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**CELL CULTURE INACTIVATED INFLUENZA VACCINES  
ANNEX TO NOTE FOR GUIDANCE ON HARMONISATION OF  
REQUIREMENTS FOR INFLUENZA VACCINES (CPMP/BWP/214/96)**

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## 1. INTRODUCTION

Current guidelines and requirements for inactivated influenza vaccines<sup>1,2</sup> have been developed for vaccines that are produced in eggs. Previously unlicensed mammalian cell substrates are now being proposed for inactivated influenza vaccine manufacture. As in the case of the current egg-derived influenza vaccines, three types may be produced: whole virion, split and subunit vaccines. Production and control of the proposed cell culture derived vaccine may be compared to other licensed inactivated vaccines produced in cell culture such as inactivated poliomyelitis, hepatitis A and rabies. Furthermore, a number of available guidelines and requirements are relevant to the cell substrates used for vaccine production, such as:

- Ph. Eur. Monographs on extraneous agents and cell substrates<sup>3,4</sup>
- ICH, WHO and CPMP guidance on cell substrates<sup>5,6,7</sup>
- Veterinary guidelines on extraneous agents (which take into account species-specificity)<sup>8</sup>
- CPMP guideline on TSEs<sup>9</sup>

Where applicable, such guidelines and requirements should be adhered to.

However, certain influenza-specific aspects, e.g. the yearly strain change and consequent time constraints, affect the applicability of existing guidance and in particular the feasibility of testing virus seeds for extraneous agents (see below). Thus, there is a need for additional specific guidance for this type of vaccine. The Ph. Eur. Monographs for Influenza Vaccines will also require adaptation to the special features of cell culture influenza vaccines.

## 2. SCOPE

The CPMP Note for Guidance on Influenza Vaccines covers Quality as well as Safety and Efficacy issues that are mainly related to the yearly change of vaccine strains in inactivated influenza vaccines produced on eggs. This Annex addresses Quality, Safety and Efficacy issues, which are pertinent to cell culture inactivated influenza vaccines including, where relevant, the issue of yearly strain changes.

## 3. QUALITY ISSUES

### 3.1 Isolation of influenza virus

Viruses to be used in vaccine manufacture may be isolated in one of the following substrates:

- embryonated hens' eggs
- cells derived from embryonated hens' eggs
- mammalian cells

In accordance with the PhEur monographs for egg-derived inactivated influenza vaccines, the origin and passage history of virus strains shall be approved by the competent authority.

### 3.2 Testing for extraneous agents of the cell substrate

- In accordance with current requirements on cell substrates<sup>4,5,6</sup> and in addition to the general testing for extraneous agents, the cell substrate used for production of virus seeds and monovalent bulks should be tested for relevant extraneous agents specific to

the species of origin of the cells<sup>8</sup> and those which may have been introduced from biological reagents used during establishment of the cell banks.

- The susceptibility of the cell substrate to various human pathogens should be investigated and this information should be used in considering a list of potential human pathogens to be included in testing for extraneous agents in working seed viruses. Pathogens to be considered could include respiratory syncytial virus, adenovirus, parainfluenza virus, coronavirus, rhinovirus, enterovirus, EBV, HSV, CMV and mycoplasmas.

### **3.3 Testing for extraneous agents in the seed viruses**

- The seed virus should be tested for extraneous agents at the level of the master seed or the working seed according to current requirements concerning testing of virus seed lots for contamination by extraneous agents<sup>3</sup>. The testing for specific viruses should take into account the susceptibility of the production cell substrate (see 3.2) and of the cells used to isolate the strain. It is recognised that whenever a strain changes, there may be time constraints which make this problematic and results of such testing may not be completely available before further processing. Notwithstanding the obligation to complete the testing according to the Ph. Eur. monograph on extraneous agents<sup>3</sup>, manufacturers of cell culture influenza vaccines are encouraged to develop assays for potential contaminating human pathogens, e.g. multiplex PCR, which could be applied effectively within the time constraints of annual vaccine manufacturing.
- Susceptibility of the cell substrate to potential contaminants is a key factor and should be investigated. If the cell substrate proves to be susceptible to a contaminating agent detected in the seed, the seed is normally not acceptable.
- If the cell substrate is not susceptible to a detected contaminating agent, steps should be in place to ensure that the contaminating agent in the working seed is removed and/or inactivated by the production process (see 3.4). In addition, appropriate and specific downstream testing at the level of each inactivated monovalent bulk should ensure that the removal and/or inactivation processes are effective and that any contaminant which may subsequently be identified in the seed virus is absent from the vaccine.

