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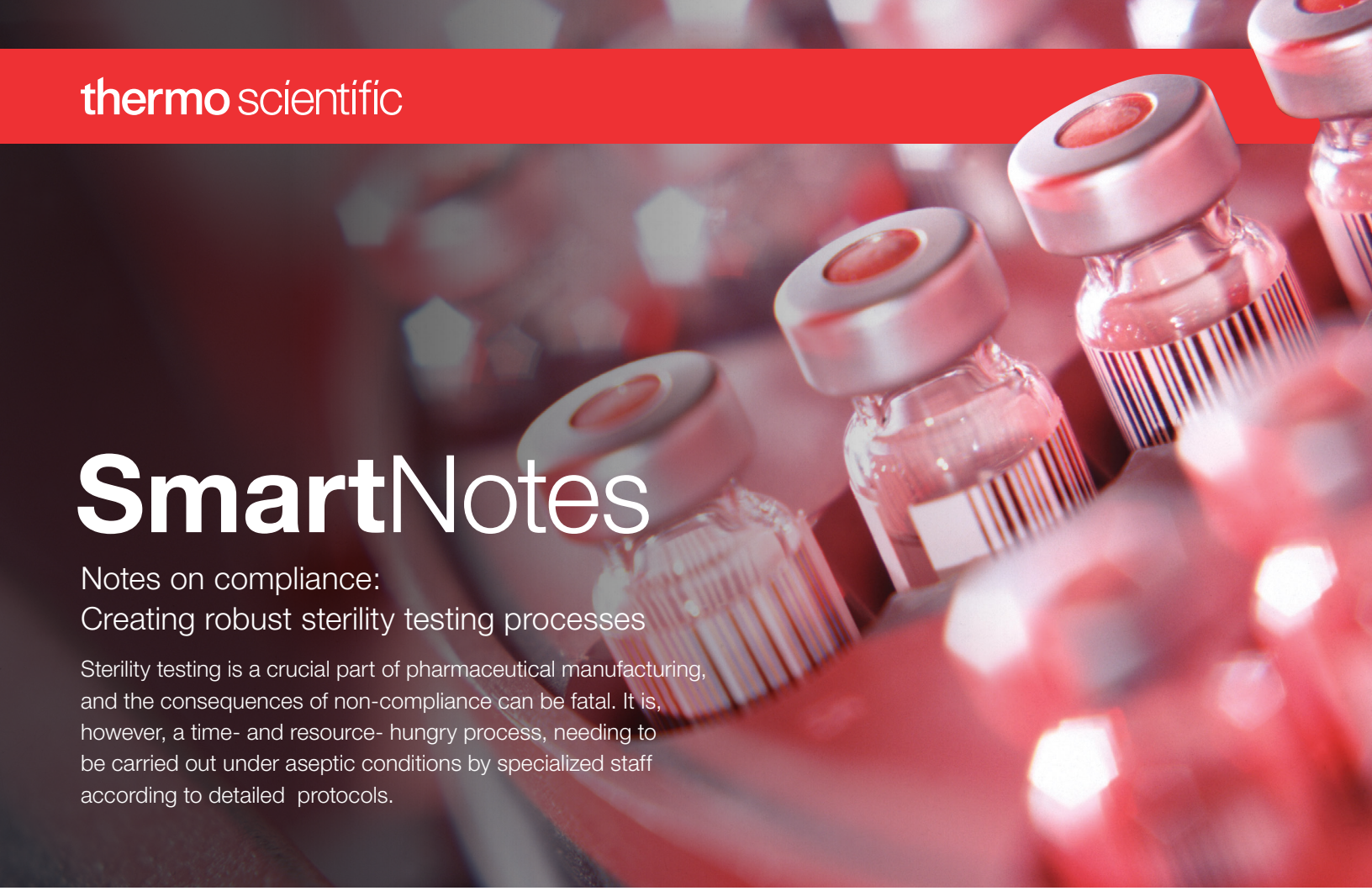

SmartNotes

Notes on compliance:
Creating robust sterility testing processes

Sterility testing is a crucial part of pharmaceutical manufacturing, and the consequences of non-compliance can be fatal. It is, however, a time- and resource- hungry process, needing to be carried out under aseptic conditions by specialized staff according to detailed protocols.

