13. OECD principles on Good Laboratory Practice (revised 1997). ENV/MC/CHEM (98) 17.

14. Requirements for the Use of Animal Cells as *in vitro* Substrates for the production of Biologicals. Annex 1. WHO TRS 878, 1998.

15. Guidelines for assuring quality of DNA vaccines. Annex 3. WHO TRS 878, 1998.

16. Guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology. Annex 3. WHO TRS 814, 1991.

17. Guidelines for the production and quality control of synthetic peptide vaccines. Annex 1. WHO TRS 889, 1999.

18. Guidance for Industry: Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product. FR; Volume: 64, No. 2, 1999, 518-519: (CBER, FDA).

19. Good manufacturing practices for pharmaceutical products. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-second Report, Geneva. World Health Organization, 1992, Annex 1 (WHO Technical Report Series, no. 823).

20. Good Manufacturing Practice: supplementary guidelines for the manufacture of the investigational pharmaceutical products for clinical trials in humans. In: WHO expert committee on Specifications for Pharmaceutical Preparations: Thirty-Four Report, 1996. Annex 7. WHO Technical Report. Series 863.

21. CPMP/SWP/465/95 - Note for guidance on preclinical pharmacological and toxicological testing of vaccines. June 1998.

22. Goldenthal KL, Cavagnaro JA, Alving CR, and Vogel FR. Safety Evaluation of Vaccine Adjuvants: National Cooperative vaccine Development Meeting Working Group. AIDS Research and Human Retroviruses. 1993, 9, supplement 1: S47-S51.

23. Guidance for Industry for the Submission of Chemistry, Manufacturing, and Controls Information for Synthetic Peptide Substances. Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER). November 1994.

24. CPMP/SWP/2145/00 - Note for guidance on non-clinical local tolerance testing of medicinal products.

25. WHO Manual of laboratory methods for testing vaccines used in the WHO Expanded Programme on Immunization. Annex 1.1997. WHO/VSQ/97.04

26. Wraith D.C., Goldman M., Lambert P.H. Vaccination and autoimmune disease: what is the evidence? The Lancet. June 2003.

27. World Health Organization. Laboratory Biosafety Manual, 2nd edition (revised) Geneva 2003.

28. European Commission Regulations No. 541/95, 542/95, 1146/98 and 1069/98.

29. Note for Guidance for Reproductive Toxicology: Detection of Toxicity to Reproduction for Medicinal Products. CPMP/ICH/386/95.

30. Note for Guidance on genotoxicity: a standard battery for genotoxicity testing of pharmaceuticals. CPMP/ICH/174/95.

31. Note for Guidance on safety pharmacology studies for human pharmaceuticals. CPMP/ICH/539/00.

32. Note for guidance on repeated dose toxicity, CPMP/SWP/1042/99.

33. ICH M3(M) Nonclinical safety studies for the conduct of human clinical trials for pharmaceuticals. November 2000.

34. Guidance for Industry: Nonclinical Studies for Development of Pharmaceutical Excipients. Draft. September 2002.

35. Note for guidance on excipients, antioxidants and antimicrobial preservatives in the dossier for application for marketing authorisation of a medicinal product. CPMP/QWP/419/03.

36. Verdier F., Patriarca C., Descotes J. (1997). Autoantibodies in conventional toxicity testing. Toxicol. 119, 51-58.

37. Note for guidance on comparability of medicinal products containing biotechnology-derived proteins as drug substance. CPMP/BWP/3207/00.

38. (2000) Recommendations for *Haemophilus influenzae* Type b conjugate Vaccines, *WHO Technical Report Series* 897.

