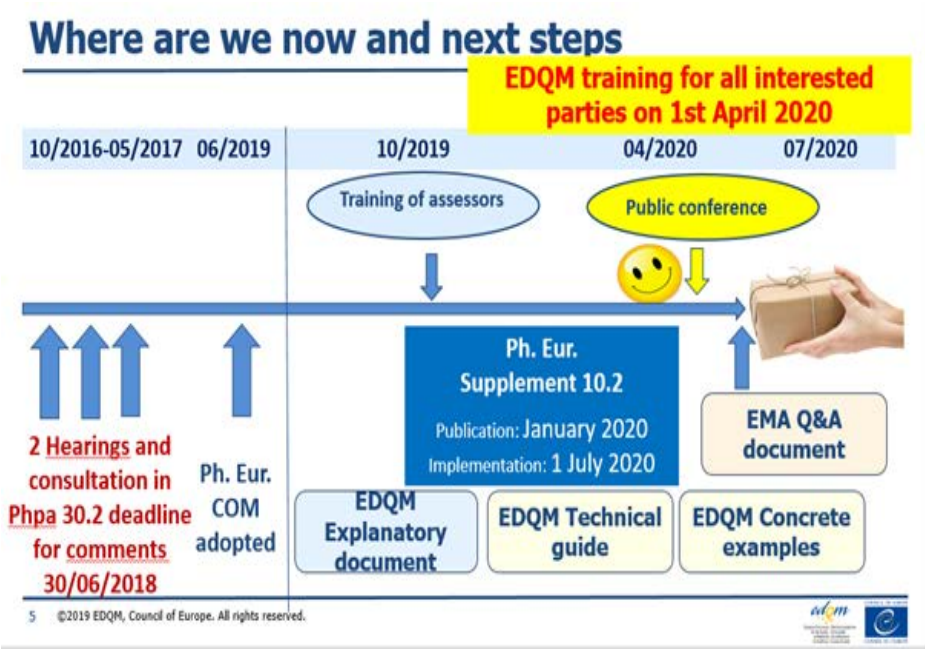
DOCUMENT SUMMARISING THE TRAINING ON THE EUROPEAN PHARMACOPOEIA'S NEW APPROACH TO THE 'MANAGEMENT OF EXTRANEOUS AGENTS IN IVMPs'

Webinar on 01 April 2020

Introduction from the EDQM before the webinar

Susanne Keitel, Director of the EDQM, welcomed the participants (180, from 18 countries) to the webinar. She said that while fighting COVID-19 had become the top public health protection priority worldwide, she was pleased that the EDQM had been able to find solutions during these unsettled times to ensure that activities can continue while respecting measures to restrict the spread of the virus. She thanked the participants for making the necessary arrangements to attend this webinar and also the speakers and scientific committee for making this new format possible. The two webinar sessions will provide manufacturers, assessors and users of the European Pharmacopoeia with a "**survival kit**" to help them prepare for the 1 July 2020 deadline for compliance with the recently revised text on management of extraneous agents in IVMPs. She hoped the participants would gain valuable knowledge from the presentations and the Question & Answer sessions and reminded them that the support documents sent by email should also help them understand the topics. On behalf of the EDQM, she thanked all the healthcare workers on the frontline combatting COVID-19 and stressed that we have to work together to win this fight.

Technical introduction to the webinars (C. Lang)



Catherine Lang, secretary to European Pharmacopoeia (Ph. Eur.) Group 15V, said that the two webinars are part of a collaborative process with stakeholders to revise the chapter on extraneous agent testing in IVMPs. This process is almost finished and these webinars are intended to help users of the Ph. Eur. to be ready to use the new approach laid out in this chapter by the **implementation date of 1 July 2020**.

The morning session explained the changes to **Ph. Eur. requirements, focusing on risk management and giving concrete examples** on how to apply these requirements. Representatives of industry were then given the floor.

The afternoon session covered the **European regulatory landscape and present guidance and Q&A documents** to facilitate the application of the new approach. The EDQM took this opportunity to thank the experts, the speakers and all the interested parties for their contribution to the work.

