30 May 2013
EMA/CHMP/BWP/596/2013
Committee for Medicinal Products for Human Use (CHMP)

Overview of comments received on 'Guideline on the use of bovine serum in the manufacture of human biological medicinal products' (EMA/CHMP/BWP/457920/2012)

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

Stakeholder no.	Name of organisation or individual
1	International Serum Industry Association
2	Life Technologies Ltd
3	Merck Sharp & Dohme (MSD)
4	Dr WBR Rolleston - South Pacific Sera Limited
5	IPFA

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1. General comments – overview

Stakeholder no.	General comment (if any)	Outcome (if applicable)
1	**#1 International Serum Industry Association** Members of the International Serum Industry Association (ISIA) are collectors, processors, and users of animal serum and animal derived products. These products are used extensively in the growth of cells in culture for research and in the production of diagnostic kits, vaccines, and other medicinal products. It is estimated that ISIA members provide more than 90% of the global supply of animal serum and animal derived products for the above technical uses. http://www.serumindustry.org **#2 Technical Use of Fetal Bovine Serum** The Fetal Bovine Serum (FBS) used in research and bio-manufacturing applications is a by-product of the meat industry. The revenue received by the meat industry for beef by-products for technical use is *extremely* small (less than 1% in total) compared to the revenue obtained from beef products for human consumption. Therefore, the meat industry has little financial incentive to change their production methods. The cost to manage BVDV would far outweigh the cost of the disease in the management of herds. As a result BVDV continues to be an unavoidable infectious agent. Moreover, this situation is made more challenging due to the high levels of antibody to BVD virus naturally present in many types of bovine sera in common use for biopharmaceutical applications. **#3 ISIA Philosophy**	The comments are noted.

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	ISIA appreciates the logical and judicious approach taken by the panel in not recommending aggressive new testing methods for which the outcomes are not yet clear. **#4 Proposed Changes** 1. We believe that the removal of the Serum Inhibitory Test will be beneficial to serum suppliers and end-users as it simplifies BVDV testing requirements and brings the new CHMP version into line with the current CVMP version and the EP Bovine Serum Monograph # 2262. 2. The ISIA requests the inclusion of a Risk Assessment provision, as detailed in the current CVMP and the EP Bovine Serum Monograph #2262, that will permit the use of sera with very high or unquantifiable levels of anti-BVDV masking antibodies where BVDV can be shown to be of negligible risk in the use of the medicinal product. This harmonization will reduce confusion. 3. The ISIA requests the inclusion of guidance on the test method(s) applicable for the detection and quantification of anti-BVDV masking antibodies as required by New Section 7.3.4. In discussion it is apparent that there is a great deal of confusion surrounding the interpretation of the various antibody tests that are currently performed. It is the opinion of the industry that the marketplace would be best served by standardization of this testing by the validated alpha serum neutralization test method where dilutions of virus are	Reference to the request of an assessment is maintained and the text 'and on the validation process' has been included on line 192 to bring this into line with the referred CVMP guideline. It is not the intention to provide specific recommendations on the test methods for BVDV antibodies.

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	exposed to undiluted serum with the result expressed as the amount of virus ($TCID_{50}$) neutralized by 1 ml of test serum." The ISIA will be recommending to its membership that this become the test of choice. Additional proposed changes from ISIA have been inserted in Table 2. on specific comments to the text. **Points for Clarification** ISIA requests clarification on the following points: Line # 189: The ISIA requests clarification of the selection of donor serum as an example in line 189 when newborn calf serum has naturally higher levels of masking antibody to BVDV. Line # 172: The ISIA requests clarification of the reason for the reference to Section 7.3.5. At this time very few laboratories are able to perform testing for Polyoma, and the test is therefore not readily available or validated. ISIA would hope that the EMA would also reissue the **EXPLANATORY NOTE ON THE TESTING OF BOVINE SERUM FOR BOVINE POLYOMA VIRUS** as was done in 2003.	Agreed. The reference to donor serum has been deleted. EMA acknowledges that current infectivity assays for bovine polyoma virus are difficult to interpret and are not widely available. As a consequence, it is not the intention of the EMA to require, for the time being, testing of bovine serum for bovine polyoma virus. The statement on bovine polyoma virus has been included to inform serum manufacturers and users about BPYV and to forewarn the that when a suitable infectivity assay or more information about contamination events becomes available, the Authorities may review their position. We do not considered necessary to reissue the explanatory note on the testing of bovine polyoma virus at this stage because the information it contained has been incorporated in the revised guideline.
2	**#1:Life Technologies Corp.**	The comments are noted.