Finished product samples must undergo a 14-day incubation period before being cleared for release onto the market. Anything less than a 100% pass rate can relegate an entire batch unable to reach the people who depend on it.

So, what can pharmaceutical laboratories do to mitigate the risk of costly contamination during the manufacturing process? Building robust, validated protocols is the key to reducing the risk of avoidable test failures and smoothing the road from component intake to product release.

Sterility – and why it matters

Patient safety is of the utmost importance in drug development, but parenteral drug products bypass many of the body's natural defenses. As such, they carry an increased risk of infection.

In 2012, for example, a multi-state outbreak in the U.S. of fungal meningitis and other infections was linked to preservative-free MPA steroid injections distributed by the New England Compounding Center in Framingham, Massachusetts.

The Centers for Disease Control was notified of more than 750 linked cases in 20 states. Tragically, 64 people died.

Cases like this demonstrate why parenteral products are so strictly regulated by the health and regulatory authorities, including the United States, European and Japanese pharmacopeias.

When it comes to sterility the stakes are high, both in terms of protecting human health and keeping supply lines open. Between 2004 and 2011, more than 75% of FDA recalls involved sterile products. Of these, 80% were linked to a "lack of sterility assurance".

Sterility testing failure, then, is not an option for organizations that are committed to protecting both human and business health.

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USP 71

USP 71, which relates to the sterility of all parenteral medicines, has been harmonised with its regulatory counterparts in Europe and Japan.

It requires drug manufacturers to ensure their end products are completely free from objectionable organisms, such as *Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa, Aspergillus brasiliensis, Eschericia coli, Clostridium sporogenes,* and *Bacillus subtilis.*

To comply to the simple presence / absence requirement, samples must be incubated in both fluid thioglycollate medium (FTM) and soya bean casein digest medium (SCDM) or tryptic soy broth (TSB) for 14 days, to check for the turbidity that may indicate the growth of colonies.

It is a statistical test that demands a 100% pass rate. In batch sizes of less than 100 containers, laboratories must test 10% or four containers, whichever is the largest figure. When the batch size is larger than 500 containers, the requirement is to test 2% or 20 containers, whichever figure equates to the fewest.

It means that manufacturers can never be completely sure that the whole batch is objectionable organism free, even if the tests are all negative. Conversely, one positive test will result in the whole batch being held back, sometimes for months, or even destroyed.

With budgets built to reflect the typical batch release lead-time of 20 to 28 days, each day of delay is considerably expensive. More importantly, it can restrict people's access to the medications they need to get on with their lives.

To avoid this, laboratories must do everything in their power to comply with USP 71 – and that includes ensuring standard operating procedures (SOPs) do not contribute to the problem. Building robust processes is the first step to eradicating avoidable sterility testing failure.

Building the process

Sterility testing may be based on producing a simple presence/ absence result, but that is just the tip of the iceberg.

Pharmaceutical laboratories first need to build and validate scientifically robust, product-specific protocols they can rely on. It is a challenging process, but it is essential if laboratories want to be sure their methods do not introduce risk.

Teams first need to carry out suitability, or growth promotion tests, and validation, or bacteriostasis and fungistasis testing, to select the right tools for their SOP.

During suitability testing, laboratories must first find a growth medium that supports the growth of viable objectionable organisms within the product. It's a process of trial and error that requires access to media, the indicated organisms, and a high level of technician knowledge and expertise.

