBS_Executive_Summary_final_20_NOV_2018.IK.pdf

Status of the TABST and LABST

Two guidelines of the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH)[7] have been recently finalized, becoming operative in May 2018:

1. Revised GL50: *Harmonization of criteria to waive target animal batch safety testing for inactivated vaccines for veterinary use;*
2. New GL55: *Harmonization of criteria to waive target animal batch safety testing for live vaccines for veterinary use.*

In 2012, the TABST was deleted from Ph. Eur. monographs for all but three veterinary vaccines: Porcine actinobacillosis vaccine, Porcine progressive atrophic rhinitis vaccine, and Tetanus vaccine for veterinary use. In these three instances, it has been renamed the: "residual toxicity test."

In 2017, the U.S. Department of Agriculture (USDA) updated its Veterinary Science Memorandum No. 800.116 to implement the VICH GL50 and 55, stating that the agency "will consider granting an exemption to target animal safety testing for specific products with a documented history of acceptable safety results and controlled manufacturing processes that have ensured batch-to-batch consistency and sterility."

Japan has implemented VICH GL50 and 55, and authorities are receptive to granting waivers following VICH guidelines.

Since 2018, the World Organization for Animal Health (OIE) refers to VICH Guidelines 50 and 55 on the removal of the test in two chapters of its *Terrestrial Manual*: "Safety tests are not required by many regulatory authorities for the release of each batch or serial where the seed-lot system is used. Other regulatory authorities may allow waiving of target animal batch safety tests in line with VICH GL50 and 55".[8]

Many countries still require the LABST for veterinary vaccines. VICH has drafted GL59 on *Harmonization of criteria to waive laboratory animal batch safety testing for vaccines for veterinary use,*[9] comparable to those for the TABST, which would allow waivers. As with the TABST, retrospective analysis of LABST[10] data revealed that the test lacks relevance, and is unable to detect problematic batches. In Europe, the LABST was removed from Ph. Eur. monographs for veterinary vaccines in 1997.[11]

---

[7] https://www.vichsec.org/en/guidelines/biologicals/bio-safety/target-animal-batch-safety.html

[8] OIE Manual of Diagnostics Tests and Vaccines for Terrestrial Animals 2018 – Chapter 3.7.2. Minimum requirements for the production and quality control of vaccines: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.7.02_MANU_SITES_VACCINE_PROD_CONTROL.pdf

[9] https://www.vichsec.org/en/activities/concept-papers/active-draft-guidelines.html

[10] Duchow et al (1996). Abnormal Toxicity: A Study in the Relevance of the Requirement V.2.1.5 of the German Pharmacopoeia for Vaccines, Immunosera and Immunoglobulins. German Ministry of Research and Technology. Project No. 0310624, Final Report. Langen, Germany: Paul-Ehrlich-Institut

[11] Schwanig M, Nagel M, Duchow K and Krämer B (1997). Elimination of abnormal toxicity test for sera and certain vaccines in the Ph. Eur.. Vaccine 15(10): 1047-1048.

Strategy

As decided during the 2019 HSI symposium, the strategy pivots around increasing stakeholder engagement and free circulation of information to create an ever-widening international dialogue where all concerns and doubts can be noted, discussed, and overcome.

HSI activities will build on the recommendations of past work by the EPAA and EFPIA, while expanding the involvement of other critical institutions and stakeholders worldwide.

To this end, the key components of the strategy will consist of:

