GUIDEGXP SERIES · PHARMA LAB

OPERATIONAL GUIDE

GMP MICROBIOLOGY LABORATORY

AND STERILITY TESTING

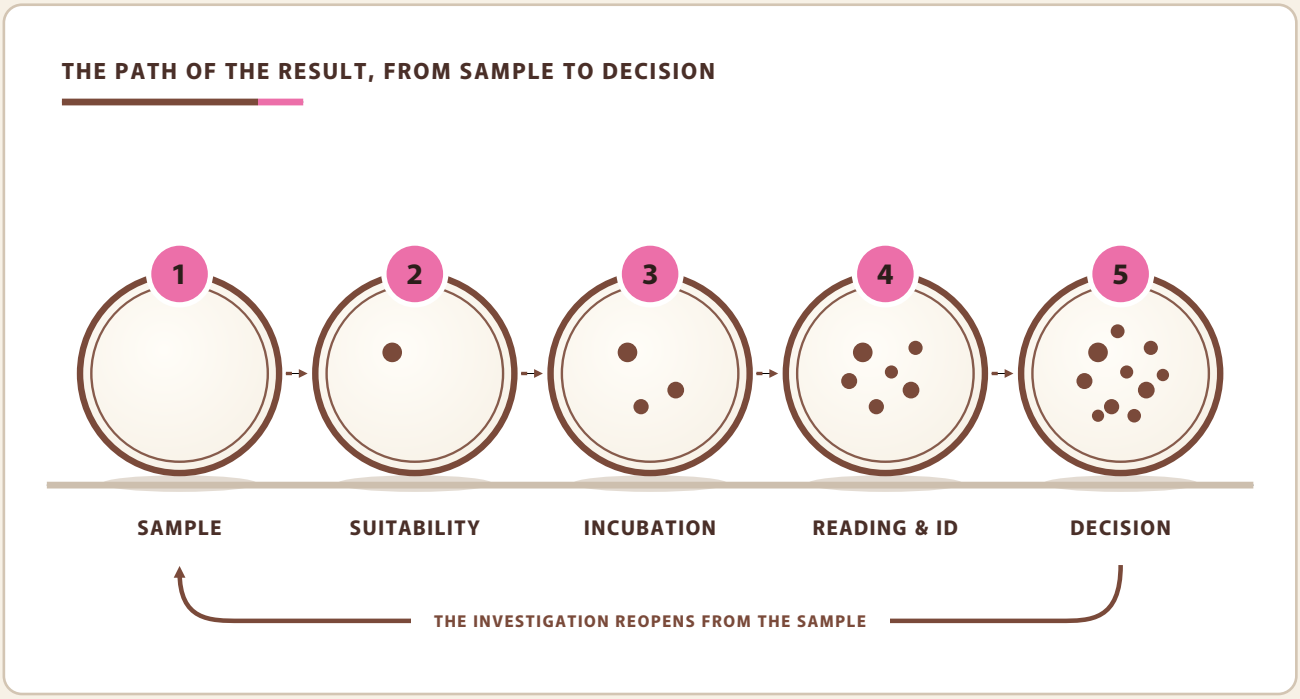
Methods, Media, Equipment, Investigations and Data You Can Defend

248 PAGES · 18 CHAPTERS · 6 PARTS · TOOLKIT OF 16 WORD + EXCEL TOOLS

FROM SAMPLE TO DECISION: MICROBIOLOGICAL RESULTS THAT STAND UP TO INSPECTION

FIRST EDITION · OCTOBER 2026 · V1.0 · CUT-OFF 04/10/2026 · ENG EDITION

FREE EXCERPT



GuideGxP
Practical compliance guides for Pharma professionals
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About this guide

The guide supports those who run a GMP QC microbiology laboratory — sterility testing and isolators, bioburden and enumeration, endotoxins, rapid methods and identification, culture media and growth promotion, reference strains, incubation, samples, equipment, microbiological OOS results and contract laboratories — from sample to decision. For every point it says which text governs it, with what force and in which jurisdiction, which value it gives (or does not give) and of what nature, what the laboratory has to decide and where the decision is recorded. No universal values: incubation times and temperatures, recovery and GPT criteria, dilutions, the number of replicates and frequencies remain laboratory decisions, taken with a written criterion.

This excerpt contains, exactly as they appear in the full edition: the regulatory and technical status box and the label system; the complete table of contents; from Chapter 4 what the sterility test can demonstrate, membrane filtration and direct inoculation, suitability, validation and verification; from Chapter 6 invalidation of a positive test and Case A in full; real pages of three toolkit tools; the list of what the purchase contains. First edition, October 2026, v1.0; regulatory cut-off 04/10/2026.

