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Human Medicines Development and Evaluation

Meeting Report - Joint EMA/EDQM workshop on improved potency assays for inactivated influenza vaccines held on 12 December 2011

European Medicines Agency, London, United Kingdom

Introduction

The lessons learnt exercise performed after the 2009 influenza pandemic recognised that the assays used for content of the hemagglutinin (HA) component in the influenza vaccine dose should be reviewed. During the pandemic and as part of the lessons learnt exercise, issues related to the interpretation due to variability/standardisation of the assays used for content determination were raised and particularly at the successive expert meetings held at the European Medicines Agency in July 2009, April 2010 and June 2010.

Quantification of HA by the single radial immunodiffusion (SRID) assay has been for many years the recognised/accepted method to monitor potency (content) of trivalent seasonal influenza vaccines for HA antigen. The current SRID method however requires, for each of the three HA antigens, strain-specific virus reagents which may not be available when the early batches of a vaccine have to be filled and released for either clinical trial confirmatory tests or to speed up the early access to pandemic vaccine doses. During the expert meetings and lessons learnt exercise, it has been raised that alternative assays to measure the content of HA should be investigated and their relative performance validated against the current SRID, before being used on a routine basis. The timing of use of such assays (e.g. at an early stage of development/qualification of the new strain vaccine until the SRID test reagents become available, and/or for a more general use as alternative assays to SRID) will need to be discussed. Also techniques for the characterisation of neuraminidase (NA) component and its assays could be considered.

The EMA guidelines on influenza vaccines are being reviewed as part of the lessons learnt exercise. In this context, an interactive technical workshop was organised to prepare the work and feedback the revision exercise of the guidelines. Furthermore the workshop was aimed to review whether there are still gaps in the development and validation of alternative assays and how these gaps can be filled.

The development of assays for HA content may impact the batch release and Official Control Authority Batch Release (OCABR) by the Official Medicines Control Laboratories (OMCLs) and potential future revision of the European Pharmacopoeia monographs for influenza vaccines.

The workshop was not considered to be a decision making meeting but rather an event where regulators, scientists and vaccine industry representatives could share information in an interactive way.

The meeting was structured in two sessions with series of presentations followed by open discussion.

Session SRID assay

Dr. Una Dunleavy (NIBSC) opened the session with an overview of the SRID assay, outlying the assay's history and preparation of official SRID reagents (antigen and antiserum). The SRID assesses a biologically relevant form of HA. Timelines for production of the reagents are tight but usually do not delay vaccine availability. Antigen standards are calibrated in international collaborative studies by WHO Essential Regulatory Laboratories (ERLs). Dr. Dunleavy indicated that a protocol for preparation and calibration has recently been adopted by the WHO Expert Committee on Biological Standardization (ECBS) which should further improve the SRID antigen reagent preparation and calibration process. For the calibration of secondary and replacement antigen reagents, NIBSC uses laboratories from the EU OMCL Network. Examples were presented of technical root causes of SRID failures such as adjuvant interference, agarose temperature and formaldehyde inactivation that prevents HA diffusion.

Dr. Francois Cano (AFFSaPS OMCL) presented the experience from the French OMCL with the SRID assay. Except for the automation of the plate reading, the SRID assay has not really evolved since its introduction in the late 1970's. However, the SRID is still considered as the "gold standard" for the measure of HA content in inactivated influenza vaccines. Although the method is relatively easy to implement and no expensive laboratory equipment is needed, the method is dependent on reagent availability, its precision is dependent on the calibration of the antigen reagent and it has a high limit of quantification. The implementation of new reagents is not a straightforward process and needs proper calibration and assay validation. Dr. Cano recommended that other stakeholders including the EDQM/OMCL network should be more involved in the calibration process. He also indicated that the WHO calibration protocol would have to be discussed in more detail with all stakeholders. The SRID was prone to improvement, for example a general SOP, a unified statistical model, and further harmonisation of the SRID reagent cross calibration could be considered. It was also recommended that work on alternative test should be initiated in parallel to SRID improvements.

During the short discussion it was clarified that the WHO calibration protocol should be seen as a first step and further technical analytical details will have to be filled in at a later stage with involvement of other stakeholders.

Dr. Tony Colegate (EVM, Novartis) noted that the SRID has important features such as a biologically relevant measure of potency, some correlation to clinical efficacy, the ability to detect individual strains in a trivalent vaccine, and stability indicating properties. Nevertheless, the time required to produce and calibrate the specific SRID reagents is seen as a primary drawback. Differences in reagent potencies have also been an issue where a vaccine is destined for different regions of the world. In a number of examples, differences in potency results were observed due to reagent calibration and SRID method issues, related to both antigen and antiserum lots and impacted stability testing results and vaccine formulation. Therefore, it is recommended to improve the robustness of reference antigen calibration, to use one set of reagents for each strain regardless of market, to use one assay method regardless of market and to work on a replacement assay for HA potency which is likely to be a long

term achievement. Further initiatives are already taken or are foreseen in this area such as the development of a reagent usage template to be used to estimate the reagent usage and discussions on how industry can contribute to replacement assay initiatives. It was recommended that activities towards the development of a single universal assay method should be coordinated and pushed on a global platform, for example by WHO.

Session Alternative assays (non-SRID) used for IPCs and/or determination of Haemagglutinin content for drug substance and finished product: used in parallel with SRID or stand-alone – pros/cons

Dr. Pascale Gonnet (EVM, Sanofi-Pasteur) discussed the list of desirable characteristics for an improved potency assay for inactivated influenza vaccines as outlined in the document presented to the WHO ECBS in October 2011.

Antibody dependent assays (current SRID, mAb SRID, ELISA, mAb EIA, bead sero-agglutination, SPR) and antibody independent assays (HPLC, SDS-PAGE, MS) were screened for these characteristics (Figure 1 & 2).

As indicated by previous speakers the SRID is a biological measure of potency, has some correlation to clinical efficacy, the ability to detect individual strains in a trivalent vaccine, and stability indicating properties. At least, the SRID provides information about the functionality (i.e. antigenicity) of the HA antigen. Whilst some of these features may be applicable to antibody dependent assays (ELISA), antibody independent assay do not measure biological properties of the HA antigen.

