Note 2: For other Listeria enrichment broths than those listed below (like Half-Fraser, Fraser...), refer to the VERIFLOW® GFP package insert instructions.

Enrichment broths compatibility:

Various broths, usually used for enrichment of the traditional method or automated detection method, (amongst the most representative bioMérieux validated detection methods), have been evaluated:

***Salmonella* methods**	BPW + supplement SX2 MKTTn RVS (occasionally, RVS broth may give a very weak positive result, which should be confirmed by other means) M broth non-fat dry milk (NFDM) with 0.01% brilliant green solution mTSB
***E. coli* O157:H7 methods**	BPW (pre-warmed at 41.5°C) + VCC mTSB (pre-warmed at 41.5°C) + novobiocine mTSB + acriflavine BPW + acriflavine
***Cronobacter* methods**	BPW + novobiocine CSB
***Listeria* methods**	LPT broth (VIDAS LPT/GENE-UP LIS /GENE-UP LMO) LMX broth (VIDAS LMX)

*RVS broth: occasionally, RVS broth may give a very weak positive result, which should be confirmed by other means

It is also possible to use VERIFLOW® GFP confirmation from a well-isolated single colony.
In this case, proceed to "Colony Sample Prep and PCR" section according to the package insert instructions.

Culture media compatibility:

Various agar, usually used for isolation or confirmation, have been evaluated, such as SALMA® and ESIA®.

The implementation of GFP confirmation does not dispense with the usual confirmation of your detection method.

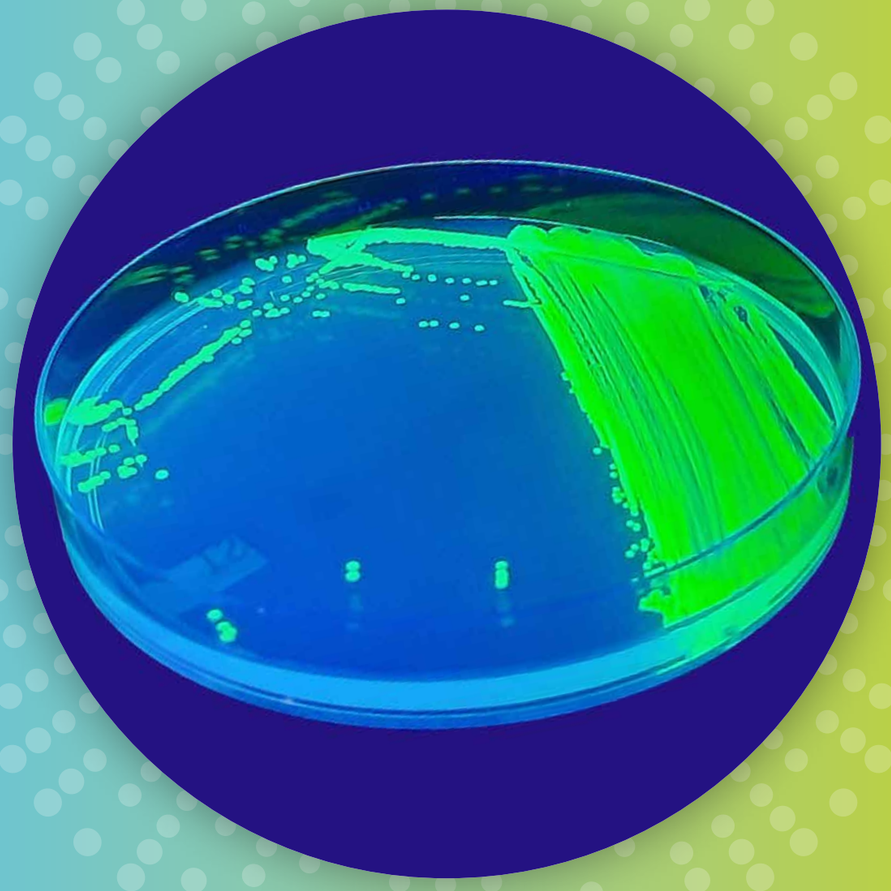
BIOBALL® LUMINATE 2.0 is a range of Genetically Modified Micro-Organisms (GMMs) and may need to comply with special regulatory requirements for a laboratory contained use in your country (e.g., European directive 2009/41/EC completed by national regulation). In this case, please refer to your competent local authority to declare or obtain an agreement before use.

