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Guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products

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7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

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Executive summary

This guideline describes the information to be considered by the manufacturer of human biological medicinal products using porcine trypsin. Although specific guidance and specification has been given for bovine sera used as cell culture reagent in manufacture of human medicinal products (CPMP/BWP/1793/02, Ph. Eur 2262 Bovine Serum), no guidance has been given so far for porcine trypsin.

1. Introduction (background)

Porcine trypsin is a reagent widely used during the manufacture of biological medicinal products. The main application is the detachment of cells from culture vessels for passaging. During manufacture of some vaccines, trypsin is added to the final culture stage of virus production for activation of a vaccine virus such as influenza virus and rotavirus. In addition, for the manufacture of specific recombinant proteins, e.g. insulin, trypsin is used as a protein-cleaving reagent during the downstream process.

Porcine trypsin, an animal derived material extracted from the pancreas of pigs, carries the risk of contamination with adventitious agents. This may especially be the case for certain viruses that are widespread among pigs and which are difficult to eliminate due to their high resistance to physicochemical treatment.

Animal-derived materials in general can be contaminated with a wide range of biological agents and therefore it is strongly recommended that all these materials are appropriately selected, tested and treated for the inactivation and/or removal of such agents before they are used for the manufacture of medicinal products. Contamination of pharmaceutical facilities with adventitious viruses may lead to a shutdown of production and to supply shortfall. Contamination can also alter growth properties of cultured cells and, theoretically, this could lead to altered properties of a biological product. For medicinal products where no virus inactivation is possible during down-stream processing steps, e.g. live vaccines, or where, in addition, testing for contaminants at the end of production is difficult, e.g. some cell based medicinal products, well-controlled reagents are essential to avoid exposure of patients to adventitious viruses or other non-viral adventitious agents.

Early in 2010 Victoria *et al.* reported the finding of DNA sequences of porcine circovirus (PCV) in a live attenuated rotavirus vaccine. Further investigation confirmed contamination of the vaccine with PCV and revealed that the PCV contamination most likely originated from porcine trypsin that was used in the development of rotavirus vaccines.

2. Scope

This guideline applies to trypsin purified from porcine pancreatic glands for use as a reagent in the manufacture of human biological medicinal products. This includes e.g. (1) trypsin used as a reagent for cell culture during manufacture of vaccines, advanced therapy medicinal products or other medicinal products produced from cell culture, (2) trypsin used to activate virus particles, and (3) trypsin used as a protein processing reagent.

3. Legal basis

This guideline has to be read in conjunction with the introduction and general principles (4) and part I of the Annex I to Directive 2001/83 as amended.

4. Types and source of porcine trypsin

Trypsin is a proteolytic enzyme obtained by activation of trypsinogen. Porcine trypsin is extracted from pancreatic glands usually obtained as a by-product of the food industry. It is prepared as a powder or liquid solution for use as a reagent in cell culture and as a protein processing reagent. Trypsin preparations used for cell cultures may contain impurities from the starting material including other pancreatic enzymes such as chymotrypsin but which do not adversely affect the performance of cell cultures. Highly purified trypsin preparations are available for certain applications in protein chemistry, e.g. as a protein processing reagent.

5. Starting Material

Selection of healthy pigs is the first step in minimizing the risk of pathogen contamination of the starting material. The pancreatic glands shall be derived from pigs fit for human consumption following ante- and post mortem inspection in accordance with European Community or equivalent (third country) conditions. Batches of raw pancreatic glands should be clearly labelled or accompanied by appropriate documentation (e.g. certificate of origin) allowing identification of the nature of the animal tissue, their origin and date of collection. Batches of raw material should be accompanied with appropriate official health certificates or equivalent appropriate documentation.

6. Testing for adventitious agents

Despite the application of control measures intended for food safety, there is a risk that an animal-derived starting material may be contaminated with transmissible agents.

Testing of starting materials or appropriate intermediates for virus contamination is an important safety measure for biological medicinal products. However, there are several limitations when considering virus testing during production of porcine trypsin. Mainly for economic and organizational reasons, it does not seem possible to test individual pancreatic glands prior to them being pooled into batches; consequently, material from a single infected animal could enter a large production batch and the sensitivity of subsequent tests may not be able to detect a diluted contaminant in the pooled material. As a general rule, testing of the pooled starting material should be performed at a stage before any virus inactivation/removal step whilst testing of the final trypsin preparation for adventitious viruses is not considered appropriate. However, this is not feasible in cases where batches of frozen pancreatic tissue are directly extracted with alcohol containing fluids. If heat or low pH is applied during extraction/precipitation steps, this might additionally inactivate a variety of enveloped and non-enveloped viruses. In addition, the pancreatic enzymes and their activity under exact conditions (pH, temperature) might have an influence on infectivity of some viruses while other viruses (e.g. porcine parvovirus (PPV) and PCV) are not affected by the enzymatic activity of trypsin.