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	Life Technologies Corporation is a leading global supplier of animal sera to both the laboratory research market and to manufacturers of human and veterinary biological medicinal products. Such uses of animal sera are typically classified as “Technical Use” by veterinary control regulations. **#2:Technical Use of Bovine Sera** Bovine Serum used in research and bio-manufacturing applications is a by-product of the meat industry. The revenue value obtained by the meat industry for beef by-products for technical use is insignificant (probably less than 1% in total) compared to the revenue obtained from meat products for human consumption. The cost of eliminating BVD virus from bovine herds would far outweigh the benefit to the meat industry. Therefore, the meat industry has little financial incentive to change their production methods to eliminate problems relating solely to product for technical use. As a consequence, BVD virus will continue to be an unavoidable infectious agent in bovine sera for technical use. Moreover, this situation is made more challenging due to the high levels of antibody to BVD virus naturally present in many types of bovine sera in common use for biopharm applications. **#3: Proposed Changes** We believe that the removal of the Serum Inhibitory Test will be beneficial to both serum suppliers and end-users as it simplifies BVD virus testing requirements and brings the proposed CHMP version into	

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	line with both the current CVMP guideline CVMP/743/00-Rev.2 and the European Pharmacopoeial Monograph #2262 “Bovine Serum”. **#4: Continuing Special Focus on Bovine Polyoma Virus** We appreciate the logical and judicious approach taken by the panel in not recommending aggressive new testing methods for potential adventitious agents for which the outcomes are not yet clear. However, since publication of the consultation document, we have seen confusion amongst serum users caused by the continuing reference to Section 7.3.5 on line 172 and hence we suggest the panel give clarification of its general intent. In addition, we continue to be concerned about the continuing inclusion in the guideline of the special focus on Bovine Polyoma Virus when the required testing methods for this agent are still not widely commercially available, and when the overall costs and benefits of such testing are not clearly understood. To minimise confusion amongst stakeholders, we believe it would be beneficial to the EMA to take the opportunity either to remove the references to Bovine Polyoma Virus or to extend Section 7.3.5 to include the guidance given in the Explanatory Note # EMEA/H/5075/03 issued in November 2003 which we believe remains relevant today.	The comment is acknowledged. A footnote in section 7.3.5 has been included (see comment above).
3	Bovine Viral Diarrhoea Virus (BVDV), a common infection of cattle, is readily horizontally and vertically transmitted. As such, there is a risk of infectious BVDV being present in fetal bovine serum, bovine calf serum, and adult bovine serum. To this end, every lot of serum used	Agreed. The guideline now states: “*The assay should be suitable to detect cytopathogenic as well as non-cytopathogenic BVDV strains and staining of cell cultures with fluorescent antibody (FA) is recommended.*“