Contact your local bioMérieux representative for more details and availabilities, and to assist you in your GMM agreement requests.

bioMérieux S.A. • 69280 Marcy l'Étoile • France • Tel.: + 33 (0)4 78 87 20 00 • Fax: +33 (0)4 78 87 20 90
www.biomerieux.com

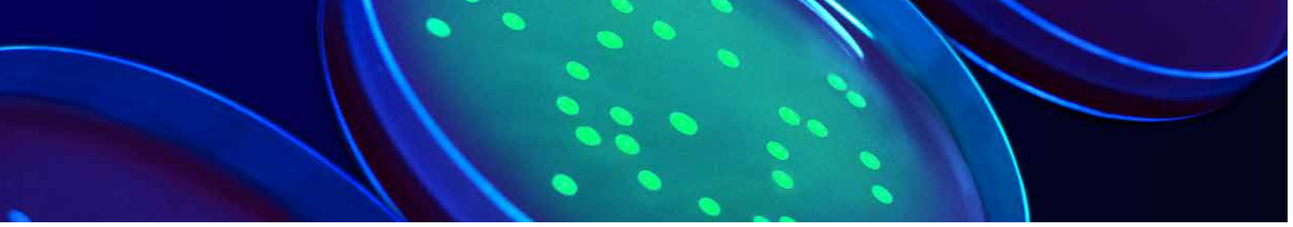
BIOBALL® LUMINATE 2.0

GFP Confirmation Techniques
For your positive tests



Your Trusted Partner in Augmented Diagnostics

PIONEERING DIAGNOSTICS



If you are using a BIOBALL LUMINATE 2.0 in your laboratory collection strains:

To release the positive results obtained on your routine food samples analysis and to confirm that this positive is from natural contamination (and not contamination resulting from cross-contamination with the BIOBALL LUMINATE 2.0 control strain), two techniques are proposed:

1. Fluorescence confirmation under UV light

After incubation of the agar culture media resulting from an analysis by a conventional method or the confirmation plate of an automated detection method, the colonies grown are observed under a UV lamp *(365-370 nm UV lamps for laboratory use)*.

The absence of fluorescence allows you to exclude the presence of possible cross-contamination due to a GFP strain collection and makes it possible to confirm that this is a valid positive sample.

Culture media compatibility: Various culture media, usually used for traditional methods or confirmation of automated detection method, have been evaluated:

- ASAP®, SALMA®, ChromID® SLM, Bismuth Sulfite Agar, Hektoen Agar, XLD Agar, COS*, TSA (*Salmonella* Typhimurium).
- CT-SMAC Agar, ChromID® EHEC, COS*, TSA (*Escherichia coli* O157:H7).
- CCI Agar, ESIA®, COS*, TSA (*Cronobacter sakazakii*).
- ALOA®, COS*, TSA (*Listeria innocua* and *Listeria monocytogenes*).

NB: PALCAM agar presents a limitation due to a natural fluorescence of the agar. Sub-cultivate onto a non-selective culture media (nutrient agar/TSA/COS*) before reading under UV light.