The list of desirable features for an alternative assay is very much influenced by the experience with SRID and it was questioned whether it should be envisaged to consider other features to fine tune the evaluation. In addition, the principles and the advantages/disadvantages of the RP-HPLC, RP-U-HPLC (column packed with sub-2μm particles) and ELISA assays were briefly outlined. It was noted that some of the alternative techniques evaluated still rely on reagents and if one of these techniques is selected as an alternative method, it will be critical to both standardize the reagents and adapt them to the selected method.

Figure 1:

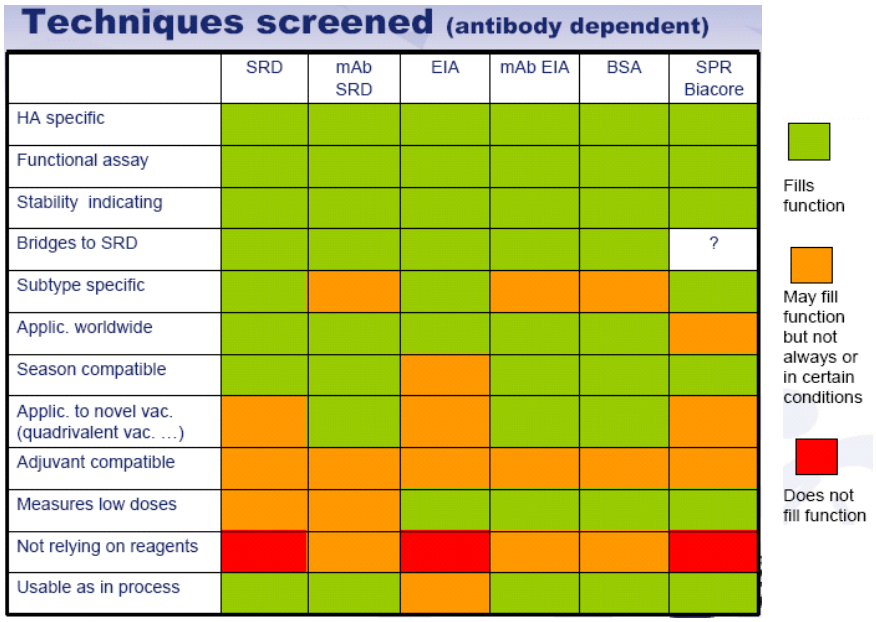
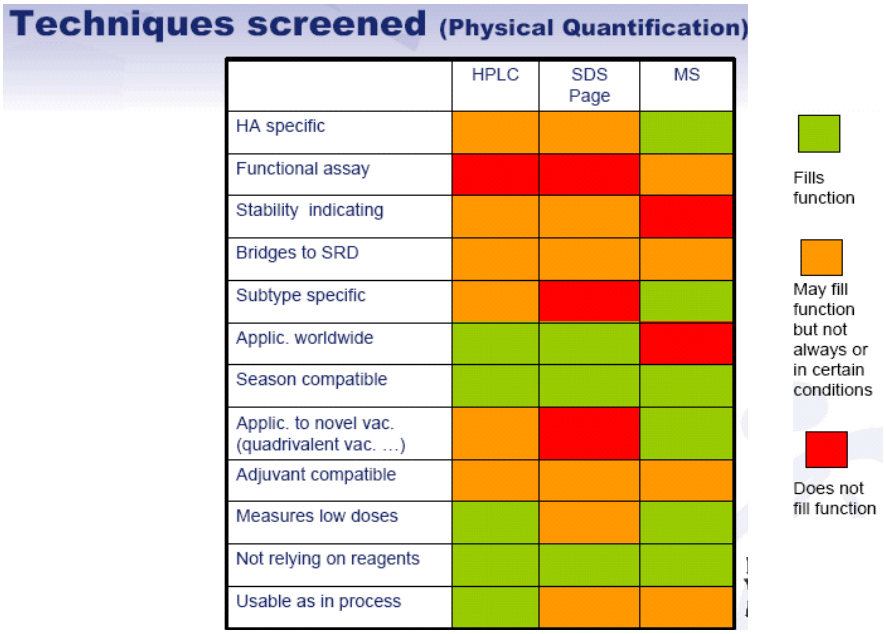


Figure 2:



HPLC

Dr. Dieter Pullirsch (AGES OMCL), Dr. Francois Cano (AFSSaPS OMCL), Dr. Hans Kapteyn (EVM, Abbott) and Dr. Michel Girard (Biologics and Genetic Therapies Directorate, Health Canada) then presented the principles and their experience with the HPLC method for determination of HA content in inactivated influenza vaccines.

The AGES OMCL was involved with the OCABR of a pandemic vaccine (cell culture derived) against the H5N1 influenza virus strain including the batch release of clinical lots. At the time no standard was available for the A/Indonesia/05/2005 strain. Comparison of HPLC and SRID results for the A/Vietnam/1203/2004 strain revealed that HPLC values were higher than SRID results for pandemic vaccines and a correlation factor was used to determine the HA concentration of the Indonesia strain. A comparable immunogenicity was shown in the mouse potency tests. Further experience was gained during the H1N1 pandemic outbreak. Three final lot batches containing the H1N1 strain for clinical studies were released based on the HPLC and batch release based on HPLC would have been possible for additional nine batches before SRID reagents were available. By using a correction factor based on the common ratio observed for several H5N1 strains in pandemic production protocols, only small differences between HPLC and SRID results for HA content for H1N1 vaccines were observed, indicating that the correlation factor was well chosen.

Since 2010 AFSSaPS has started to work on the use of HPLC for HA content determination in influenza vaccines. A feasibility study was conducted, based on publications from Kapteyn *et al*. Different samples were tested using reference antigen preparations obtained from NIBSC and TGA, split virion inactivated vaccine (monovalent pandemic and trivalent seasonal vaccines from different seasons and manufacturers), and trivalent seasonal surface antigen vaccines. Depending on the vaccine strain composition it was difficult to obtain good peak separation whatever the type of vaccine, i.e. surface antigen or split virion vaccine. Initial results showed overall good correlation between SRID and HPLC results but varied between strains. Based on the feasibility study analysis it was concluded that the HPLC was not (yet) applicable to trivalent influenza vaccine but it is expected that technical improvements could be implemented, e.g. use of different columns. However, because the HPCL is a protein based assay and not a biological assay which provides information about conformation, it may not be suitable as a stability indicating test. However, HPLC may be suitable as a batch release test possibly in combination with orthogonal methods. It was discussed whether specific antigen reagents such as a reagent based on the PR8 strain could be used for standardisation and this may indeed be considered. Also, it was noted that for reference preparations qualification general protein assays are being used with relative large test variability which impact both SRID and HPLC testing results. More absolute protein determination methods such as mass spectrometry could be envisaged to solve this problem.