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	to manufacture human vaccines is (1) treated to inactivate adventitious agents (e.g. gamma irradiated) and (2) tested for the presence of infectious BVDV in cell based assays prior to inactivation. Most strains of BVDV are cytopathic, however, there have been reports of BVDV strains that do not cause CPE in cell culture. For this reason, 9CFR 113.53 requires testing the final culture via fluorescent antibody (FA) for BVDV. Since BVDV antibodies are broadly cross reactive (Veterinary Virology, 3rd edition, Academic Press, San Diego, CA (USA), p. 563-564), FA testing on the final cultures will detect a broad range of BVDV strains in the absence of CPE, providing the Agree necessary confidence that infectious BVDV is not entering the vaccine manufacturing process. Instead of instituting PCR analysis for BVDV detection, we instead recommend that FA testing is included in the guideline.	
4	The following general comments are made to provide context and background to the specific comments on sections 7.3.3. and 7.3.4. The paragraph containing lines 92-100 in section 4 (Types and Sources of Serum) appear to apply exclusively to Donor Bovine Serum however the comments on exotic diseases are just as applicable to abattoir derived serum. There is no rationale for the recommendation not to vaccinate donor herds against BVD or recognition that BVDV antibody free serum is achievable through the use of specifically controlled donor herds. There is a risk manufacturers may misinterpret the intent of this statement.	This has been revised. Requirements for exclusion of exotic diseases refer to both abattoir derived as well as donor herd derived serum. Some additional wording will be introduced: “It is recommended not to vaccinate these herds against BVD, in order to prevent any impact of vaccine-derived antibodies on the herd health control strategy

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	At the same time this section of the guideline provides no explanation as to why the “health status of the Donor Herd should be well defined and documented”. The implication is that the herd status of abattoir derived serum does not have to be well defined and documented. In other words a higher standard is being applied to Donor Bovine Serum than that applied to abattoir derived serum without any indication that this would provide added protection to the manufacturer. The guideline should provide guidance to the manufacturer which enables them to appreciate the opportunity to reduce risk in relation to viral contamination from viruses such as BVD by using Donor Bovine Serum from herds of animals controlled for BVD virus and antibody. The EP monograph on Bovine Serum 01/2008:2262 is instructive in this respect. Absence of BVD virus and antibody in specifically controlled Donor Bovine Serum overcomes the issues sections 7.3.3 and 7.3.4 are trying to address. We are not advocating removal of these important control steps from the guideline but the guideline should accurately reflect the lower relative risk of specifically controlled Donor Bovine Serum in comparison to serum collected from the abattoir with respect to BVDV.	The comments are noted. For abattoir derived serum such extensive control of the herds of origin is not possible. Animals from which serum is sourced at slaughter have to pass ante- and post mortem inspection and must be declared “fit for human consumption”. Recommendation of specific types of sera is not within the scope of this NfG. It has to be considered that age of the animals, from which the blood is sourced, has an impact on particular quality characteristics of the serum. Therefore choice of a particular type of serum has to be made by the manufacturers.
5	There is no comment on the scope not approach of this draft guideline. In line with the Guideline on the use of bovine serum for immunological veterinary medicinal products.	The comment is noted.

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
63	5	Comment: ”Serum that is used minimally” Proposed change (if any): ”Limited use of serum”	Accepted.
66	5	Comment: ” This guideline is applicable to serum manufactured after” A batch of serum is the result of manufacturing of the serum manufacturer Proposed change (if any): ” This guideline is applicable to the batches of serum manufactured after”	Accepted. The references to applicability of manufactured batches of serum have been removed from the text as this guideline does not request any stricter requirement compared with the previous version. The revised guideline is applicable to batches of serum manufactured after the date when this revised guideline comes into effect. Serum that has been manufactured before this date but which has not yet been used by the serum user, is outside the scope of this revised guideline and can be used by the serum user, provided that it complies with the previous version of the guideline.
66-67	5	Comment: “Serum which has been manufactured before this date but which has not yet been used by the serum user, is outside the scope of this guideline.” Some clarification is needed: Does this mean that serum batches which have been manufactured before this date can be used by the serum user? Proposed change (if any): “Serum batches which have been manufactured before this date are outside the	Accepted. See above.