The slides are available on EDQM website (page "European Pharmacopoeia Training Resources"):

The following abbreviations will be used throughout this text:

European Pharmacopoeia: Ph. Eur.
Immunological Veterinary Medicinal Products: IVMPs
Extraneous agents: EAs
Good Manufacturing Practice: GMP

Morning Session - Setting the scene

Setting the scene for the changes to the Ph. Eur. requirements for the management of extraneous agents in IVMPs

Member of Ph. Eur. Expert Group 15V – **A-M. Brady**

The purpose of this introductory session was to outline the background to the changes as well as the challenges, opportunities and benefits of the new risk management approach.
There were a number of drivers for change within the wider regulatory landscape, which led Ph. Eur. Group 15V to review the current requirements. These included revisions within the EU guidance; the impetus of the consistency approach (moving quality control upstream thereby removing reliance on end-product batch testing); the routine implementation of Good Manufacturing Practice (GMP) standards; new state-of-the-art methodology allowing increased sensitivity and a reduction in the use of *in vivo* tests. The Group also noted that recent experiences of handling emerging infectious agents highlighted the need for a flexible dynamic framework and tools for both industry and regulators in order to respond in a timely manner.

The limitations of the existing approach were explored. These included the lack of transparency and harmonisation in the current texts as a result of the gradual development of the Ph. Eur. texts over time. It is also prescriptive, concentrating on a rigid framework of laboratory testing that does not take into account the impact of good manufacturing standards on risk reduction and providing limited scope for recourse to newer methodologies. It does not provide sufficient flexibility for the management of new infectious agents. These limitations mean that the current framework is no longer fit for purpose and would be unsuited to underpinning a risk management approach that provides a dynamic mechanism and tools for industry and regulators.

Risk management is a step-wise process by which risks are identified, evaluated and assessed, allowing them to be controlled and mitigated and resulting in elimination or reduction of the risk to a negligible or acceptable level. There are three main steps in the approach to managing EAs:

- Identify: biological starting materials and those used during the process
- Mitigate and control: sourcing and treatment/process mitigation
- Assess and evaluate risk: may lead to removal of tests for risk agents in final product if absent or negligible

New chapter *5.2.5* builds on its existing risk management requirements and provides a framework and step-wise approach. It introduces new methods as well as retaining existing methods. This allows a “mix and match” approach, allowing the use of old and new methods tailored to individual product risk evaluation and justification to authorities rather than a tick-box approach which may result in unnecessary testing. A comprehensive list of agents to be considered within the risk assessment is provided as well as a decision tree to enable identification of mitigations and control steps. New chapter *2.6.37* includes general principles and examples of parameters to be taken into account when considering whether a methodology will support a risk management approach.
It was acknowledged that implementing the new approach held challenges for both industry and regulators. However, the risk management approach was based on authorisation experience

gained with both old and new products at European level where a mix and match risk assessment approach had been used. To ensure clarity and consistency, Group 15V and the EU's Immunological Working Party (IWP) had worked together to prepare assessors' training and Question & Answer documents for industry. It was clarified that there was no immediate requirement to have new methodology in place for all listed agents by July 2020 (date of implementation of the new approach). It was also emphasised that a risk assessment should be conducted to establish which agents remained a risk after sourcing and process mitigations and controls. Any existing methods already in place for those agents should comply with requirements of chapter *2.6.37*.

The opportunities brought by the new approach were identified. These included a reduction in testing during manufacture and deletion of unnecessary tests for EAs in final products as well as a flexible and dynamic mechanism for handling new infectious agents which may not have a practical risk of infection. The increased sensitivity of newer methods was also seen as an advantage.
The new texts are harmonised across the Ph. Eur. and the EU which means that the requirements are centralised with no duplication and have a transparent rationale.
This allows regulators and industry to work together using fit-for-purpose methodology in a cost-effective manner to provide robustly safe and efficacious IVMPs.

"*Management of extraneous agents in IVMPs*" - the new general chapter *5.2.5*
Member and former Chair of Ph. Eur. Expert Group 15V – **C. Lorteau**

The purpose of this presentation was to illustrate the changes, new structure and content of chapter *5.2.5*, which becomes the core chapter for the management of EAs in IVMPs. This chapter, which was previously dedicated solely to substances of animal origin used in the production of IVMPs, already followed the risk management approach. The original template was kept but the scope and content of the chapter were extensively revised.

