1. Scope

Animal immunoglobulin or immunoserum for human use is prepared from serum or plasma of immunised animals. Purified products consist mainly of immunoglobulin G. Immunosera are at least partially purified products and could thus contain serum components other than immunoglobulins. These medicinal products contain a mixture of different antibodies but are enriched in specific antibodies against a particular target antigen.

The clinical targets of these products are diverse. The preparations in use include anti-lymphocyte/T-cell immunoglobulins/sera, anti-toxins against microbial and other toxins (e.g. *C. botulinum*, digitalis), anti-sera against bacterial and viral agents and anti-sera against the venoms of snakes, scorpions and spiders. In general, the development of immunosera dates back to the beginning of the 20th century when they were the only available treatments for certain life-threatening conditions. In fact, this is still the case for most of these products.

In general, animal immunoglobulins and immunosera are used infrequently and in very few patients. However, the anti-lymphocyte immunoglobulins/sera product group is still important and routinely used in the prophylaxis and treatment of acute rejection episodes in organ transplantations, for the treatment of GvHD in bone marrow transplantations and in the therapy of aplastic anaemia. New developments include immunosera produced from the yolk of immunised hens, for example for the treatment of diarrhoea caused by parasites in AIDS patients. Animal immunoglobulins/immunosera are administered intramuscularly, subcutaneously or intravenously. Some products are diluted in large volumes of physiological solutions before infusion.

The first products consisted of crude sera, which were replaced by purified immunosera as required by the relevant Ph. Eur. monograph. These early products were purified by precipitation, often consisted of complete antibodies and may contain serum components other than immunglobulins. The manufacturing processes of more recently developed products include more effective purification steps. Products are available with active substances consisting of purified $F(ab')_2$ or Fab immunoglobulin fragments produced by pepsin or papain digestion of complete immunoglobulin molecules.

In this document, the requirements for animal immunoglobulins/immunosera for therapeutic use in humans are outlined. Animal immunoglobulins/immunosera to be used for diagnostic purposes *in vitro* are not the concern of this note for guidance. Those products intended for use in the purification of other products, e.g. by immunoaffinity columns should be shown to be pure and free from adventitious agents by the methods described.

Important considerations for the clinical use of animal immunoglobulins/immunosera include the risk of sensitisation of the recipient, the need of preparations with sufficient purity, the viral and TSE safety aspects, adverse effects caused by additives, pyrogens, cell or complement-active aggregates or immune complexes and variability in the specific potency. Therefore, there is a need to use improved manufacturing processes to reduce the amount of heterologous protein, to remove aggregates, to ensure viral safety and to develop appropriate control methods.

The quality of animal immunoglobulins/immunosera should be considered on a case-by-case basis taking into account the individual character of each product, the clinical indication and the availability of alternative products.

Reference is made to the general Ph.Eur monograph Immunosera ad Usum Humanum, 0084, and to the monographs available for a number of specific immunosera (Immunoserum botulinum, 0085; Immunoserum diphthericum, 0086; Immunoserum gangraenicum, Cl. novyi, 0087; Cl. perfringens, 0088, Cl. septicum, 0089; Immunoserum gangraenicum, mixtum, 0090; Immunoserum contra venena viperarum europaearum, 0145, Anti-T-lymphocyte

immunoglobulin for human use, animal, 1928).

2. Species used for the Production of Animal Immunoglobulins for Human Use

Animal immunoglobulins/immunosera are obtained from sera of different species. Currently, these sera are collected from rabbit, horse, goat and sheep. Other species, like hens, could additionally be used. In general, it is desirable to have alternative products available from sera of different species for use in patients in the event of intolerance against heterologous protein.

Specific and general requirements for the animals used in the manufacturing process are set out under 3.1. General reference is made to the relevant Commission Directives. For those species where a TSE risk is known, the requirements specified in the EU Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy via Human and Veterinary Medicinal Products (CPMP/BWP/410/01, rev. 1) should be considered.