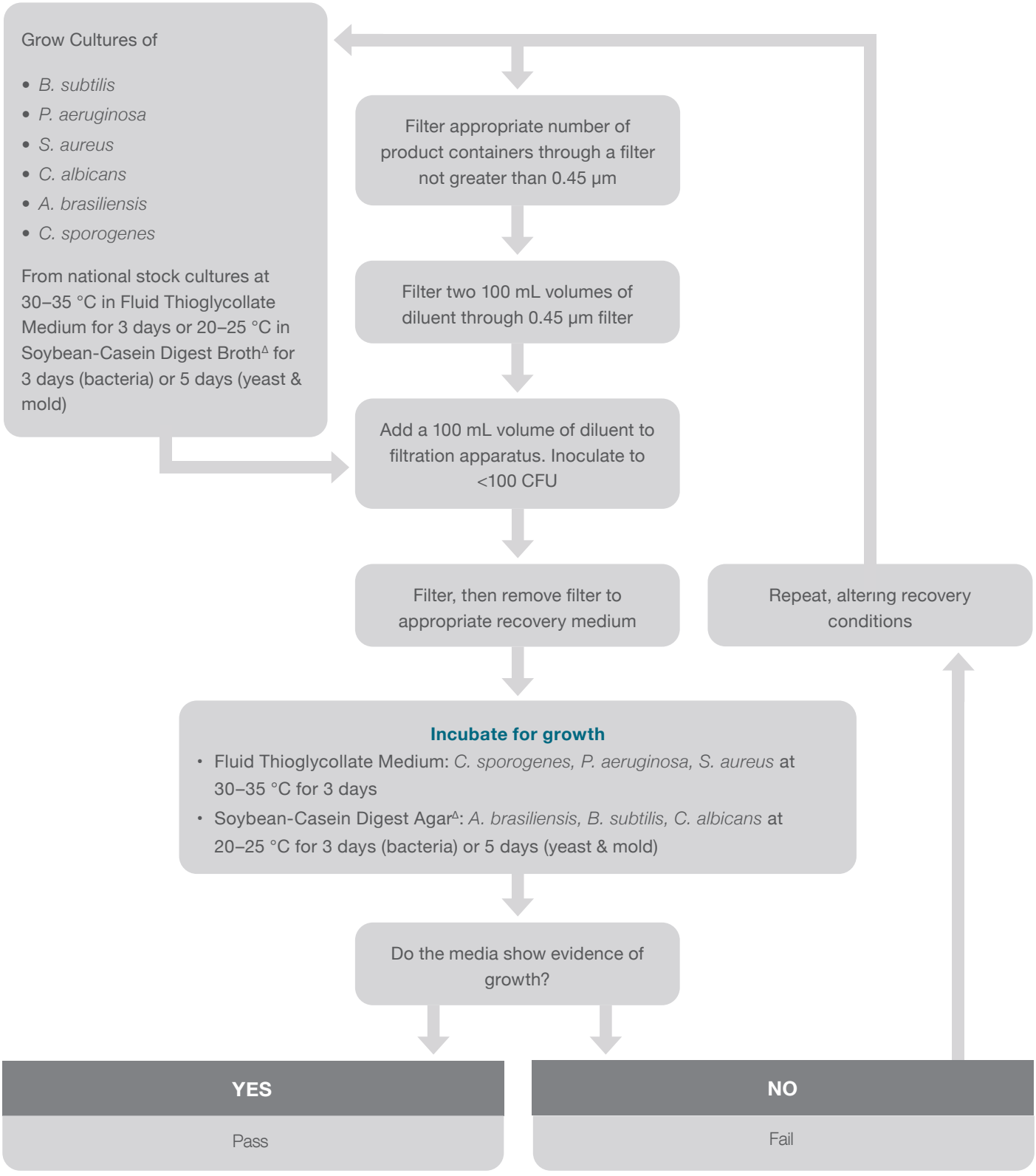
Next, that medium itself must be incubated and assessed for sterility. (Please refer to the workflow for Sterility Test: Method Suitability Study Design.)

Laboratories must also perform validation, to ensure the test sample will not inhibit the growth of the microorganisms in the selected media. The aim is to ensure the active ingredients of the product are neutralized to allow the microorganisms to grow, rather than inhibiting their growth.

The way these tests are performed will depend on the method of the final sterility testing, which will tend to be membrane filtration, for liquid pharmaceuticals, or direct transfer, for medical devices.