### **3.4 Production issues related to extraneous agents**

The influenza vaccine inactivation step, along with any other steps considered to contribute to virus inactivation/removal should be evaluated for the inactivation/removal of a wide range of potential contaminants of the vaccine seeds in accordance with existing guidance on virus validation<sup>2,6,10,11</sup>. This information should be a part of the core dossier for Marketing Authorisation (MA).

### **3.5 Issues related to standardisation**

- The current standardisation of influenza vaccine potency is based on the immunochemical SRD assay. There is preliminary evidence that it is possible to use current ‘egg-derived’ standards (e.g. WHO/NIBSC antigens and antisera) for cell culture vaccines manufactured using a virus seed derived and passaged in eggs<sup>12</sup>. However, in future, problems may arise if a vaccine strain is isolated, passaged and produced on a mammalian cell substrate, as this is a process that can select for viruses that are antigenically distinct. Studies to evaluate the need for “cell-derived” standards by using them in parallel with “egg-derived” standards (antigens and antisera) are recommended.

- The results of the mandatory yearly clinical trials should be used to confirm the applied standardisation.

### 3.6 Other issues

- Tests for the effectiveness of vaccine virus inactivation may be performed using the cell substrate or any other cell system provided that there is adequate validation of this test for sensitivity.
- Endotoxin levels are expected to be far less than for egg-derived vaccines and the limits should be based on the results of batch analysis.
- Depending on the type of cell substrate used and on the type of influenza vaccine (whole virion, split virion or subunit), there may be a need to develop appropriate and adequately validated tests for residual host cell protein and residual DNA.
- The core dossier should contain information on the equivalence of cell culture and egg-produced vaccines including antigenic characterisation, cross-reactivity of specific antisera and animal protection studies.
- Consideration should be given to the possibility that vaccine strains chosen at the annual meeting of the BWP Working Group on Influenza (i.e. egg-derived viruses) may show inadequate propagation on mammalian cells.

## 4. SAFETY AND EFFICACY ISSUES

For Marketing Authorisation of a cell culture-derived inactivated influenza vaccine, the principles of the CPMP Note for Guidance on the Clinical Evaluation of New Vaccines<sup>13</sup> and of the CPMP Note for Guidance on Harmonisation of Requirements for Influenza Vaccines are applicable. The extent to which the recommendations of the New Vaccines guideline<sup>13</sup> have to be fulfilled depends upon the extent to which the cell-derived vaccine is shown, in pre-clinical studies, to be similar to an equivalent egg-derived vaccine. In vitro studies concerning similarity should be addressed in the Part II Quality documentation, following the recommendation of section 3.6 (4<sup>th</sup> bullet point) of this Annex. Standardisation studies (see section 3.5) may also contribute to the demonstration of similarity. Evidence of similarity should be based on studies of more than one strain of a given subtype.

### 4.1 Efficacy

From available *in vitro* comparisons between viruses/vaccines derived from mammalian cell culture and those from eggs<sup>12,14,15,16,17</sup>, it is anticipated that the criteria defined in the Influenza Vaccines guideline<sup>1</sup> for egg-derived vaccines (i.e., seroprotection, seroconversion and sufficient increase in GMT), based upon *in vivo* tests<sup>18,19</sup> are also appropriate for cell-derived vaccines. Consequently, for MA, these criteria may be used if the cell-derived vaccine has been demonstrated to be similar to an egg-derived vaccine and in that case it is not anticipated that clinical protection studies will be required.

