EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee for Medicinal Products for Human Use (CHMP)

Overview of comments received on 'Guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products' (EMA/CHMP/BWP/814397/2011)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation:

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1. General comments – overview

Stakeholder	General comment (if any)	Outcome (if applicable)
EDQM	This guideline complements the general requirements of chapter *5.1.7* for viral safety with considerations specific to trypsin in a very helpful manner. The link between this guideline, chapter 5.1.7 and the European Pharmacopoeia trypsin monograph is clearly highlighted. It is gratefully acknowledged that the guideline will contribute to a better understanding of the use of general chapters/monographs of the Ph. Eur. with regard to viral safety.	Accepted. No modification of guideline is necessary.
Neova Technologies Inc.	Neova Technologies Inc ("NTI") appreciates the opportunity to review and respectfully submit the following comments in response to the Draft "Guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products" issued 21 February 2013 by the Committee for Medicinal Products for Human use ("CHMP") under EMA/CHMP/BWP/814397/2011. NTI is a manufacturer of Trypsin products which are used as processing aids in food and pharmaceutical products internationally. NTI has been manufacturing trypsin since 1990 and has been selling trypsin to manufacturers of human biological medicinal products since the mid 1990's.	Accepted. No modification of guideline is necessary.
Regulatory Affairs & Biological Safety Consulting	The guideline provides a scientific approach to perform a risk assessment and demonstrate that the porcine trypsin product is suitable for the intended use in the manufacturing process of a medicinal product for human use. This is highly acknowledged and - having this intention in mind – the guideline is well written. Considering however the high level of analysis which is required (considering for instances the epidemiological situation in the country	Partly accepted. This guideline outlines important scientific/technical aspects to be considered when using porcine trypsin at production. It is agreed that a risk assessment concerning porcine trypsin and that changes in the quality of some trypsin-products or documentation of currently-produced trypsin may be needed. Nevertheless, this is considered necessary.

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	of origin, i.e. identification of viral contamination risks related to the area of sourcing of porcine tissue) it might be very difficult to meet these requirements. This will be explained later in more detail. Furthermore, considering the current low level of information that is provided by the suppliers of porcine trypsin and the request to implement if possible two steps of virus reduction and provide adequate data, it may need some time to establish the data base that is needed to meet the expectations laid down in this guideline. It is therefore proposed to use a longer transition period and extent the normal range of six month after adoption by the CHMP for the implementation of this guideline	Extension of timeline for implementation is agreed. Text has been included in the guideline.
Vaccines Europe	Vaccines Europe welcomes the opportunity to provide comments in the consultation on the 'Guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products'. Vaccines Europe would like to highlight that harmonisation between this guideline and the monograph of the European Pharmacopoeia on trypsin would be welcome. In addition, we would like to suggest that the EMA liaises with the EDQM to promote such a harmonisation. In addition, we would like to point out that some statements could be better explained and supported by literature references. Please refer to our detailed comments. Finally, definition of starting material, raw material, reagent should be part of this guideline. This would allow clearly distinguishing the roles and responsibilities of manufacturers of trypsin from those of medicinal product manufacturers.	 Not agreed. Harmonisation with European Pharmacopoeia has already been considered and EDQM has been contacted. Specific statements are discussed below. Not agreed. As already outlined in the Guideline, the scope is trypsin used as a reagent at manufacture of the medicinal product. (see Section 2 of the Guideline) It has been outlined in Section 11 that the marketing

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		authorisation holder/applicant of the medicinal product performs the risk assessment. The manufacturer of the medicinal product is responsible for the safety of the medicinal product.
ISIA	Focus of this guidance: ISIA understands that this guidance is largely focused on the presence of porcine circovirus in porcine trypsin. There are therefore two points to note: 1) Circovirus is a common virus found in pigs throughout the world, **nearly every herd of swine is seropositive worldwide**. Neither the USDA nor OIE monitors the incidence of disease since it is so prevalent and difficult to test for. There is no treatment for the disease. There are currently four commercial PCV2 vaccines available worldwide. In most of the world the vaccination rate is very high (over 80%, with the US being about 98%). Only China and Vietnam have low rates of vaccination, being below 5%. In addition, the pork industry has changed animal management practices to reduce the incidence of the disease. But the fact **remains nearly every herd of swine is seropositive worldwide**. 2) The issue that has resulted in this guidance emerged in 2010 and involved the presence of Circovirus DNA in attenuated rotavirus vaccines for humans. Since the identification of the circovirus DNA in vaccine, the FDA, EMA and WHO have all evaluated the risk of circovirus DNA in these vaccines and determined the risk profile allowed for the continuation of vaccination for rotavirus in every	Not agreed. It is recognized that PCV contamination of rotavirus vaccine triggered the creation of this guideline. However, it is not agreed that this Guideline focusses on porcine circovirus (PCV). Ad1) Not agreed. It is well known that PCV-Infection is highly prevalent in pigs and that it is difficult to find antibody-negative herds, considering also vaccination against PCV2. However, this Guideline does not make the requirement to source pancreatic glands only from antibody-negative herds. The guideline requires a comprehensive risk assessment addressing not only sourcing of animals but additional safety measures such as virus testing, virus inactivation/removal, testing of cell banks etc. Ad2) Not agreed: The recommendation for contaminated rotavirus vaccines considered the specific public health benefit of Rotavirus live Vaccine as well as its specific use (oral application). This recommendation must not be extrapolated to all medicinal products. It is much more difficult to predict a risk to humans from exposure to the