Sterility Test: Method Suitability Study Design

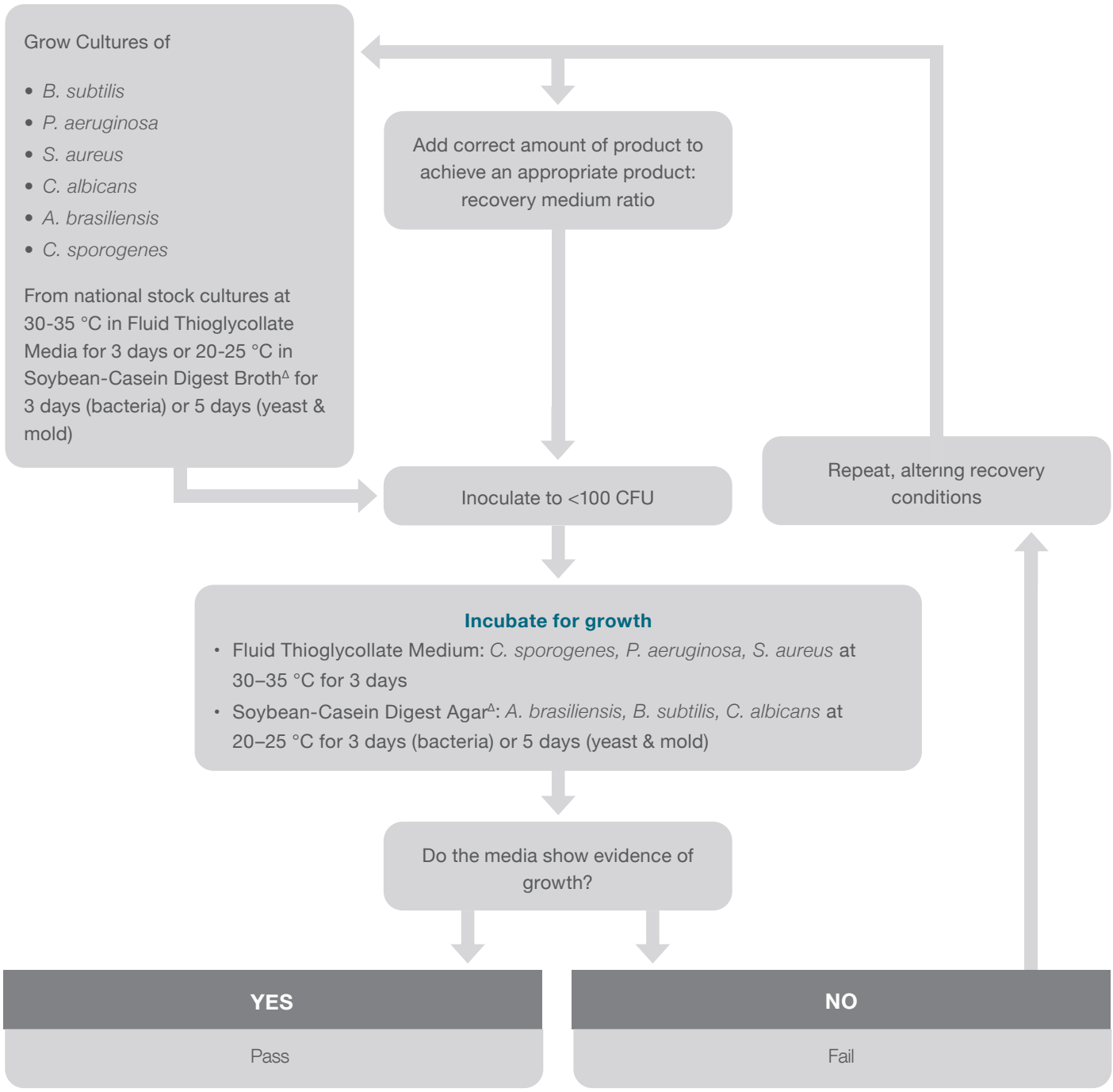
Membrane Filtration



[Δ] Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

Sterility Test: Method Suitability Study Design

Direct Transfer



[Δ] Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

To reduce the risk of contamination during sterility testing, the procedure should be carried out in a closed system under aseptic conditions. Most organizations interpret this as using an ISO-regulated laminar airflow cabinet within an ISO-regulated clean room, or an isolator in a controlled environment.