Regulatory and technical status

⚠ NOTICE

Regulatory and technical status as of 04/10/2026

- **EU.** CURRENT REGULATORY REQUIREMENT in the EU: EU GMP Annex 1, Chapters 1, 3, 4, 6, 7, Annex 2, 11, 15, 17, 19 (revised, from 24/09/2026); veterinary GMP from 16/07/2026. CURRENT GUIDANCE in the EU: EMA guidelines and Q&As (see Chapter 1).
- **PIC/S** CURRENT GUIDANCE: PE 009-18 (from 24/09/2026), PI 006-4 (from 01/10/2026), PI 023-2, PI 041-1; PI 012-3 and PI 014-3 from 2007, whose values are historical guidance (see Chapters 1, 4, 5).
- **WHO.** TRS 961, Annex 2 CURRENT GUIDANCE is the guideline for the microbiology laboratory; TRS 1052, Annex 4 excludes it (see Chapter 1).
- **ICH** CURRENT GUIDANCE: Q4B(R1) and Annex 4A(R1), 4B(R1), 4C(R1), 8(R1), 14; Q9(R1); Q10.
- **USA.** 21 CFR 211; 610 and 600.3 (licensed biologics) CURRENT REGULATORY REQUIREMENT in the USA; FDA aseptic processing guidance (October 2004), pyrogen and endotoxins Q&A Edition 2 (March 2026), OOS (May 2022), CCIT (February 2008), CGMP Q&As CURRENT GUIDANCE in the USA; the 1993 inspection guide and the FDA laboratory manuals are not guidance (see Chapter 1).
- **Pharmacopoeias.** As of 04/10/2026 the applicable texts are Ph. Eur. 12th Edition up to Issue 12.3 and USP–NF 2026, Issue 5 (official from 01/10/2026); Ph. Eur. Issues 13.1, 13.2 and 13.3 have been published but apply only from 01/01/2027, 01/04/2027 and 01/07/2027 respectively, and until then the previous text remains in force. Ph. Eur. texts were not consulted directly: this guide reports their number, title and status from official EDQM sources and describes the structure of the decisions; for requirements, methods and criteria the current Ph. Eur. text prevails. The Ph. Eur. is mandatory in the states that apply it; in the EU the USP becomes a mandatory reference only through a dossier commitment or a quality system decision (see Chapter 1). Status of 2.6.32, method G, 2.6.8, 5.1.6, 5.1.9 and USP ⟨86⟩: table below (see Chapters 4, 8, 9).
- DRAFT. 2025 revisions of EU GMP Annex 11, Chapter 4 and Chapter 1 DRAFT GUIDANCE: consultations closed, final texts not published; the 2011 Annex 11 and Chapter 4 and the 2013 Chapter 1 remain in force (see Chapter 1). Pharmacopeial Forum proposals for USP ⟨1117⟩, ⟨1113⟩, ⟨1085⟩, ⟨1119⟩, ⟨1119.1⟩; FDA drafts of 2020 and 2021; Rev. 1 of the EMA reflection paper on the 3Rs: none of these is current text.
- IN DEVELOPMENT. Revision of Annex 15 (draft planned for December 2026); ISO/DIS 11737-1, ISO/DIS 14644-7, ISO/FDIS 11133, EN 12469 parts 3 and 4; integration of rCR into the Ph. Eur.
- ~~SUPERSEDED~~. Ph. Eur. 2.6.8; ISO 14698-1 and -2; the June 2012 FDA Q&A (table below); WHO TRS 957, Annex 1, by TRS 1052, Annex 4; the 2008 FDA draft on rapid methods for cellular and gene therapy products, withdrawn on 06/05/2015; NSF/ANSI 49-2024, by 49-2026; PI 006-3, by PI 006-4.
- CURRENT TECHNICAL STANDARD. ISO 11737-1:2018, 11737-2:2019, 13408-6:2021, 14644-7:2004, 11138-1 and -3:2017, 14937:2009, ISO/IEC 17025:2017; EN 12469-1, -2, -5:2025; NSF/ANSI 49-2026.
- **GB.** Human Medicines Regulations 2012; the formal status of the 2022 Annex 1 must be checked against MHRA sources.

- **What is not there.** No regulation sets a single value for incubation times and temperatures, recoveries, frequencies or numbers of replicates: the criterion is defined for the method, the sample and the intended use through a documented assessment based on laboratory data and the applicable text.
- **To be re-verified** (D-00.1 · **who decides:** head of the microbiology laboratory with laboratory QA · **on what basis:** re-verification events listed in the front matter and status of the sources as of 04/10/2026 · **where it is recorded:** Source_Review (MB-TK-16)): Ph. Eur. 13.1–13.3; USP–NF 2026 Issue 6 and 2027 Issues 1–2; Pharmacopeial Forum proposals; final texts of the EU drafts and the Annex 15 draft; standards in development; new EU pharmaceutical legislation; Annex 1 in GB; NSF/ANSI 49-2026. Periodic laboratory review: D-18.3 (see Chapter 18).

Document	Status	Dates not to be confused	Jurisdiction	Source
EU GMP Annex 1	CURRENT	published 25/08/2022; operational from 25/08/2023; 8.123 from 25/08/2024	EU	FS-01
Ph. Eur. 5.1.13; 2.6.8	CURRENT; ~~SUPERSEDED~~	5.1.13 from 01/07/2025; 2.6.8 suppressed from 01/01/2026	Ph. Eur. member states	FS-83
Ph. Eur. 2.6.32; method G of 2.6.14	CURRENT; no label	2.6.32 until 31/12/2026; method G from 01/01/2027	Ph. Eur. member states	FS-82
Ph. Eur. 5.1.9; 5.1.6	CURRENT; revision without label	5.1.9 revised from 01/01/2026; 5.1.6 revised from 01/04/2027	Ph. Eur. member states	FS-81, FS-85
USP ⟨86⟩	official, not 'applicable'	May 2025	USA	FS-65
FDA pyrogen and endotoxins Q&A	CURRENT; Edition 1 ~~SUPERSEDED~~	Edition 2 March 2026; Edition 1 June 2012	USA	FS-48
ISO 14698-1 and -2	~~SUPERSEDED~~	withdrawn on 16/03/2026	—	FS-91

How to read the labels

Four labels give the status, seven the nature and one marks examples; status comes first, then nature, with the jurisdiction outside the brackets ("CURRENT in the EU").