First, a brief overview of the requirements of the Ph. Eur. applicable until July 2020 was given: information on these requirements was scattered amongst the different texts of the Ph. Eur., and organised differently depending on the origin (poultry, mammals or fish) and material tested. Examples were then given of how revised chapter *5.2.5* offers a single and harmonised approach for IVMPs, whatever the material at risk and the species considered.

New chapter *5.2.5* encompasses the whole production process, from starting materials (including seeds) to final product, and takes into consideration the production conditions. Following the principles of risk management, the list of EAs to be tested for in the final product is limited to those that cannot be excluded by other means.

This list of EAs to be considered appears in annex I to the chapter. In agreement with the EMA/CVMP/IWP, a single list was compiled and is now published in the Ph. Eur. It will be maintained by Group 15V. Where mammalian and fish materials are concerned, the list was extracted directly from the EMA guideline in force (EMA/CVMP/IWP/206555/2010-Rev.1). For poultry materials, Group 15V revised and updated the information already present in the Ph. Eur. (from chapter *5.2.2*, specific monographs of avian vaccines and former chapters *2.6.24* & *2.6.25*); the lists were then drawn up following a similar template (table of viral/bacterial agents – species by species).

For each material - master seed lot, substrate, substance and final product - the acceptance limits for contamination by EAs were gathered together in this chapter and clarified.

The risk assessment approach and risk control measures were then discussed in detail, illustrated by 2 slides. A third slide presented Annex II which is an example of a decision tree for substrates; this tree may be used in the risk assessment approach. A brief demonstration was given of how the approach may lead to alleviated testing at final product level, provided absence of contamination is guaranteed by a production process carried out under GMP conditions and using a seed lot system together with cell lines, substances and a Master Seed Virus all established as free of EAs.

A reminder of control measures applicable to the starting materials (caution at sourcing, quality of embryonated eggs, etc.) and in production (seed lot system and GMPs; in-process inactivation or EA removal steps, etc.) was also provided.

The new requirements for methods of detection were then introduced. The principle is to move from compendial tests expected to afford a broad detection of EAs, to the use of any fit-for-purpose method for the target EA at risk of contaminating the material. These methods must provide adequate sensitivity and specificity, as well as appropriate controls.

New information was provided regarding molecular methods which may be used either as an alternative to *in vivo* tests or as a supplement/alternative to *in vitro* culture tests. Appropriate interpretation is required and further investigation may be necessary, for example where a positive signal is obtained; indications were given on how to deal with divergent results.

The chapter ends with cross references to the chapters of interest, where additional information exists in the Ph. Eur. (e.g. *2.6.1. Sterility*, *2.6.7. Mycoplasmas*, *2.6.21. Nucleic acid amplification techniques*). These chapters include the new text on the principles for the detection of extraneous viruses in IVMPs using culture methods (*2.6.37*).

Chapter *5.2.5* concludes with a global, risk management-based approach for EAs, applicable to all IVMPs. The chapter includes practical information for implementation (e.g. lists of agents to be considered, risk control measures and information on testing).

New approach for extraneous agent testing - Concrete examples
Member of Ph. Eur. Expert Group 15V – **M-J Ferrer**

Marketing authorisation applicants should ensure that the product is free of EAs throughout the manufacturing process and up to final product release. This presentation focused on the new approach followed for the management of EAs during the development and production of a live bovine vaccine.
This was an example based on a real case which showed that some manufacturers were already using the revised approach.
The vaccine virus grows in bovine cell cultures and different starting materials of animal origin are used: viral seed, cell line and different raw materials.
Only two of all these materials of animal origin were chosen to be included as examples in the presentation: the Master Seed Virus - MSV (as a starting material not listed in a pharmacopoeia) and peptone (as a starting material listed in a pharmacopoeia). Since the management of EAs must include the entire production process, the final product was also addressed.

