WHICH QC STRAINS TO USE FOR MINIMIZING RISK OF FALSE POSITIVES DUE TO CROSS-CONTAMINATION IN A LAB?

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INTRODUCTION

The use of traceable culture collection strains, including pathogenic ones, is necessary in food as well as water and environmental laboratories for various applications, including:

- Daily or regular **Quality Control (QC)** of methods, mandatory for accredited labs
- **Performance testing** of culture media and reagents
- **Verification** and **in-house validation** of methods (e.g., according to ISO 16140-3)

However, the use of (pathogenic) strains, which often require culture and inoculum preparations, can lead to **cross-contamination of routine samples.**

Handling control strains in the same lab where routine samples are tested increases the risk of cross-contamination leading to **false positive results,** with financial and time-consuming consequences (re-testing, sequencing…).

When a positive result is found in samples, it may be necessary for the lab to quickly establish that the result is from a true contamination and not caused by laboratory cross-contamination with the culture collection strain. They may have to investigate or retest the sample while taking measures (sometimes unnecessarily) that delay the release of food products.

Consequences for the customer whose sample was contaminated by the laboratory may include holding stock and withholding release of product to market, stopping production, stripping down and cleaning equipment and in the worst case an unnecessary product recall, with loss of reputation and impact on brand image.

PURPOSE

To facilitate the confirmation of positive results, detect false positives and help to quickly exclude or identify the control strain, labs are looking for reliable alternatives to minimize the impact of cross-contamination due to the use of lab culture collection strains.

Ideally, alternative collection strains should:

- Be **easily distinguishable** from natural contaminants.
- Have the **same characteristics** (e.g., molecular, phenotypical…) as the strains currently used.

Food manufacturers have alerted the standardization bodies about the risk of using classical collection strains, especially pathogens, and have asked them to study the possible use of such strains that are the same target organisms but which are easily distinguishable from the target (wild type) strains.

ISO/TC34/SC9 and CEN/TC463 agreed to create an Ad hoc group to study this topic and to propose recommendations.

This poster aims to:

- Present the work and conclusion of this group;
- Clarify definitions and conditions of use of such strains;
- Propose alternative control strains to labs and their addition in ISO standards;

METHODS

As mentioned, food laboratories are facing the **risk of false positive results** due to their control strains and have **difficulties confirming true positives**.

To help food industry control this risk, the committee ISO/TC34/SC9 dedicated to horizontal methods in the field of microbiological analysis of the food chain, established an Ad hoc group D – "Use of distinguishable target control strains".

It was created to propose the following: more complete definitions and precise characteristics of these strains to be easily differentiated from natural contaminations, conditions and advantages for their use and investigations needed to confirm between true positive and false positive detections.

The group drafted recommendations, validated by ISO/TC34/SC9 and provided them to the ISO/TC34/SC9/WG7 in charge of the revision of ISO 7218 "General requirements and guidance for microbiological examinations".

ISO 7218 will include the possibility of using these distinguishable target control strains.

The same recommendations will now be provided to joint WG5 of the ISO/TC34/SC9 (Food microbiology) and ISO/TC 147/SC4 (Water microbiology) in charge of the revision of ISO 11133 "Preparation, production, storage and performance testing of culture media" to take this use into account.

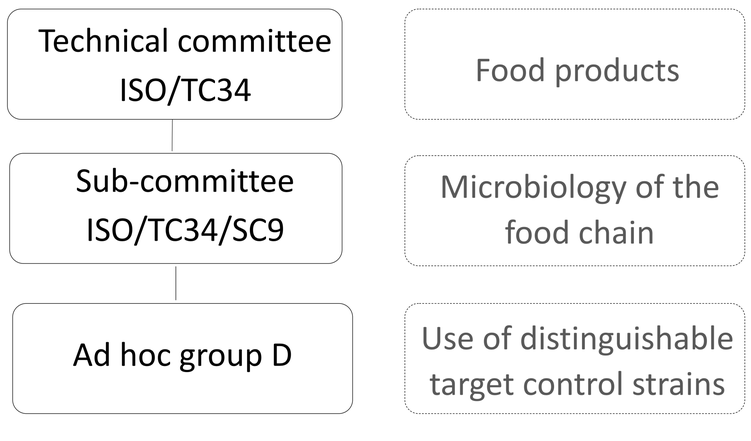

Fig 1. Organization of ISO/TC34/SC9 and Ad hoc group.