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		scope of this guideline, and can be used by the serum user"	
155	5	Comment: IBRV stands for infectious bovine rhinotracheitis virus Proposed change (if any): infectious bovine rhinotracheitis virus (IBRV)	Accepted.
174	4	Comment: the statement in Section 7.3.3 which reads "BVDV is a highly prevalent infection of cattle and its presence in bovine serum cannot completely be avoided." is incorrect. BVDV can be avoided using donor herds which are specifically controlled for BVDV. South Pacific Sera has been producing such BVDV free serum for more than 15 years. Proposed change (if any): BVDV is a highly prevalent infection of cattle and its presence in bovine serum cannot completely be avoided except in serum from specifically controlled donor herds. In any case the presence of BVDV in a batch of serum should be tested before any viral inactivation/removal treatment is performed by an accepted assay for infectious virus.	Accepted.
177	5	Comment: "RT-PCR can be useful the detection of virus" Proposed change (if any): "RT-PCR can be useful the	Partly accepted. The text has been reworded to refer that: "*The assay should be suitable to detect cytopathogenic as well as non-cytopathogenic*

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		detection of virus (viral nucleic acid) "	*BVDV strains and staining of cell cultures with fluorescent antibody (FA) is recommended. Direct RT-PCR has limited value in the detection of infectious virus."*
178 - 180	2	Comment: We agree with the inclusion of the words "and must be below the level … for inactivation treatment". Gamma Irradiation is clearly the preferred inactivation method of choice for viruses including BVD virus. Industry experience shows that a highly contaminated lot of bovine serum would contain no more than $10^2 – 10^3$ $TCID_{50}$ virus particles per ml. Virus inactivation studies using spiked bovine serum have shown that a minimum dose of 30 kGys of gamma irradiation will inactivate at least 10^6 $TCID_{50}$ BVD virus particles per ml, ensuring that this new requirement can be met. Proposed change (if any): None	The comment is noted.
176 – 183	3	Comment: Direct RT-PCR analysis of serum is not a meaningful tool for deciding the disposition of a serum batch. Since BVDV is a common bovine infection, the presence of BVDV RNA in serum is expected. However, the presence of BVDV RNA cannot be correlated to the presence of infectious BVDV. RT-PCR analysis would only be meaningful as an infectivity assay input and endpoint readout. Since 9CFR already requires fluorescent antibody analysis at the end of the BVDV infectivity assay, and BVDV antibodies are broadly cross-reactive, incorporating RT-PCR into the BVDV	Partly accepted. See comment above (line 177).

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		infectivity assay is not needed. Every batch of serum is tested for infectious BVDV prior to inactivation. Any batch that is positive for infectious BVDV, regardless of concentration, is rejected and not used in vaccine manufacturing. Quantifying the level of BVDV via RT-PCR will not provide meaningful data since BVDV RNA cannot be correlated to infectious BVDV. Serum batch disposition should be based on the detection of infectious BVDV prior to inactivation, not on RNA levels. Proposed change (if any): To replace RT-PCR analysis by FA analysis	
182-183	1	The ISIA proposes the deletion of the words “and serum inhibition” from lines 182 and 183 since this test requirement has been deleted from the guideline.	Accepted.
182 - 183	2	Comment: The original requirement for a serum inhibition test has been deleted from the proposed revised guideline hence continuing reference to it is no longer relevant. Proposed change (if any): We propose the deletion of the words “and serum inhibition” from lines 182 and 183.	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
183	2	Comment: We propose the inclusion of a Risk Assessment provision, as detailed in the current CVMP and the EP Bovine Serum Monograph # 2262, that will permit the use of sera that naturally contain very high levels, sometimes higher than quantifiable levels, of anti-BVDV masking antibodies where BVD virus can be shown to be of negligible risk in the intended use of the medicinal product. This harmonisation will reduce confusion among end-users regards compliance. Proposed change (if any): Addition of the words: “No single measure, or a combination of measures, can guarantee complete viral safety but they can reduce the risk involved in the use of serum in the manufacture of medicinal products. It is therefore recommended for the manufacturer of a medicinal product to take account of this when choosing a serum for a particular use by making a risk assessment.”	Partly accepted. This has been taken care in the modification of sections 7.3.3. and 7.3.4.
181-187	1	The ISIA proposes that Lines 181-187 are already covered in section 7.3.4 and should be deleted	Partly accepted. Lines 184-187 have been deleted.
184-187	1	The ISIA proposes the deletion of lines 184 to 187 from New Section 7.3.3 since the requirements are covered by New Section 7.3.4.	Accepted.
184 - 187	2	Comment: The comments and recommendations in this paragraph are covered by lines 189 – 192 in the New Section 7.3.4.	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): We propose the deletion of lines 184 to 187 from New Section 7.3.3.	
184-187	5	Comment: Already mentioned in paragraph 7.3.4 Proposed change (if any): To be suppressed	Accepted.
189	2	Comment: Donor serum is highlighted as a special case for concern regards antibody masking of BVD virus but higher levels of antibody masking are routinely seen in other types of bovine sera hence it is not logical to put special focus on donor serum. Proposed change (if any): We propose the deletion of the words “such as donor serum”.	Accepted.
189	4	Comment: Rather than highlighting Donor Bovine Serum as a likely source of BVD antibodies, section 7.3.4 of the guideline should make it clear that BVD antibodies can be avoided in controlled donor herds. Proposed change (if any): Anti-BVDV antibodies can be avoided in specifically controlled donor herds. Anti-BVDV antibodies in bovine serum, such as abattoir derived serum and uncontrolled donor serum, may mask the detection of BVDV in an infectious virus assay.	Partly accepted. The sentence has been reworded to remove any reference to the type of serum or donor.
190	2	Comment: New Section 7.3.4 requires that a validated test be performed on bovine serum to detect /quantify any antibodies to BVD virus that may be present. However, as with the current guideline version, no	Not accepted. The guidance is intended to provide general guidance on the principles of the detection of anti-BVDV antibodies. It is not the