The stage where testing is performed should be clearly defined and justified. The batch size of the tested product intermediate as well as the size of samples subjected to virus testing should be defined and needs to be considered when assessing the benefit of virus testing.

A comprehensive literature-based risk analysis of potential porcine viruses that may contaminate porcine trypsin and could pose a risk to humans has been published (Marcus-Sekura et al., 2011). In summary, 55 porcine virus species from 17 different families have been identified with a documented or potential human host range as indicated by reports of natural human infections, detection of antibodies in humans and/or ability to infect human cells in culture. Sixty percent of these viruses can replicate in Vero cells and a variety can be detected in porcine cells. Therefore a general *in vitro* test is recommended using two distinct cell lines, one of which should be of human or primate origin (e.g.

Vero) and the other of porcine origin. The cell lines should be capable of detecting haemadsorbing viruses and cytopathic viruses. Cells should be cultivated in a manner that allows detection of viral replication.

Specific tests for porcine viruses that are not detected by a general cell culture test should be considered on a case-by-case basis following a product-specific risk analysis (see Chapter Risk assessment) considering more product specific documents where relevant (e.g. WHO 2010, Ph.Eur 5.2.3). All potentially-contaminating porcine viruses should be considered. Specific consideration should be given to widely distributed viruses which are difficult to inactivate (e.g. PCV and PPV) and with zoonotic potential (e.g. HEV). The risk analysis takes into consideration the whole manufacturing process, the testing capability and the use of the medicinal product. For example, consideration of a specific geographic origin or demonstration of highly effective virus inactivating/removing manufacturing steps can justify why testing for certain viruses might be omitted. Generally, if an infectious virus contaminant is detected, the trypsin batch should not be used for the manufacture of human biological medicinal products unless a careful risk assessment demonstrates that the infectious virus will be reliably inactivated or removed by virus reduction steps. Although this is not in the scope of this Guideline, care should be taken to prevent spread of the virus in the facility or to other medicinal products when using virus positive materials.

Virus testing may be performed by the trypsin supplier, by the manufacturer of the medicinal product, by a contract laboratory or by more than one of these. It is the responsibility of the marketing authorisation holder of the medicinal product to ensure that testing is carried out to the required standard.

Trypsin used as reagents for cell culture or activation of virus particles should comply with the Ph. Eur. test for sterility and be free of mycoplasmas (Ph. Eur 2.6.7 or validated equivalent test, e.g. USP, JP, CFR, etc.). Trypsin used for other applications should comply with microbiological tests required in Ph. Eur. 0694 Trypsin (or equivalent tests).

7. Manufacture

Trypsin is usually obtained by extraction of pools of frozen pancreatic glands with additional optional purification steps such as precipitation or chromatography. Production frequently includes a prolonged incubation at low pH. Data presented at conferences (Yang et al., 2013 and Lackner et al., 2014) indicate that both PCV and PPV might be significantly inactivated during prolonged incubation at pH 1.7, room temperature. It has been reported that commercially available trypsin retains its activity after prolonged treatment (18-24h) at a pH of 1.0 at 4°C (Melnick and Wallis, 1977) and can be used for cell culture after such treatment. In addition, a final pathogen inactivation step such as gamma irradiation (minimum dose 30 kGy), e-beam irradiation, or UV irradiation can be applied. Other methods (e.g. virus filtration) or novel methods for virus inactivation/removal can be used alternatively or in addition to the methods described above. Given the limitations on the control of raw materials and limitations on testing for viruses, it is advisable to incorporate two complementary virus reduction steps, unless otherwise justified. In any case, the manufacturing process should be appropriately controlled with respect to critical parameters that affect virus reduction, or the purity and activity of the enzyme preparation.

Appropriate and validated cleaning measures should be implemented in order to minimize the risk of batch-to-batch cross contamination and cross contamination with other materials of animal origin. Each batch of manufactured trypsin product should be uniquely identified and a certificate of analysis should be generated for each batch. Trypsin should be manufactured under a quality system such as GMP, ISO, or an HACCP-compatible system.

8. Validation of the virus-inactivation/removal capacity of the manufacturing process

Inactivation/removal of microbiological agents is considered as a major factor contributing to adventitious agent safety of trypsin. Therefore, selected process steps should be carefully validated with respect to their virus inactivation/removal capacity. Reference is made to the 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).

Although the enzymatic activity of trypsin and possibly other porcine pancreatic enzymes may contribute to the inactivation of some viruses, this does not apply to resistant viruses such as the non-enveloped porcine parvovirus and porcine circovirus. Therefore, special consideration should be given to assure adequate clearance for this type of contaminant and the inclusion of more than one virus inactivation or removal step is warranted unless such viruses are controlled by other safety measures (e.g. testing). As an option for an additional, dedicated virus inactivation step, gamma or UV-C irradiation, or virus filtration should be considered.

For the validation of irradiation steps, virus filtration and for low pH steps, an animal parvovirus (e.g. porcine parvovirus) should be included as these viruses are small DNA non enveloped viruses considered to be resistant to physico- and chemical treatments.