For the MSVs and peptone, the EAs shown to be absent were those on the list of bovine EAs included in the current *Guideline on requirements for the production and control of immunological veterinary medicinal products* (EMA/CVMP/IWP/206555/2010-Rev.1):

- For bacterial agents, appropriate and validated tests were performed.
- For viral agents, the first step consisted in identifying those agents for which testing could be considered unnecessary. In these cases, evidence that the viruses were not present in the country of origin – supported by official sources or solid scientific publications – or that they could not grow in the bovine cell line when the vaccine virus has been neutralised, was used to prove their absence. As the second step, the remaining viruses on the list were tested and validation of the methods was provided.

The decision to test the final product was taken on the basis of a risk assessment which took into account whether or not all the following general conditions had been met: the vaccine had been produced under GMP conditions, it had been shown that the MSV and all the other materials used in the production process were free of EAs.

When the risk assessment ensures that the product is free of viral EAs at final product stage, no additional tests for EAs need be performed on the final product. Based on this, the final product test for EAs was not performed.

New approach for extraneous agent testing - Concrete examples
Member of Ph. Eur. Expert Group 15V – **R. Kovacova**

The combined testing and risk assessment approach is not new. It has been applied over the last 20 years to cell seeds and vaccine virus seeds and has been accepted during various marketing authorisation procedures. As an example, for some of these vaccines, the decision not to test for potential EAs was based on solvent treatment of master seed viruses during isolation and preparation. Viruses that might have survived the treatment were tested in cell cultures and by molecular methods. In master cell banks, it was possible not to perform tests for some viruses on the basis of a risk assessment taking into account the geographical origin of the target or source species.
Three concrete examples were given.

The first focused on risk management for vaccine components produced using eggs from healthy chicken flocks. When considering the risk of EAs being present in a healthy flock, the available information and results of testing were taken into account. Chapter *5.2.13* refers to general chapter *5.2.5* with regard to the risks related to using eggs from healthy chicken flocks. The risk assessment focused on vertically transmitted agents listed in Ph. Eur. general chapter *5.2.2* for SPF chicken flocks. The risk of contamination was assessed both during production and during the inactivation procedure.

The second example touched on the sensitivity issue observed with the test for avian reticuloendotheliosis virus (REV). The validation report for the R–PCR for REV was provided. Parameters such as the limit of detection (LOD), accuracy, precision, specificity and robustness were examined in line with guidelines for validation of analytical procedures (VICH 1 and 2). Positive and negative control samples were used. The LOD of the method for REV was found to be lower than expected compared to LODs determined for other viruses using molecular detection methods. A means of improving the sensitivity of the REV detection method was provided.

The limits given in the presentation are examples based on current state of the art technology and suitable product specific justifications. Such examples do not set precedents for acceptable LOD for such viruses. These will be evaluated by the Competent Authority on a case by case basis taking account of the current state of the art technology and product specifications.

The last example concerned a risk assessment of Schmallenberg virus contamination in a working cell bank (WCB). Based on geographical origin and bibliographic data, the risk of contamination of the WCB by this virus was considered zero by the applicant.

The voice of industry
Animal Health Europe – **F. Descamps**

This presentation gives the point of view of veterinary vaccine manufacturers (members of Animal Health Europe) on the recent Ph. Eur. updates specific to the management of extraneous agents in IVMPs. It points out that the current set of Ph. Eur. monographs have worked very well, with no major concerns reported over the last 20 years, and that the Ph. Eur. updates (will) have a big impact on the Industry. As the risk assessment approach may be perceived differently by different assessors (depending on their expertise and experience), the Ph. Eur. updates decrease the predictability of outcomes (versus current texts). While one objective of the EDQM was to reduce the amount of testing required for viral safety (at least for finished products), in the case of new seeds (i.e. first time use in a commercial product), the updated Ph. Eur. texts may oblige Industry to conduct more extensive testing to increase the predictability of approval of the product in question and avoid the need for additional testing very late in development. The updates also increase the regulatory risks related to the use of an existing seed for the development of a new product (a very common practice in the veterinary vaccine sector), which may lead to some new development projects being shelved. This has already started to happen and will have a negative impact on the availability of new products. The presentation highlights other issues and then provides general recommendations (essentially, to increase predictability of risk assessments) and specific recommendations. Overall, continued dialogue and collaboration between the European Regulatory Authorities and Industry will be the key to a smooth and harmonised implementation of the new approach.