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WHO GUIDELINES ON NONCLINICAL EVALUATION OF VACCINES

This document provides guidance to National Regulatory Authorities (NRAs) and vaccine manufacturers on the nonclinical evaluation of vaccines by outlining the international regulatory expectations in this area. It should be read in conjunction with the guidelines on clinical evaluation of vaccines: regulatory expectations, in order to complete the understanding of the whole process of vaccine evaluation (4). Vaccines present a diverse class of biological products and their nonclinical testing programs will depend on product specific features and clinical indication. Therefore, the following text is written in the form of guidelines instead of recommendations. Guidelines allow greater flexibility than Recommendations with respect to specific issues related to particular vaccines.

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Introduction

Recent progress in biotechnology and basic immunology is leading to the development of a broad range of novel vaccines raising exciting possibilities for the prevention of infectious diseases (1,2). In addition, improvements to already licensed vaccines are also being considered leading to new products as well as the introduction of new adjuvants. However, the complexity and novelty of these products presents scientific and regulatory challenges, as criteria for their safety, potency and quality assessments may not always exist. Because of product diversity and since new approaches, technologies and methodologies develop over time, it is emphasized that judgement based on the best science available should always form the basis for deciding on the type and extent of nonclinical evaluation for these products.

Nonclinical evaluation plays an essential part in the overall development of vaccine candidates. There is at present limited guidance regarding nonclinical evaluation programs for these products. In this guidance document, the general principles of nonclinical evaluation of vaccines are discussed, with particular attention being given to the regulatory expectations for new and novel vaccines.

Preclinical testing is a prerequisite to move a candidate vaccine from the laboratory to the clinic and includes all aspects of testing, product characterization, proof of concept/ immunogenicity studies and safety testing in animals conducted prior to introducing the product into humans. Nonclinical evaluation, within the context of this document, means all *in vivo* and *in vitro* testing performed before and during clinical development of vaccines. For example, nonclinical evaluation may be

necessary when changes in the manufacturing process or product formulations are made or to further evaluate potential safety concerns that may have arisen from phase 1 and 2 trials or have been described in the literature with similar products.

1. General Remarks

Nonclinical studies are aimed at defining the *in vitro* and *in vivo* characteristics of candidate vaccines including safety and immunogenicity evaluations. Non clinical studies in animals are valuable tools to identify possible risks to the vaccinees and help to plan protocols for subsequent clinical studies in human subjects. However, in all cases, when safety testing in animals is performed, there should be a clear rationale for doing so and the study should be performed in compliance with the National and International laws for the protection of laboratory animals (25), biosafety requirements (27) and with Good Laboratory Practice (GLP) (13). However, there may be situations where full compliance with GLP is not possible. If the study or part of the study were not conducted in compliance with GLP, areas of noncompliance should be defined and a statement of the reason for the noncompliance should be justified and reported.

Potential safety concerns for a vaccine product include those due to inherent toxicities of the product, toxicities of impurities and contaminants, and toxicities due to interactions of the vaccine components present in the vaccine formulation. In addition, the immune response induced by the vaccine may lead to undesired toxic side effects.

Despite efforts to maximize the predictive value of nonclinical toxicity studies there are always limitations to reducing uncertainties of risk. The limitations of

animal testing in reflecting clinical safety and efficacy in humans should be recognized since pathogenesis and immune responses are frequently species-specific. Moreover, potential safety concerns identified during animal testing may not necessarily indicate a problem in humans. However, any signal observed in nonclinical toxicity studies should be carefully addressed in human clinical trials and may require additional nonclinical testing. Likewise, a lack of detectable toxicity does not necessarily mean a vaccine will be safe in humans. Potential safety concerns related to specific types of vaccine candidates are considered in section 6.

The development and subsequent validation of *in vitro* tests as alternatives to nonclinical evaluation of vaccine candidates in animals is encouraged as it may lead to the improvement of nonclinical testing as well as to the reduction of animal usage.

The need and extent of nonclinical testing will depend on the product under consideration. For example, for a product for which there is no prior nonclinical and clinical experience, nonclinical testing would be expected to be more extensive than for those vaccines previously licensed and used in humans. In some cases, it may not be necessary to perform preclinical safety studies prior to the initiation of Phase 1 clinical trials. For example, in the case of transfer of technology, where the access to database of the originally developed vaccine exists, data from nonclinical bridging studies (e.g., physico-chemical characterization and abreviated *in vivo* studies) may be acceptable to further develop the product.