RECOMMENDATIONS

According to the experts of Ad hoc group D, when using strains alongside testing, labs can control the risk of cross-contamination and limit false positive results by:

- **Implementing preventive measures and good practices** such as inoculating the control strains in a zone or a biosafety cabinet separated from the tested samples or separated in time (e.g., inoculating the control strains for QC at the end of sample testing, in a specific part of the lab).
- **Preferably choosing specific micro-organisms that are easily distinguishable** from typical sample isolates, for example by choosing uncommon or genetically modified (altered) strains with easy-to-detect characteristics, such as fluorescence.

Which strains are considered distinguishable?

- **Uncommon control strains** are strains **rarely or never encountered** in foods or samples tested in food labs, having specific **characteristics that are easily distinguishable** from more common strains (e.g., by serology for *Salmonella*).
- **Genetically altered control strains** are strains with specific characteristics that can be **easily and rapidly distinguished** from common strains (e.g., **luminescence** or **fluorescence** after the GFP (Green Fluorescent Protein) gene has been inserted).

How fluorescent strains are obtained?

The use of highly fluorescent strains was made possible thanks to huge progress in molecular biology over the last years. Fluorescent reference strains carrying a stable integrated copy of the GFP gene have been developed. In addition, these strains were then improved by modifying the GFP gene to have a brighter fluorescence **[1].**

Today, the insertion of the GFP gene is even possible into the chromosome of the host directly, providing a better stability of the gene in bacteria and a high intensity of fluorescence.

These breakthroughs now make it possible to use these genetically altered strains to distinguish true positives from false positives.

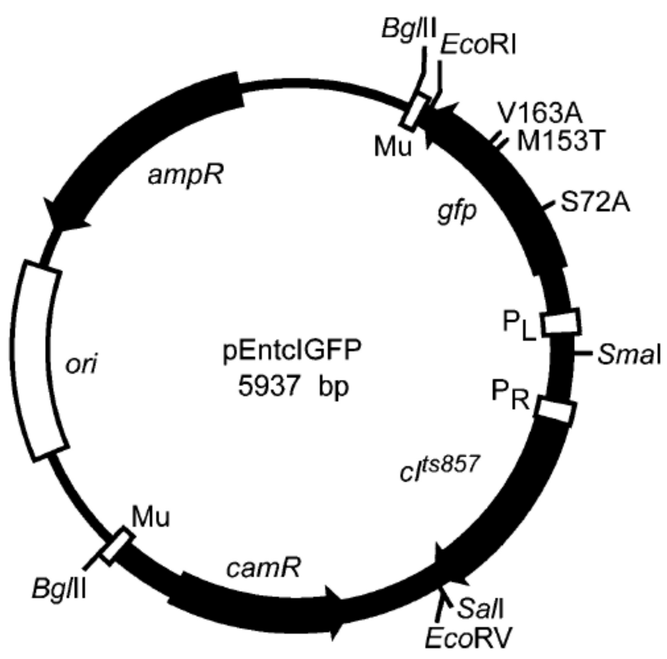

Fig 2. Example of plasmid construction inserted in bacteria to produce fluorescence.

How to use these strains?

Uncommon strains and genetically altered control strains must be:

- Used with the **same rules and precautions** for their culture and preparation as conventional control strains, especially if they are not ready-to-use.
- **Sufficiently evaluated beforehand** to demonstrate that they are suitable for the chosen method control.
- In some countries, genetically altered control strains are considered as GMO/GMM (genetically modified organisms/ genetically modified microorganisms) and as such specific rules apply to labs for their use and handling.

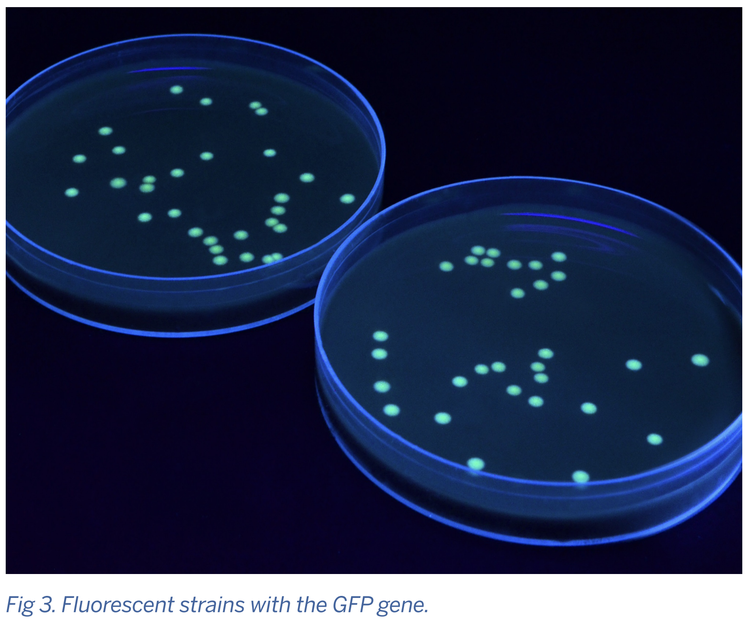

Fig 3. Fluorescent strains with the GFP gene.