In case another selective media is used, it is necessary, beforehand, to:

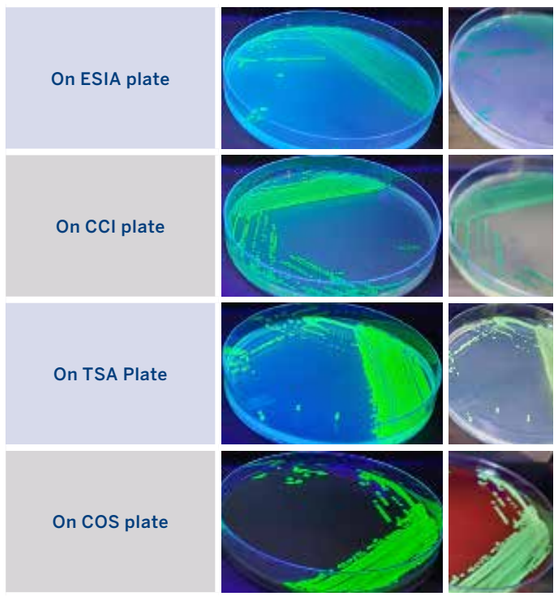
- Evaluate the absence of natural fluorescence of the agar.
- Evaluate the fluorescence of the corresponding GFP strain.

In case of natural fluorescence of the agar and/or absence of correct fluorescence of the strain on this agar, sub-cultivate onto a non-selective culture media (nutrient agar/TSA/COS*) before reading under UV light.

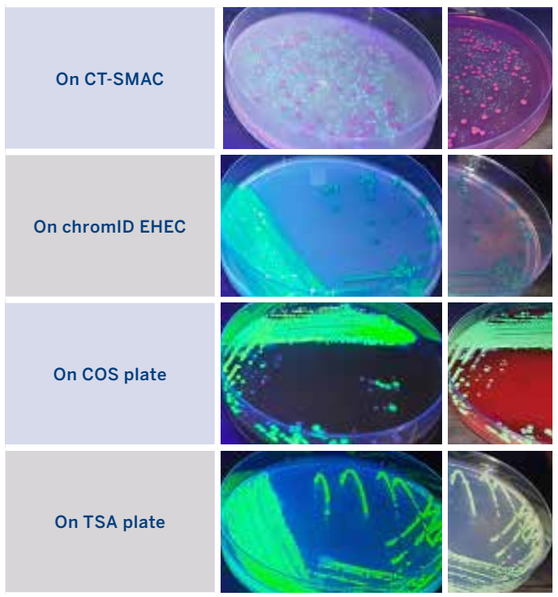
*COS Columbia blood agar.

Example of fluorescence: Under UV light (in a black box on the left side).