The voice of industry - Focus on risk assessment
Genera Inc./ Dechra Pharmaceuticals PLC Group, Zagreb, Croatia - **M. Weber Susanj**

Risk analysis, combined with risk control, can replace traditional testing for EAs in poultry live viral vaccines. An Ishikawa diagram can be useful tool for risk analysis. All potential risks for extraneous agents should be listed in diagram and analysed. Five steps for EAs should be considered: virus seed, substrate for production, substances of animal origin, media for vaccine production and production conditions. An analysis of these topics with an emphasis on the likelihood of introducing EAs into the vaccine is crucial. The outcome of a risk analysis can lead to additional testing in cases where the presence of EAs cannot be excluded during the risk assessment. Completion of the risk analysis, with or without testing, corresponds to the result pass/fail of the vaccine specification parameter - EAs.

Close of webinar 1

Afternoon Session - Regulatory landscape

EMA Guidelines (including need for revision or elaboration of new guidelines) and Q&A document as a tool for harmonised assessment

Chair of the EMA IWP – **E. Werner**

The purpose of this session was to outline how the newly revised text on management of EAs in IVMPs is embedded in the European regulatory landscape and what kind of guidance is foreseen to facilitate the application of this approach.

The requirement to test for potential infectious contaminants for any IVMP placed on the market in the EU is specified in Directive 2001/82/EC and in the Ph. Eur.

It was pointed out that prevention of potential contamination through EA testing embraces the entire production process, from raw (starting) materials to the final product. This includes reliable sourcing and testing of raw materials; standardised, controlled production processes using GMP in order to assure consistent production; and tests confirming the quality of starting and in-process materials as well as the final product.

Therefore, the testing applies to all components of animal origin (cell substrates, virus seeds, substances of animal origin), in-process materials and the final product, as specified in the respective legislation, Ph. Eur. and relevant guidelines.

The *Guideline on requirements for the production and control of IVMPs: "Annex 2 - The approach to demonstrate freedom from extraneous agents as part of the production and control of IVMPs for mammalian species and finfish*" (EMA/CVMP/IWP/206555/2010-Rev.1) came into force on 1 May 2017. A revised EU approach was laid down in this Guideline, moving away from prescribing the test methodology that must be used for a particular agent or substrate towards describing the general approach in order to demonstrate suitability of tests applied to show freedom of the relevant substrate from specified EAs.

Annex 2 of this guideline is applicable to IVMPs for mammalian species, salmonids and other finfish; it includes a 2-step risk assessment identifying those EAs for which testing is necessary and defines the following:

- Substances, substrates, starting materials, intermediates and final products for which testing is required.
- Criteria by which the requirement to demonstrate freedom of a material from particular EAs may be waived and examples of justification for not carrying out a test for a specific EA.
- How tests may be shown to be suitable for EA testing in terms of performance criteria (sensitivity, specificity, etc.).
- Lists of EAs per species, which must be taken into account when considering which test for EA is appropriate.

In addition, a CVMP reflection paper on methods found suitable within the EU for demonstrating freedom from EAs of the seeds used for the production of IVMPs (EMA/CVMP/IWP/251741/2015) was prepared. This document contains examples of suitable cells and methods for testing for freedom for a range of EAs. It is currently limited to porcine, bovine, canine and feline species, but work is ongoing with additional species (ovine, equine).

The approach for EA management (moving from a prescriptive to a scientifically sound and targeted approach) is now included in Ph. Eur. monographs and is restricted to living replicative EAs. It covers all starting materials of animal or human origin and the entire production process, from the sourcing of raw materials to the final product stage. The approach based on risk management and including risk assessment and risk control allows the use of any suitable culture or other fit-for-purpose method capable of detecting specified EAs but with a focus on *in vitro* methods. Furthermore, it provides an updated single reference list of EAs to be considered in the risk assessment.

