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Appendix 1: Sartorius Stedim FMT Single Use Biosafety Strategy

Contents

1. Purpose of the document

To guarantee the safety of manufactured products in their expected conditions of use, and to support their clients, Sartorius had defined a strategy of biosafety evaluation. As regulations and standards evolve over time, this strategy is updated in alignment with the last regulatory requirements. As explained in this document, references for cytotoxicity evaluation and position on animal experiments are the points of evolution of the Sartorius Stedim FMT Biosafety strategy.

This document is applicable to FMT and STR® products.

2. USP <87> vs. ISO 10993-5: Why use ISO instead of USP?

2.1. Comparative analysis of both references

Currently, several references are available to evaluate the cytotoxicity potential of a material and its manufacturing process. The most used are USP <87>, the current method at Sartorius, and ISO 10993 part 5. These references expose different technical methods, which have similarities and differences. Sartorius had compared both references and their methods in Appendix 1. Based on this comparative analysis, USP describes three different technical methods, when ISO 10993-5 details four methods.

Three of the four ISO 10993-5 tests are equivalent to the USP ones, when we compare the management of samples, the test method or the acceptance criteria. Few technical parameters are diverging from a process to the other. However, those are not considered to have an impact on final results obtained and thus, on the assay conclusion regarding the cytotoxic potential. Additionally, ISO 10993-5 gives informative appendices with concrete cytotoxicity test methods as guidelines for laboratories.

These three equivalent methods allow a qualitative evaluation. The ISO 10993-5 additional test is a quantitative method, which is preferred by authorities as directly mentioned in section 8.5.1.: "Determine cytotoxic effects by either qualitative or quantitative means. Quantitative evaluation of cytotoxicity is preferable. Qualitative means are appropriate for screening purposes." Quantitative evaluation limits the uncertainty linked to operators reading and interpretation. Unfortunately, USP <87> does not provide a quantitative method.

This analysis has been the starting point for updating the reference for cytotoxicity assessment at Sartorius. It has evidenced the equivalence of USP <87> and ISO 10993-5 thanks to literature review.

2.2. Equivalence demonstrated by testing data for Sartorius products

To technically demonstrate the equivalence of ISO 10993-5 and USP <87>, Sartorius has performed cytotoxicity testing under both methods on film S40 (Sartorius Qualification report No. 2202072), S80 (Sartorius Qualification report No. 2209960) and on film S71 (Sartorius Qualification report No. 2210257) as presented on the Table below. These trials were performed in the same test conditions as much as technically possible. Under the conditions of testing, both methods showed compliant results. No condition has demonstrated different conclusions about the cytotoxic potential.

Summary of Comparative Testing Data			
FIlm	Ageing Condition	Testing Method	Conclusion
S40	T0	USP <87>	Compliant
		ISO 10993-5	Compliant
S40	T36 months	USP <87>	Compliant
		ISO 10993-5	Compliant
S80	T0	USP <87>	Compliant
		ISO 10993-5	Compliant
S80	T12 months	USP <87>	Compliant
		ISO 10993-5	Compliant
S80	T36 months	USP <87>	Compliant
		ISO 10993-5	Compliant
S71	T36 months	USP <87>	Compliant
		ISO 10993-5	Compliant

These technical data confirm the equivalence between USP <87> and ISO 10993-5 under real experimental conditions. Sartorius products could thus be evaluated by either method.

2.3. Recognition by Worldwide authorities

Each authority has its own recommendation regarding standards and guidelines. The authorities of interest, based on Sartorius market, are United States Food and Drug Administration (US FDA), the European Commission (EC), the National Medical Products Administration (NMPA) of China, and the Ministry of Health, Labor and Welfare (MHLW) of Japan.

US FDA and EC have officially published their list of approved standards: "Recognized Consensus Standards" for US FDA, and "Harmonised standards" for EC. Both lists are referring to ISO 10993 standards series, including part 5, as standards admitted to evaluate the biosafety of materials used for the medical industry. Only US FDA had published a specific Guidance on ISO 10993 part 1 to add details on requirements for the biological evaluation.

Chinese NMPA also recommends using ISO 10993 standards series for the biological evaluation of medical products, while MHLW published their own official evaluation methods based on ISO 10993 standards evaluation methods, permitting to consider that MHLW admits them as valid reference.

Regarding USP, US FDA and 140 countries around the world recognize this guideline. In Europe, this is known as a reliable reference in biosafety evaluation. Nevertheless, Europe has its own Pharmacopeia and referential, and thus, its authorities would follow them. This is also applicable to China and Japan, which have their own pharmacopeia too.

Additionally, pharmacopeias are designed for evaluation and management of medicines and their packaging. They are not always adapted to the materials used for the medical industry. ISO 10993 standards series, as initially dedicated to medical devices, bring methods that are more applicable to solid products.