3. Characterisation of the Animal Immunoglobulin/Immunoserum during Development

The active ingredient of any new immunoglobulin/immunoserum should be characterised by chemical and biological methods. Particular attention should be paid to use a wide range of analytical techniques for exploring different physico-chemical properties of the immunoglobulin. A clear difference should be made between the analytical tests performed during development in order to fully characterise the immunoglobulin, and tests performed routinely on each batch of the finished product.

It should be demonstrated that the product has a characteristic pattern of antigen-binding. Desired or undesired secondary processes known to be induced after the binding with the target antigen should be investigated. It should be shown that the product consists of a defined immunoglobulin G concentration. The content of other immunoglobulin classes should be investigated

The product should not contain antibodies that cross-react with human tissues to a degree that would impair clinical safety. In the event that erythrocytes were used for absorption, the level of content of haemoglobin should be demonstrated to be low. The protein content, the composition of protein, the degree of aggregation and molecular fragmentation of the immunoglobulin should be determined. When human blood cells have been used for absorption, the content of haemagglutinins and haemolysins should be demonstrated to be low.

The immunoreactivity of the immunoglobulin should be assessed. The specific activity of the purified immunoglobulin should be determined.

4. Points to Consider in Manufacture

Most of the techniques used to manufacture anti-venoms or anti-toxin immunosera are based on the data published on tetanus and diphtheria antitoxins, i.e. ammonium sulfate precipitation, peptic digestion, thermocoagulation and aluminium gel absorption. Other products like anti-lymphocyte immunoglobulins/immunosera are produced by combinations of chromatographic and precipitation steps. Because of the large variety of methods used for manufacturing, the quality of the products varies widely.

The main manufacturing steps consist of the preparation of the immunisation antigen, the immunisation of animals, collection of serum, absorption of undesired antibodies, purification including steps for removal or inactivation of viruses, formulation and filling. The absorption of unwanted antibodies may involve human tissue or cells.

Several of the requirements relating to establishments in which biological products are manufactured apply to the manufacture of animal immunoglobulins/immunosera (e.g. WHO,

Technical Report Series 822, 1992: Annex 1 Good Manufacturing Practices for Biological Products; EU Guide for Good Manufacturing Practice for Medicinal Products and Annex I to the EU Guide to GMP: Manufacture of sterile medicinal products). Specific information can be found in WHO Technical Report Series, 413, 1969: Annex 2: Requirements for Immunosera of Animal Origin.

4.1 Animals used in the Manufacturing Process

The marketing authorisation holder of the immunoglobulin/immunoserum has the responsibility for ensuring that the starting material comes from documented and recorded sources, and should perform regular audits of the farms supplying animals. The animals used should be a species approved by the competent authority, healthy and exclusively reserved for production of immunoserum. The supplier of animals should be subject to routine legal supervision by the competent veterinary authority.

The donor animals should be held in a closed breeding and production colony, whenever possible. The strain, origin and number of the animals should be specified. Transport and introduction of the animals into production should follow specified procedures, including definition of quarantine measures. If different requirements apply to breeding and production animals this should be specified in the Marketing Authorisation Dossier. For large animals, the differentiation between breeding and production animals may not be applicable. Source, identity and control of animals taken to complete the herd should be recorded. The feed should originate from a controlled source and no animal proteins should be added.

If the animals are treated with antibiotics there should be a suitable withdrawal period before collection of blood or plasma. The animals must not be treated with Penicillin antibiotics. If a live vaccine is administered to the animals, a suitable waiting period is imposed between the vaccination and collection of plasma for Immunoserum/immunoglobulin production.

A regular health monitoring system should be in place which ensures that the animals are subject to continuous and systematic veterinary and laboratory monitoring for freedom from specified infectious agents. This should include constant monitoring of the animal colonies by the veterinarian, routine pathological examination of randomly selected animals, serological analysis for a range of viruses, bacteria and parasites and the examination of the health status of all animals by the responsible veterinarian, or a person under the responsibility of this veterinarian, prior to bleeding.

The Annex to this guideline provides examples of viruses which the company should consider when establishing a system of health control of the animals used as plasma/serum donors for their specific product. The number of animals which should be tested for and the frequency of testing depend on various factors and should be specified for each product depending on the epidemiology of the agent, the size of the herds and the incidence of infections. Testing for viruses should be performed in laboratories with experience in routine virus testing.