Dr. Girard presented data showing that by using RP-HPLC, the identification of HA strains is possible irrespective of the sample matrix based on the differences in HA1 retention times. Interference of the detergent Triton has previously been reported but this can be overcome by optimization of elution conditions. Good correlation could be shown between HA content as determined by biochemical assays (e.g. Western blot) and RP-HPLC. The RP-HPLC method is able to separate HA1 subunits from different strains based on retention times which make this method applicable directly to influenza bulk preparations and vaccines. The RP-HPLC method developed at HC is not patented.

Overall, good HPLC assay linearity for H1N1 pandemic vaccines was illustrated based on data from two manufacturers. It was noted that considering the precision of the standard calibration by SDS-PAGE and SRID the variances in the HPLC response were not significant. Precision was shown to be acceptable and on average percentage coefficients of variation (CV%) of 1.9% and 6.2% for repeatability and intermediate precision respectively, were observed. The CV% for HPLC accuracy was shown to be <5% for H1N1 vaccines. Consistent ratios between SRID and HPLC results were obtained

for several lots of influenza strains but a systematic deviation between HPLC versus SRID of approximately 20% could be shown between A and B strains. Several potential factors may have affected the HPLC-SRID ratio such as differences in inactivation processes (BPL vs. formaldehyde) between vaccines and the NIBSC standard, the percentage of HA0 which is proteolytically cleaved into HA1 and HA2 (at the activation cleavage site) or a combination of these factors.

Improved versions of the RP-HPLC such as U-HPLC are under development. In this case, the column is packed with sub-2μm particles. With U-HPLC, the identification and quantification of the HA content in trivalent vaccines is possible because of increased peak separation but needs further optimization.

HPLC methods can be successfully applied in mass balance studies to select the high yield virus strain in the early production phase or process validation studies using multi-factorial design of experiments (DOE) virus yield optimization studies when SRID reagents are not yet available. In addition, data were presented that showed that HPLC could also be applied for HA integrity studies. For example, an effect of BPL-inactivation on HA integrity resulted in changes in HPLC chromatogram (double HA1 peak) coinciding with a decrease in SRID response, while no significant changes could be detected when the samples where analysed by SDS PAGE.

In summary, the HPLC assay has high assay sensitivity and a broad assay range, good performance (in terms of precision, robustness and reproducibility), can be standardized world-wide, has limited matrix interference, is not very labour-intensive, is relatively inexpensive and may be useful for process optimization and process validation studies. However, HPLC results are not predictive for biological activity and the method is measuring different aspects of HA when compared to SRID. As such, it cannot be considered as a real potency assay. Additionally, whether any particular analytical method can accurately measure the occurrence of aggregation needs to be considered. In those cases where strains cannot be separated further optimisation may be necessary. Specific antigen reagents such as a reagent based on the PR8 strain may be considered for standardisation. More absolute protein determination methods such as mass spectrometry could be envisaged to limit the relative large test variability due to the calibration process.

It was mentioned that HPLC methods may have patent implications. Should this be the case it may prohibit the incorporation into the Ph. Eur. and would require follow-up in due time.

ELISA and SPR

Dr. Ethan Settembre (EVM, Novartis) presented the concept of ultra-filtration-based conformational screen that could mimic the size 'sieving' of agarose as applied in the SRID. This concept is based on the key SRID functionality to separate and measure the 'good' conformational HA. After 'sieving' the HA antigen, it is then quantified by an ELISA assay. In principle multiple sieving formats are possible that could effectively be applied as an initial physical separation of active and inactive HA preceding the quantification by almost any of the conformationally insensitive assays, yielding an assay specific for active HA.

Three different approaches for an ELISA assay were presented; a lectin based ELISA that detects conformational epitopes, a polyclonal serum based bridging ELISA, and a monoclonal antibody based bridging ELISA. Binding studies with sheep antisera and denatured HA antigen showed that the antisera contain antibodies that recognize improperly folded HA and that SRID conformational specificity does not primarily rely on IgG selectivity.

The lectin based ELISA is a sensitive and highly reproducible assay that uses a universal capture and a specific detection antibody, and can be used in-process even when samples are too low to be distinguished by SRID. The presented lectin ELISA depends on traditional strain specific reagents, however, more broadly reactive monoclonal antibodies could be used.

Feasibility studies have been performed with bridging ELISAs using CBER antiserum and monovalent bulks as a standard. Whilst specificity, acceptable recovery and good correlation with SRID could be demonstrated, interference was observed between some B-strains and precision was limited. The method requires strain specific reagents and a standard with an assigned potency, is not applicable for all strains and may need more extensive validation in case of a strain change when compared to SRID.

The monoclonal antibody based ELISA uses monoclonal antibodies that capture the antigen and peroxidase bound monoclonal antibodies for detection. This ELISA enables detection of trimeric and higher order HA complexes. The method has high sensitivity within a broad analytical range and high accuracy (90-115%). The methods correlate well with SRID when used for release and stability testing.

Dr. Kendra Frederick (Protein Sciences) presented key features of Surface Plasmon Resonance (SPR) (Biacore) as a potential alternative for SRID. SPR detects protein-protein binding through changes in mass due to adsorption to the chip surface. In-house antisera and reference antigen preparations are used. Accuracy and precision have been shown to be in the range of approximately 98-120% and 4-12%, respectively. A drawback frequently mentioned is the high cost due to high turn-over. However, in this case chips were usable for more than 140 cycles. Reproducible results can be obtained when new chip sets are used. There is good agreement between SPR and SRID results when identical reagents are used. When performed in a competitive format, the method is able to detect aggregates. The method needs specific reagents (antibodies). Dr. Frederick also presented data about the company's ELISA which is based on a variable capture strategy (i.e. either antibody capture, antigen adsorbed to well, or fetuin capture that contains terminal sialic acids which are the cellular receptors of HA) and one detection strategy based on detection of bound antigen by a primary antibody and detection by HRP-linked secondary antibody. This method correlates well with SRID (SRID:ELISA ratio: 100-125%), is sensitive (LOQ: 10 – 200 ng/mL) and precise (RSD <6%).