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	national immunization program for children world-wide (10% of children world-wide die of rotavirus infections). Unless replicating virus is present the presence of DNA does not constitute a high risk. ISIA understands and supports the need for control of adventitious agents but is concerned that overregulation could lead to shortage of material	virus via parenteral route.
	Roles of supplier and manufacturer 3) The business plan of the trypsin supplier and the manufacturer of the medicinal product are not the same. The guidance appears to assume that the trypsin supplier produces the material exclusively for use in the manufacture of medicinal products. In general, this is not the case. Some slaughterhouses and firms may exit this business if requirements become too specific, since this is a small portion of their overall production, being produced from a minor by product of the meat packing process. ISIA recognises that, in the view of any regulatory agency, it is the responsibility of the manufacturer of the medicinal product to assure that the requirements laid down in this guideline are fulfilled. It is also clear in this case that the trypsin manufacturer must fulfil the requirements in Section 7 and 8, but it is the responsibility of the manufacturer of the medicinal product to assure that these requirements are met. If not, then the manufacturer of the medicinal product must perform additional testing or inactivation prior to use of the trypsin. Clearly the responsibility for Section 11 lies with the manufacturer of the medicinal product. This distinction may need to be further clarified in the guidance	It is well known that many reagents used at production of medicinal products (e.g. amino acids, peptones, tallow derivatives, bovine blood-derivatives etc.) are not exclusively manufactured for use in pharmaceutical industry and that they may have a broader application in food industry, cosmetics or technical applications. The intention has been well understood. It is considered essential that the manufacturer/supplier of trypsin appropriately informs their customer about intended use and/or potential risks from their products.
	Inactivation Methods	Partially agreed. It is agreed that novel methods can be used.

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	ISIA believes that this guidance should not list specific inactivation methods but require inactivation steps be robust and effective in normal manufacturing environments, as laid down in ICH Q5A guidelines and EU Guideline CHMP/BWP/268/95. The manufacturer should be able to validate such methods. The guidance should also state that novel methods may be used if it can be shown that they are effective in inactivating a broad range of viruses or specific virus(es) of concern.	No requirements for a specific method for virus inactivation/ removal have been made in this guideline. Nevertheless this Guideline mentions some methods which are used or can be used at production of trypsin. It is an integral part of this guideline to provide advice on feasible methods for virus inactivation/removal. It will be clarified in the revised guideline that it will be possible to use other or novel methods.
SciencePharma	It is considered that the guideline would be adequate for any reagent/substance of animal origin used in the manufacture of human biological medicinal products. Only minor requirements or advices, specific only for porcine trypsin, can be found in this draft guideline. It would be highly advisable to provide, for example, a list of viruses specific to porcine trypsin that should be considered for safety analysis. Moreover, the guideline seems to be prepared mostly for cell cultures and vaccines. Detailed requirements specific for trypsin used as a protein-cleaving reagent would be helpful. For example, the section 6 "Testing for adventitious agents" is currently focused on cell cultures/vaccines only. It is proposed to emphasise that an adequate risk analysis should be performed, justifying a range of tests and controls to be conducted, depending on whether the trypsin is used for cell cultures or as protein-cleaving reagent.	The possibility to define a list of viruses for testing has been intensively discussed. Reference to relevant literature (listing 55 porcine virus species from 17 different families with a documented or potential human host range) has been made. It was not considered appropriate to specify a list of viruses to be tested considering the possibilities to introduce virus inactivation/removal steps as well as the many different applications of trypsin during manufacture of human medicinal products.
EBE (European Biopharmaceutical Enterprises)	EBE appreciate the opportunity to comment the draft guideline and agree that the use of porcine trypsin for manufacture of medicinal products introduces a potential risk for contamination of the medicinal product with viruses coming from the pancreas glands used as starting	No modification necessary.

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	material for trypsin manufacture. We appreciate the approach of • justifying the stage where testing is performed • evaluating the use of specific tests for porcine viruses on a case-by-case basis following a product-specific risk analysis, where the whole manufacturing process and use of the medicinal product are taking into consideration	
	Harmonisation between this guideline and the monograph of the European Pharmacopoeia on trypsin would be welcome. We suggest that the EMA liaises with the EDQM to promote such a harmonisation.	Agreed. Liaising has been already performed.
	The document outlines adventitious agent risk considerations as specific for trypsin. Our belief is that firms should consider using the document for other porcine derived reagents used in the manufacturing process that present the same viral risk profile. The primary consideration on this point is that testing "alone" should be considered a limited viral risk mitigation activity. Rather, testing at an appropriate stage of the raw materials production "combined" with knowledge of the manufacture of trypsin (or other porcine materials) along with inactivation strategies supported by data should always be considered. It might be worthwhile to consider adding a paragraph in this regard in the guideline.	Partially agreed. It is agreed that that testing "alone" is a limited viral risk mitigation activity and the limitations have been already outlined in this document. It is agreed that the general principles of this guideline can be applied to the porcine materials. However, this guideline has been specifically designed for porcine trypsin and some statements about manufacture and virus inactivation (e.g. low pH treatment) might not be feasible for other porcine materials (enzymes).
	A general concern relates to the guideline equalling the demands on trypsin (as a raw material) with that of the final medicinal product for which trypsin was used as a raw material (by reference to CHMP/BWP/268/95). Detailed manufacturing information is necessary in order to evaluate	The principles of Guideline CHMP/BWP/268/95 apply to all stages of the biological production chain. It is agreed that validation of manufacturing steps requires careful consideration.

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	virus clearance of the trypsin production process; information which is proprietary information of the trypsin manufacturer. This change can be time consuming if change of supplier / raw material is needed and we therefore ask for an appropriate date for coming into effect e.g. 2 – 3 years for products on the market.	Extension of timeline is agreed.
	Definition of starting material, raw material, reagent should be part of this guideline. That would allow clearly distinguishing the roles and responsibilities of manufacturers of trypsin from those of medicinal product manufacturers.	See same comment from ISIA above.
	Some statements should be better explained and supported by literature references. Please refer to our detailed comments.	See detailed comments below.
	Finally we would like to emphasize that cell banking processes no longer require trypsin since non-adherent cell culture is employed.	Not agreed: Adherent cells are still being used or for some medicinal products.
Parenteral Drug Association (PDA)	PDA appreciates the opportunity to provide comments on this guideline submitted for public consultation. PDA is a non-profit, international, professional association of more than 10,000 individual member scientists having an interest in the fields of pharmaceutical, biological, and device manufacturing and quality. Our review was completed by an international group of expert volunteers with experience in virology, regulatory affairs and GMPs on behalf of our Regulatory Affairs and Quality Advisory Board. PDA realizes this response is being sent after the posted deadline but took extra time to ensure we reached scientific consensus on the comments and ask that CHMP still consider the attached recommendations in working towards a final guideline.	
	PDA recognizes this guideline provides a scientific approach to assess	Agreed.