Label	Microbiology example
CURRENT	Ph. Eur. 5.1.9 in the revision applicable from 01/01/2026
DRAFT	proposed revision of USP ⟨1117⟩: 'the proposal provides for'
IN DEVELOPMENT	revision of Annex 15, concept paper only
~~SUPERSEDED~~	Ph. Eur. 2.6.8 'Pyrogens' from 01/01/2026
REGULATORY REQUIREMENT	one sterility test per sub-batch (Annex 1, 10.6) in the EU
COMPENDIAL REQUIREMENT	Ph. Eur. 2.6.1 in the Ph. Eur. member states
GUIDANCE	sterility testing environment (WHO TRS 961, Annex 2, 2.4.3)
TECHNICAL STANDARD	EN 12469-1:2025 on biological safety cabinets
SCIENTIFIC PRINCIPLE	$P = 1 - (1 - p)^n$ for detection
GEP/QRM	second reading of plates decided on risk

Label	Microbiology example
GUIDEGXP RECOMMENDATION	Annex 1, 4.21–4.22 applied by analogy to the testing isolator
DEMONSTRATION EXAMPLE	GPT ratio for a local isolate at a fictitious company

Texts published but not yet applicable carry no status label; the borderline cases are in the table above.

The nature of a value is written out in words next to the number.

Nature of the value	Value and object	Document and point	Source
requirement without a value	shelf life of media "established, documented and scientifically justified"	EU GMP Chapter 6, 6.24	FS-02
guidance with context	NMT 10 CFU/100 ml (TAMC), bulk before sterile filtration	EMA sterilisation guideline, 4.1.5	FS-17
historical guidance	certification of the testing environment "at least annually"	PIC/S PI 012-3 (2007), 8.1.1.2	FS-23
WHO contextual guidance	50–200% recovery after inoculation of not more than 100 CFU, media only	WHO TRS 961, Annex 2, 5.2.2	FS-29
convention	use within five passages of the ATCC culture; countable ranges 30–300, 25–250, 15–300	ATCC recommendation; BAM	FS-98, FS-61
literature	pre-moistened swabs 14.3%, dry swabs 4.4% (spores on steel)	Rose et al. 2004	FS-99
example	20 units from a batch with 0.1% contaminated: 98% probability of passing the test	FDA 2004 guidance, XI.B	FS-47
principle	0 positives out of 20: rates compatible at 95% up to 13.91% DEMONSTRATION EXAMPLE SCIENTIFIC PRINCIPLE	exact binomial limit	FS-100

DEMONSTRATION EXAMPLE Hypothetical data, calculated only to illustrate the method: they are not acceptance criteria or results of a real laboratory.

Full edition — complete table of contents: the map of the manual

The full edition comprises 18 chapters in six parts, named after the six themes of the Microbiology Laboratory and Sterility Testing hub, plus six appendices, over 248 A4 pages. Chapters 2–18 follow the same seventeen-section path: the problem and the decision; scope and boundaries; data to collect; technical principles; requirements and sources; interpretation; risk; decision; reasoned procedure; evidence; audit readiness; example (or integrated case); errors to avoid; chapter checklist; toolkit tools and related articles; the decisions the chapter produces; chapter sources. Page numbers refer to the full edition, not to this excerpt.

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CHAPTER 4

Sterility test method: membrane filtration, direct inoculation, method suitability

Excerpt from Chapter 4, exactly as it appears in the full edition: the problem and the decision and, from the technical principles, what the test can demonstrate, membrane filtration and direct inoculation, suitability, validation and verification, and the decision tree. In the full edition the chapter continues with harmonised texts and sampling, short shelf life, requirements and sources, interpretation, risk, procedure, evidence, audit readiness, example, checklist and the D-04.n decisions.

The problem and the decision

A test with no growth can hide a method that has lost its sensitivity: antimicrobial activity that was never removed, critical interventions that were never sampled, suitability shown on a formulation that no longer exists. The decision is which method, which samples and which suitability evidence give a defensible sterility test result for a given product, knowing that the test does not demonstrate sterility. The sterility testing and isolator lead and the laboratory head decide, with sterility assurance and laboratory QA; regulatory affairs keep the dossier aligned (D-04.1, D-04.2, D-04.3).

Technical principles

What the test can demonstrate

The sterility test is the last in a series of critical controls: because of its statistical limitations it can detect only gross contamination and cannot demonstrate the sterility of the batch, which is assured through process design, validation and control. No growth in a test or a zero count means that, under the conditions of the method, no microorganism was detected in the quantity examined, not that the material is free of microorganisms; the result is reported as 'not detected' in the defined unit.

EU GMP Annex 1 CURRENT REGULATORY REQUIREMENT in the EU (10.5) is explicit: "The sterility test applied to the finished product should only be regarded as the last in a series of critical control measures by which sterility is assured." The reasons (few samples, media that do not support every organism) are set out in Annex 17 CURRENT REGULATORY REQUIREMENT in the EU (4.2) and in FDA guidance 'Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice' (October 2004) CURRENT GUIDANCE in the USA (XI).

The text of USP ⟨71⟩ and USP ⟨1211⟩ is available only by subscription or purchase: this guide reports its title, number, edition, status and, where available, the public preview, and does not reproduce its content, tables or numerical criteria. USP ⟨1211⟩ CURRENT GUIDANCE points out that sterility cannot be demonstrated without destroying every unit of the batch.