SDS-PAGE

Dr. Jane King (EVM, Novartis) briefly outlined the use and characteristics of the SDS PAGE for use in the qualification of the HA antigen in influenza vaccines. The primary use of this method is the characterization of total protein and measurement of what proportion of protein is HA. Whilst the method has clear advantages (i.e. no strain specific reagents required, estimation of HA and potential of NA content when deglycosylated SDS PAGE is used, simple implementation, less variable than SRID), it does not measure antigenic content of HA, is not stability indicating and not suitable for testing of trivalent vaccines. Therefore it is less suitable for release or stability testing although it may be of value as an in-process control method.

CBER experience on alternative assays

Dr. Maryna Eichelberger (CBER, US FDA) presented an overview of the HHS initiatives to accelerate influenza vaccine manufacturing. One of the projects that is being run within the US Biomedical Advanced Research and Development Authority (BARDA) is the vaccine potency assay development with topics concerning the accelerated antigen calibration and alternative potency assays.

Studies are on-going in order to standardize reference reagents faster using Isotope dilution mass spectrometry (IDMS). Primary standard preparations will be compared using IDMS and conventional methods like PAGE/densitometry.

Further progress is being made in the area of alternative potency assay using Immunoaffinity Capture (IC)-IDMS and ELISA. In the ELISA system, wells are coated with subtype specific mAbs to capture the antigen and polyclonal rabbit anti-HA is used to detect bound antigen. The current reference antigen is

used as standard. A fairly good agreement between SRID and ELISA is obtained and results obtained so far demonstrate that the assay is stability-indicating.

Other research projects comprise Surface Plasmon Resonance and Antibody-independent label-free MS potency assay methods.

In general, the goals are to demonstrate that the method(s) are stability-indicating and that these methods can be applied to seasonal and pandemic vaccines. If an approved reference antigen is not used as the standard, bridging studies will have to be performed to establish correlation with the SRID assay.

Comparison of alternative methods

Dr. Othmar Engelhardt (NIBSC) presented information on methods that are being explored and established at NIBSC which comprise SRID, SPR, ELISA, HPLC and SDS-PAGE. The competition ELISA uses single domain VHH alpaca antibodies that are cross-reactive to HA of several subtypes in phylogenetic group 1 and recognise conformationally sensitive epitopes. Overall, good agreement between ELISA and SRID results are obtained. With SPR it is possible to detect and to quantify conformational correct HA.

He then presented results of studies performed by NIBSC to assess whether various existing or suggested potency assays can discriminate between native HA and conformationally compromised HA. Different vaccine materials (pandemic vaccine final product, seasonal and pandemic strains monovalent bulks) were treated in a way that may damage its antigenicity/immunogenicity (acid conditions, BPL treatment without pH control, freeze/thaw cycles, and heat). Treated and un-treated samples were tested using various methods (SRID, SDS PAGE, HPLC, immunisation of mice). Based on the results it was concluded that SRID correlates with immunogenicity in mice, HPLC and SDS-PAGE do not correlate with immunogenicity in mice, and SRID and the selected physico-chemical methods measure different aspects of HA protein.

Session Potential assays for investigating the Neuraminidase content

Dr. Elisabeth Neumeier (EVM, GlaxoSmithKline Biologicals) introduced the topic by summarising the current requirements as regards NA qualification for influenza vaccines. There is evidence that antibodies to NA contribute to protection against influenza disease. However, current regulations (e.g. Ph. Eur.) do not require NA content nor is the manufacturing process standardised on the basis of NA antigen content. In fact, the proportion of HA and NA is determined by the virus strain and the manufacturing process used and the process can only be standardised on one antigen. NA enzyme activity and kinetics may vary between influenza strain (sub)types and are not well suited to study vaccine antigen stability. A precise correlation between the enzymatic activity and antigenic structure has not been determined. As for the HA content determination, standardized strain specific reagents are needed to determine the amount of NA antigen.

Novartis have been evaluating two versions of an ELISA assay for NA antigen qualification based on the NA enzymatic activity. The first qualitative ELISA is used as an identity assay which confirms the virus subtype. The presence of the NA antigen is assessed against a trivalent standard (whole virus). This assay has been shown to be problematic for low yielding (pandemic) strains and some N2 subtypes. The second quantitative ELISA uses neuraminidase from *Vibrio cholerae* as a standard to provide quantitative analysis. In both cases, no strain specific reference standard is as yet available.

Dr. Sean Li (Biologics and Genetic Therapies Directorate, Health Canada) also made reference to literature suggesting that viral NA is correlated with immune protection in animal and human studies. Conversion rate data in influenza neuraminidases and epitope binding studies indicate that the HCA-2 and HCA-3 epitopes may be the relevant antigenic epitopes for NA quantification. Antibodies generated against these epitopes bound to different subtypes of influenza A and B neuraminidases and demonstrated good specificity against viral neuraminidase. Comparative analyses of pandemic H1N1 vaccines from different manufacturers show significant differences in NA enzymatic activity. Immunoassay data indicate that the NA content between lots produced by one manufacturer is on average relatively constant but the ratio HA:NA may vary between lots.

A quantitative sandwich ELISA is also being developed using a universal antibody against NA that is coated to the ELISA plate. NA-subtype specific antibodies are then used as a secondary antibody. By using this sandwich ELISA, differences in NA content could be determined between vaccines produced by different manufacturers and significant differences in NA:HA ratio are discernable between vaccine lots from different manufacturers. The reasons for variable NA content in the different vaccines could be multi-factorial. However, aggregates and un-processed HA precursor (HA0) were sometimes accompanied with lower levels of NA antigens. As noted above, the unprocessed HA0 in vaccine preparation might also complicate the quantification of HA content by HPLC and SRID. No studies have been performed to relate the NA antigen content to (animal) immunogenicity.