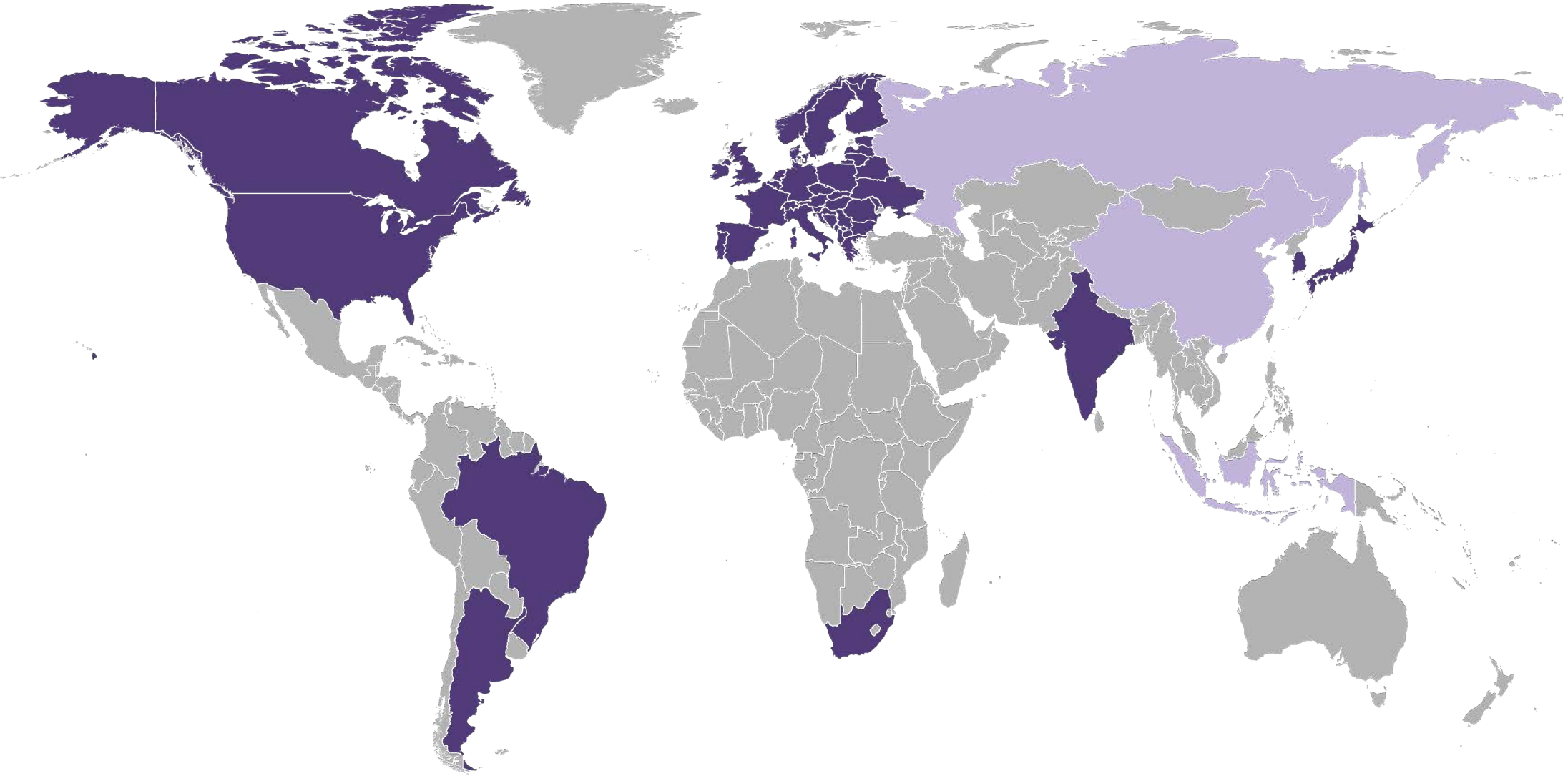
1. **Providing interested and engaged stakeholders with:**
    - key messages to use and disseminate;
    - key publications, and access to case studies and leading experts.
2. **Defining country- and stakeholder-specific action plans, including:**
    - Analysis of the current state of the regulatory environment, requirements, and activities in specific countries;
    - Identification of relevant stakeholders and their roles;
    - Fostering opportunities for discussion on the tests, creating the proper climate to inform and help decision-makers embrace change.

Follow-up activities will be tailored for each of the countries represented at the March 2019 symposium through country-specific strategies and action plans. Additional outreach activities will be executed to engage other key stakeholders in Asia, Africa, Central, and South America.

