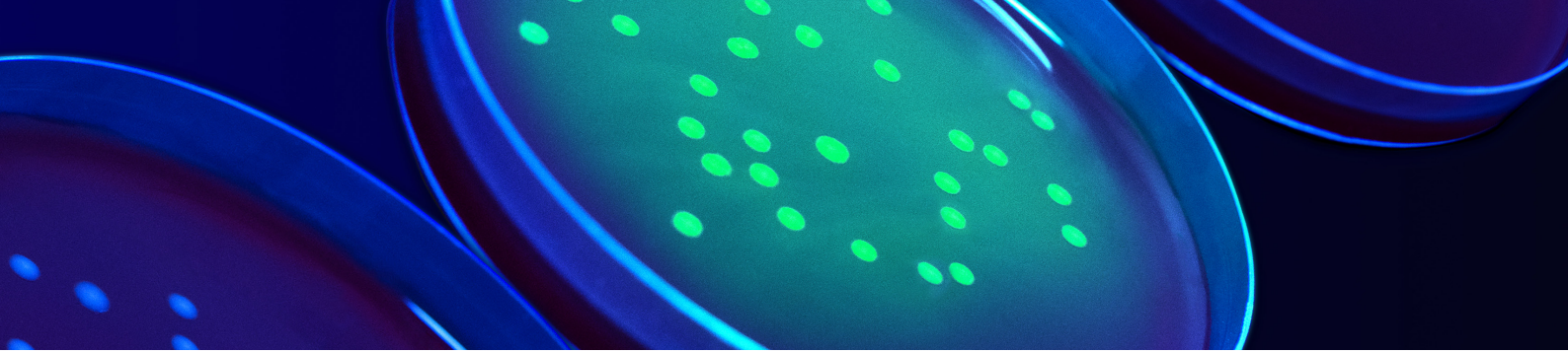
BIOMÉRIEUX

BIOBALL® LUMINATE 2.0

GFP CONFIRMATION TECHNIQUES
For your positive tests

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.

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.

UV Lamp characteristics: *365-370 nm UV lamps for laboratory use*

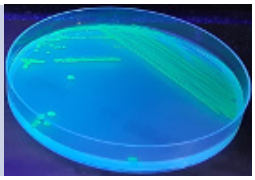
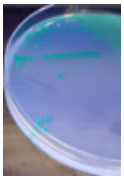
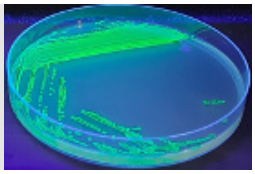
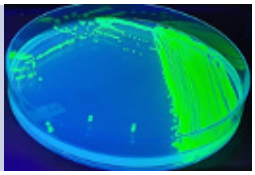
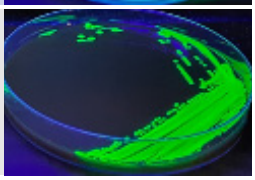
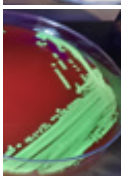
Various culture media, usually used for traditional methods or confirmation of automated detection method, have been evaluated:

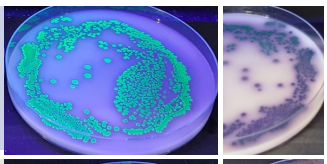
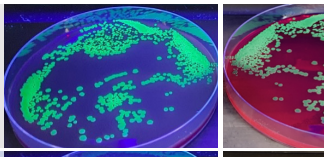
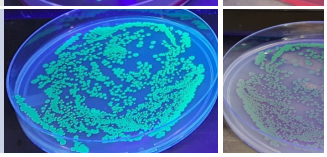
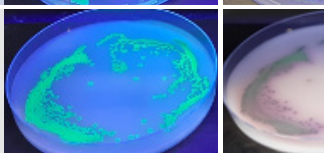
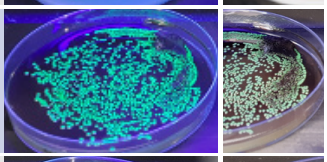
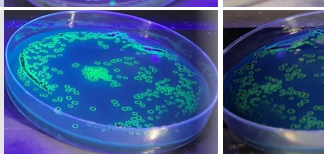
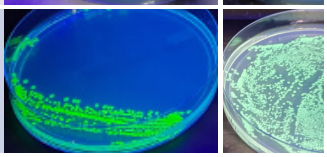
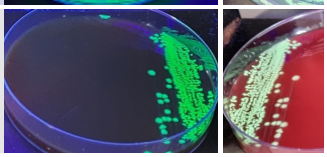
- *Salmonella Typhimurium* - SALMA, ASAP, ChromID SLM, XLD, Bismuth sulfite agar, and Hektoen
- *Escherichia col*i O157:H7 - CT-SMAC, ChromID EHEC
- *Cronobacter sakazakii* - ESIA, CCI
- Non-selective media (nutrient agar/TSA/Columbia blood agar...)
- In progress for *Listeria innocu*a and *Listeria monocytogenes* - ALOA and PALCAM

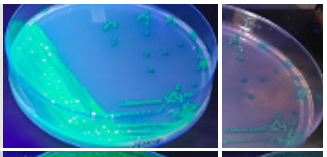
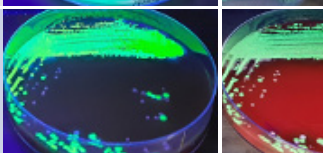
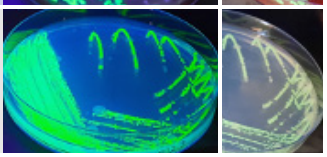
In case of the use of another selective media, it is necessary to:
1- Evaluate the fluorescence reading of the corresponding GFP strain before use, or
2- Sub-cultivate onto a non-selective culture media (nutrient agar/TSA/Columbia blood agar) before reading

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

***Cronobacter sakazakii* LUMINATE 2.0**

On ESIA plate:		
On CCI plate:		
On TSA Plate:		
On COS plate:		

***Salmonella Typhimurium* LUMINATE 2.0**	
On SALMA plate:	
On XLD plate:	
On chromID SLM plate:	
On ASAP plate:	
On Bismuth sulfite plate:	
On Hektoen plate:	
On TSA plate:	
On COS plate:	

***Escherichia coli* O157:H7 LUMINATE 2.0**	
On CT-SMAC:	
On chromID EHEC:	
On COS plate:	
On TSA plate:	

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 @ -20°C

- IS6012 - VERIFLOW GFP KIT BOX (cassettes, buffer, and tubes) (24 tests) - storage @ room temperature

- ISTC002 - VERIFLOW THERMOCYCLER (PCR endpoint)

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

Follow sampling and enrichment that corresponds to the detection method.

From enriched culture for all sample types, pipette 500 µL to provided 1.5 mL sampling tube, and invert to mix contents.

Note 1: Specifically, from non-fat dry milk (NFDM) with 0.01% brilliant green solution, pipette 50 µL to provided 1.5 mL sampling 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.

Proceed to Sample Prep and PCR section according to the package insert instructions.

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

For *Listeria* ASSAYS
Evaluation in progress

Note 2: 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.

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.

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