Dr. Maryna Eichelberger (CBER/FDA) indicated the rational for the development of a NA potency assay. Though studies have shown that NA antibodies correlate with protection, no protective titer has yet been established as Neuraminidase Inhibition (NI) titers are not routinely measured. The quantity of NA antigen that is required or its conformation to induce a protective NI titer is unknown. For new vaccines developments, a different strategy may be chosen where the vaccine can have a large proportion of NA and clinical efficacy may be correlated with NI titers. In developing new NA potency assay it is important to take into account the confirmation and content of the NA in the vaccine and to which extent the NI titer correlates with protection and the required dose to achieve this titer. Different methods have been or are being developed to measure the potency (antibody-dependent ELISA) or response to the NA antigen (TBA methods, enzyme-linked lectin assay). Results were presented of NA determination in monovalent H1N1 vaccine preparations using label-free mass spectrometry analyses which indicated good consistency between different lots from one manufacturer. Significant differences in HA:NA ratio of egg-grown influenza virus preparations have been determined between different A and B strains.

Session Moving forward - all participants - discussion

Feedback from the Vaccine Working Party: clinical relevance of alternative assays

Dr. Michael Pfleiderer (Chairman of the CHMP's VWP, PEI) gave feedback from the Vaccine Working Party which was held on November 29th and 30th, 2011, with the aim to demonstrate why it is important to mediate between quality (BWP), non-clinical and clinical issues (VWP, PDCO) of potency assays for influenza vaccines.

Scientific key considerations were presented concerning clinical issues of influenza vaccination, particularly related to vaccine potency determination. The clinical performance, mainly of seasonal influenza vaccines, in different age and risk populations is not very well understood and a better definition of the clinical mode of action of individual influenza vaccines is needed. Furthermore, the current generic concept of "influenza vaccination" should be supplemented by more product specific

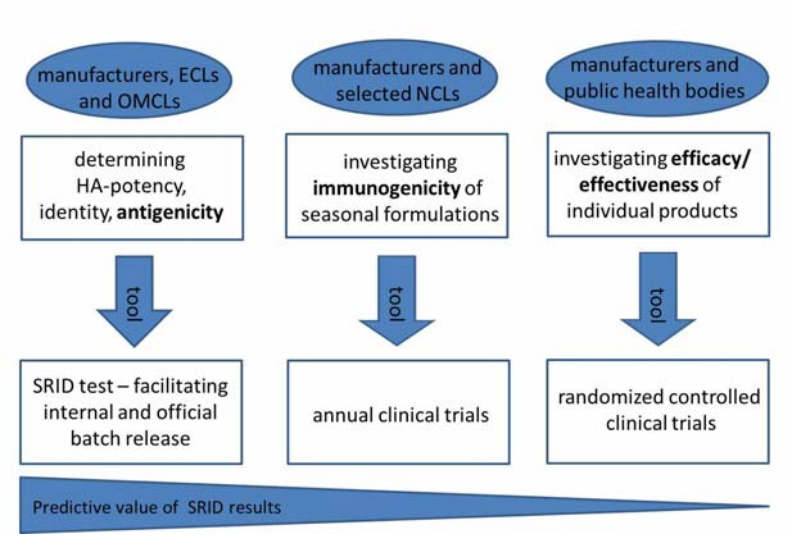
considerations. Predictive immunological markers for effective booster response from varying levels of pre-existing immunity and clinical effectiveness have to be identified. In this regard Dr. Pfleiderer has announced that a VWP/BWP Workshop on serological aspects will be organized in Q1 2012 for more detailed discussion of these highly complex issues.

Large gaps in the understanding of efficacy/effectiveness of influenza vaccines exist for the use in the paediatric population. The relevance of immunological acceptance criteria for inactivated influenza vaccines has been established for adults but it is unproven for infants and children. In contrast, the benefit of Live Attenuated Influenza Vaccines (LAIVs) is very well established for children >2 years of age while no robust clinical data are available justifying the use of inactivated influenza vaccines in this age group. In the paediatric population the optimal biological effect level of antigen/adjuvant ratios have to be explored in order to have maximum benefit and minimal risk of vaccination. This requires a precise assay to determine the potency of influenza vaccines. However, recent experience feeds concerns on the precision of the SRID test. In addition, the robustness of primary immunization has to be verified by booster studies. The efficacy/effectiveness of inactivated (pre-) pandemic influenza vaccines can only be extrapolated from appropriate clinical data generated with appropriate seasonal formulations. Age specific immunological correlates for protection have to be matched with efficacy/effectiveness through extensive serological analyses by relevant assays.

Dr. Pfleiderer stated that for the time being there is a tendency to look at quality issues and non-clinical/clinical issues independently. As regards the review of the guideline on influenza vaccines as a holistic document it is important to achieve a mutual understanding between quality, non-clinical and clinical issues. The SRID assay has been for many years the accepted method to determine the potency of influenza vaccines. Indeed, the SRID assay is a tool to determine HA antigen content which is a release specification of influenza vaccines. But is SRID potency testing a reliable, robust and sustainable method ensuring vaccine safety and efficacy across strains, seasons and products? In order to answer this question, Dr. Pfleiderer provided an overview on positioning SRID potency testing of trivalent inactivated influenza vaccines within the EU regulatory framework of evaluating quality, safety and efficacy of medicinal products (Figure 3). Current understanding, resulting from many years of technical experience as well as from numerous meta-analyses on the efficacy of seasonal influenza vaccines, is suggesting that there is insufficient evidence that potency values determined by SRID testing indeed consistently correlate with efficacy for different target populations.

Therefore, the development of clinically relevant alternative assays, even product specific assays, is strongly encouraged for a better understanding and reliable outcomes of influenza vaccination. Such assays might be run side by side with conventional SRID testing – without a mandatory need to cross calibrate both assays - in order to address both, quality as well as clinical aspects of influenza vaccines.

Figure 3: Overview on positioning SRID potency testing of trivalent inactivated influenza vaccines within the EU regulatory framework of evaluating quality, safety and efficacy of medicinal products



ECL: Essential Control Laboratory (= WHO Essential Regulatory Laboratory), OMCL: Official Medicines Control Laboratory, NCL: National Control Laboratory

A) Alternative potency assays

Based on the presentation of Dr. Pfleiderer it was discussed whether there was a need for product specific assays instead of a universal method like the current SRID method.

Industry representatives indicated that there is a clear need for a reliable test that can be used for vaccine formulation and can be applied as early as possible for release of clinical trial material and release of early commercial product. It is clear that calibration of reagents is a time consuming effort and it is not (always) possible to wait for the outcome before formulation of vaccine (for clinical trials). As an alternative to the SRID, the HPLC method may be used when linked to the clinical outcome.