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		specific guidance is given regards test methodology or the interpretation of results. We believe this has caused confusion in the past and will lead to confusion in the future. Proposed change (if any): We propose that standardisation of the test methodology by specifying the use of the classical Virus Neutralisation method (also known as the Serum Neutralisation method) recommended by the OIE in the Manual of Diagnostic Tests 5th Edition 2004 Vol II Chapter 2.10.6 pp 1056-1057. In this test, dilutions of serum are exposed to a standard amount of virus to quantify the amount of neutralising antibody present.	intention to provide specific recommendations on the test methods. (see general comment #1)
189-195	3	Comment: Since BVDV is a common bovine infection, the presence of BVDV neutralizing antibodies is the serum is possible. If neutralizing antibodies and infectious BVDV are present in the serum, the infectious BVDV may not be detected in the standard cell-based adventitious agent test methods thereby introducing infectious BVDV into the vaccine manufacturing process. However, the current infectious assays coupled with standard inactivation methods and vaccine manufacturing practices provide assurance that infectious BVDV is not present in the final product. 1. If both infectious BVDV and BVDV neutralizing antibodies are present in the serum preventing the detection of infectious BVDV, there is no	Not accepted. The proposed approach is not considered acceptable to assure viral safety.

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		published data indicating the neutralizing antibodies would dissociate from the virus when used in vaccine manufacturing. As such, the antibody-bound BVDV would not infect the cells in the vaccine manufacturing process and would be removed during normal operating procedures. 2. If BVDV neutralizing antibodies only partially neutralize infectious BVDV, the non-antibody bound BVDV will be detected in the infectivity assay, and the lot would not be used in vaccine manufacturing. 3. If the level of infectious BVDV is sufficiently low (either from a very small amount of infectious virus being present or partial neutralization) such that it is not detected in the infectivity assay, the infectious BVDV would be readily inactivated during inactivation of the serum (e.g. gamma irradiation). For these reasons, testing bovine serum for the presence of BVDV antibodies is not required; assurance that infectious BVDV is not present in the final product is provided through 9CFR testing, serum inactivation, and routine vaccine manufacturing operations. Proposed change (if any): Removal of serum antibody testing from the guidance.	
194-195	5	Comment: "The assessment should therefore take into account the estimated safety margin (virus	Accepted.

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		burden before inactivation treatment vs. virus clearance by inactivation treatment).” There is no clear definition for “safety margin”. Here I would use term “residual virus (infectivity)” in the product Proposed change (if any): “The assessment should therefore take into account the estimated residual virus in the product (virus burden before inactivation treatment vs. virus clearance by inactivation treatment).”	