Stakeholder	General comment (if any)	Outcome (if applicable)
	the virus safety of trypsin produced from porcine pancreatic tissue. It contains both, guidance for the acceptance of trypsin used as a reagent for production of medicinal products and it provides, in addition, current knowledge. PDA supports inclusion of both areas but suggests clearly separating the two within the document for enhanced clarity.	See specific comments below.
	The guideline requires a high level of documentation of sourcing, testing and manufacturing including adventitious agent inactivation of trypsin that may be difficult to implement in a short time frame and might possibly lead to potential supply issues.	Agreed. Extension of timeline is agreed.

2. Specific comments on text

Line no.	Stakeholder	Comment and rationale; proposed changes	Outcome
184 - 189	Biogenomics Limited.	Comment: *Usage of "Recombinant Trypsin" shall be recommended to replace Porcine Trypsin.* Proposed change (if any): *Usage of "Recombinant Trypsin" shall be recommended if the product quality and suitability is properly evaluated and complies in terms of the activity , Identity, purity, HCP , HCD, and absence of pathogenic organism in the final product.*	Not accepted. Given that adequate safety measures are performed, the risk from porcine trypsin is found minimal. It has been already stated in this Guideline in section 10 that "*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*." This statement is considered sufficient.
Section 6. 118-121	Eisai	"*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*" Eisai question: Could you please clarify what would be needed to be provided by a MAA applicant, if the virus testing is performed by the porcine trypsin supplier? Does this statement in the guideline mean that, if the virus testing is performed by the trypsin supplier, the MAA applicant can use a certificate of conformity to standard provided by this supplier without performing any additional testing on the porcine trypsin purchased?	It is agreed that no re-testing by MAA is obligatory. The information required is considered in Section Regulatory Aspects.
122 and 123	Neova Technologies	Comments:	Accepted.

Line no.	Stakeholder	Comment and rationale; proposed changes	Outcome
	Inc.	NTI has some concerns about the restrictions to comply only with the EP test. The change to the sentence (as written below) recognizes that a variety of manufacturers of trypsin exist worldwide and that the tests for sterility and mycoplasma presence among government agencies could be considered equivalent to determine that sterility and freedom from mycoplasma. Proposed change: The testing for sterility and mycoplasma presence of trypsin used as reagents for cell culture or activation of virus particles should follow the EP (Ph. Eur 2.6.7) or equivalent test (e.g. CFR, USP, JP, etc).	Trypsin used as reagents for cell culture or activation of virus particles should comply with EP test on sterility and be free of mycoplasmas (Ph. Eur 2.6.7) or equivalent test (e.g. USP, JP, CFR, etc.).
129 and 130		Comment: The sentence in this section gives some examples of typical virus inactivation techniques; however it may be more inclusive to include a reference to a commonly used virus removal technique (i.e. nanofiltration) as well. This would broaden the scope of potential techniques that could be applied and recognize that some trypsin products are sold in a liquid format where nanofiltration or UV-C irradiation could then be the technique of choice for those manufacturers. Proposed change: In addition, a final virus inactivation (e.g. gamma, e-beam or UV-C irradiation) or removal (e.g. nanofiltration) step can be applied.	Accepted. Modification: *Other methods (e.g. virus filtration) or novel methods for virus inactivation/removal might be used alternatively or in addition to the methods described above.*

Line no.	Stakeholder	Comment and rationale; proposed changes	Outcome
152 and 153		Comments: The techniques mentioned in this section should include commonly employed techniques for the inactivation and removal of virus. This would broaden the scope of potential techniques that could be applied and recognize that some trypsin products are sold in a liquid format where nanofiltration or UV-C irradiation could then be the technique of choice for those manufacturers. Proposed change: As an option for an additional, dedicated virus inactivation or removal step, irradiation (e.g. gamma, UV-C or e-beam) or nanofiltration should be considered.	Accepted. *(see modification above)*
Chapter 6, Lines 109-117	Regulatory Affairs & Biological Safety Consulting	Comment: The current text requires the thorough analysis of occurrence of porcine viruses which may be of risk according to the source of tissue (epidemiologic situation in the country of origin, health control of animals etc.). The publication of Marcus Secura et al., 2011, contains a long list of viruses which may be of concern and some of them are not detectable in common virus assays using Vero cells or porcine cells like primary porcine kidney cells or the PK13 or ST cell lines. It is very difficult to get information about virus infections of pigs which are endemic in areas/countries but not included in the OIE list of notifiable diseases; also ProMed mail may not provide a complete picture of virus infections. To get such information from national	Partially agreed. PPV, HEV and PCV have now been flagged for specific consideration is Section 6 on testing for adventitious agents. However it was not considered appropriate to define a unique list considering the different uses at manufacture of medicinal products and preparations of porcine trypsin.

Line no.	Stakeholder	Comment and rationale; proposed changes	Outcome
		authorities is very difficult as well so that the occurrence/absence of specific virus infections might remain speculative. It is proposed therefore to take a similar approach as provided in the 'Guideline on the use of bovine serum in the manufacture of human biological medicinal product (EMA/CHMP/BPW/457920/2012 rev1)' where requirements on testing for specific viruses are included. They shell base (1) on the ability of the general test(s) to detect specific viruses and (2) the current epidemiological situation in the country of origin. But in addition to these general requirements, specific viruses are listed which needs to be considered for testing. A similar approach would be beneficial also for porcine trypsin. If the trypsin would not be used directly for production of the medicinal product and the manufacturing process provides several steps which have a capacity for virus removal/inactivation, the general level of testing considering the detection of hemadsorbing and cytopathic agents, detection of the specific porcine viruses and the inactivation of trypsin might provide a sufficient high safety level. In other cases, e.g. the use of trypsin as a medium supplement in the production of life vaccines, the demonstrated safety of trysin, i.e. the established levels of control, could be higher considering more in detail the epidemiological situation in the country of origin and providing a thorough analysis of the probability of virus contamination of the medicinal product.	