Samples must be prepared as per the detailed protocol and incubated for 14 days. If a sample appears to indicate the presence of pathogens within that period, incubation must continue for the full two weeks before teams can take steps to quantify the results or identify the microorganism.

If growth is confirmed, a re-test is only permitted if an investigation finds cause to invalidate the results.

Sterility Test: Direct Transfer

Add validated amount of product to achieve an appropriate product:recovery medium ratio. Test all required units of product in both Soybean-Casein Digest Broth[Δ] and Fluid Thioglycollate Medium

↓

Incubate for growth 14 days
Fluid Thioglycollate Medium: 30–35 °C
Soybean-Casein Digest Broth[Δ]: 20–25 °C

↓

Do the media show evidence of growth?

YES	NO
Fail	Pass

Sterility Test: Membrane Filtration

Filter appropriate number of product containers through 0.45 µm filter

↓

Validated volume of appropriate diluent through 0.45 µm filter

↓

Filter, then remove filter to appropriate recovery medium

↓

Incubate for growth for 14 days
Fluid Thioglycollate Media: 30–35 °C
Soybean-Casein Digest Agar [Δ]: 20–25 °C

↓

Do the media show evidence of growth?

YES	NO
Fail	Pass

Maximum stress test

Robust sterility testing protocols are essential to protecting patient safety and keeping manufacturing on time and on budget.

To build effective strategies, pharmaceutical laboratories need skilled staff, high manufacturing standards, and access to all a wide range of media, rinses, and quality control microorganisms.

Thermo Scientific™ sterility testing workflow:

- Diluting and rinsing fluids*:
  - o Fluid A
    - R112490 100mL serum bottle
    - BO0833M 100mL vial
  - o Fluid D
    - R112321 300mL serum bottle
  - o Fluid K
    - R112332 100mL vial
- Growth Media*:
  - o Fluid Thioglycollate Medium
    - BO0368M 100mL vial
    - R112641 100mL vial
  - o Fluid Thioglycollate Medium, Dehydrated
    - R453452 500g
  - o Soy Casein Digestor Tryptic Soy, Dehydrated
    - R455052 500g
  - o Tryptic Soy Broth
    - BO0369M 100mL vial
    - R112731 100mL vial
  - o Vegetable Peptone Broth, Dehydrated
  - o VG0101B 500gCold Filterable Tryptic Soy Broth for Sterile Media Fills
    - BP1065C 10L
  - o Cold Filterable Vegetable Peptone Broth for Sterile Media FIlls
    - BP0104C 10L
- Thermo Scientific™ Quanti-Cult Plus™ Quality Control Organisms
  - o Guaranteed to return <100 colony forming units per 0.1ml
  - o Ease of use – simply rehydrate, mix and incubate, then inoculate
  - o 100 tests per kit
  - o Strains derived from ATCC cultures:
    - R4717016 *Staphylococcus aureus* ATCC® 6538™
    - R4711221 *Bacillus subtilis* ATCC® 6633™
    - R4715210 *Pseudomonas aeruginosa* ATCC® 9027™
    - R4711700 *Clostridium sporogenes* ATCC® 19404™
    - R4711503 *Candida albicans* ATCC® 10231™
    - R4711100 *Aspergillus brasiliensis* ATCC® 16404™

*Visit thermofisher.com for a more complete portfolio of related products which support USP 71

References

[1] https://www.fda.gov/drugs/drug-safety-and-availability/fda-advises-drug-manufacturers-burkholderia-cepacia-complex-poses-contamination-risk-non-sterile

[2] https://www.cdc.gov/hai/organisms/bcepacia.html

[3] https://www.cdc.gov/hai/organisms/bcepacia.html

[4] https://www.fda.gov/media/88801/download

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Contact us at **microbiology@thermofisher.com** for a confidential discussion on your sterility testing workflows, or visit **thermofisher.com/pharmamicrobiology.**

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