The results of the health monitoring of the animal colonies should be well documented and newly emerging serious veterinary diseases should immediately be reported to the competent authorities.

4.2 Starting Materials

Biological materials used in the production

Any reagents of biological origin used in the production of the immunoglobulin/immunoserum should be monitored for microbial contaminants such as mycoplasma, fungi and bacteria. Special consideration should be given to possible viral contaminants and tests for relevant viruses should be performed. Bovine sera used as supplement, e.g. in culture media used for culture of the cell line providing the immunisation

antigen should be checked and found negative for potential virus contaminants (at least bovine viral diarrhoea, infectious bovine rhino-tracheitis and para-influenza 3 virus). Preferentially, inactivated bovine serum should be used[1]. In addition, bovine sera and other bovine-derived biologicals used as supplements during the manufacturing procedure should comply with the requirements in the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy via Human and Veterinary Medicinal Products

Antigens for immunisation

A number of different antigens is used, e.g.

- human antigens like thymocytes or permanent lymphocyte cell lines to produce anti-lymphocyte-cell sera,
- venoms from snakes, scorpions and spiders to produce anti-venoms
- toxins to produce anti-toxins
- viral and bacterial antigens

The antigens should be appropriately characterised. Information on the source and method of preparation should be provided. If appropriate, identification and sanitary status, age of the animal from which the antigen originates should be known. If the antigen is derived from a human donor, information concerning the health of the donor should be provided. Antigens derived from human tissues should be shown to be free of infectious agents. When a cell line is used, this cell line should be characterised according to the relevant requirements, e.g. CPMP/ICH/294/95 Note for Guidance "Derivation and characterisation of cell substrates used for production of biotechnological/biological products" and should be shown to be free of adventitious agents according to the CPMP/ICH/295/95 Note for Guidance.

Material used for absorption of undesired antibodies

There are products whose manufacture includes steps for absorption of cross-reacting or unwanted anti-human antibodies. For this purpose, material from human tissues and/or blood is mainly used. The human materials should be shown to be free of infectious agents. The donors of the human material for absorption should comply with the requirements for donors of blood and plasma according to the Ph. Eur. monograph „Human plasma for fractionation". The origin, time of collection and testing should be specified. Any deviation from these requirements should be justified. It is preferred to subject these materials to viral inactivation.

4.3 Production Process

Immunisation of the animals

The animals are immunised with antigens according to a defined scheme with booster injections at regular intervals. The use of adjuvant agents is permitted. The serum collection and the immunoglobulin/immunoserum production should be performed in separate rooms. The animals from which the serum is collected may be anaesthetised. They should be thoroughly examined, particularly for evidence of infections. If an animal shows any pathological lesions, relevant to the use of the serum in the preparation, it should not be used, nor any remaining animals of the group concerned, unless it is evident that their use will not impair the safety of the product.

[1] If adopted by the CPM, the Note for guidance on the use of bovine serum in the manufacture of human biological medicinal products (CPMP/BWP/1793/01) should be considered.

Blood or plasma collection

Collection of blood or plasma from animals should be made by venepuncture or intracardiac puncture. The area surrounding the point at which insertion is made into the vein should be cleaned and disinfected. The blood should be collected in such a manner as to maintain sterility of the product. If the blood/plasma is held for any period before further processing, it should be treated and stored in such a way as to exclude microbial contamination. Further storage before processing should be validated to ensure that the quality of the product is not influenced.

Pool-Testing

The freedom of contaminating viruses should be supported by testing either the serum pool or in the event that the manufacturing procedure contains a step of absorption, the first defined manufacturing step after absorption. The earliest step at which the serum obtained from all animals is assembled should be defined as serum pool. The pool should be tested for the absence of specific and adventitious viruses using appropriate *in vitro* and, if appropriate, *in vivo* tests. The program to be established to test for absence of specific viruses depends on the individual manufacturing process. Thus, when human blood is used for absorption of unwanted antibodies and/or for immunisation the absence of human viruses, at least HCV, HIV 1 /2 and HBV, should be demonstrated.