Knowing this information, ISO 10993 standards series would allow to evaluate the biosafety of Sartorius manufacturing products within the requirements of authorities which administrate the Sartorius market.

2.4. Management of existing data

Sartorius performed safety evaluation for several years. Many data were generated and used to evaluate safety of Sartorius products. Products assessments were performed according to applicable pharmacopoeias, regulations and standards. Regarding cytotoxicity evaluation, tests were performed according to USP <87>. Based on the technical equivalence between USP <87> and ISO 10993-5, all existing testing data remains usable.

These data will continue to support the Client's project when required. They also would be used for Sartorius studies and historical data management when applicable.

2.5. Selection criteria of Sartorius' suppliers

Sartorius has many suppliers that provide raw materials, manufacturing adjuvants and equipment. All these companies are selected according to several criteria listed in document reference OP-004. One of those is the cytotoxicity evaluation of raw materials and adjuvants.

As USP <87> and ISO 10993-5 are considered as equivalent, Sartorius accepts data generated under both references. If data are considered as not sufficient to manage biological risks, additional tests are performed by Sartorius to comply with all their regulatory commitments, that must include, but are not limited to, the following regulations related biosafety:

- Medical Device Regulation 2017/745, if applicable (depends on type of product)
- European Pharmacopeia 3.1.5., 3.1.7. and 3.1.9.
- ICH Q3D Elemental impurities
- USP <661>
- ISO 10993-5 or USP <87> Cytotoxicity evaluation

All regulatory commitments and compliant data are available on Sartorius' Product Compliant Statements. This global evaluation allows managing risks linked to suppliers and secure the list of materials.

2.6. Conclusion on the use of ISO 10993-5 as reference for cytotoxicity evaluation

As demonstrated above, ISO 10993-5 is a key reference in the medical industry to evaluate cytotoxicity of medical products. Beyond its technical equivalence with USP <87>, it brings technical advantages such as technical methods adapted for solid products and quantitative evaluation of the cytotoxic potential.

This standard is also recognized by the most influential worldwide authorities in the biomedical industry, allowing Sartorius to offer global support to their clients.

ISO 10993-5 is now considered as Sartorius reference in terms of cytotoxicity testing, without excluding USP <87> as valid reference for the management of existing data and the selection of suppliers.

3. Animal experiments: Why limit it?

3.1. Regulation and ISO 10993 series recommendations

3.1.1. European legal requirements

In Europe, animal experiments are regulated according to Directive 2010/63. This Directive is transcribed into French law by the 2013-118 decree. These regulatory texts derive from the 3R rule: "Reduce, Refine, Replace". This rule in details consists to:

- Reduce, the number of animals used;
- Refine, to decrease constraints and pain;
- Replace, by other methods when possible.

Europe has implemented it to increase ethics in biomedical research, to respond to citizens' demands and to guarantee the reliability of generated scientific data. Thanks to this European approach, research and industry have developed a new management of experimentation focused on animal welfare.

Like Europe, ISO 10993 standard series has included animal protection in their evaluation strategy with the part 2. This part is aligned with legal requirements and provides guidelines on animal management.

3.1.2. ISO 10993-1: A risk management depending on the patient contact with the medical product

ISO 10993-1 describes the strategy of evaluation of biological risks for the patient associated with the use of a medical product. The risk assessment will depend on several factors: the type of medical product, the type of contact between the medical product and the patient, the cumulated duration of contact and the existing scientific and clinical data.

Sartorius products are considered as having an indirect contact with patients. They are intended to be used in the manufacturing of medicinal products which are intended to be in direct contact with patients. It could be incubated from -80°C to +45°C or +5°C to +45°C depending on the product range and used for maximum of three years, except for particular products which are limited to one or two years (see label information). Even though they are not a component of the final-finish product, their key role in the manufacturing process requires an assessment of the potential transfer of hazardous leachable into the medicinal product. Nevertheless, this evaluation should be adapted to evaluated products, especially about

testing to perform. Accordingly, ISO 10993-1 is not recommended to perform *in vivo* tests for such devices as FMT and STR® products.

3.1.3. Cytotoxicity test as the adapted test for evaluation of Sartorius' products

Based on the information above, *in vitro* testing methods are thus the most adapted ones. The common *in vitro* test to all biological safety evaluation, regardless the type of device, of contact or its duration, is the cytotoxicity test. This test allows a quick and sensitive evaluation of potential hazardous leachable.

Due to its sensitivity, cytotoxicity test allows to assess materials and manufacturing process, and to give a good overview of the risk associated with the use of Sartorius products in the manufacturing of medicinal products by their clients.