PIC/S PI 012-3 is a PIC/S recommendation or aide-memoire GUIDANCE written for inspectors: it is not binding on industry and becomes binding only if adopted by the competent authority. PIC/S PI 012-3 (2007) and PI 014-3 (2007) GUIDANCE predate the 2022 Annex 1 and, for PI 012-3, are based on the 1998 Ph. Eur.: the values they give are historical guidance, to be reconciled with the current Annex 1 and pharmacopoeia. The document CURRENT refers (6.3) to "significant statistical limitations".

Detection probability and the contamination rate still compatible with zero positives are calculated with the binomial model SCIENTIFIC PRINCIPLE (see Example).

Membrane filtration and direct inoculation

Filtration separates the product from the organisms and washes it away with the rinses; direct inoculation dilutes or neutralises it in the medium SCIENTIFIC PRINCIPLE. ICH Q4B Annex 8(R1) CURRENT GUIDANCE (2.1.1) excludes diluting and rinsing fluids that have antibacterial or antifungal properties. The 'usual' membrane porosity and material are supplier conventions. No regulation sets a single value for rinse volumes, number of rinses and neutralisers: the criterion is defined for the method, the sample and the intended use through a documented assessment based on laboratory data and the applicable text; the applied chapter gives the parameters as a method requirement, not reproduced here; laboratory parameters: `<to be defined>` (D-04.4).

Aspect	Membrane filtration	Direct inoculation	Main risk	Suitability test that covers it
Antimicrobial activity	Removed by rinsing	Diluted or neutralised in the medium	Residual inhibition	Product versus control, per strain and medium
Adsorption	On the membrane and canister	Not relevant	Organisms retained or inhibited	Test with the routine rinses
Turbidity; quantity	Product kept out of the medium	Product in the medium	Unreadable result	Routine reading and routine quantity

Table GUIDEGXP RECOMMENDATION.

Suitability, validation, verification

A compendial method is considered validated: the laboratory verifies its applicability under its own conditions and demonstrates its suitability for the specific product (recovery of microorganisms in the presence of the product); full validation is required for non-compendial, alternative or modified methods. WHO TRS 961, Annex 2 CURRENT GUIDANCE (3.1): "However, the specific test method to be used by a specific laboratory for testing of a specific product needs to be shown to be suitable for use in recovering bacteria, yeast and mould in the presence of the specific product." Annex 15 CURRENT REGULATORY REQUIREMENT in the EU (9.2), USP ⟨1227⟩ CURRENT GUIDANCE and the 2012 preamble CURRENT point the same way; under the preamble, verification lasts as long as the method is in use.

> **▲ COMMON MISTAKE**
>
> "Pharmacopoeial method, so already validated: no testing on the product": WHO TRS 961, Annex 2, 3.1, does say that standard methods "are considered to be validated", and in the very next sentence asks for recovery to be shown with the specific product. The answer is one suitability report per product (D-04.3).

Ph. Eur. 2.6.1 CURRENT COMPENDIAL REQUIREMENT in the Ph. Eur. member states: the text was not consulted and the current text prevails (see Chapter 1). Ph. Eur. 5.1.9, in the revision in force from 01/01/2026, allows alternative methods according to 5.1.6 and no longer states the class or location of the cabinet for the sterility test; the chapter is CURRENT GUIDANCE. Strains: those prescribed by the applied chapter (species not reported); inocula and the comparable-growth criterion are those of the chapter, not reproduced; laboratory choices: `<to be defined>` (D-04.5). The GPT qualifies the medium, not the method (see Chapter 10); events that trigger renewal of suitability: `<to be defined>` (D-04.3).

Regime	Text	What is demonstrated	Nature
EU	Annex 1, 10.5; Annex 15, 9.2; Chapter 6, 6.15	Recovery not affected by the product; verification	REGULATORY REQUIREMENT in the EU
USA, drugs	21 CFR 211.165(e); 211.194(a)(2)	Suitability under actual conditions of use	REGULATORY REQUIREMENT in the USA
USA, licensed biologics	21 CFR 610.12(b)(2)–(b)(3)	Validation; verification of the test and its components	REGULATORY REQUIREMENT in the USA
WHO	TRS 961, Annex 2, 3.1	Suitability for the specific product	GUIDANCE

Decision

Decision tree GUIDEGXP RECOMMENDATION:

1. What is the product type and regime?
   - Short shelf life → **Outcome:** alternative method to be assessed (see Chapter 9; D-04.1).
   - Licensed biologic in the USA → 21 CFR 610.12 also applies; go to 2.
   - Aseptically filled or terminally sterilised → go to 2.
2. Can the product be filtered and is its activity removed by the rinses?
   - Yes → filtration, preferably with a closed system (D-04.1, D-04.4); go to 4.
   - No → go to 3.
3. Can the activity be controlled in the medium, with a readable result?
   - Yes → direct inoculation (D-04.1); go to 4.
   - No → **Outcome:** alternative method (see Chapter 9).
4. Has suitability been demonstrated according to the applied chapter (D-04.5)?
   - No → **Outcome:** no routine use.
   - Yes → go to 5.
5. Does the plan cover sub-batches, start, end and critical interventions (D-04.2)?
   - No → **Outcome:** plan to be revised before release.
   - Yes → **Outcome:** method in use, renewed when the defined events occur (D-04.3).

Option	Advantages	Limitations	Dependencies	When to choose it
Filtration, closed system	Few aseptic openings	Adsorption	Supplier certificates; pump	Filterable products
Filtration, open system	Flexibility	More manipulations	Test environment (D-02.2)	Formats with no closed system available
Direct inoculation	No filtration step	Product stays in the medium	Demonstrated dilution	Products that cannot be filtered
Alternative method	Time to result	Equivalence to be demonstrated	Dossier	Short shelf life (see Chapter 9)

Table GUIDEGXP RECOMMENDATION.