In the event of viral contamination detection in the pooled serum, evidence should be presented that this viral contamination has been eliminated or inactivated during the manufacturing process.

Purification

The batch of a product intended for further processing should be clearly defined. Methods used to purify the product and their in-process controls including their specification limits should be described in detail, justified and validated. It is important to ensure that purification procedures do not impair relevant immunobiological features of the immunoglobulin/ immunoserum.

The flow-charts and descriptions of the manufacturing procedure should be detailed. Any optional variations of the manufacturing procedure should be validated. Criteria for reprocessing of any intermediate or of the final bulk should be carefully defined, and the procedure of reprocessing should be validated and justified.

The parallel purification of several intermediate serum pools is possible. The maximum number of these intermediate pools and their volume should be defined.

All possible steps should be taken to prevent aggregation. The residues deriving from the purification procedure should be tested for. It is important that the techniques used to demonstrate purity be assessed using as wide a range of methods as possible, including physico-chemical and immunological techniques. These should include tests for protein contaminants of the host and - if relevant – of human origin, as well as tests on materials derived from the purification process. The level of contamination with host proteins considered as acceptable should be justified and criteria of acceptance or rejection of a production batch should be given. Assays for endotoxin levels should be carried out.

The effectiveness of the manufacturing process to inactivate or remove potential viral contaminants is important for product safety. Unless otherwise justified, effective step(s) which inactivates or eliminates potential viral contaminants should be incorporated. Examples are solvent-detergent treatment, pasteurisation or appropriate filtration methods. Any inactivation process should not compromise the biological activity of the product.

Procedures, which make use of chromatography, should be accompanied by appropriate measures to ensure that column substances or any additional potential contaminants arising from their use do not compromise the quality and safety of the final product. Data on the characterisation of column material or material used for the precipitation of the protein including data on the purification, cleaning, storage and repeated use of these materials should be provided.

The composition and source of any cell-culture media, buffers, other products and substances used for production should be recorded.

Residues remaining from the purification process should be tested for and relevant specifications should be defined. The stability of intermediates should be demonstrated.

Validation of the purification procedure

The capacity of the purification procedure to remove unwanted host derived proteins, additives used as part of the purification, viruses and other impurities should be investigated thoroughly. The reproducibility of the purification process with respect to its ability to remove specific contaminants should be demonstrated.

Specific studies should be performed to investigate the capacity of the purification process to inactivate or remove viruses. The principles of the Note for Guidance CPMP/268/95 "Virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses" have to be applied. If human material are used for immunisation and absorption, human viruses should be considered in addition to species-specific viruses in selecting appropriate viruses for spiking studies. The validation of the purification process should also include justification of the working conditions such as column loading capacity, column regeneration and sanitisation and length of use of the columns as well as the use of any other substances such as precipitation substances.

Antimicrobial agents

Although antimicrobial agents are allowed to be added according to the Ph. Eur. monograph „Immunosera", they should not be included in the manufacture unless their use is justified by quality and/or safety considerations. They must never be used as a substitute for any aspect of GMP. In particular, this should be taken into account for products to be administered intravenously and in large doses.

In selecting a preservative system the applicant should consider its effectiveness against potential microbial contaminants, possible interaction with the formulation or container and possible effects on testing in biological systems.

If replacement of preservatives is considered on the basis of side effects or for other reasons, a risk/benefit evaluation should be made, taking into consideration that such a change implies a new formulation with the need for additional studies for sterility, potency, stability and their clinical implications on a case by case basis.

5. The Final Bulk Product

The quality of all components of the final preparation forming the final bulk product should comply with the specifications of the relevant monographs of the Ph. Eur., when available. The amount of active substance should be adjusted according to the protein concentration or activity. The bulk product should be shown to be free from bacterial, fungal and other microbial contamination.