For MA, the primary clinical endpoint should be defined more stringently than the criterion for the yearly update of influenza vaccines. Thus, all three criteria, seroprotection, seroconversion and sufficient increase in GMT, should be met, with seroprotection and GMT being the most important. These correlates of protection observed in a clinical trial of a cell-derived vaccine should be non-inferior to that obtained with an equivalent egg-derived vaccine and the number of subjects within a trial should be adequate to ensure that the data is statistically valid (see New Vaccines guideline<sup>13</sup>). Stratification should ensure that a cross-

section of the population is studied, taking into consideration that sufficient numbers are needed in certain groups who may respond to the vaccine differently.

For all comparative trials predefined in- and exclusion criteria are required. These include stratification in age categories reflecting relevant intervals for efficacy evaluation, influenza vaccination history, co-morbidity and co-medication. These stratification methods should be described in the protocols.

If the cell-derived vaccine is not considered similar, it should be handled as a new vaccine for which the New Vaccines guideline<sup>13</sup> applies and the correlates for protection as defined in the Influenza Vaccines guideline<sup>1</sup> are only applicable when validated.

Following MA, yearly updates should be evaluated according to the Influenza Vaccines guideline<sup>1</sup>, provided that the criteria for the immunological correlates for protection (see above) apply as such for the cell-derived influenza vaccine.

## **4.2 Safety**

Irrespective of whether the New Vaccines<sup>13</sup> or the Influenza Vaccines<sup>1</sup> guideline is considered for immunogenicity (i.e. accepting the criteria and ultimately uncontrolled trials in the yearly updates), a control arm is necessary for safety evaluation for MA, including a sufficient number of individuals. This means that, in all instances, comparative data are necessary. Although an open label design for immunogenicity evaluation is defensible, for safety evaluation at least a single (investigator) blinded and preferentially a double blind design should be recommended to detect differences with frequencies in the range of 1 – 10%.

Follow-up for safety and collection of safety data should be in accordance with recommendations in the New Vaccines guideline<sup>13</sup>. This implies a follow up of at least 6 months and a full assessment of the safety profile over this period in comparative trials and using the causality classifications defined in the New Vaccines Guideline. This safety evaluation includes also all parameters requested in the Influenza Vaccines guideline<sup>1</sup>, as requested for the subsequent annual updates.

## **4.3 Studies on specific target populations depending on the claims made in the Summary of Product Characteristics (e. g. paediatric use, allergies to egg proteins)**

Claims for specific target populations (e.g. patients with allergies to egg proteins), either for efficacy or for safety reasons need to be substantiated.

Specific efficacy claims in defined high risk populations (e.g. the elderly, cardiovascular disease, respiratory disease, metabolic disease, renal disease, nursing home residents, immunocompromised patients) need to be justified with immunogenicity data. Such data may contribute to further characterisation of the immunogenicity profile of the vaccine despite the fact that no standard criteria for protection have been defined for these groups. Considering the relatively small numbers of subjects in studies for annual updates and the variability observed in these studies, data deriving from them is insufficient to address safety issues in specific risk groups.

Children are a special target group. When a cell-derived vaccine is to be used in children, trials for MA should include a sufficient number of children in the claimed age categories and in the specific risk groups, for both efficacy and safety reasons. The criteria for efficacy assessment in children are not described in the Influenza Vaccines guideline<sup>1</sup>. Specific criteria used for seroprotection, seroconversion and significant increase in GMT thus need to be substantiated in the protocols.