CHAPTER 6

Execution, aseptic controls and investigation of the positive test

Excerpt from Chapter 6, exactly as it appears in the full edition: the problem and the decision, invalidation of a positive test from the technical principles, the decision tree and Case A in full. Case A is one of the five integrated cases of the book (A positive sterility test, B testing isolator, C endotoxins, D growth promotion failure, E count OOS), linked to the toolkit with the same identifiers. Torvenza Pharma S.p.A. is a fictitious company: data, dates and codes are hypothetical.

The problem and the decision

Faced with a turbid canister, the temptation is to look straight away for a source inside the laboratory. The decision is which evidence makes a positive sterility test invalid because of laboratory error and when, without conclusive evidence, the batch is non-conforming and the investigation moves to manufacturing; and how the session is run so that such evidence exists at all. The laboratory proposes, laboratory QA approves or rejects the invalidation, QA management and the Qualified Person decide on the batch and on the extension of the investigation (D-06.1...D-06.5).

Technical principles

Invalidation

A positive sterility test is invalid only if the growth is unequivocally ascribed to a laboratory error with documented evidence; if the evidence is inconclusive the batch does not conform to sterility requirements and the investigation extends to manufacturing and other batches. FDA 2004 (XI.C): "When available evidence is inconclusive, batches should be rejected as not conforming to sterility requirements." A deviation known during the session changes the path: "If any deviation is considered to have compromised the integrity of the sterility test, the test should be invalidated immediately without incubation." (D-06.1).

Ph. Eur. 5.1.9, in the revision in force from 01/01/2026, allows alternative methods according to 5.1.6 and no longer states the class or location of the cabinet for the sterility test. In that chapter CURRENT GUIDANCE the recommendations on invalidation are no longer confined to condition (d) of Ph. Eur. 2.6.1 CURRENT COMPENDIAL REQUIREMENT: texts not consulted, the current text prevails (see Chapter 1). The text of Ph. Eur. 5.1.9 and 2.6.1 is available only by subscription or purchase: this guide reports its title, number, edition, status and, where available, the public preview, and does not reproduce its content, tables or numerical criteria. PI 012-3 (Note 3 to 13.1), historical guidance, rules out repeat testing based on morphological or biochemical characteristics; its invalidation criteria are not current.

For licensed biological products 21 CFR 610.12 REGULATORY REQUIREMENT in the USA allows the sterility test to be repeated only once, only if an investigation definitively ascribes the positive result to a laboratory error or faulty materials, using the same method and a comparable sample. The rule, CURRENT for biologics, does not extend to drugs or to the EU. The test does not demonstrate sterility; a positive result is direct evidence that has to be explained (see Chapter 4).

Evidence	What it demonstrates	What it does not demonstrate	Source
Deviation known before incubation	Compromised test	The status of the batch	FS-47
Documented error, strain and route within the laboratory	Attribution to the laboratory	Sterility of the batch	FS-47
Negative control with growth	A session problem to be assessed	On its own, invalidity	FS-55
Same species in the test and in the laboratory	A hypothesis	Strain and route	FS-47, FS-23
Growth in one medium only	A positive result	A false positive	FS-101

Regime	Text	What it allows	Nature
USA, licensed biologics	21 CFR 610.12(f)	One repeat after definitive attribution	REGULATORY REQUIREMENT in the USA
USA, drugs	FDA 2004, XI.C; 21 CFR 211.192	Invalidation only for an unequivocal error	GUIDANCE; REGULATORY REQUIREMENT in the USA
EU	Annex 1, 3.2; Ph. Eur. 2.6.1, 5.1.9	Investigation before certification	REGULATORY REQUIREMENT in the EU; COMPENDIAL REQUIREMENT in the Ph. Eur. member states (2.6.1); GUIDANCE (5.1.9)

> ▲ COMMON MISTAKE
>
> "Growth in one medium only: the positive is not confirmed": each medium has its own sensitivity, and the 2004 FDA guidance (XI.C) allows invalidation only for an unequivocal error. The turbid canister is a positive result (D-06.1).

Decision

Decision tree GUIDEGXP RECOMMENDATION:

1. Is the product a licensed biologic in the USA?
   - Yes → 21 CFR 610.12(f) regime; go to 2.
   - No → go to 2.
2. Did a deviation known during the session compromise the test?
   - Yes → **Outcome:** immediate invalidation without incubation, documented (D-06.1).
   - No → go to 3.
3. Is a laboratory source being invoked?
   - No → **Outcome:** test valid; batch non-conforming; investigation extended (D-06.3).
   - Yes → go to 4.
4. Do species identification by the same method and strain typing link the isolate to a laboratory source with a documented route (D-06.2)?
   - No or inconclusive → **Outcome:** test valid; batch non-conforming; investigation extended (D-06.3).
   - Yes → go to 5.
5. Which regime applied at node 1?
   - Licensed biologic in the USA → **Outcome:** invalidation approved by QA; one repeat, same method (21 CFR 610.12(f)).
   - Other → **Outcome:** invalidation documented and approved by QA (D-06.1); CAPA (see Chapter 17).

Option	Advantages	Limitations	Dependencies	When to choose it
Species, same system	Fast	Cannot separate strains	Library (D-09.5)	Always, as the first step
External genomic typing	Separates strains	Turnaround time, contract	Isolates retained	When the laboratory is invoked as source
No typing	No cost	No conclusion on origin	—	Only when the laboratory is not invoked

Table GUIDEGXP RECOMMENDATION.