This raised the question whether there should be a requirement to correlate alternative (potency) assays with the SRID or whether the alternative assay would only have to be correlated with the clinical readout measured by relevant and reliable serological assays. This could speed up the developments and implementation of alternative methods for HA and NA antigen quantification. Whilst standardisation of the alternative assay is considered important this could be done at a later stage.

It was mentioned that the SRID readout does not necessarily provide the right information for each and every target population, i.e. non-primed individuals, and a more precise assay is needed for correct dosing. A participant commented that the use of an alternative method will however not improve the vaccination strategy. If a dosing schedule had to be modified this should be done by using a common/standardised method to determine the HA content. If in this case an alternative method is used with no link to the common/standardised method, it may even be more difficult to interpret the clinical outcome.

Some industry participants indicated that an assay correlated to the established SRID might be easier to accept by the regulators. In case of a completely independent potency assay, full clinical validation would be needed which will take far longer. It was noted that it might not be possible to power the analyses for the alternative assay based on clinical data. It was reminded that the SRID is still relevant

for biological activity and has clinical correlation. Another participant commented that any *in vitro* test will not measure vaccine immunogenicity but, like the SRID, will at most measure antigenicity. Moreover, the SRID is stability-indicating which is considered an important attribute.

Generally, it was agreed that there should be no requirement to strictly correlate the alternative method to the SRID, e.g. by defining a correlation factor. However, there should be a link between the HA antigen content as measured by the alternative method and SRID in order to monitor how both methods compare to each other.

An industry participant indicated that it is important to consider that current vaccines are licensed products and produced in accordance with the approved license dossiers. Any change in requirements, including changes to the analytical methods should be taken step-by-step. Supportive data will have to be generated which will take some time. It was suggested to build a platform which could be taken forward in a "company-independent" way. A WHO representative noted potential implications that will have to be considered from a more global perspective. Solid data are required to decide whether it is needed to follow an individual approach or to follow different approaches from vaccine clusters. Most participants felt that no immediate changes were needed but standardisation (e.g. calibration of SRID reagents) should be optimised and alternative methods could be developed in parallel.

It was acknowledged that with the introduction of product specific methods, i.e. each manufacturer developing its own alternative method, there is a potential for disharmonisation in vaccine standardisation. An EDQM representative mentioned that the change to individual assays would render it difficult to compare individual vaccines. It was also mentioned that vaccines may not be interchangeable which may be particularly important in case of a pandemic situation where Member States may decide to buy vaccines from different companies. It would also be more difficult to compare the clinical results of different vaccines due to content differences.

Another point raised is the fact that the performance (e.g. precision) of the alternative assays is being evaluated against the performance of the SRID which is known to have some variability. Enhanced data sets will be needed to evaluate the performance of new methods. Based on sound science and improved standardisation, different vaccines (i.e. split, purified surface antigen with adjuvant or whole virion) could be developed for different target populations.

The question was then raised how to validate a stand-alone alternative method or a combination approach. What would be desirable and what would be a feasible approach? In general, it was agreed that antibody independent based methods can be useful for in-process testing and possibly as a QC release test for HA content at the stage of the monovalent drug substance. Nevertheless, identity and correct antigenic conformation would still need to be assured at the drug product level by additional methods. A participant mentioned that drug product quality could also be assured by a strictly validated (GMP ruled) process and confirmatory clinical qualification. In this case, a antibody independent based method such HPLC could be sufficient to assure correct HA antigen content. However, the stability is still to be determined by a method measuring the correct antigenic conformation. It was commented that as long as the SRID is considered the "gold standard", companies will be hesitant to standardise their processes on the HPLC. In addition, the characteristics of the influenza virus (sub)types vary and some may be less stable. Therefore, it is not possible to rely on process validation and the company's experience, and a functional assay is needed for drug product release and stability testing. Divergent opinions were presented on the acceptability of the HPLC as a QC release test for the drug product. Some felt though that this could be acceptable provided additional methods are applied to assure correct antigenic conformation. With this respect, reference was made to the presentation on sieving in combination with HPLC.

It was also mentioned that the HPLC method for HA antigen content determination is patented. The EDQM indicated that this may prevent the inclusion of such a method in the Ph. Eur. It was

recommended that EDQM follow up the matter and determine if HPLC could be introduced in the Ph. Eur.

B) Improvements to SRID

The SRID has been for many years the recognised/accepted method to monitor potency (content) of trivalent seasonal influenza vaccines for HA antigen. This assay is capable to discriminate vaccine batches with acceptable antigenicity from batches with impaired antigenicity.

If the SRID was to be maintained, which is likely to be the case for the years to come, it is recommended to produce a sufficiently large quantity of reference material to obviate the need to replace the reagents during the campaign. Moreover, efforts should be made to improve the SRID standardisation, both from a reagents perspective and the method itself. It was suggested that additional bodies, such as additional OMCLs, could be involved in the reagents calibration in addition to the four ERLs. However, it should be recognised that if more laboratories are introduced there will be a potential risk for further delay to the calibration procedure which is unwanted. It was felt that further harmonisation between ERLs is needed most ERLs are using their own methods of reagent calibration. It was recognised that the WHO has already made efforts to harmonise the calibration methods for ERLs. An OMCL representative mentioned that it was not the intention to involve more OMCLs in the laboratory work but that the OMCLs could be involved in the preparation of, for example, an SOP and in discussion on how the calibration could be optimised. Also, communication between the ERLs and companies could be increased at an early stage in the calibration procedure for their feedback and possible scientific input. An industry participant indicated that manufacturers should be involved at an early stage to see whether the reagents work with all products.

Influenza vaccine production is a wordwide effort. At present, different sets of reagent are supplied in different regions which is not optimal. On the other hand, a single set of reagents might pose problems in terms of supply or unexpected issues associated with the use of these reagents.

The EDQM representative mentioned that any suggestion to introduce a common assay for the HA antigen determination (improved SRID or alternative method) into the Ph. Eur. would be welcome. EDQM is willing to run collaborative studies to support this. Industry commented that the same method would have to be agreed by the US and it is unsure whether the FDA would follow the EU approach. The WHO may pick up the wish for the development of an universal method. EDQM responded that the FDA could also be involved in the EDQM collaborative studies, an approach already followed for other biological assays.