6. The Finished Product/Release Testing

Quality control tests should be carried out routinely on each batch of finished product according to the GMP guidelines. The aim of the release testing of a given batch is to show that this batch is consistent with and equivalent to the successive batches produced and to batches that have been shown to be safe and efficacious in clinical trials in man. The tests should be performed as laid down in the Marketing Authorisation. Among all tests included in the specifications for release testing, most have to be carried out on the product in its final container

Identity

A selection of tests used to characterise the immunoglobulin should be used to confirm product identity for each batch. The methods employed should include tests for biological activity as well as physicochemical and immunological methods. Using antisera specific to plasma proteins of each species of domestic animal commonly used in the preparation of materials of biological origin in the country concerned, it should be demonstrated that the product is shown to contain only proteins originating from the species used for the immunoglobulin production. The typical protein composition should be specified and tested for.

Purity

The degree of purity will depend on several factors; these include the method of its production and purification and the degree of consistency of the production process. The purity of each batch should be established and be within specified limits. The product should be shown to be free from microbial contamination. Pyrogenicity should be tested for. Particular attention should be given to assessing the degree of aggregation or molecular fragmentation of the immunoglobulin. The protein content of the product must be as low as possible relating to its specific activity. The content of characteristic protein impurities or stabilisers e. g. albumin should be specified.

Potency

The biological activity of the animal immunoglobulin/immunoserum should be established by a biological assay. The test for potency should give information on the functional property of the immunoglobulin. Currently, most of the tests are based on protective or therapeutic effects of the animal immunoglobulin/immunoserum determined in animals. For example, the dose necessary to protect 50% of a group of mice challenged with a specified, normally lethal, dose of venom or toxin may be determined. It is highly desirable to avoid the use of animals by substituting *in vitro* methods. Thus, the potential use of *in vitro* methods should be investigated. However, correlation to the protective or therapeutic effect should be established.

Other parameters which should be tested for include sterility, pH and content of antimicrobial preservatives.

Stability

Stability studies should be performed to provide data to support the requested storage period for either drug substance (bulk material) or drug product (final container product). The data should be based on real-time and real-condition investigations. Depending on the product, it may be feasible to obtain data on the stability of the product during transport and storage at higher temperatures. If loss of activity during storage is revealed by stability studies, a shelf life specification should be established.

Specifications and reference material

The studies described in Section 4 will contribute to a definitive specification list for the product, when justified by the information obtained from the examination of successive batches and results of batch analyses as indicated in Section 6.

When an international reference preparation is not available, an in-house reference preparation should be produced. This should originate from a suitable batch of the product which has been clinically evaluated and fully characterised in terms of chemical composition, purity, potency and biological activity. Criteria for establishing the reference preparation and criteria for re-testing and prolongation of the shelf-life should be stated.

7. Consistency of the Manufacturing Process

Evidence should be provided on the consistency of production on at least three consecutive full-scale production batches. This should include information on the final bulk, finished product as well as on in-process controls. The studies should include biological, chemical and immunological methods to characterise the animal immunoglobulin/immunoserum as well as methods to detect and identify impurities.

Annex: Potential Viral Contaminants

Tables 1 to 3 give examples of viruses that a marketing authorisation applicant should consider when establishing a system of health control of the animals used as plasma donors. This system should be established individually for each product taking the following into account for the concerned species:

- the epidemiology of infectious disease in the country or geographical region where the production animals are maintained
- the use of a strict barrier system which effectively protects the animals from contact with wild animals, including rodents,
- the provision of a reliable system of veterinary control,
- the testing of donor animals or randomly selected animals before entering the colony and at regular intervals thereafter.

The occurrence/absence of infectious diseases in the country of origin should be substantiated by an official certificate of a legal veterinary authority .In this certificate the legal authority should also confirm that a compulsory notification of suspected cases of infectious diseases including clinical and laboratory verification, is in place.

In general, the company should routinely monitor the epidemiological situation in the country of origin of its plasma and in particular take note of any new emerging veterinary diseases and amend its list of considered viruses accordingly, if necessary.