Line no.	Stakeholder	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): Implement requirements for testing of trypsin for specific viruses; require that testing should be done before inactivation. Maintain the differentiation in the control of trypsin according to the intended use.	
Chapter 7, Lines 130-134		Comment: It is requested 'to implement two complementary virus reduction steps, unless otherwise justified. 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.' It is a goal that possibly needs some time to be implemented. Currently the information provided by the supplier is very limited; it will need some time to get such information or to generate the data which are required (e.g. virus validation studies). Proposed change: The text should be maintained but a longer period should be considered for the implementation of this guideline (one year or even more).	Agreed longer timeline for implementation.
Chapter 8, Lines 152-169		Comment: low pH, gamma irradiation and UV-C treatment are considered as virus reduction steps. Virus removal by small size virus retentive filters (so-called parvovirus filters) should in addition be taken into consideration.	Agreed, text to be modified, see responses to comments above.

Line no.	Stakeholder	Comment and rationale; proposed changes	Outcome
		The filters are reliable tools for removal of viruses larger than the pore size of the filters. PPV should be used for the control of the filtration process. It is expected that PCV is too small to be removed by current commercial available filters so that the absence of PCV must be assured by other means. Testing could be considered or the use of a combination with other methods (UV-C?). Proposed change (if any): Implement filtration as an additional tool for virus removal. Add the concerns related to PCV.	
Chapter 11, Lines 196-210		Comment: The text provides the different categories of data which needs to be considered in the risk assessment. It provides also a strategy for performing the risk assessment. This is highly appreciated. However, MA applicants may have a problem to get data related to point (1) up to point (4). Especially if only small quantities of trypsin are used by a company it may be difficult to get such information from trypsin suppliers; the willingness to share detailed information about sourcing and manufacturing is very limited if not driven by business aspects. Proposed change (if any): The difficulties to get such information (point 1-4) should be considered especially in cases where trypsin is not used in routine manufacturing and virus	Agreed. It is recognised that it may be difficult to obtain information on all the points listed. According to Ph. Eur. 5.1.7 the listed factors are examples and it remains a case-by-case decision which factors are relevant. The wording has been modified according to Ph. Eur. 5.1.7. The risk assessment should follow the general principles outlined in Ph. Eur. 5.1.7 Viral Safety. Such risk analysis ~~should consider~~ takes into consideration relevant factors, for example:

Line no.	Stakeholder	Comment and rationale; proposed changes	Outcome
		contamination is unlikely because of the analysis of the aspects considered in points (5) to (8).	
74-76	Vaccines Europe	Comment: The guideline states that '*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*.' Does the EMA expect to receive Certificates of Origin for this reagent? Is it sufficient if the received Certificates of Analysis from the suppliers indicate the origin? Proposed change: Please clarify which documents are expected.	Agreed. Certificates of origin might be accepted. However no modification of the Guideline text was found necessary.
75		Comment: "…or equivalent" is too vague. Pigs can be checked in some developing countries and found fit for consumption but the standards may still be a lot lower than those of EC. Difficult to evaluate. Proposed change: Add a precision such as " …or at least equivalent"	Not agreed. The wording "equivalent" is considered sufficient to indicate that the standards should not be significantly lower. The same wording has been used in Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3)
82-98		Comment: This paragraph discusses several limitations when considering virus testing during production of porcine trypsin, however it is not clear where the limitations begin and end within this discussion. Reorganization of	Agreed. The paragraph has been re-structured.

Line no.	Stakeholder	Comment and rationale; proposed changes	Outcome
		the main points should be considered as detailed below. Proposed change: Propose to re-organize the limitations more succinctly, and how these limitations drive the need for more comprehensive virus testing that follows in the next paragraph.	
93 –94		Comment: The fact that trypsin is known to inactivate many viruses has been recently reported in the publication from C Markus-Sekura (Carol Marcus-Sekura et al - Biologicals 39 (2011) 359 – 369) without providing scientific data nor references scientific article. It is the first time that this statement is used to contribute to support the viral safety of the trypsin of porcine origin used as raw material. Proposed change: Please add bibliographical references to support this statement.	Accepted. It is recognised that the available literature about inactivation of viruses by trypsin is rare and that few has been published about porcine viruses. Trypsin may have inactivating effects, activating effects, or no significant effect on infectivity of virus particles. Moreover, studies performed with purified trypsin are not directly relevant to product intermediates containing mixtures of pancreatic enzymes. Therefore it is considered difficult to make statements on inactivation of specific viruses in this Guideline. Stability of PPV and PCV in trypsin has been reported by several parties and it is found helpful to mention this in the Guideline. Modification: In addition, the pancreatic enzymes and their activity under exact conditions (pH temperature) might have an influence on infectivity of virus particles while other viruses such as porcine parvovirus (PPV) and PCV are not affected by the enzymatic activity of trypsin. ~~trypsin itself is known to inactivate many viruses, e.g. retroviruses, due to its intrinsic enzymatic~~

Line no.	Stakeholder	Comment and rationale; proposed changes	Outcome
			~~activity. However, this activity is dependent on the exact pH and conditions of manufacture~~
104-106		Comment: This sentence recommends a "general *in vitro* test that uses two distinct cell lines, one of which should be of human or primate origin (e.g. Vero) and the other of porcine origin." This statement provides an example of the primate cell line but does not provide any direction for the human cell lines that are suggested. It also does not provide any direction that would help to determine if the human or primate origin would be best to use. Generally speaking, 9CFR113.53 provides recommendations on how to perform the infectivity tests using these different cell lines. This regulation is widely used in the biopharma industry and it would be important that the European guideline being developed keeps as much consistency with it. Proposed change: Propose to add at least one example of a human cell line and provide direction on making a determination of which to choose, human or primate. That way one could expect a consistent approach in detector cell line determination. Consider modifying the sentence as follows: "…and the other of porcine origin (as recommended per 9CFR113.53)."	Not agreed. There is currently no sufficient scientific database which would justify a recommendation for a distinct human cell line. Nevertheless the option for a human cell line has been included in order to allow manufacturers flexibility to develop/design their own appropriate assays. Regulation 9CFR113.53 is recognized as a widely used test. The proposed is in line with following the 9CFR1553.51 regulation and is not in contradiction to this procedure.