Case A — Positive sterility test: same species, different strain and batch rejected

Scenario built for teaching purposes. Simulated case: Torvenza Pharma S.p.A. is a fictitious company; its microbiology laboratory, batches and data are hypothetical.

DEMONSTRATION EXAMPLE

Context. TZP-02, batch 26-117, filled on 06/05/2026 on LF-TZ-01 with a critical intervention: the glove print of an operator in grade A recovers 1 CFU, identified by IDS-TZ-01 as *Staphylococcus capitis*; DEV-TZ-26-022 is opened under the EM programme (Annex 1, Table 6, note (c): "any growth should result in an investigation"); this guide stops here on this point: management of production-area EM deviations is covered in the GuideGxP Environmental Monitoring guide. Samples in three separate sets, each filtered through its own pair of canisters (D-04.2). ST-TZ-26-0412, started on 11/05/2026 in ISO-TZ-02 (CYC-TZ-02-0388 within parameters; gloves within the internal criterion): at the end of the session the glove print from port 3 recovers 1 CFU of *S. capitis*.

Problem. At the reading of 20/05/2026 the soya-bean casein digest canister of the "after intervention" set is turbid; the thioglycollate canister of the same set and the other sets show no growth. The laboratory proposes invalidation: same species in the test and on the isolator glove.

Risk. Invalidating a true positive releases a contaminated batch; the species demonstrates neither strain nor route; growth in one medium only does not make the positive false; an investigation weighted towards the laboratory is the topic of letters MARCS-CMS 576961 and 719568.

Decisions. D-04.2 (three sets); D-06.1 (conclusive evidence; typing whenever the laboratory is invoked); D-06.2 (IDS-TZ-01 to species; genomic typing at LAB-EXT-TZ-02); D-06.3 (same campaign and interventions of the same type); D-06.4 (six-monthly review, rate and 95% upper limit). Torvenza internal criterion, demonstration choice of a fictitious company: it is not a recommended or reference value.

Activities and data. Negative controls: 0 out of 412, upper limit 0.72%. A test with no growth on 20 units would be compatible, at 95% confidence, with rates of up to 13.91%; a positive is direct evidence that has to be explained. Species identifications with IDS-TZ-01, the QA rejection and the typing of the three isolates are in the chronology.

DEMONSTRATION EXAMPLE Hypothetical data, calculated only to illustrate the method: they are not acceptance criteria or results of a real laboratory.

> ▣ EVIDENCE EXPECTED
>
> INV-TZ-26-024 with the LAB-EXT-TZ-02 typing report on the three isolates and the hypothesis matrix (laboratory: excluded; manufacturing: confirmed).

Date	Event	Record	Who
06/05/2026	Filling of TZP-02, batch 26-117, with a critical intervention; glove print in grade A: 1 CFU, *S. capitis*	Batch record; DEV-TZ-26-022	PRD-TZ-01; EM-TZ-01
11/05/2026	ST-TZ-26-0412 in ISO-TZ-02, three separate sets; glove print from port 3 at the end of the session: 1 CFU, *S. capitis*	ST-TZ-26-0412; CYC-TZ-02-0388; laboratory EM	QC-STE-01; QC-AN-MB
20/05/2026	Turbidity in the soya-bean casein digest canister of the "after intervention" set; subculture on solid medium (PI 012-3, 12.1)	DEV-TZ-26-024; INV-TZ-26-024	QC-AN-MB; QC-STE-01
22/05/2026	Identification by the same method (IDS-TZ-01) of the test isolate and the isolator glove isolate: both *S. capitis*; the laboratory proposes invalidation	INV-TZ-26-024	QC-MB-01
27/05/2026	QA-LAB-TZ rejects the proposal: species identity not conclusive, route not demonstrated; typing of the three isolates at LAB-EXT-TZ-02	INV-TZ-26-024	QA-LAB-TZ; SA-TZ-01
10/06/2026	Typing result: test isolate ≠ isolator glove isolate; test isolate = production glove print isolate of 06/05/2026 (same strain)	LAB-EXT-TZ-02 report	SA-TZ-01
12/06/2026	Test valid; batch 26-117 does not conform to sterility requirements and is rejected; investigation extended to manufacturing and to batches 26-116 and 26-118 of the same campaign	DEC-TZ-26-024	QA-TZ-01; QP-TZ-01
15/07/2026	Laboratory CAPA: typing mandatory whenever a laboratory source is invoked; SOP-TZ-MB-011 rev. 5	CAPA-TZ-26-024	QA-LAB-TZ
30/11/2026 (planned)	Effectiveness check of CAPA-TZ-26-024	CAPA-TZ-26-024	QA-LAB-TZ

Documents. SOP-TZ-MB-011 rev. 4 and 5; SOP-TZ-MB-010 rev. 7.

Evidence. ST-TZ-26-0412; CYC-TZ-02-0388; INV-TZ-26-024; LAB-EXT-TZ-02 report; DEC-TZ-26-024; CAPA-TZ-26-024.

Outcome as of 04/10/2026. Batch 26-117 rejected; ST-TZ-26-0412 valid. Batches 26-116 and 26-118 are within the scope of the manufacturing investigation; this guide stops here on this point: the general deviation and CAPA process is covered in the GuideGxP Deviations, Self-Inspections and CAPA guide. Effectiveness check of CAPA-TZ-26-024 planned for 30/11/2026 (date after the 04/10/2026 cut-off: activity not yet performed).