How to confirm that a positive sample is not due to a contamination with distinguishable target control strains?

The use of genetically altered control strains allows the lab to easily confirm its control strain by using a specific method, including:

- Fluorescence reading of colonies isolated on plates under UV light.
- Adapted **PCR (Polymerase Chain Reaction)** tests (e.g., detecting Green Fluorescent Protein).

- If the confirmation for GFP is **positive,** the lab can conclude that the positive sample result was caused by cross-contamination and a full root cause investigation should be initiated.
- It is crucial that labs using genetically altered strains must not use conventional strains of the same species in the same area. Indeed, genetically altered strains should totally replace the same conventional species used for method control operations.

The use of uncommon strains for method control allows the lab to confirm positive samples by confirming the identity of this strain (e.g., using serology for *Salmonella*)

- Although detection of this uncommon strain is unlikely, the lab is not exempted from confirming the presence of the same uncommon strain in the sample tested.
- Consequently, additional investigations (with a root cause analysis) up to a whole genome sequencing (WGS) may be required before concluding a cross-contamination occurred in the lab.

Alternatively, if confirmation of the **uncommon** or **genetically altered strain** is **negative** in the tested sample, the lab can confirm that the positive result obtained was due to a natural contamination (true positive) and continue testing the sample.

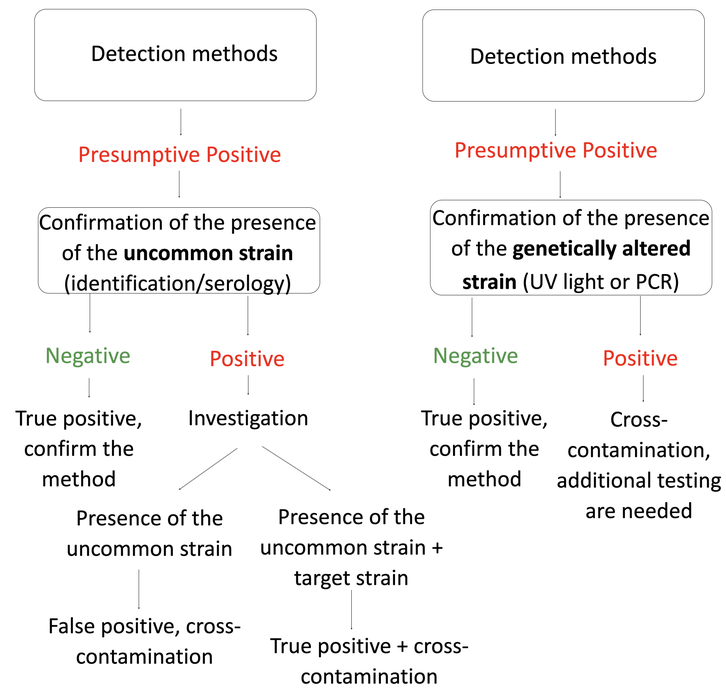

Fig 4. Use of distinguishable strains to (dis)confirm positive results or not.

To summarize, the use of these specific strains helps to distinguish false positive from true positive results, reducing the need for extensive complementary analyses for confirmation, root cause investigations, action plans and increases the reliability of the released results.

CONCLUSION

- **The use of uncommon or genetically altered control strains for microbiological control of culture media and methods enables laboratories to quickly and easily confirm laboratory cross-contamination by their control strain.**
- Their presence in a food tested by the laboratory confirms cross-contamination (after additional analyses) and therefore confirms a false positive result.
- These easily distinguishable strains increase the reliability of the results while limiting the cost of testing by avoiding unnecessary extra-work.
- **ISO 7218, which is currently under revision, will soon introduce the possible use of these distinguishable control strains in food testing labs to reduce the impact of laboratory cross-contamination.**
- Similarly, **ISO 11133** (Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media), whose revision process has just begun, should also introduce the possible use of distinguishable strains.

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[1] *Pinheiro, L.B., Gibbs, M.D., Vesey, G. et al. Appl Microbiol Biotechnol (2008) 77: 1287. https://doi.org/10.1007/s00253-007-1253-9. Fluorescent reference strains of bacteria by chromosomal integration of a modified green fluorescent protein gene*