Line no.	Stakeholder	Comment and rationale; proposed changes	Outcome
106-107		Comment: This sentence states that the cell lines used for testing adventitious agents "should be capable of detecting haemadsorbing viruses and cytopathic viruses." These endpoints are not specific and, even some critical viruses such as porcine parvovirus (PPV) and porcine circovirus (PCV) could be missed. Proposed change: Propose to use more specific endpoints in this sentence such as Fluorescent Antibody staining or PCR targeting some specific viruses of concerns such as PPV, PCV and Hep-E. It would also be very useful to provide more guidance about the following testing details: amount of trypsin powder to be tested (eg, 5g, 10g, etc), duration of the test (eg, 21 or 28 days; culture sub-passages during the culture period (eg, at least 2 passages). This would ensure consistency in testing and data generated.	The purpose of this test is non-specific general virus screening and not testing for specific viruses. HEV is unlikely to grow on the suggested indicator cells, so a read out for HEV would make no sense. A read out for PCV and PPV could be performed if the risk analysis indicates that specific tests should be performed. As outlines in lines 112-114, "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)". Defining a specific amount of tested sample (as in 9CFR113.53) does not make sense if the size of the whole batch has not defined.
109 - 110		Comment: The risk analysis should be performed virus by virus, to establish the list of specific viruses to test based on Risk assessment, taking into account the manufacturing process, the sensitivity of in vitro test and the use of the medicinal product. Proposed change: Please consider the following text revision:	Partly agreed. It is possible to use "risk management methodology" but this will not be required.

Line no.	Stakeholder	Comment and rationale; proposed changes	Outcome
		"….considering more documents where relevant (e.g. WHO 2010, Ph Eur 5.2.3.). The list of virus to test should be established using Risk management methodology, performed virus by virus, and taking into account the whole manufacturing process, the sensitivity of in vitro test and the final use of the medicinal product. For example, …	
114-117		Comment: This sentence indicates that a trypsin batch should not be used if an infectious virus is detected unless it can be demonstrated that the virus will be reliably inactivated or removed. It does not provide any possible solutions on acceptable ways to inactivate or remove a virus. Proposed change: Suggest adding language in this statement that would bring up the possibility of nano filtration as a solution.	Accepted. Option for nanofiltration has been introduced.
125-134		Comment: For the manufacture of trypsin it discusses virus reduction steps but it does not list any of them specifically. Proposed change: Suggest adding nano filtration as a possible solution for a step that could be added to the process to reduce or remove viruses	Accepted. It is not the intention of this guideline to request a specific virus inactivation/removal method as this could preclude development of novel methodologies (see comments above). Virus filtration (nanofiltration) has been added to the list of applicable methods.

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129-130		Comment: Inactivation treatments of biological materials by gamma irradiation are performed with a range of doses. It is not clear on what basis the value of 45 kGy is proposed. For example, a minimum of 25 kGy is required for gamma irradiation of serum for human use (required by USP1024 on serum) and 30kGy is required by EMA guideline for serum used for veterinary products (CPMP/BWP/1793/02). Experience gathered for years and literature data point out that trypsin irradiation should be efficient at doses between 25 and 35 kGy. Moreover this range is aligned with those required by Ph. Eur. 5.1.1. and ISO 11137-1. It is therefore proposed to put a range between 25-45 kGy. Proposed change: Please remove reference to "45 kGy" or modify sentence as follows: "In addition, a final pathogen inactivation step such as gamma irradiation (~~45 kGy~~ 25-45 kGy), e-beam irradiation, or UV irradiation can be applied."	Not agreed/partially agreed. PH Eur. 5.1.1 and ISO 11137-1 have been designed for viable microorganisms and not for virus particles. Many viruses are resistant to 25-30 kGy. An upper limit which can be applied to trypsin has so far not been explored. A minimum of 30kGy has been specified in CPMP/BWP/1793/02 in order to inactivate a reasonable range of viruses including pestiviruses. However trypsin might tolerate higher doses. 25 kGy is not sufficient to effectively inactivate all viruses and even 45kGy might not be sufficient for complete inactivation of very small viruses such as PPV and PCV. 30Gy should give reasonable inactivation of a broad range of viruses. However, recognizing the limitations of irradiation steps, this guideline advises a second step for virus inactivation.
142-175		Comment: The option of nano filtration is not mentioned in this section. Proposed change:	Agreed (see comments above).

Line no.	Stakeholder	Comment and rationale; proposed changes	Outcome
		Suggest adding nano filtration as a possible step that can be used in the trypsin manufacturing process at the end.	
155-156		Comment: Porcine parvoviruses are not resistant to pH treatment (12h at pH2). Validation data from trypsin suppliers have demonstrated that PPV is very sensitive to this pH treatment with a log reduction of more than 7. Proposed change: Modify sentence as follows: "For irradiation steps and for low pH steps also, an animal parvovirus, e.g. porcine parvovirus, should be included in the study ~~as these are pH resistant and have relatively low sensitivity to gamma irradiation~~ as these viruses are small DNA non enveloped viruses considered to be resistant to physico and chemical treatments and often used as model viruses for inactivation treatments."	Partially agreed: Efficient inactivation (7 logs!) of parvoviruses at pH2 has not been repeatedly/convincingly published/demonstrated. Paroviruses belong to viruses that are most resistant against low pH. It is recognised that parvoviruses are not the most resistant viruses for gamma or UV irradiation. Nevertheless, the proposed wording is considered appropriate. Sentence modified as follows: "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 are pH resistant and have relatively low sensitivity to gamma irradiation~~ as these viruses are small DNA non enveloped viruses considered to be resistant to physico- and chemical treatments."
157		Typo: lyophilized instead of lypohylized	Accepted.
163-169		Comment: This paragraph does not appear to add value to this guideline. Proposed change: We would propose removing this paragraph	Not agreed. Spiking of virus into lyophilized preparations is considered critical.
184-186		Comment:	Not agreed. The limitations (i.e. BSE risk from bovine trypsin