Early communications between the vaccine manufacturer and the respective National Regulatory Authority to agree on the requirement and type of nonclinical testing are recommended.

1.1 Scope

For the purpose of this document, vaccines are considered a heterogeneous class of medicinal products containing immunogenic substances capable of inducing specific, active and protective host immunity against infectious disease.

While the majority of vaccines are being developed for pre- and post- exposure prophylaxis, in some cases, they may be indicated for therapeutic use against infectious diseases, e.g., HIV, HPV etc. Both prophylactic and therapeutic vaccines for infectious disease indications are considered in this document.

Vaccines for human use include one or more of the following: micro-organisms inactivated by chemical and/or physical means that retain appropriate immunogenic properties; living micro-organisms that have been selected for their attenuation whilst retaining immunogenic properties; antigens extracted from micro-organisms, secreted by them or produced by recombinant DNA technology; chimeric micro-organisms; antigens produced *in vivo* in the vaccinated host following administration of a live vector or nucleic acid or antigens produced by chemical synthesis *in vitro*. The antigens may be in their native state, truncated or modified following introduction of mutations, detoxified by chemical or physical means and/ or aggregated, polymerised or conjugated to a carrier to increase immunogenicity. Antigens may be presented

plain or in conjunction to an adjuvant, or in combination with other antigens, additives and other excipients.

Therapeutic vaccines for non infectious diseases (e.g., certain cancer vaccines) and monoclonal antibodies used as immunogens (e.g., anti-idiotipic antibodies) are NOT considered here.

2. Characterization of candidate vaccines

2.1 Vaccine production

The biological nature of the starting materials, the manufacturing process and the test methods needed to characterize batches of the product are important elements for the design and the interpretation of nonclinical testing of vaccines. Many vaccines are produced using prokaryotic or eukaryotic micro-organisms and subtle changes in these organisms may radically affect the vaccine product. Therefore, the establishment of a seed lot system is essential for vaccine production. Moreover, the quality, safety and potency of these products are usually sensitive to changes in manufacturing conditions. The quality and safety of vaccine preparations cannot be assured solely by end product testing, but depends on the strict control of the manufacturing process following principles of Good Manufacturing Practice (12). This includes demonstration of the purity and quality of the starting material (raw materials and seeds), in process control testing, testing for process additives and process intermediates and the development and establishment of lot release tests. Moreover, as the relationship between physical and chemical characteristics, and the

immunogenicity and efficacy of these products is frequently not completely understood, biological characterization through the use of biological assays should always complement the physical and chemical product characterization. The development of appropriate laboratory methods to characterize a vaccine formulation with respect to its components, as well as its safety and potency, is a prerequisite to the clinical use of any new or novel bacterial, viral, or parasite vaccines.

Consistency of production is of paramount importance, and the demonstration that the product does not differ from vaccine lots that have been shown to be safe and adequately immunogenic and protective in clinical studies is a crucial component of vaccine evaluation, licensing and batch release. For that reason, manufacturers should make all effort to characterize these clinical lots and try to keep some of these lots for future reference if possible.

Where no appropriate animal model exists for testing potency or where direct serological or immunological correlates of clinical protection are not available, the challenge is to ensure that each production batch has the same protective efficacy as those batches shown to be protective in clinical trials. In such cases, emphasis is increasingly being placed on assuring the consistency of production using modern physical, chemical and immunological methods that enable characterization of some products to a degree of precision not previously possible.

The vaccine lots used in preclinical studies should be adequately representative of the formulation intended for the clinical investigation and, ideally, preclinical testing should be done on the same lot as proposed for the clinical trials. If this is not

feasible, then these lots should be comparable with respect to physico-chemical data, stability, formulation etc.