Regulatory challenges/concerns regarding the implementation of the approach to EA testing have been identified and these could threaten the availability of veterinary vaccines. According to industry, the new approach would introduce uncertainty into the development cycle of both new vaccines using new seed materials and new vaccines using existing seeds that have been previously approved. Unpredictability and the risk that EA status of existing seeds may be questioned could have an impact on the availability of vaccines in the future. These aspects are considered to be outside the scope of Ph. Eur. texts, particular where matters for authorisation are concerned.

Therefore, it was agreed to prepare a Q&A document to address comments and concerns received during public consultation of the Ph. Eur. texts on the revised approach for EA management and which are matters for the authorisation of the products, and to provide clarification on the mentioned aspects.

A revision of the guideline EMA/CVMP/IWP/206555/2010-Rev.1 is also foreseen for the future.

The particular case of old master seeds used for the production of new vaccines: re-testing of well-established cell banks and master seeds? What can be used to justify no re-testing?
Member of Ph. Eur. Expert Group 15V- **I. Lemke**

Manufacturers of veterinary vaccines currently use master cell seeds and master seed viruses that have been tested by methods prescribed so far in Ph. Eur. 0062 and Ph. Eur. 5.2.4. These seeds have already obtained marketing authorisation approval. Now, questions have been raised as to whether these approvals would also be maintained for new products without the need for re-testing, i.e. do existing cell seeds/master seeds used for the production of new vaccines need to be re-tested extensively even if they are “well-established”.
It was made clear that the new and revised Ph. Eur. requirements based on the risk management approach do indeed apply retroactively. In any case, the risk of contamination of cell seeds/master seeds with EAs must be assessed using the new approach laid down in Ph. Eur. *5.2.5*

New products as well as new cell seeds/master seeds to be introduced must fulfil these requirements. For existing cell seeds/master seeds currently used in approved products, re-testing is normally not required. However, if an existing seed is to be used in a new product, a full risk assessment is needed.

Test methods used up until July 2020 for existing cell seeds/master seeds are in most cases found to be acceptable. In this context, it is stated that the documentation for these currently performed tests and existing data based on those test methods can generally be used. These methods can still be considered with the new approach but under the fit-for-purpose condition. However, new developments in methodology should be taken into account as well.

Re-testing of existing cell seeds/master seeds may only be necessary in part and combined with the risk assessment. Thus, EAs which are included in Annex II of the 'new' Ph. Eur. *5.2.5* but which cannot be excluded by justification should be tested.

In any case, justification for not performing a test for specific EAs (or diseases) in cell seeds/master seeds should be provided and supported by reliable, independent sources and scientific evidence. In this context, some examples for types of justification which may be considered, e.g. official data, scientific literature or references, independent statements, results of any tests performed and documentary evidence/certificates, were given.

It was also pointed out that any new EAs identified since the current tests had been performed should be subject to risk assessment or testing, if not excluded by risk assessment. For master seeds derived by recombinant DNA techniques, the risk assessment should also include the materials that were/are used to produce the active substance together with a thorough justification.

Historical value of the previous and detailed testing methods as described in the Ph. Eur.: can the "old" detailed protocols now available in the Ph. Eur. archives still be used?
Chair of the EMA IWP – **E. Werner**

The focus of this presentation was how to handle the previous requirements and detailed testing methods described in earlier versions of the Ph. Eur. (before Supplement 10.2). Can they still be used? What is expected with regard to the fit-for-purpose aspects of these test methods? What documentation has to be provided to demonstrate adequate sensitivity and specificity?

It was pointed out that the detailed descriptions of the test methods for EAs in materials which are used during the manufacture of IVMPs and that are referred to in monograph *0062*, chapter *5.2.4*, chapter *2.6.24* and chapter *2.6.25* have been deleted from the new texts. The detailed protocols for these methods which have been deleted from the current version of the Ph. Eur. can be found in the Ph. Eur. archives.

The new approach to managing EAs is now described in Ph. Eur. chapter *5.2.5* and chapter *2.6.37*. New chapter *2.6.37 Principles for the detection of extraneous viruses in IVMPs using culture methods* maintains the methods used by the applicant for decades (cell cultures, chicken materials such as embryonated eggs & primary cells) but in less detail and it has to be shown that they are fit-for-purpose. The Q&A document, which is under elaboration, will give more information.