Table 1: Rabbit

Rabbit rotavirus
Reovirus type 3*
Poxviruses:
- Rabbitpox (RPXV)*
- Myxomatosis virus (MYXV)
- Shope fibroma virus

Rabbit haemorrhagic disease virus (RHDV)
Rabbit papillomaviruses (e.g., Shope papillomavirus)
Lapine parvovirus (LPV)
Rabbit kidney vacuolating virus
Herpes cuniculi
Adenovirus
Encephalomyocarditis virus
Borna disease virus*

Sendai virus*
Simian Parainfluenza (SV-5)*

Pneumonia virus of mouse (PVM)

* Virus classified as pathogenic for humans

Table 2: **HORSE**

Eastern, Western & Venezuelan Equine encephalitis viruses*
St Louis encephalitis virus (SLEV)*
Japanese B encephalitis virus*
Vesicular stomatitis virus (VSV)*
Equine herpesvirus, type 1-4*
West Nile fever virus (WNFV)*
Equine morbilli virus (Hendra)*
Borna disease virus*
Reovirus type 1-3*
Equine influenza virus*
Equine rotavirus
Equine and bovine papillomaviruses (EqPV 1-2 and BPV 1-2)
Equine infectious anaemia virus (EIAV)
Equine arteritis virus
African Horse Sickness(Orbi)
Equine parvovirus

* Virus classified as pathogenic for humans

Table 3: SHEEP & GOAT

Foot and mouth disease virus (FMDV)*
Wesselbron virus*
Louping ill virus (LIV)*
Rift valley fever complex*
Tick-borne encephalitis virus (TBEV)*
Bluetongue virus (BTV)*
Vesicular stomatitis virus (VSV)*
Poxviruses:
- Parapoxvirus (Orf)*
- Capripox virus*
- Cowpox virus*

Parainfluenza virus type 3 (PIV-3)*
Borna disease virus*
Reovirus 1-3
Respiratory syncytial virus
Rotavirus
Akabane virus
Ovine herpes virus 2
Bovine herpes virus types 1,2,4
Border disease virus (BDV)
Ovine/bovine papillomavirus (OPV)
Bovine viral diarrhoea virus (BVDV)
Retroviruses:
- Caprine arthritis encephalitis virus (CAEV)
- Maedi-Visna virus (MVV)
- Jaagsiekte virus (OPAV)
- Bovine leukemia virus (BLV)

Epizootic haemorrhagic disease virus
Peste des petits ruminants (Morbillivirus)
Adenoviruses
Nairobi sheep disease
Ross river virus

* Virus classified as pathogenic for humans

Technical Report Series No.

WHO RECOMMENDATIONS FOR PRODUCTION AND CONTROL OF SMALLPOX VACCINE * REVISED 2003

* Previously called Requirements for Smallpox vaccine

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

Part B. Recommendations for National Control Authorities

INTRODUCTION

The Recommendations (formerly Requirements) for Production and Control of Smallpox Vaccines were last revised in 1965 (1). Since that time an intensified global eradication program from 1967 to 1980, led by WHO, resulted in the global eradication of smallpox (2). This was achieved by the globally coordinated use in national immunization programs of effective vaccines that met the quality specifications in the 1965 Requirements. The last naturally occurring case of smallpox was in 1977. In addition to the availability of effective vaccines a well functioning infrastructure was established world-wide in relation to vaccine production, vaccine supply and vaccine administration. Surveillance, diagnosis of disease, training and public health information were additional important elements to successfully combat smallpox.

After human-to-human transmission of smallpox was interrupted everywhere the Global Commission that certified eradication believed that the likelihood of reintroduction of smallpox from laboratories or natural or animal reservoirs was negligible. Nevertheless it recommended that it would be prudent for WHO and national health authorities to be prepared for unforeseen circumstances. One measure was to ensure that adequate reserves of potent vaccine were available (3). Accordingly a global

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stockpile of vaccine was established, as were national stockpiles. In addition seed lots of vaccinia virus suitable for the preparation of smallpox vaccine were maintained in a designated WHO collaborating centre. However in March, 1986, the Committee on Orthopoxvirus Infections concluded that the maintenance of a global reserve by WHO was no longer indicated (4), and interest rapidly declined. A survey conducted by WHO in 2001 found that only small amounts of stockpiled smallpox vaccines exist. These stocks are distributed quite unevenly around the world and accessible to only a very selected part of the global population. Additional production would be needed to meet any major demand on vaccine supply such as might follow an intentional release of smallpox vaccine.