Three lessons. Species is not strain. Strain is not route. QA rejects an invalidation proposal that lacks conclusive evidence.

D codes. D-04.2, D-06.1, D-06.2, D-06.3, D-06.4.

Tools used. MB-TK-05; MB-TK-08 for identification; MB-TK-15 for the effectiveness check.

The toolkit: real pages of three tools

The images are pages of the delivered files — Torvenza filled-in examples from the 07_Examples folder, English edition — converted to PDF without retouching. In the clean templates of folders 01–06 the same sheets are empty: no criterion is preloaded and no sheet declares a method suitable, a batch compliant or a result valid: the outcomes are "Comparison with the entered criteria" or "Assessment required".

MVD — maximum valid dilution and highest 2ⁿ dilution within the MVD

Operational Guide to the GMP Microbiology Laboratory and Sterility Testing — toolkit item linked to Chapters 8; 13, 17

MB-TK-07 rev. 1.0 · First edition · October 2026 · v1.0 · cut-off 04/10/2026 · [DEMONSTRATION EXAMPLE]

Simulated case: Torvenza Pharma S.p.A. is a fictitious company; its microbiology laboratory, batches and data are hypothetical. [DEMONSTRATION EXAMPLE] Hypothetical data, calculated only to illustrate the method: they are not acceptance criteria or results of a real laboratory.

MVD = EL·C/λ (1:n), from the data of the Product sheet (one row per Product row); highest 2ⁿ dilution within the MVD = 2^FLOOR($\log_2$ MVD). λ = 0 → "calculation not possible". Calculation-only sheet: the "Data status" column shows "Assessment required" with the reason, otherwise it stays empty. The MVD is the maximum dilution at which the endotoxin limit can still be detected: it is not the routine testing dilution; an out-of-specification result at the MVD that cannot be attributed to a testing error leads to rejection of the lot according to the FDA Q&A (Edition 2).

No.	Product (code)	EL (value, in the EL unit)	EL unit	C (concentration on the EL basis, per mL)	λ (EU/mL)	MVD (1:n)	Highest 2ⁿ dilution within the MVD (1:n)	Data status	Reason
1	TZP-08	0.500	EU/mg	2.000	0.005	1:200.0	1:128		
2									

MB-TK-07 — Endotoxin Calculator (MVD, Dilutions, Positive Product Control, Interference) (Excel) · MVD and dilutions from the limit, the concentration and the λ of the method

Survivor_Curve — D value from the survivor curve and by segments

Operational Guide to the GMP Microbiology Laboratory and Sterility Testing — toolkit item linked to Chapters 5; 6, 16

MB-TK-04 rev. 1.0 · First edition · October 2026 · v1.0 · cut-off 04/10/2026 · [DEMONSTRATION EXAMPLE]

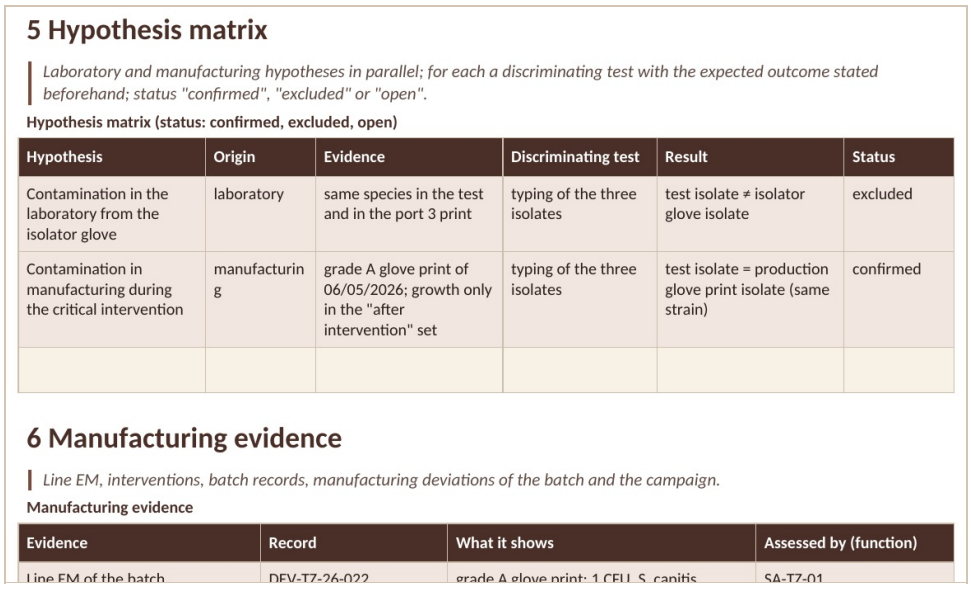
Simulated case: Torvenza Pharma S.p.A. is a fictitious company; its microbiology laboratory, batches and data are hypothetical. [DEMONSTRATION EXAMPLE] Hypothetical data, calculated only to illustrate the method: they are not acceptance criteria or results of a real laboratory.

Points: one row per time of each series (3–12 points per series, up to 120 rows); $\log_{10}$N calculated. Series: one row per series (up to 10) with the segment points chosen by the user (optional). Least-squares slope b of $\log_{10}$N on t (as SLOPE); D = −1/b; R^2 (as RSQ); two-point D between the first and the last point; D of a segment = (t_j − t_i)/($\log_{10}$N_i − $\log_{10}$N_j). The flag "non-linear curve" is informative and appears only if the user enters the threshold of the ratio of the final to the initial segment D (no default). Calculation-only sheet. A single D value is misleading when the curve is biphasic; F_0 applies to moist heat, not to peroxide.