At a minimum, candidate vaccines for clinical trials should be prepared under conditions of Good Manufacturing Practice for clinical trial material (20). However full GMP will be required at the later stages of clinical development (12,19).

Any change in the manufacturing process during vaccine development should be considered carefully to evaluate the impact on the quality, safety and efficacy of the vaccine and the possible need for additional nonclinical and clinical investigations.

Subsequent change in production methods or scale-up following product licensure will necessitate further product characterisation to demonstrate comparability with the original lot(s) used to demonstrate safety and efficacy of the product. The extent of comparability testing needed depends on the nature of the changes implemented (37). These should be documented and the National Regulatory Authority consulted regarding all changes. Regulatory authorities should clearly define and implement into regulations what changes require only a notification and what changes require a formal approval before implementation (28).

The procedures used in the characterization and control of existing licensed traditional vaccines are likely not applicable to newer products developed using state of the art technology to protect against the same infection. For example, specific guidelines have been developed for the production and control of acellular pertussis vaccines that differ from those applied to whole cell pertussis vaccine (5). Likewise,

the tests applied to the characterization and control of traditional inactivated cholera vaccine for parenteral use are not necessarily applicable to the new inactivated whole cell cholera vaccine intended for oral administration, and an appropriate potency test for the oral vaccine needs to be developed.

2.2 Potency

Potency tests measure biological activity of a vaccine but do not necessarily reflect the mechanism of protection in humans. Potency measurement is often used to verify the consistency of the manufacturing process. The initial concept of potency testing for vaccines was to quantify the biological activity of the vaccine in comparison with reference preparation of known bioactivity, where the antigenic component(s) were not well defined.

Classical challenge studies in animals immunized with a vaccine under consideration have been developed into routine potency assays (e.g., for Diphtheria and Tetanus toxoids). In the case of the whole cell pertussis potency assay, which consists of intracerebral challenge of immunized and nonimmunized animals, a correlation was established with clinical protection in humans (5). Where no suitable animal challenge model exists, potency is often based on measurement of immune responses, usually serological (e.g., influenza and Hepatitis B vaccines).

More recently, recombinant DNA methodology and modern physico-chemical techniques have resulted in the manufacture of highly purified products that can be better characterized than classic biologicals. However, the ability to measure the

"relevant" biological activity for such products may still be lacking. For these products, physico-chemical characterization, such as amount of antigen, size of the antigen, protein content or other physico-chemical parameters can be used as a measure of consistency, but not necessarily of the potency of a vaccine.

For live attenuated vaccines, the approach to potency measurement is generally different. The potency of live viral vaccines is usually based on titration of the minimum infective dose in cell culture or chicken embryos, which in fact, may be considered as a surrogate marker of potency, but not potency itself. A similar approach is taken to the potency measurement of live attenuated bacterial vaccines, BCG, and typhoid vaccine (live Ty21A oral), where the number of live organisms present is the measure of potency.

For vectored vaccines that express inserts encoding heterologous vaccine antigens, it is not sufficient to determine the "biological activity" of the entire construct by measuring Colony Forming Units (CFU) or infectious titre. In these cases, considerations should be given to other alternative methods such as the quantitation of the expression of the insert, or the evaluation of the effective dose (ED_{50}) of the vectored vaccine.

2.3 Stability

The stability evaluation of vaccines is complex, as they are very susceptible to inactivation by environmental factors. Potency as defined in the glossary, should be measured as a part of the stability testing, except in those cases where potency testing

based on biological activity is not available. Physical and chemical product characterization should be included in the stability evaluation. For a product entering human clinical trials, sufficient data should be generated to support the stability of the product for the duration of the preclinical and clinical trial. In certain cases, accelerated stability data may be used to support preliminary data generated at the normal storage temperature. Stability data to support licensure should be carried out under the proposed storage conditions and should be based on long-term, real time stability studies. Finally, the stability of standards and reference materials also need to be considered in order to ensure that procedures used to measure relevant parameters are reliably standardized.