Implementation of the country-specific strategies will receive support from HSI, which will assume responsibility for creating discussion opportunities, facilitating dialogue, and securing mutual understanding and agreement among stakeholders.

Countries where HSI has active connections and activities.

Countries where HSI has initiated stakeholder engagement activities.



Key Messages

Supporting Deletion of the ATT, TABST, and LABST

The following key messages are based on the work of experts and organizations that have been promoting removal of those tests over the last decade. We wish to acknowledge EDQM, EFPIA, and EPAA for their work and contribution in this context.

1. These tests do not provide added value to the quality-control of medicines or to the safety of patients or animals.
2. These tests have a long history, dating back to the development of the general safety test in the early 1900s, when production processes and QC for biological products were poorly established.
3. Originally, the ATT was established as a mouse test to identify phenols-derived preservatives in diphtheria sera. Successively, a guinea pig test was introduced as biological indicator for the presence of tetanus toxin in antiserum preparations.
4. Over time, the ATT was adopted into a host of regulations as a safety test intended to detect product or process contaminants to avoid batch-to-batch differences in quality. In this role it has often needed substantial modifications to avoid complete failure. As a result, the test's original purpose and limitations were forgotten, and its use extended well beyond its original scope.
5. Beyond the original purpose of these tests, there is no evidence that the ATT, TABST, or LABST are useful to predict or control harmful batches or adverse events; on the contrary, there is substantial evidence that their results on the same batch might be unpredictable and contradictory.
6. Numerous reviews of historical test results have revealed that no reliable conclusions could be drawn from the ATT, TABST, or LABST, because they are variable, non-reproducible and non-specific.
7. These tests do not conform to International Conference on Harmonization (ICH) validation criteria for a QC test, i.e. specificity, reproducibility and detection limit. Beyond their ethical implications, there is simply no way they could be validated today.
8. There are inherently non-specific factors other than contaminants that can influence results, e.g. animal species, strain, and body weight.
9. Identical batches tested in different laboratories have, over time, been known to produce significantly different test results. Failed tests never showed a correlation to an effective product quality issue and/or contamination. Additionally, misinterpretation of responses caused by the active ingredients themselves, or their formulation components, may lead to false-positive results, since administered concentrations are unrealistically high compared to the human ones.

Controls for Contaminants

(specific to biological products)

Schutte et al. *Biologicals*, 2017; O. Garbe, et al. J. Pharm. Sci., 2014

Measures currently available to detect and control different types of contaminants include the following:

- Extended product characterization during process development and validation, where degradation profiles are investigated.
- Advanced process understanding, in-process controls, validation of the manufacturing process, and release testing complying with international standards are part of modern product development.
- Contaminants are controlled via a number of validated and specific tests that aim to detect microbiological contaminants or residual contaminants (mass spectrometry, sterility test, bioburden), pyrogens (MAT), and endotoxins (BET/LAL or recombinant factor C).
- Manufacture according to Good Manufacturing Practices (GMP).
- Routine QC release testing, which verifies batch-to-batch consistency and that a specific batch has been manufactured according to the previously validated process.

10. Due to their inherent unreliability, false-positive results may occur, causing delays in batch release, and therefore delays in patient access to life-saving medicines.
11. In the very few instances of actual batch safety concerns to have occurred in the last decades involving human or veterinary vaccines,[12] the harmful batches had all passed the ATT or TABST or LABST. For example:
    - Measles vaccine deaths in India in 2008 were caused by products which had passed both the ATT and the sterility test;[13]
    - A study[14] reported that hundreds of cattle died after vaccination in the Netherlands, because the infectious bovine rhinotracheitis (IBR) vaccine used had been contaminated with bovine viral diarrhea (BVD) virus. The IBR vaccine had been tested according to the Ph. Eur. monograph, passed the release tests, and had been released.
12. Modern pharmaceutical manufacturers have appropriate quality-assurance and control (QA/QC) in place, and comply with GMP rules, which together cooperate to prevent any risk of contamination. The existence of an established pharmacovigilance system is another guarantee of safety through post-marketing control of the products.
13. Currently, some regulators no longer require the ATT, TABST or LABST for most product classes, implicitly (or explicitly) recognizing that product quality can be ensured via quality control measures and state-of-the-art analytical techniques.
14. Since the deletion of the ATT in Europe and the counterpart GST in the USA, there has been no increase in safety concerns related to products that are no longer animal-tested using these procedures.
15. Ongoing requirements for the ATT result in unjustified use of a substantial number of animals with a negligible increase in product safety.