Threshold of the ratio final D/initial D for the flag (optional) — Value decided by the user, no default: without a value the flag does not appear.

Example row	Series (location, cycle)	t (min)	N (CFU/carrier)	$\log_{10}$N value	Valid point (1/0)	t of the valid point (min)	$\log_{10}$N of the valid point	Point no. in the series	Key series|point (support)	Data status	Reason
EXAMPLE	*reference location (cycle development)*	*0*	*1.8E+06*	6.255	1	0	6.255	1	reference location (cycle development)|1		
EXAMPLE	*reference location (cycle development)*	*2*	*2.6E+05*	5.415	1	2	5.415	2	reference location (cycle development)|2		
EXAMPLE	*reference location (cycle development)*	*4*	*3.1E+04*	4.491	1	4	4.491	3	reference location (cycle development)|3		
EXAMPLE	*reference location (cycle development)*	*6*	*5.0E+03*	3.699	1	6	3.699	4	reference location (cycle development)|4		
EXAMPLE	*reference location (cycle development)*	*8*	*6.2E+02*	2.792	1	8	2.792	5	reference location		

MB-TK-04 — Isolator Bio-decontamination Cycle Log and Analysis (Excel) · D value from the survivor curve of cycle development (Case B)



5 Hypothesis matrix

Laboratory and manufacturing hypotheses in parallel; for each a discriminating test with the expected outcome stated beforehand; status "confirmed", "excluded" or "open".

Hypothesis matrix (status: confirmed, excluded, open)

Hypothesis	Origin	Evidence	Discriminating test	Result	Status
Contamination in the laboratory from the isolator glove	laboratory	same species in the test and in the port 3 print	typing of the three isolates	test isolate ≠ isolator glove isolate	excluded
Contamination in manufacturing during the critical intervention	manufacturing	grade A glove print of 06/05/2026; growth only in the "after intervention" set	typing of the three isolates	test isolate = production glove print isolate (same strain)	confirmed

6 Manufacturing evidence

Line EM, interventions, batch records, manufacturing deviations of the batch and the campaign.

Manufacturing evidence

Evidence	Record	What it shows	Assessed by (function)
Line EM of the batch	DEV-TZ-26-022	grade A glove print: 1 CFU, S. capitis	SA-TZ-01

MB-TK-05 — Positive Sterility Test Investigation Form (Word) · section 5: hypothesis matrix of Case A

What the purchase contains

The purchase is digital: after payment you download a ZIP package with the guide and the toolkit in the language you chose. Choose one variant: **ITA - Italiano** or **ENG - English**; the two editions have the same structure, the same tools and the same codes.

- **The complete PDF guide** — 248 A4 pages, 18 chapters in six parts, five integrated cases and six appendices (ITA/ENG glossary, source matrix, chapter–toolkit–article matrix, toolkit instructions, assumptions and limitations, revision); clickable table of contents and bookmarks.
- **16 editable tools** — 8 Word templates and 8 Excel workbooks, codes MB-TK-01...16, arranged in the six folders of the parts (list below).
- **16 filled-in examples** on the simulated Torvenza Pharma laboratory, with the case datasets (folder 07_Examples).
- **Test Log and checks** of the Excel workbooks (folder 08_Verification).

Folder and group	Code	Tool	Format
01 · Laboratory Organisation	**MB-TK-01**	Laboratory Test, Workflow and Responsibility Matrix	Excel
01 · Laboratory Organisation	**MB-TK-02**	Personnel and Aseptic Technique Qualification Programme	Word
02 · Sterility Testing and Isolators	**MB-TK-03**	Sterility Test Method Suitability Protocol	Word
02 · Sterility Testing and Isolators	**MB-TK-04**	Isolator Bio-decontamination Cycle Log and Analysis	Excel
02 · Sterility Testing and Isolators	**MB-TK-05**	Positive Sterility Test Investigation Form	Word
03 · Microbiological Methods and Technologies	**MB-TK-06**	Enumeration, Recovery and Method Suitability Calculator (Poisson, MPN)	Excel
03 · Microbiological Methods and Technologies	**MB-TK-07**	Endotoxin Calculator (MVD, Dilutions, Positive Product Control, Interference)	Excel

Folder and group	Code	Tool	Format
03 · Microbiological Methods and Technologies	**MB-TK-08**	Rapid Method, Identification System and Colony Counter Evaluation Protocol	Word
04 · Media, Strains and Incubation	**MB-TK-09**	Culture Media Batch and Growth Promotion Register	Excel
04 · Media, Strains and Incubation	**MB-TK-10**	Reference Strain Management Model Procedure	Word
04 · Media, Strains and Incubation	**MB-TK-11**	Incubation Excursion Calculator	Excel
05 · Sampling and Sample Management	**MB-TK-12**	Sample Receipt, Chain of Custody and Hold Time Checklist	Word
05 · Sampling and Sample Management	**MB-TK-13**	Air Sampler and Surface Recovery Calculator	Excel
06 · Equipment and Result Reliability	**MB-TK-14**	Laboratory Autoclave, Biosafety Cabinet and Incubator Qualification Protocol	Word
06 · Equipment and Result Reliability	**MB-TK-15**	Microbiological OOS Investigation and CAPA Form	Word
06 · Equipment and Result Reliability	**MB-TK-16**	Contract Laboratory Assessment and Periodic Review	Excel

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