Gamma irradiation is generally performed on frozen liquid trypsin. Applying irradiation to lyophilised powder requires careful investigation of virus inactivation. During validation of virus inactivation, it should be considered that it is difficult to achieve a homogeneous distribution of viruses when spiking liquid virus preparations directly into the hydrophobic trypsin powder. Therefore it is recommended to spike the pre-lyophilised intermediate with a liquid virus preparation and to determine the virus titre after lyophilisation; this can then be used as the load titre for the irradiation step.

As regards UV-C irradiation, virus inactivation is mainly attributed to direct interaction with nucleic acids, and most of the photoproducts are generated on pyrimidines. However, study data show that virus inactivation is not simply predictable by the genetic composition or type of nucleic acid genome (RNA/DNA, ss/ds). It should also be considered that repair mechanisms mediated by cell based infectivity assays, which are used for measuring virus inactivation, may reduce the observed lethal effects, especially for viruses possessing double-stranded nucleic acids. Generally, Adenovirus is considered to be rather resistant to UV-C irradiation as well as herpes virus (PRV) and should be considered for evaluation of virus inactivation.

Due to the proteolytic nature of trypsin, controls assessing the stability of a spiked virus in trypsin-containing test material and controls for cytotoxicity and interference of the test matrix in a virus assay are important for this product.

Adverse effects on trypsin quality or performance should be assessed when considering implementing a viral inactivation or viral removal step in an existing trypsin manufacturing process.

9. Quality Controls

It is recommended that identity and activity testing for porcine trypsin follow the Ph Eur requirements in the Trypsin monograph (Ph. Eur. 0694 Trypsin) or equivalent test. The pancreatic starting material contains a variety of proteases but no general recommendation for purity of trypsin used as a cell culture reagent can be given as the presence/absence of other enzymes is variable between lots of trypsin and this may be tolerated in its use in cell culture. On the other hand, purity can be important for other applications such as protein processing steps where specific cleavage of proteins is required.

10. Use of alternative reagents in cell culture

Proteolytic enzyme preparations other than porcine trypsin are available, e.g. recombinant bacterial or plant-derived trypsin, enzymes from invertebrates, bovine trypsin that could be an alternative for use in cell culture. The use of bacterial or plant derived recombinant trypsin minimises in principle the risk for animal virus contamination and the application of such alternatives is therefore encouraged. However, no general recommendation to replace porcine trypsin can currently be given considering that these alternatives need a careful assessment of suitability, quality, sterility and performance characteristics as well as associated risks such as other adventitious agents such as prions from bovine species or invertebrate viruses. When bovine trypsin is used, the bovine virus safety needs to be carefully considered and the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01) in its current version is to be applied.

11. Risk Assessment

This Guideline provides a general quality specification for porcine trypsin, especially with respect to viral safety, and various measures that should be applied during the production of porcine trypsin to minimize the viral risk are described. No combination of the measures outlined below can guarantee complete viral safety, but rather they reduce the risk involved in the use of trypsin in the manufacture of medicinal products. It is therefore necessary for the manufacturer of a medicinal product to take account of this when choosing the trypsin for a particular use by making a risk assessment. The risk assessment should follow the general principles outlined in Ph. Eur. 5.1.7 Viral Safety. Such a risk analysis takes into consideration relevant factors, for example: (1) the epidemiology and control of the animals from which the starting material is sourced (2) the availability of suitable virus test methods and the stage at which such testing is implemented, for instance testing on the animals, production intermediate or final batches of trypsin, or testing at any other stage of production of the medicinal product (3) virus inactivation by trypsin itself, (4) the virus inactivation/removal during manufacture of the trypsin, (5) the stage of manufacture of the medicinal product at which trypsin is used as a reagent, (6) the risk of virus replication in cell cultures used for production of the medicinal product, (7) additional virus inactivation/removal steps applied during the manufacture of the medicinal product, (8) the amount of trypsin to produce a dose of medicinal product, and (9) the route of administration of the medicinal product.

12. Regulatory Aspects

The Marketing Authorisation Holder/Applicant of the medicinal product should have sufficient information on the trypsin to allow a comprehensive risk assessment and provide a sufficient data package to the competent authority for assessment. This should include a description of testing methods and the stage at which virus testing is performed, as well as the volumes and sensitivity of the virus tests. Study reports validating virus reduction steps should be provided according to Guideline CPMP/BWP/268/95. In the case of a change of supplier of trypsin, data as outlined above should be provided for the new trypsin.

This guideline is for prospective implementation, i.e. for new marketing applications. However it is advisable to consider the risk of contamination from porcine trypsin already at early stages of product development. It is recognised that it may take some time to implement the recommendations in this guideline, therefore, a transition period of a maximum of two years from the date of adoption of this guideline is set. Nevertheless, in the interim phase, a re-assessment of the virus safety is

recommended for authorised live virus vaccines and cell based medicinal products that use porcine trypsin and do not incorporate viral inactivation/removal steps in the manufacturing process.

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