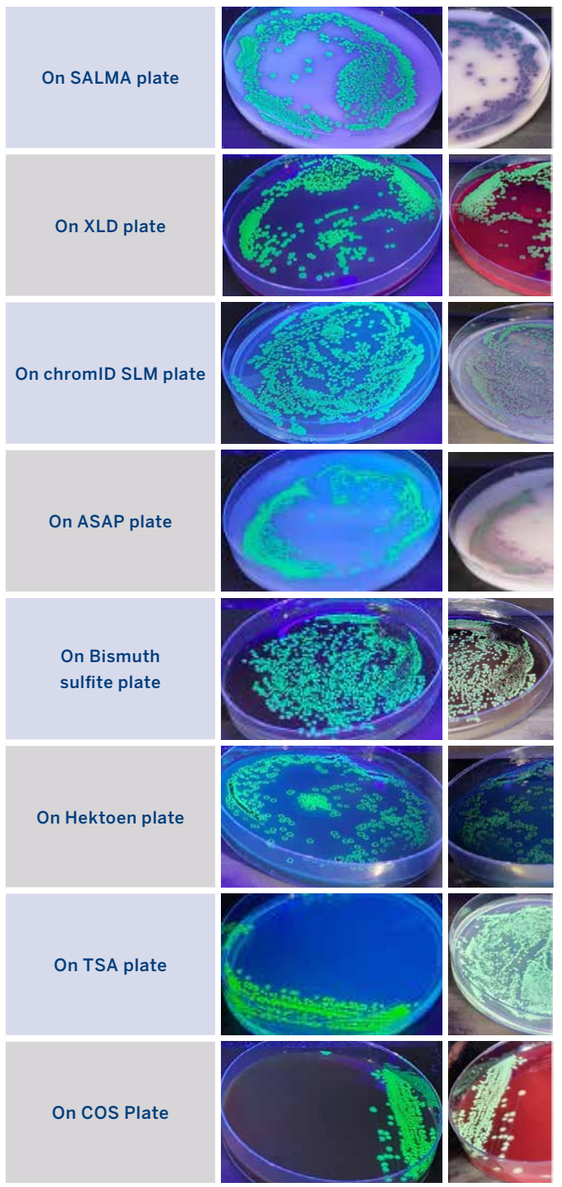
Cronobacter sakazakii LUMINATE 2.0



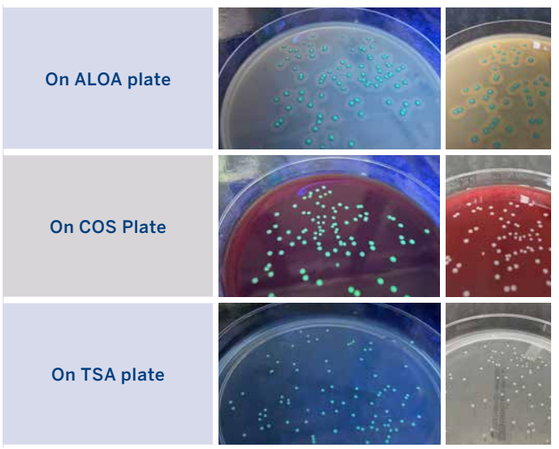
Escherichia coli O157:H7 LUMINATE 2.0



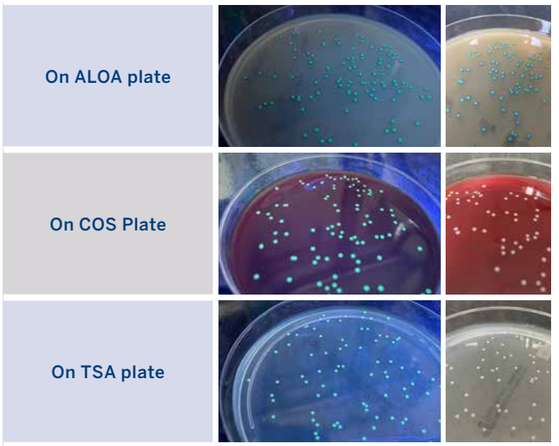
Salmonella Typhimurium LUMINATE 2.0



Listeria monocytogenes LUMINATE 2.0



Listeria innocua LUMINATE 2.0



2. VERIFLOW® GFP confirmation

VERIFLOW® Green Fluorescent Protein (GFP) is a molecular-based assay for detecting GFP coding sequence of BIOBALL® LUMINATE 2.0 strains. It's an easy-to-use Invisible Sentinel® kit for rapid confirmation on the VERIFLOW® platform.

- IS0506200 - VERIFLOW GFP PCR REAGENT (24 PCR tests) - storage at -20°C
- IS6012 - VERIFLOW GFP KIT BOX (cassettes, buffer, and lysis tubes) (24 tests) - storage at room temperature
- ISTC002 - VERIFLOW THERMOCYCLER (PCR endpoint)

For Salmonella, E.coli O157:H7, Cronobacter and Listeria ASSAYS

- Follow sampling and enrichment that corresponds to the detection method.
- From enriched culture for all sample types, pipette generally 500 µL to provided 1.5 mL sampling lysis tube, and invert to mix contents.
- Proceed to "Sample Prep and PCR" section then to "Cassette sample analysis" according to the VERIFLOW® GFP package insert instructions.

Note 1: Specifically, from non-fat dry milk (NFDM) with 0.01% brilliant green solution, pipette 50 µL to provided 1.5 mL sampling lysis tube, and invert to mix contents. The transfer of a larger volume of enrichment, such as 500 µL, can cause a pink coloration of the cassette, which can interfere with the reading. However, this will not modify the interpretation.