2.4 International and National guidelines

The World Health Organization (WHO), through considerable international consultation, develops Recommendations and Guidelines on the production and control of vaccines and other biologicals of significance (6), and these form the basis for assuring the acceptability of products globally. These documents specify the need for appropriate starting materials, including seed lot system and cell banks; strict adherence to established protocols; tests for purity, potency, and safety at specific steps during production; and the keeping of proper records. Guidelines allow greater flexibility than Recommendations with respect to specific issues related to particular vaccines.

WHO also provides guidance on the establishments in which vaccines are manufactured. Recommendations can be found in the WHO document on good

manufacturing practice for biologicals (12). Particular attention should be given to developing documented standard operating procedures for both production processes and testing procedures. These should be introduced as early as possible during the development of a vaccine and be well established by the time Phase III clinical studies are undertaken and an application for marketing authorization is filed. The basic principles for the production and control of vaccines can be found in WHO Technical Report Series (TRS) (14,3,15,16,17). Specific WHO guidelines and recommendations for particular vaccines are also available (6) and should be consulted where appropriate.

WHO Recommendations and Guidelines are intended to be scientific and advisory in nature and to provide guidance for national regulatory authorities and for vaccine manufacturers. These documents may be adopted by national health authorities as definitive national regulations or used as the basis of such regulations. They are also used as the basis for deciding the acceptability of vaccines for purchase by United Nations agencies such as the United Nations Childrens Fund (UNICEF) for use in global immunization programmes. Regulatory requirements for vaccines and other biologicals are also developed by other bodies, such as the European Agency for the Evaluation of Medicinal Products (EMEA) and the Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA) (18) and these documents can be found on the appropriate web sites (www.emea.eu.int and www.fda.gov/cber). In addition, pharmacopoeial requirements, such as those of the European Pharmacopoeia, are also established for vaccines and are available at the following web site www.pheur.org.

For newly developed products, specific WHO, national, or pharmacopoeial requirements may not be available and a national regulatory authority will need to agree on specifications with the manufacturer on a case-by-case basis during the evaluation of products for clinical trials and for licensing. For some of these novel products general Guidance from WHO for production and control can be found in relevant documents, such as those describing DNA and peptide vaccines (15,17), as well as Recommendations on animal cell substrates used for production (14).

In addition, information on how to assure the quality of biologicals in general and on procedures for approving manufacture and for setting up a national control laboratory, can be found in appropriate WHO guidelines (9,10). For vaccines for global marketing, the development of which also involves much international collaboration, it will be essential to ensure consistency of a regulatory approach for novel products such as HIV preventive vaccines (11).

2.5 Batch release and Independent Laboratory evaluation

The potential variability of biological production methods has led to the establishment of national and international requirements to define procedures for assuring the quality of vaccines and for assessing consistency both amongst manufacturers and over long time periods. Licensed vaccines are subject to independent batch release by a National Regulatory Authority/ National Control Laboratory, before release onto the market. Independent evaluation entails at least an evaluation of manufacturer's batch release data (protocol review) but in many

instances it also includes independent laboratory testing in addition to that carried out by the manufacturer.

Batch or lot release tests are those tests chosen during full product characterization to demonstrate the purity, safety and potency of the product. Lot release testing provides one measure of assurance that a lot can be manufactured consistently. Validation and establishment of lot release tests and specifications is a process that continues throughout product development and should be finalized prior to licensure.

In some countries, samples of vaccine for clinical trials are required by the National Regulatory Authority, as a part of clinical trial approval. Vaccine developers are encouraged to consult the appropriate regulatory agency early during the development of a vaccine.

2.6 Standards and Reference Materials

Standards and reference materials play a vital part in the licensing and quality control process, their role ranging from use in specific antigen recognition tests to assays of vaccine toxicity, immunogenicity, and potency. The standardization of the methods used to evaluate vaccines, as well as to evaluate immune responses to vaccine antigens, is also vital so that results may be compared directly between laboratories both within and between countries and between clinical trials.