[12] AGAATI, 2002

[13] Times of India, 8/5/2008

[14] Falcone et al. 1999.

Case Studies

1 A retrospective survey and statistical analysis of data on use of the ATT for human and veterinary vaccines was performed by the Paul-Ehrlich-Institute (PEI) and vaccine manufacturers between August 1993 to September 1994. The study concluded that it was not possible to demonstrate any correlation between identified deficient final batches and deviations in the ATT results, and that the ATT demonstrated little predictive value in consideration of the chosen animal model, and questionable transferability of results to target species.

2 In a 2002 publication in Biologicals by the Advisory Group on Alternatives to Animal Testing in Immunobiologicals (AGAATI), "The Target Animal Safety Test—Is it Still Relevant?", data were collected from 14 manufacturers over the period 1997 to 1999. A total of 11,386 batches were tested in the TABST, of which 215 passed after retesting and 7 failed. Although only 30% of the European Official Medicinal Control Laboratories (OMCLs) provided data, and the data of the manufacturers are not complete, they clearly indicate that the TABST does not contribute to the safety of veterinary vaccines and should therefore not be required as a routine batch test.

Case Studies

3 Within the VICH Outreach Forum, training materials are available about the implementation of GL50 and GL55 for waiving the TABST in Japan. GL55 was implemented in 2018 and is applicable to live vaccines for veterinary use, where the seed-lot system is implemented in the production of the product and there are existing data proving successful passage through the TABST for 10 consecutive batches.

In Japan, manufacturers are required to provide the following for the 10 most recent batches:

- manufacturing records;
- batch release testing data;
- information on a defective batch (if available);
- revision history of the dossier;
- rational explanation of waiving the TABST using the data presented; and
- overall safety assessment, including pharmacovigilance data.