Global resumption of smallpox vaccine production would benefit from modern concepts of production and control, and modern regulatory expectations should be adhered to in the licensing process. These general principles should apply to new vaccine manufactured in embryonated eggs, or in primary or continuous cell lines, or animal skins. In addition contemporary international reference materials to determine the potency of new vaccines and their immunogenicity in vaccinated individuals would be beneficial. The WHO Secretariat has followed these principles in this reformulated document and included changes to bring the document into line with other WHO Recommendations published since the last revision.

GENERAL CONSIDERATIONS

Since the cessation of routine smallpox vaccination after the successful eradication of the pathogen, population susceptibility has increased with each unvaccinated birth cohort. After the terrorist events of 11 September 2001 in New York and the subsequent anthrax related incidents in the United States, there has been heightened concern from Member States about potential intentional release of

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microorganisms. Even though the risk of the deliberate use of smallpox against civilian populations is considered to be low, there is consensus of the need to develop a coordinated response effort, to include sufficient numbers of doses in a strategic reserve of vaccines, an enhanced public health infrastructure, as well as disaster mitigation capacity.

Since the last revision of the WHO requirements for smallpox vaccines the principles governing regulation of medicinal products, in particular biological medicinal products including vaccines, have evolved fundamentally in response to increased knowledge in this field and to advanced technologies. New vaccine production should take into account these developments and all new vaccines, including those intended for strategic reserves, should be subject to current national licensing processes for biological medicines. It is recognized however that the use of vaccines stockpiled from the eradication era or new vaccines still under evaluation may be justified, if supported by an appropriate risk-benefit analysis, in cases of urgent national emergency.

Licensing of a new vaccine usually requires the demonstration of efficacy against the natural infection in a clinical trial. This is not possible in the case of new smallpox vaccines where the natural infection has been eradicated. One approach that has been taken is to develop a new vaccine that is phenotypically similar to a vaccine known to be successful in the eradication initiative, but other approaches are possible too. Immunological correlates of protection are not defined for vaccinia virus. However pock formation in humans after smallpox vaccination is a marker of vaccine effectiveness. The formation of papules, vesicles and pustules within a predefined time frame and with the appropriate appearance may be used as a marker to support the acceptability of a new smallpox vaccine. Other parameters such as levels of neutralising (NA) or haemagglutination inhibiting (HI) anti-vaccinia virus antibodies can presently only be considered as supportive information. Further research is needed in this area. Challenge studies in relevant animal model

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(mouse/vaccinia virus and monkey/monkeypox virus) may provide additional evidence on the protective efficacy of new smallpox vaccines.

Many general and specific aspects for (live) viral vaccines, such as the origin, quality and certification of starting materials, cell substrate issues, Specific Pathogen Free status of eggs, viral safety, method and process validation, testing procedures and GMP-principles are not adequately covered by the 1965 WHO guideline. All of these issues are addressed in this current revision.

The 1965 requirements on smallpox vaccines focuses on production on animal skin, in embryonated eggs and in chick embryo fibroblast cells (CEFs). Production in other cell lines is not covered by the guideline. There is considerable interest in new smallpox production in either Vero cells, human diploid cells or primary rabbit kidney cells, and other cells such as quail cells have been used in the past, so a new section is introduced for production and control of cell substrate produced vaccine. Cell culture vaccines were not used in areas endemic for smallpox during the eradication campaign and their efficacy against smallpox is not demonstrated. Therefore an important parameter to establish is that the cell substrate does not have a negative effect on the safety and/or efficacy of the vaccine virus. This is not predictable and considerable efforts are thus needed to show that a new cell culture derived vaccine has similar pre-clinical and clinical properties to a comparator vaccine with a known safety and efficacy profile.