Session Wrap-up / Concluding remarks

The chairman indicated that detailed and very useful information about several analytical methods to determine the HA and NA antigen in influenza vaccine was shared. At current, it seems that there is no obvious candidate to replace the SRID which is still considered an acceptable test. However it is recognised that the SRID is dependent on strain specific reagents and the calibration of these reagents is a time consuming effort. Therefore, in the short term, it is recommended to improve the early availability of these reagents and the calibration procedure. Efforts should be made on a global level (e.g. WHO). Meanwhile, alternative methods could be further developed. These alternative methods could be used in parallel and then when more experience is gained, it could be investigated whether

these methods could replace the SRID in due time. To date it seems premature to recommend the dropping of the SRID test.

Useful information has also been provided on how different antibody-dependent and antibody-independent methods for HA determination can be compared in terms of functionality, HA specificity, applicability, stability-indicating, etc. The EMA drafting group might consider this information for the influenza guidance document which is currently under preparation.

The message of the VWP is well appreciated and industry and regulators need to work together to precisely determine the HA content in the vaccine to determine which dosing strategy is best used in various vaccine strategies, i.e. in a seasonal, pre-pandemic, or pandemic situation.

Whilst it is recognised that the significance of NA content in vaccines is still being investigated, manufacturers are encouraged to investigate the NA content of their vaccines and the analytical tools that could be applied to determine NA content in parallel with the their work on the standardisation of the HA antigen.

The chairman concluded that further meetings might be considered to discuss the progress in the field of SRID and alternative potency assays.

Attachments

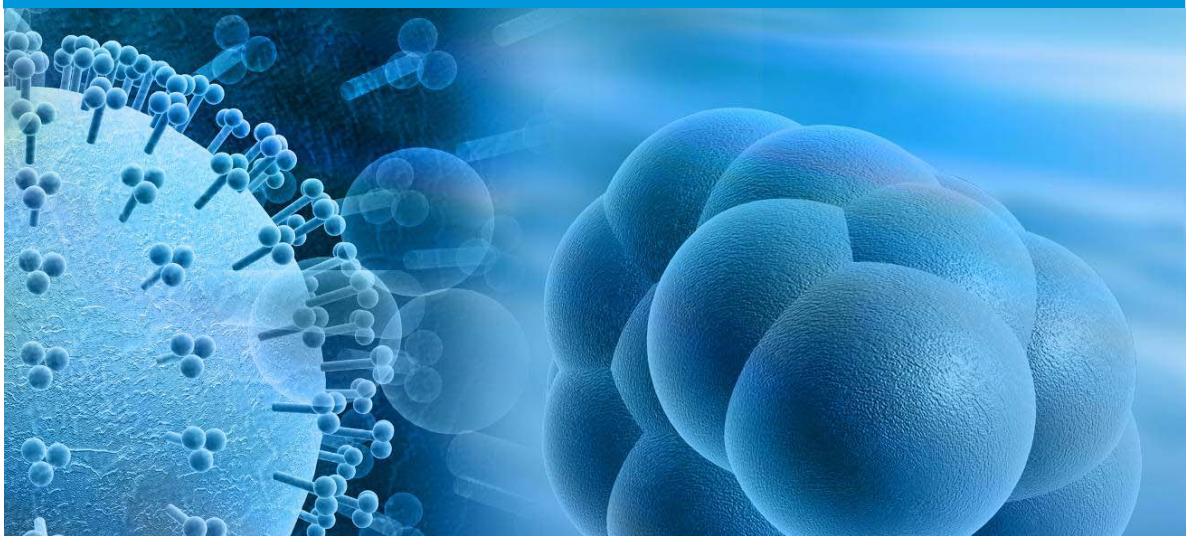
1. Programme overview and workshop agenda

2. List of participants

Joint EMA/EDQM workshop on improved potency assays for inactivated influenza vaccines

Workshop programme

12 December 2011, 8.30-17.00 (UK time)
European Medicines Agency, London, United Kingdom



See websites for contact details

European Directorate for the Quality of Medicines & HealthCare www.edqm.eu
Council of Europe www.coe.int
European Medicines Agency www.ema.europa.eu

Programme overview

Scope

The lessons learnt exercise performed after the 2009 influenza pandemic recognised that the assays used for content of the hemagglutinin (HA) component in the influenza vaccine dose should be reviewed. During the pandemic and as part of the lessons learnt exercise, issues related to the interpretation due to variability/standardisation of the assays used for content determination were raised and particularly at the successive expert meetings held at the European Medicines Agency in July 2009, April 2010 and June 2010.

Quantification of HA by the single radial immunodiffusion (SRID) assay has been for many years the recognised/accepted method to monitor potency (content) of trivalent seasonal influenza vaccines for HA antigen. The current SRID method however requires, for each of the three HA antigens, strain-specific virus reagents which may not be available when the early batches of a vaccine have to be filled and released for either clinical trial confirmatory tests or to speed up the early access to pandemic vaccine doses. During the expert meetings and lessons learnt exercise, it has been raised that alternative assays to measure the content of HA should be investigated and their relative performance validated against the current SRID, before being used on a routine basis. The timing of use of such assays will need to be discussed (e.g. at an early stage of development/qualification of the new strain vaccine until the SRID test reagents become available, and/or for a more general use as alternative assays to SRID). Also techniques for the characterisation of neuraminidase (NA) component and its assays could be considered.

The guidelines on influenza vaccines are being reviewed as part of the lessons learnt exercise. In this context, it is proposed to hold a technical workshop to prepare the work and feedback the revision exercise of the guidelines. Furthermore it will be reviewed whether there are still gaps in the development and validation of alternative assays and how these gaps can be filled.

The development of assays for HA content may impact the batch release and official control laboratory network (OMCL) testing and potential future revision of the European Pharmacopoeia monographs for influenza vaccines.

Chairs and rapporteurs

Jean Hugues Trouvin (workshop co-chair)
Chairman of the CHMP's
Biologicals Working Party / AFSSaPS

Phil Minor (workshop co-chair)
NIBSC

Ton van der Stappen (Rapporteur)
Member of the CHMP's
Biologicals Working Party / MEB

Constanze Göpfert (Rapporteur)
PEI

Programme details

Monday, 12 December 2011 – Room 4A

8.00 Registration and coffee

Please register with reception (ground floor) to receive your security badge.