Looking Ahead

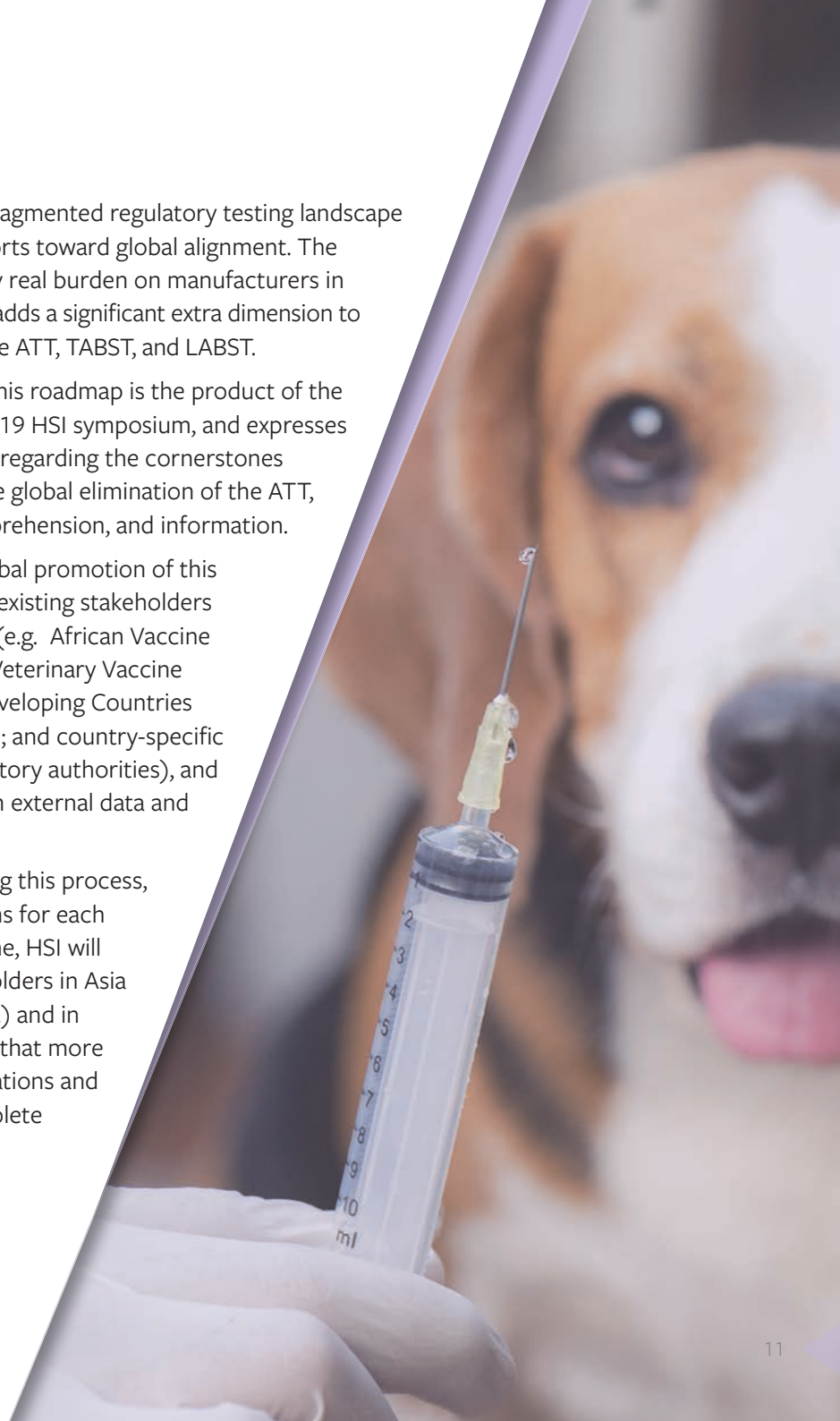
This roadmap highlights the diverse and fragmented regulatory testing landscape for vaccines, and the need for greater efforts toward global alignment. The current lack of consistency imposes a very real burden on manufacturers in terms of costs and complexities, and also adds a significant extra dimension to the challenge of achieving elimination of the ATT, TABST, and LABST.

Together with its accompanying actions, this roadmap is the product of the agreement of all the participants in the 2019 HSI symposium, and expresses the consensus reached on a shared vision regarding the cornerstones that must underpin a strategy to attain the global elimination of the ATT, TABST, and LABST: dialogue, mutual comprehension, and information.

Symposium participants agreed to the global promotion of this strategy, striving to engage both new and existing stakeholders through each participant's own networks (e.g. African Vaccine Regulatory Forum - AVAREF; Pan African Veterinary Vaccine Centre of African Union - AU-PANVAC; Developing Countries Vaccine Manufacturers Network - DCVMN; and country-specific pharmacopoeia committees and/or regulatory authorities), and to further reinforce the key messages with external data and case studies.

HSI offered to take a lead role in facilitating this process, including helping to define targeted actions for each stakeholder or institution. At the same time, HSI will continue in its activities to engage stakeholders in Asia (China, Japan, South Korea, Indonesia, etc.) and in South America (Brazil, Argentina, etc.), so that more can be known of their regulations, expectations and commitment towards the removal of obsolete animal tests.

Please follow our progress at:
afsacollaboration.org



AFSA COLLABORATION

Key Publications

A 4R concept for the safety testing of immunobiologicals. Dev. Biol. Stand. 101. Cussler, K. (1999).

Abnormal toxicity - a relevant safety test under GLP- and GMP- conditions in the production of vaccines? ALTEX 11 (5). Duchow, K. & B. Kramer (1994).