3.2. Sartorius position as informed Single Use System (SUS) supplier

As already mentioned, Sartorius supplies its products to manufacturers of medicinal products and biotechnologies. This position limits the regulatory requirements applicable to Sartorius in terms of product safety. However, the company wants to produce safe devices and support their clients with safety data, even if the responsibility of the safety evaluation remains to the final-finish product manufacturer.

To provide data applicable to Clients' regulatory expectations and that are representative of products and processes, Sartorius chose to follow applicable regulatory and standards within their limits of action. In this regard, Sartorius Product Compliance team has a regular watch on worldwide sources of information and regularly update documentation with the latest information to provide efficient support to their clients.

3.3. Case Study: Biocompatibility investigation of POM material

In 2020, Sartorius had a recent case with one of materials they used in the product manufacturing: Polyoxymethylene (POM). This component is used only on a small part of Flexsafe STR®, identified as ball bearing reference BR112562, and in contact with fluids contained inside the product. During a periodic review of the components conformity for one of their clients, POM was demonstrated as not biocompatible by *in vitro* and *in vivo* testing, after irradiation at 50 kGy. The safety evaluation included cytotoxicity test (*in vitro*) according to USP <87>, acute systemic toxicity test, intracutaneous irritation test and intramuscular implantation test (*in vivo*) according to USP <88>.

All the investigation process, including the testing results, are detailed into Sartorius document reference GP-100A. Following this investigation and an evaluation of the benefit-risk ratio, Sartorius decided to exclude POM from their list of materials for fluids contact to first guarantee patient safety, and then, improve the quality of the products they proposed to their clients.

If we look at the evaluation strategy used, the cytotoxicity test provided results that allowed them to detect potential hazardous materials and to begging a risk evaluation on the use of POM. This case study confirms that cytotoxicity methods are reliable and sufficient for the detection of potential biological risks linked to SUS.

3.4. Conclusion on the use of *in vivo* testing for biological risk evaluation at a Sartorius standpoint

As described above, European regulation evolves to new practices for animal experiments, and Sartorius wants to be part of this evolution. SUS has an indirect contact with patients, which do not need an *in vivo* testing assessment when alternative *in vitro* methods are available, according to ISO 10993-1. Moreover, due to its SUS supplier position, Sartorius is only able to evaluate a part of biological risks but wants to help its clients in this process with data-based support in alignment with European legal requirements.

In this regard, and following the POM case study, even if *in vivo* testing provides additional data on risks for patients, Sartorius considers now that only a non-compliance of cytotoxicity testing will trigger a risk assessment and that *in vivo* testing is therefore not deemed necessary anymore.

4. Conclusion on the update of Sartorius Biosafety Strategy

Because regulations and standards evolve over time, the biomedical industry should also evolve and update its practices. Within this context, Sartorius has edited this document to explain the latest updates on their biosafety evaluation strategy.

Currently, USP <87> is the cytotoxicity evaluation reference. After a literature and experimental comparative study, and a review of the worldwide authorities' recommendations, ISO 10993-5 now appears as the most appropriate reference for the assessment of FMT and STR® products. USP <87> will remain an acceptable reference for existing and supplier data, due to its technical equivalence with ISO 10993-5.

This update allows to also develop the Sartorius position regarding *in vivo* studies. Animal experiments are more and more questioned by the public. In alignment with Europe, Sartorius has evaluated biological risks associated with their products, their impact as a SUS supplier and how they can improve this point of concern. After this global assessment, they conclude that *in vivo* testing (USP <88> as much as ISO methods) is not necessary anymore to guarantee the safety of their products in the intended conditions of use, as ISO 10993-5 cytotoxicity methods are a key tool to evidence hazardous substances.

As a reminder, this strategy is applicable to FMT and STR® products. For any new product range, a global evaluation will be done to identify associated risks and verify the applicability of this biocompatibility evaluation strategy.

5. References

ISO 10993-1: 2018 - Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process

ISO 10993-2: 2006 - Biological evaluation of medical devices — Part 2: Animal welfare requirements

ISO 10993-5: 2009 - Biological evaluation of medical devices — Part 5: Tests for *in vitro* cytotoxicity

USP <87> 44 NF 39 BIOLOGICAL REACTIVITY TESTS, IN VITRO

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USP <88> 44 NF 39 BIOLOGICAL REACTIVITY TESTS, IN VIVO

DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes

Decree No. 2013-118 of 1st February 2013 on the protection of animals used for scientific purposes.

FDA Recognized Consensus Standards database

Official Journal of the European Union L 90 I - COMMISSION IMPLEMENTING DECISION (EU) 2020/437 of 24 March 2020 on the harmonized standards for medical devices drafted in support of Council Directive 93/42/EEC