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		Making a reference to bovine trypsin as an alternative material to porcine trypsin reads strange. Proposed change: Suggest removing the example or to describe cases when this alternative may be envisaged.	as well as the virus risk) have been outlined below in the same section.
204-205		Comment: The risk assessment should take into account the testing of trypsin itself. It should describe the manufacturing stages evaluated. Proposed change: Consider revising item 2 (line 204) as follows: "the availability of suitable virus test methods and the stage at which such testing is implemented, for instance on donor animals, on the raw material, during production and on final batches of trypsin …"	Partially agreed. The risk assessment addresses the medicinal product and not only the porcine trypsin and therefore the testing is not limited to testing of the trypsin itself. In addition, There are not live donor pigs for porcine pancreas. Modification: Clarification will be given: (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 any other stage from production of the medicinal product).
212-217		Comment: The guideline recommends: *"The MAH 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 CHMP/BWP/268/95."*	Partially agreed. It is agreed that the recommendation in the guideline necessitates cooperation from reagent manufacturers to access key information allowing for comprehensive risk evaluation and subsequent submission to Competent Authorities. The information is considered essential for the manufacturer of a medicinal product in order to allow a comprehensive risk assessment. The cited ICH Guidance (ICHQ5A) is not applicable to all medicinal products which are in the scope of

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		This recommendation implies a significant data package submitted as part of the initial MAA specifically for trypsin reagent, including a risk assessment for using the reagent. Given that adventitious agent testing is performed to support the risk/benefit profile of the medicinal product in accordance with ICH and safety testing is performed routinely for in-process controls of biological products and as release tests, it is difficult to discern the need for a separate substantial data package supporting the trypsin reagent. This is complicated as manufacture and/or testing details performed by a supplier are often restricted by proprietary rights to confidentiality in which the MAH may not have full access to such data packages. This recommendation in the guideline necessitates cooperation from reagent manufacturers to access key information allowing for comprehensive risk evaluation and subsequent submission to Competent Authorities. Proposed change: Clarification on the magnitude and type of data (beyond the virus testing, and assay parameters suggested) to be provided to the competent authority on the reagent alone would be appreciated. We suggest that only a summary of the tests and validations performed to allow a proper risk	this guideline.

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		management is provided in MAAs. Detailed raw data would be available on request. In addition, does the EMA foresee that this risk assessment should be discussed in the Risk Management Plan as a potential medicinal product risk?	Inclusion of the risk assessment for porcine trypsin in the risk management plan is not necessary.
218-220		Comment: The meaning of "prospective implementation" is not clear. Proposed change: Please clarify what the expectations are for the application of these requirements.	Accepted. Clarification will be given: This guideline is for prospective implementation, i.e. for new marketing applications.
76 - 78	ISIA	Comment: ISIA believes that the requirement for specific labeling would be very difficult to implement, but the requirement for traceability of all batches should be required. In addition, the USDA does not issue health certificates for materials shipped within US boundaries. It is not clear from this guidance what the health certificate should attest to. Also, the USDA cannot attest to the inspection of animal products in accordance to EU conditions. Within the EU Veterinary Certificates do not commonly accompany raw materials. With regard to material imported into the EU, there is no health certificate required for enzymes such as trypsin. In fact enzymes are often excluded because of Tariff Code 3507.	Accepted Batches of raw material should be accompanied with appropriate official health certificates or equivalent appropriate documentation.

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152		Comment: ISIA believes that the requirement for two methods of inactivation when either one has been validated in the literature is unacceptable. Porcine parvovirus is the virus of choice for demonstrating inactivation as it is the most difficult to inactivate and hence should be a part of any inactivation study. Viral Inactivation studies must be performed by the trypsin manufacturer, and should occur post manufacturing to ensure effective management of source availability and risk management	Not agreed. As outlined in this Guideline, various/multiple procedures for virus inactivation/removal can be applied to trypsin. Given the many limitations on sourcing and testing of starting materials, application of virus inactivation/removal methods to trypsin is a key element for producing safe trypsin.
152		Comment: Gamma or UV-c irradiation: ISIA would not recommend using these methods on lyophilized material even given the recommendation for treatment prior to irradiation.	Partially agreed. Performing UV-C on lyophilized powder has not been recommended in this draft guideline. The difficulties with gamma irradiation have been outlined. Modification: Gamma irradiation is generally performed on ~~the lyphylized trypsin powder or~~ frozen liquid trypsin. Applying irradiation to lyophilised powder requires careful investigation of virus inactivation.
185		ISIA would recommend removing bovine trypsin from this list	Not agreed. Requirements on virus safety and prion safety have been outlined.
39	SciencePharma	Comment: A list of the most problematic viruses specific/typical to porcine trypsin regarding their virulence or difficulty in	See also comments above.

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		inactivation could be provided, with addition of general elimination recommendations.	
67-71		Proposed change: It is prepared as a powder or liquid solution for use as a reagent in cell culture and as a protein processing reagent. ~~The~~ 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. Higher purified trypsin preparations are available for ~~analytical purposes and~~ other applications in protein chemistry, e.g. as a protein processing reagent.	Accepted.
82-84		Comment: It would be highly advisable to describe how and in what range the drug product manufacturer should verify the manufacturer/supplier of trypsin, regarding safety. Regarding the above, what data/documents should be provided in a MA application dossier. Could an adequate declaration be sufficient?	This is considered in Section regulatory Aspects.
118-121		Comment: It is noticed that the information provided in this paragraph are in contrast to the recommendations given in lines 88-90 that "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	Accepted. The statement saying that "many viruses are inactivated by trypsin has been revised. A clear recommendation to perform in vitro testing has been given.