Alternatives to animals in development and control of biological products for human and veterinary use. The role of the Ph. Eur.. Dev. Biol. Stand. 101. Artiges, A. (1999).

Alternatives to Animal Testing in the Production and Control of Vaccines: Present Practice and Perspectives. Bilthoven. Castle, P. (1993).

Alternatives to Animal Testing in the Quality Control of Immunobiologicals: Current Status and Future Prospects. Hendriksen, C.F.M. ATLA 22. (1994).

Animal experiments in the context of quality control of pharmaceuticals. ALTEX 21 (2). Sponer, G. (2004).

EDQM's 3R Activities in the Field of Quality Control of Vaccines. Milne, C. & K.H. Buchheit (2012). Altex Proceedings 1/12.

Elimination of abnormal toxicity test for sera and certain vaccines in the Ph. Eur.. Vaccine 15(10): 1047-1048. Schwanig M, Nagel M, Duchow K and Krämer B (1997).

Expert Committee on Biological Standardization, Fortieth Report. TRS, Vol. 904. WHO, Geneva. World Health Organization (2002).

Global harmonization of vaccine testing requirements: Making elimination of ATT and TABST a concrete global achievement. Viviani et al. Biologicals (2019).

Historical Data Analyses and Scientific Knowledge Suggest Complete Removal of the Abnormal Toxicity Test as a Quality Control Test. Garbe, J.H.O., (2014). J. Pharm. Sci. 103.5.

International Symposium on Regulatory Testing and Animal Welfare: Recommendations on Best Scientific Practices for Biologicals: Safety and Potency Evaluations. ILAR J. 43 (Suppl. 1). Cussler, K., J. Kulpa & J. Calver (2002).

Is the abnormal toxicity test still relevant for the safety of vaccines, sera and immunoglobulins? ALTEX 13 (1). Kraemer, B., M. Nagel, K. Duchow, M. Schwanig & K. Cussler (1996).

Is the test for abnormal toxicity, general safety or innocuity necessary for vaccines? Vaccine 14. Gupta, RK. (1996).

Modern science for better quality control of medicinal products "Towards global harmonization of 3Rs in biologicals": The report of an EPAA workshop. K. Schutte et al. Biologicals 48 (2017).

On developing a process for conducting extractable–leachable assessment of components used for storage of biopharmaceuticals. T. Reynolds, K. Mazzarella, E. Hoff, S. Gomez & S. Martin-Moe (2010).

Protein–excipient interactions: Mechanisms and biophysical characterization applied to protein formulation development. Adv. Drug Deliver. Rev. 63. Kamerzell, T.J., R. Esfandiary, S.B. Joshi, C.R. Middaugh & D.B. Volkin (2011).

Reactive Impurities in Excipients: Profiling, Identification and Mitigation of Drug–Excipient Incompatibility. AAPS Pharm. Sci. Tech. 12. Wu Y., J. Levons, A. S. Narang, K. Raghavan & V.M. Rao (2011).

Reduction of animal use in human vaccine quality control: opportunities and problems. Vaccine 20. Metz, B., C. Hendriksen, W. Jiskoot & G. Kersten (2002).

Replacement, Reduction, Refinement (3Rs): Animal Welfare Progress in Ph. Eur. Monographs. Pharmeuropa 19 (3). Castle, P. (1997).

Three Rs achievements in vaccinology. AATEX 14 (Special Issue). Hendriksen, C. (2007).

US FDA, Revision to the General Safety Requirements for Biological Products. Feb. 26, 2003.

US FDA, Revocation of General Safety Test Regulations That Are Duplicative of Requirements in Biological License Applications. Aug. 22, 2014.

WHO activities towards the three Rs in the development and control of biological products. Dev. Biol. Stand. 86. Milstien, J, V. Grachev, A. Padilla & E. Griffiths (1996).

WHO Good Manufacturing Practices for Pharmaceutical Products: Main Principles. TRS, No. 961 (Annex 3). World Health Organization (2011).