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- <sup>1</sup> Committee for Proprietary Medicinal Products (CPMP). Note for Guidance on Harmonisation of Requirements for Influenza Vaccines (CPMP/BWP/214/96).
- <sup>2</sup> European Pharmacopoeia Commission 1997. Influenza Vaccine (Split Virion, Inactivated), Monograph 0158. Influenza Vaccine (Surface Antigen, Inactivated), Monograph 0869. Influenza Vaccine (Whole Virion, Inactivated), Monograph 0159.
- <sup>3</sup> European Pharmacopoeia Commission 1999. Tests for Extraneous Agents in Viral Vaccines for Human Use (General method N° 2.6.16.). European Pharmacopoeia 3rd edition Supplement: 50-51.
- <sup>4</sup> European Pharmacopoeia Commission 1998. Cell Substrates for the Production of Vaccines for Human Use (PA/PH/Exp. 15/T (94) 25, 3R, ANP). *Pharmeuropa* **10**, 629-632.
- <sup>5</sup> CPMP Note for Guidance on Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates used for the Production of Biotechnological/Biological Products (CPMP/ICH/294/95).
- <sup>6</sup> WHO Expert Committee on Biological Standardisation 1998. Requirements for the Use of Animal Cells as *in vitro* Substrates for the Production of Biologicals (Requirements for Biological Substances N° 50). *WHO Technical Report Series* **878**, 19-56.
- <sup>7</sup> CPMP Position Statement on the use of tumorigenic cells of human origin for the production of biological and biotechnological medicinal products (CPMP/BWP/1143/00)
- <sup>8</sup> European Commission, DG III, 1999. Table of Extraneous Agents to be Tested for in Relation to the General and Species Specific Guidelines on Production and Control of Mammalian Veterinary Vaccines. The Rules Governing Medicinal Products in the European Union, Volume 7B: Guidelines/Veterinary Medicinal Products/Immunologicals, quality, p. 99.
- <sup>9</sup> CPMP Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products (CPMP/BWP/1230/98).
- <sup>10</sup> CPMP Note for Guidance on Virus Validation Studies. The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses (CPMP/BWP/268/95).
- <sup>11</sup> CPMP Note for Guidance on Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnological Products Derived from Cell Lines of Human or Animal Origin (CPMP/ICH/295/95).
- <sup>12</sup> Wood J. M., Dunleavy U., Newman R. W., Riley A. M., Robertson J. S. and Minor P. D. 1999. The influence of the host cell on standardisation of influenza vaccine potency. In "Inactivated Influenza Vaccines Prepared in Cell Culture". Eds. Brown F., Robertson J. S., Schild G. C. and Wood J. M. *Dev. Biol. Stand.* **98**, Karger, Basel, pp183 – 188.
- <sup>13</sup> CPMP Note for Guidance on the Clinical Evaluation of New Vaccines (CPMP/EWP/463/97).
- <sup>14</sup> Brands R., Visser J., Medema J., Palache A.M. and van Scharrenburg G.J.M. 1999. Influvac<sup>TC</sup>: a safe Madin Darby Canine Kidney (MDCK) cell culture-based influenza vaccine. In "Inactivated Influenza Vaccines Prepared in Cell Culture". Eds. Brown F., Robertson J. S., Schild G. C. and Wood J. M. *Dev. Biol. Stand.* **98**, Karger, Basel, pp93-100.
- <sup>15</sup> Kistner O., Barrett P.N., Mundt W., Reiter M., Schober-Bendixen S., Eder G. and Dorner F. 1999. Development of a Vero cell-derived influenza whole virus vaccine. In "Inactivated Influenza Vaccines Prepared in Cell Culture". Eds. Brown F., Robertson J. S., Schild G. C. and Wood J. M. *Dev. Biol. Stand.* **98**, Karger, Basel, pp101-110.
- <sup>16</sup> Palache A.M., Scheepers H.S.J., de Regt V., van Ewijk P., Baljet M., Brands R. and van Scharrenburg G.J.M. 1999. Safety, reactogenicity and immunogenicity of Madin Darby canine kidney cell-derived inactivated influenza subunit vaccine. A meta-analysis of clinical studies. In "Inactivated Influenza Vaccines Prepared in Cell Culture". Eds. Brown F., Robertson J. S., Schild G. C. and Wood J. M. *Dev. Biol. Stand.* **98**, Karger, Basel, pp115-125.
- <sup>17</sup> Percheson P.B., Trépanier P., Hassell T., Nestruck A.C., Dugré R. and Mabrouk T. 1999. A phase I, randomised controlled clinical trial to study the reactogenicity and immunogenicity of a new split influenza vaccine derived from a non-tumorigenic cell line. In "Inactivated Influenza Vaccines Prepared in Cell Culture". Eds. Brown F., Robertson J. S., Schild G. C. and Wood J. M. *Dev. Biol. Stand.* **98**, Karger, Basel, pp127-132.
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- <sup>19</sup> Al-Khayatt R., Jennings R. and Potter C.W. (1984). Interpretation of responses and protective levels of antibody against influenza A viruses using single radial haemolysis. *J. Hyg. (Camb)* **92**, 301-312.