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		considered appropriate". A clear recommendation should be provided.	
130-132 150-151		Comment: It could be clarified which type/method of virus reduction step can be recognized as complementary. It is considered that in some situations/cases one reduction step could be sufficient. What analysis should be performed and what data should be provided for justification to use one reduction step?	Not agreed. No further clarification of the term 'complementary' is considered necessary.
206-209		Comment: Exemplary issues to be considered when conducting a risk assessment are recommended to include use of trypsin as protein processing reagent. Proposed change: (5) the stage of manufacture of the active substance or the medicinal product at which trypsin is used as a reagent, (6) the risk of virus replication in the course of the manufacturing process of the active substance or in cell cultures used for production of the medicinal product, (7) additional virus inactivation/removal steps applied during the manufacture of the active substance or the medicinal product, (8) the amount of trypsin to produce a dose of active substance or medicinal product	Not agreed. It is not clear to what the term "active substance" refers to (i.e trypsin preparation or "active drug substance" component of the medicinal product). As outlined in this section, the risk assessment is performed by the manufacturer of the medicinal product and addresses the specific medicinal product including manufacture of the drug substance.
211-201		Comment: It would be advisable to add relevant information on range of data required in case of a change of supplier/manufacturer of trypsin as material for which	Agreed clarification is given. In the case of a change of supplier of trypsin, data as outlined

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		an assessment is required of viral safety.	above should be provided for the new trypsin.
77/78	Abbott Laboratories GmbH	Proposed change: Batches of raw materials should be accompanied with official health certificates or equivalent appropriate documentation. Comment: According to Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption effective since April 2011together with its executive law Reg. (EC) 142/2011 a "veterinary health certificate" is not foreseen for intra-European trade. The only legally required documentation which has to be provided with each consignment is a trading document containing information on the origin of the material and species, i.e. mainly traceability related information. Any additional statement with the character of an official veterinary certificate e.g. statements about freedom of certain notifiable diseases is redundant since this general quality attribute of the animal-by product is already covered by the fact that EU slaughterhouses are approved by the relevant national authorities and are operating under supervision of these authorities in compliance with applicable EU legislation regarding notifiable animal diseases, slaughter hygiene and animal by-products legislation. For that reason for intra-EU deliveries the trading document, in some cases	Accepted: Batches of raw materials should be accompanied with official health certificates or equivalent appropriate documentation.

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		also called delivery document is regarded as equivalent, ensuring the same quality and full traceability.	
114		Comment: The term infectious virus is not clear. Proposed change: The term should be replaced by "human pathogenic virus".	Not agreed. It is difficult to predict whether an animal virus, which has been generally classified as "non human pathogenic",will be pathogenic to humans in the context of medicinal products (i.e considering intra venous or intra cranial application).
130		Comment: No rationale or reference for the suggested irradiation of 45 kGy is provided. Proposed change: Delete "45 kGy" or add a reference/justification for this number.	See comments above.
140/141		Comment: ICHQ5A consistent term "virus clearance" should be used. Proposed change: "Validation of the virus clearance capacity of the manufacturing process"	The term virus inactivation/removal will be used in order to be consistent with other European guidelines (CPMP/BWP 268/95) and EMA/CHMP/BWP/706271/2010).
143 / 144		Comment: The text mixes the terms "virus-reducing capacity", Inactivation/removal of microbiological agents", and	Agreed.

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		"pathogen reduction". It should be clarified that validation of process steps with respect to pathogen reduction only applies to viruses and not to bacteria or fungi. Proposed change: "Virus inactivation / removal steps are considered a major factor contributing to adventitious agent safety of trypsin. Therefore, selected process steps should be validated with respect to their virus inactivation or removal capability."	Inactivation/removal of microbiological agents is considered a major factor contributing to adventitious agent safety of trypsin. Therefore, selected process steps should be validated with respect to their virus inactivation/removal capacity. ~~pathogen reduction.~~
51	EBE (European Biopharmaceutical Enterprises)	Comment: "…exposure of patients to adventitious viruses." Proposed change: We suggest adding: "…exposure of patients to adventitious viruses **or other non-viral adventitious agents.**"	Agreed.
72ff.		Comment: Should some counties or regions be excluded due to the presence of disease related to the porcine population? Proposed change: Please clarify.	No general recommendation can be given.
74-76		Comment: The guideline states that '*pancreatic glands shall be derived from pigs fit for human consumption following ante- and post mortem inspection in*	Same comment as from Vaccines Europe, see above.

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		accordance with European Community or equivalent (third country) conditions.' Does the EMA expect to receive Certificates of Origin for this reagent? Is it sufficient if the received Certificates of Analysis from the suppliers indicate the origin? Proposed change: Please clarify which documents are expected Please clarify what "equivalent" means and give reference/ source for the "conditions" if possible.	
82-98		Comment: This paragraph discusses several limitations when considering virus testing during production of porcine trypsin, however it is not clear where the limitations begin and end within this discussion. Reorganization of the main points should be considered as detailed below. Proposed change: Propose to re-organize the limitations more succinctly, and how these limitations drive the need for more comprehensive virus testing that follows in the next paragraph.	Same comment as from Vaccines Europe, see above.
93–94		Comment: the fact that trypsin is known to inactivate many viruses has been recently reported in the publication from C Markus-Sekura (Carol Marcus-Sekura et al - Biologicals 39 (2011) 359 – 369) without providing scientific data nor references scientific article. It is the	Same comment as from Vaccines Europe, see above.

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		first time that this statement is used to contribute to support the viral safety of the trypsin of porcine origin used as raw material. Proposed change: please add bibliographical references to support this statement.	
104-106		Comment: This sentence recommends a "general *in vitro* test that uses two distinct cell lines, one of which should be of human or primate origin (e.g. Vero) and the other of porcine origin." This statement provides an example of the primate cell line but does not provide any direction for the human cell lines that are suggested. It also does not provide any direction that would help to determine if the human or primate origin would be best to use. Generally speaking, 9CFR113.53 provides recommendations on how to perform the infectivity tests using these different cell lines. This regulation is widely used in the biopharma industry and it would be important that the European guideline being developed keeps as much consistency with it. Proposed change: Propose to add at least one example of a human cell line and provide direction on making a determination of which to choose, human or primate. That way one could expect a consistent approach in detector cell line	Same comment as from Vaccines Europe, see above.

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		determination. Consider modifying the sentence as follows : "…and the other of porcine origin (as recommended per 9CFR113.53)."	
106-107		Comment: This sentence states that the cell lines used for testing adventitious agents "should be capable of detecting haemadsorbing viruses and cytopathic viruses." These endpoints are not specific and, even some critical viruses such as porcine parvovirus (PPV) and porcine circovirus (PCV) could be missed. Proposed change: Propose to use more specific endpoints in this sentence such as Fluorescent Antibody staining or PCR targeting some specific viruses of concerns such as PPV, PCV and Hep-E. It would also be very useful to provide more guidance about the following testing details: amount of trypsin powder to be tested (eg, 5g, 10g, etc), duration of the test (eg, 21 or 28 days; culture sub-passages during the culture period (eg, at least 2 passages). This would ensure consistency in testing and data generated.	Same comment as from Vaccines Europe, see above.
109 - 110		Comment: the risk analysis should be performed virus by virus, to establish the list of specific viruses to test based on	Same comment as from Vaccines Europe, see above.