WHO International Biological Standards and Reference Reagents form the primary standards globally. In addition, individual national regulatory authorities and manufacturers establish their own national or working standards for establishing the quality of each batch, where appropriate, calibrated against the International Standard. Where appropriate the WHO International Standard is referenced. Of concern is that multiple standard preparations, may result in "drifting" from the International Standard. Therefore regional working standards are being produced on large scale in an attempt to further harmonize vaccine quality. For example, the European Department for the Quality of Medicines of the Council of Europe, has been active in establishing working standards for vaccines that are calibrated against the WHO international standards, where appropriate. The complete listing of WHO International Standards and Reference Reagents can be found on the WHO web site www.who.int/ biologicals.

3. Immunogenicity and other pharmacodynamic studies

A pharmacodynamic study for a vaccine product essentially means evaluation of the immunogenicity. However, pharmacodynamic study may also extend to actual drug pharmacology of an adjuvant.

Immunization studies in animal models should be conducted since they may provide valuable "proof of concept" information to support a clinical development plan. In addition, immunogenicity data derived from appropriate animal models are useful in establishing the immunological characteristics of the product and may be of help to select the doses, schedules and routes of administration to be evaluated in

clinical trials. Nonclinical immunogenicity studies should assess the relevant immune response, e.g., humoral and/or cell mediated immune response, induced in the vaccinated animals. Depending on the immune response induced, such studies may include an evaluation of seroconversion rates, geometric mean antibody titers, or cell-mediated immunity in vaccinated animals. Nonclinical studies should, where possible, be designed to assess relevant immune responses, including functional immune response (e.g., neutralizing antibodies, opsonophagocytic activity, etc.) leading to protection. These studies may also be designed to address interference between antigens and/or live viruses. If a vaccine consists of more than one defined antigen, the response to each antigen should be evaluated (e.g. acellular pertussis vaccine consisting of 3-5 protein products). Where appropriate, challenge/protection studies with the corresponding infectious agent may be conducted to confirm the relevance of the animal models. Of primary concern in interpreting the data obtained from such studies should be how closely the animal model resembles the human disease and human immune response. It should be recognized that frequently, animal models may not predict immunogenicity and efficacy in humans.

4. Toxicity assessment

4.1. Basic toxicity assessment

The non-clinical safety assessment of vaccines needs to be viewed in the context of the evolving field of vaccine development. Thus, judgement based on the best science available should always form the basis for any decisions regarding the need for nonclinical safety studies, type of study(ies) as well as study designs. Similarly,

scientific judgement should be applied to the interpretation of pre-clinical data, whereby the risk/benefit ratio, animal model, dosing etc. should be considered. For example, hypersensitivity reactions in an animal model may not necessarily prevent proceeding to clinical trials, but may indicate the necessity for careful monitoring of a particular clinical parameter.

- Section 4.1 provides a general framework for designing a pre-clinical toxicity study for a vaccine. The parameters set out in this section are considered a minimum safety assessment prior to the initiation of clinical trials in humans, in situations where preclinical safety studies are deemed necessary. As the design of any toxicity study is product specific and indications based, modifications to the framework outlined below may be necessary depending on product features, availability of animal models, methodologies, etc.

- Section 4.2 provides additional considerations for performing special toxicity assessments that may be required on a case-by-case basis.

4.1.1 Study design

The preclinical toxicity study should be adequate to identify and characterize potential toxic effects of a vaccine in order to conclude that it is reasonably safe to proceed to clinical investigation. Parameters to be considered in designing animal toxicology studies are the relevant animal species/strain, dosing schedule and method of vaccine administration, as well as timing of evaluation of endpoints (e.g., sampling for clinical chemistry, antibody evaluation, necropsy, etc.). The route of