8.30 Welcome and introduction

Jean Hugues Trouvin (workshop co-chair)
Chairman of the CHMP's Biologicals Working Party / AFSSaPS
Phil Minor (workshop co-chair)
NIBSC

8.45 SRD (current) assay

Reagents, origin, supply, timelines, calibration, antigen issues, antiserum issues
Una Dunleavy *(15 minutes)*
NIBSC

Usage of SRD/comments
Francois Cano *(15 minutes)*
AFSSaPS OMCL
Tony Colegate *(15 minutes)*
EVM (Novartis)

9.30 Alternative assays (non-SRD) used for IPCs and/or determination of Haemagglutinin content for drug substance and finished product: used in parallel with SRD or stand-alone – pros/cons

Characteristics for an improved potency assay & how the different methods compare in light of these characteristics
Pascale Gonnet *(20 minutes)*
EVM (Sanofi-Pasteur)

HPLC *(60 minutes)*
Dieter Pullirsch *(10 minutes)*
AGES OMCL
Francois Cano *(10 minutes)*
AFSSaPS OMCL
Hans Kapteyn *(30 minutes)*
EVM (Abbott)
Michel Girard *(note: presentation to take place later in the morning) (10 minutes)*
Biologics and Genetic Therapies Directorate, Health Canada

10.50 – 11.10 Coffee break

Coffee will be available outside the meeting room.

Other methods such as ELISA, SDS-PAGE, MS, SPR *(50 minutes)*

– ELISA
Ethan Settembre
EVM (Novartis)
Kendra Frederick
Protein Sciences

– SDS-PAGE
Jane King
EVM (Novartis)

CBER experience on alternative assays
Maryna Eichelberger *(15 minutes)*
Center for Biologics Evaluation & Research, US Food and Drug Administration

Comparison of alternative methods
Othmar Engelhardt *(15 minutes)*
NIBSC

12.30 Potential assays for investigating the Neuraminidase content

Elisabeth Neumeier *(10 minutes)*
EVM (GlaxoSmithKline Biologicals)

Towards qualitative and quantitative analyses of Neuraminidase in influenza vaccines - an update *(10 minutes)*
Sean Li
Biologics and Genetic Therapies Directorate, Health Canada

Assessment of Neuraminidase in vaccines – update from CBER *(10 minutes)*
Maryna Eichelberger
Center for Biologics Evaluation & Research, US Food and Drug Administration

13.00 – 14.00 Lunch

14.00 Moving forward - all participants - discussion

Feedback from the Vaccine Working Party: clinical relevance of alternative assays *(10 minutes)*
Michael Pfleiderer
Chairman of the CHMP's Vaccine Working Party / PEI

A) Haemagglutinin

i. Value/limitation of currently published studies on alternatives

ii. Combination approach: - company specific assay for IPC + internationally agreed immunogen assay, or - internationally agreed protein assay + internationally agreed immunogen assay?

iii. Validation of an improved assay strategy
– How to validate a stand-alone assay?

– How to validate a combination approach?

– Clinical qualification

– Is a measure of HA protein content sufficient? If not, what else would be required (validation)?

iv. Develop Ph.Eur. mononographs?

v. Industry experience and comments

vi. Consideration of SRD

– How to improve variability (including the calibration of reagents) /timelines, can they be?

vii. Future assays for novel vaccines

viii. Recommendations for future studies/collaborative studies

B) Neuraminidase

16.00 – 16.30 Coffee break

Coffee will be available outside the meeting room.

16.30 Wrap-up / Concluding remarks

List of participants

Svein Rune Andersen	Norwegian Medicines Agency, Norway
Kalman Bartha	National Centre for Epidemiology, Hungary
Joep Bergers	RIVM, the Netherlands
Koenraad Brusselmans	Scientific Institute of Public Health, Belgium
Karl-Heinz Buccheit	EDQM
Laura Campitelli	Istituto Superiore di Sanità, Italy
Francois Cano	AFSSAPS OMCL, France
Michel Chevalier	Sanofi-Pasteur
Tony Colegate	Novartis
Christoph Conrad	WHO
Mirjam Cramer	Swissmedic, Switzerland
Giuseppe Del Giudice	Novartis
Françoise Denamur	GSK
Una Dunleavy	NIBSC, UK
Maryna Eichelberger	CBER, U.S. FDA
Othmar Engelhardt	NIBSC, UK
Hans Engelmann	GSK
Kendra Frederick	Protein Sciences
Anne-Marie Georges	Novartis
Ed Geuns	Abbott
Constanze Goepfert	PEI OMCL, Germany
Pascale Gonnet	Sanofi-Pasteur
Yoshikazu Hayashi	MHLW/PMDA Liaison at EMA
Claire Huson	MedImmune
Hans Kapteyn	Abbott
Christoph Kefeder	AGES PharmMed OMCL, Austria
Jane King	Novartis
Otfried Kistner	Baxter
Holger Kost	Novartis
Dominique Labbé	GSK
Daniela Mattei	Istituto Superiore di Sanità, Italy

Christian McLarnon-Riches	MedImmune
Clifton McPherson	Protein Sciences
Catherine Milne	EDQM
Philip Minor	NIBSC, UK
Marc Mosimann	Crucell
Elisabeth Neumeier	GSK
Brijesh Patel	MHRA, UK
Michael Pfleiderer	PEI, Germany
Augustin Portela-Moreira	Spanish Agency of Medicines and Medical Devices, Spain
Dieter Pullirsch	AGES PharmMed OMCL, Austria
James Robertson	NIBSC, UK
Sylke Roth-Eichhorn	GSK
Ethan Settembre	Novartis
Daniel Stalder	Crucell
Ton van der Stappen	Medicines Evaluation Board, the Netherlands
Tricia Stewart	CSL
Pieter Torfs	GSK
Jean Hugues Trouvin	AFSSAPS, France
Fokke Venema	Abbott
Lodewijk Venhuizen	Crucell

Via teleconference:

Navneet Ahluwalia	BGTD, Health Canada
Jessica Amell	BGTD, Health Canada
Tania Dalla Pozza	TGA, Australia
Nathalie Fortin	BGTD, Health Canada
Michel Girard	BGTD, Health Canada
Sean Li	BGTD, Health Canada
Helen MacDonald-Piquard	BGTD, Health Canada
Aline Rinfret	BGTD, Health Canada

EMA Secretariat:

Gwenaël Cirefice	EMA

Nick Gate	EMA
Elisa Pedone	EMA
Peter Richardson	EMA
Robin Ruepp	EMA
Ragini Shivji	EMA