Furthermore, guidance can be found in the CVMP reflection paper on suitable cells/substrates and methods of detection for the listed cells and substrates for specific EAs (historic data).

It was discussed whether detailed testing methods for EAs described in previous versions of the Ph. Eur. would simply be accepted without justification (as had been the case for many years) or if justification should be provided or positive controls included.

The test methods for EAs described in previous versions of the Ph. Eur. (before Supplement 10.2, applicable from July 2020) performed originally for existing cell seeds/master seeds (used in approved and new products) are, in most cases, found acceptable. They had been used by applicants for decades and were accepted by regulatory authorities in marketing authorisations for IVMPs. These detailed test methods can still be used with the new approach if they have been

shown to be fit for purpose (i.e. suitable sensitivity, specificity, repeatability and use of appropriate controls).

Reference was also made to the presentation on validation.

Validation of new test techniques - expectation with regard to validation and documentation in the dossier
Member of the EMA IWP – **C. Guittré**

The purpose of this presentation was to illustrate the management of EAs in IVMPs focusing on the validation of new techniques with regard to the documentation that should be provided in the marketing authorisation dossier.

In principle, the concepts presented in VICH GL1 (Validation: definition) and VICH GL2 (Validation: methodology) are applicable to the analytical methods used for the detection of EAs as the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose and that the test results obtained are relevant. Nevertheless, due to their complex nature, analytical procedures for biological products may be approached slightly differently and not all validation characteristics mentioned in the guidelines may be necessary.

As the detection of EAs corresponds to a qualitative test, the following parameters are considered the most important:

- ✓ Specificity of the assay, which is the ability to distinguish unequivocally an EA in the presence of components that may be expected to be present.
- ✓ Sensitivity of the assay, which is the ability to detect the presence of the EA enabling as accurate as possible a measurement. The limit of detection (LOD) is a measure of the analytical sensitivity of the method. It corresponds to the lowest amount of EA in a sample that can be detected but not necessarily quantitated as an exact value.

The method is considered specific if it is able to differentiate an EA from similar organisms or other interference from matrix elements that could have a positive or negative effect on the assay value. In addition, the method should have the ability to detect relevant EAs representing temporal and geographical diversity (field virus strains).

The validation assay should be adapted to the method of detection on a case-by-case basis. For example, if the method is an ELISA assay, the specificity should be demonstrated by testing other relevant microorganisms and showing that there is no cross-reaction. In the case of a PCR assay, the details of the primers and probes should be provided and their specificity to detect relevant EAs should be investigated by comparing the chosen sequences with sequences in published data banks. There should be no major homology found with sequences unrelated to the EA. Independently of the method, the use of quality control samples (positive controls [including positive controls around the cut-off to confirm test performance]/negative/internal controls) is necessary in each run to validate the results and evaluate test performance.

For the LOD, the same approach applies and no acceptable cut-off can be defined a priori. A justification of the cut-off limits should be given with relevancy to the test method used for the particular agent being tested (in a relevant matrix) and the target species (literature references could be used to support this and/or the minimum infectious dose if available from known challenge studies). The relevance of the results obtained in the validation assay should be assessed with regard to the risk of contamination by living replicative EAs.

If a new method for which limited validation data or experience exists is used and a positive result is obtained, the result should be confirmed using another relevant/reliable method (e.g. cytopathic effect, haemadsorption, immunostaining) in an attempt to verify the result. Further investigation may be needed and an appropriate method for differentiation between live (replicative/non-replicative) or killed agents should be implemented. The biological significance of any confirmed positive result must be discussed in terms of potential contamination by a particular EA.

Generally, it is never possible to achieve 100% specificity and sensitivity of the method. It is necessary to maintain a balance between specificity and sensitivity with respect to the potential contamination by the agent under investigation. Therefore, the applicant should justify the proposed parameters of the methodology with regard to the EA to be tested for. Test controls should always be included.

The development of new detection techniques is encouraged (for example, broad molecular methods such as next generation sequencing techniques). Collaboration and dialogue between industry and regulatory authorities are necessary to facilitate the development, standardisation and regulatory acceptance of new techniques for the detection of EAs in IVMPs.

Close of webinar 2