Adventitious agent testing for viruses in the vaccine virus seeds and product intermediates is complicated due to the fact that complete neutralization of vaccinia virus is difficult to achieve. Testing for viral adventitious agents in eggs, animals and tissue culture might give ambiguous results. Whilst these tests remain the gold standard, supplementary specific testing for viral adventitious

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agents using validated PCR-methodology or immunochemical methods is envisaged. The scope of the test program depends on multiple parameters such as nature and origin of the virus seed. For smallpox vaccines produced on animal skin special attention should be given to the health status of the animals. Viral adventitious agent testing for animal skin vaccine should depend on the species, the

origin and on epizooiological considerations in the source country of the animals. Transmissible spongiform encephalopathy (TSE) guidelines are referenced and should be adhered to. The bioburden of new vaccines produced in animal skin can be lowered by state of the art animal husbandry in dedicated facilities. A revised (lower) specification for bioburden in the final product is introduced in this revision. However, since the production process on animal skin may be very difficult to validate, consistent sterility of the finished product may be difficult to achieve. The use of non-sterile final product may be justified since smallpox vaccine is administered in a very low volume by scarification rather than by intramuscular or intravenous inoculation, and specified pathogens are excluded from the vaccine. In addition the history of use of the product defined its safety profile and unambiguously demonstrated its efficacy.

An animal model to predict neurovirulence of vaccine virus has been introduced. It had been shown in the past that vaccinia viruses can be classified according to their high, medium and low neuropathogenic potential in man (5). Although the histologic pattern is different in the mouse brain following intracerebral inoculation of different vaccinia strains compared to the histologic changes found in the human brain following post vaccinal encephalitis, the mouse model reflects the neuropathogenic potential of vaccinia strains in man. The mouse model is introduced for phenotypic characterisations of both the seed material and also each lot of bulk suspension produced until sufficient validation data is available to reduce the test frequency.

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Future research is anticipated on highly attenuated vaccinia virus strains, or other approaches such as inactivated vaccines, subunit vaccines or DNA vaccines, to facilitate the safe and effective immunization of vulnerable sectors of certain populations (such as the immunocompromised, the elderly, pregnant women and children with eczema). If such strains or approaches do not induce pock formation, then alternative markers of efficacy will be needed. Vaccinia strains that do not induce pocks are not covered in this revision.

The term "national regulatory authority" and "national control laboratory", as used in these recommendations, always refer to the country in which the vaccine is manufactured.

PART A MANUFACTURING RECOMMENDATIONS

1. DEFINITIONS

1.1 *International name and proper name*

The international name shall be "Vaccinum variolae". The proper name shall be the equivalent name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the recommendations formulated below.

1.2 Descriptive definition

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

Vaccinum variolae is a preparation of live vaccinia virus grown in the membranes of the chick embryo or in *in vitro* cultures of suitable tissues or in the skin of living animals. The preparation shall satisfy all the recommendations formulated below.

1.3 International standard and reference preparations

To allow for standardization of potency assays an International Reference Preparation of smallpox vaccine was established in 1962 (6,7). This standard was derived from the Lister strain of virus and was produced on the flanks of sheep before lyophilization and storage at -20°C. Initially held at the

Statens Seruminstitut in Copenhagen the Reference Preparation was passed to the National Institute for Biological Standards and Control (NIBSC) (UK) in 1997. This Reference Preparation is available from NIBSC for calibration and establishment of in-house potency reference materials.

An international collaborative study of two new candidate International Reference Preparations is in progress, one of which will later be selected to replace the dwindling stocks of the current International Reference Preparation. The study is expected to be completed in 2003.

An International Standard for Anti-Smallpox Serum was established in 1966. (8). Initially held at the Statens Seruminstitut in Copenhagen the Reference Preparation was passed to the National Institute for Biological Standards and Control (NIBSC) (UK) in 1997. Subsequently this material was found to be contaminated with hepatitis B virus and was destroyed.

A WHO informal consultation in 2002 recommended that a replacement preparation be obtained and studied for suitability in an international collaborative study. Such a standard would be used for the assay of variola and vaccinia antibodies and to calibrate vaccinia immunoglobulin preparations.

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