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		Risk assessment, taking into account the manufacturing process, the sensitivity of in vitro test and the use of the medicinal product. Proposed change: Please consider the following text revision: "….considering more documents where relevant (eg WHO 2010, Ph Eur 5.2.3.). **The list of virus to test should be established using Risk management methodology, performed virus by virus, and taking into account the whole manufacturing process, the sensitivity of in vitro test and the final use of the medicinal product.** For example, …	
114-117		Comment: This sentence indicates that a trypsin batch should not be used if an infectious virus is detected unless it can be demonstrated that the virus will be reliably inactivated or removed. It does not provide any possible solutions on acceptable ways to inactivate or remove a virus. Proposed change: Suggest adding language in this statement that would bring up the possibility of nano filtration as a solution.	Same comment as from Vaccines Europe, see above.
116-117		Comment: The risk assessment might not prevent the contamination to spread to different equipment and facilities even if initial contamination might be	Clarification will be given: Generally, if an infectious virus contaminant is detected, the trypsin batch should not be used for the manufacture of

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		eventually removed or inactivated by the downstream process. Thus a contaminated trypsin batch should not be used at all or it should be stated that the scope of the risk assessment should ensure implementation of preventive measures to avoid contamination in the facility where needed.	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 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 raw materials.
118 - 121		Comment: None Proposed change: The possibility should be added to test cell lines that are used for medicinal products and which have been exposed to porcine trypsin by appropriate viral tests where information on adventitious agents testing of the trypsin is not available.	Accepted. This option has been considered in the section risk assessment: Modified text. (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 from production of the medicinal product.
124		Comment: Are there any requirements for the proposed manufacturing inactivation proposals, e.g., UV-light? Proposed change: We suggest adding a phrase that inactivation claims should be based on data.	Accepted. Requirement for validation data has been clearly outlined in Section 8 of this Guideline.
125-134		Comment: For the manufacture of trypsin it discusses virus reduction steps but it does not list any of them specifically.	Agreed. The option of nanofiltration has been mentioned. (see comments above).

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		Proposed change: Suggest adding nano filtration as a possible solution for a step that could be added to the process to reduce or remove viruses Add an additional sentence to line 129 before "In addition, …": "It was demonstrated that both porcine parvovirus and PCV were significantly inactivated by a low pH of 1.7 (*Yang et al. and Kreil et al., 2013 PDA Europe: Virus and TSE Safety Forum*)	Production frequently includes a prolonged incubation at low pH. Data presented at conferences (Yang et al. 2013 and Lackner et al. 2014) indicate that PCV and PPV might be significantly inactivated during prolonged incubation at pH 1.7, room temperature.
130		Comment: Inactivation of biological material by gamma radiation is generally performed within a range of doses from 25 – 40 kGy. Proposed change: Refer to a range of doses or refer to case by case evaluation of the dose based on the biological material being inactivated.	See comments above.
131		Comment: It is not clear why two complementary virus reduction steps are required. Proposed change: Change the sentence on line 131 "it is advisable to incorporate two complementary virus reduction steps, …" to "it is advisable to incorporate at least one virus reduction step, …".	Not agreed. As explained in the guideline and is response to comments above, there are many limitations at sourcing and testing of materials. Therefore robust concept for virus inactivation is considered a key element for safety of trypsin. The robust nature of trypsin and small size of trypsin allows application of multiple virus inactivation/removal steps.

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142		Comment: First sentence should be edited as suggested below. Proposed change: "Inactivation/ removal of microbiological agents should be considered a significant method to reduce the risk of adventitious agent safety of trypsin."	Not agreed. See comment above.
155		Comment: Porcine parvoviruses are not resistant to pH treatment (12h at pH2). Validation data from trypsin suppliers have demonstrated that PPV is very sensitive to this pH treatment with a log reduction of more than 7. Proposed change: Please remove "..as these are pH resistant and have relatively low sensitivity to gamma irradiation" and replace by "as these viruses are small DNA non enveloped viruses considered to be resistant to physico and chemical treatments and often used as model viruses for inactivation treatments"	Same comment as from Vaccines Europe, see above.
163-169		Comment: This paragraph does not appear to add any value to this guideline. Proposed change: Suggest removing it.	Same comment as from Vaccines Europe, see above.
184-186		Comment:	Same comment as from Vaccines Europe, see above.

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		Making a reference to bovine trypsin as an alternative material to porcine trypsin reads strange. Proposed change: Suggest removing the example or to describe cases when this alternative may be envisaged.	
203		Comment: It is not clear what is meant with epidemiology and control of the animals. Proposed change: The wording "epidemiology and control of the animals" should be replaced by "confirmation that the pigs were fit for human consumption" in order to be in line with section 5 of this draft guideline.	Not agreed. Not all viruses are excluded when declaring pigs as 'fit for human consumption".
204-205		Comment: The risk assessment should take into account the testing of trypsin itself. It should describe the manufacturing stages evaluated. Proposed change: Consider revising item 2 (line 204) as follows: "the availability of suitable virus test methods and the stage at which such testing is implemented, for instance on donor animals, on the raw material, during production and on final batches of trypsin …"	Same comment as from Vaccines Europe, see above.
212-217		Comment: Please give details on the magnitude and type of data	Same comment as from Vaccines Europe, see above.

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		expected to be included in the data package for assessment by the competent authorities. As stated in the general comments it might be problematic for the MA holder to provide such information on the basis of proprietary rights.	
217		Comment: "CHMP/BWP/268/95" Proposed change: Change to: "**CPMP**/BWP/268/95"	Accepted.
218-220		Comment: The meaning of "prospective implementation" is not clear. Proposed change: Please clarify what the expectations are for the application of these requirements.	Same comment as from Vaccines Europe, see above.