5.2. Methods

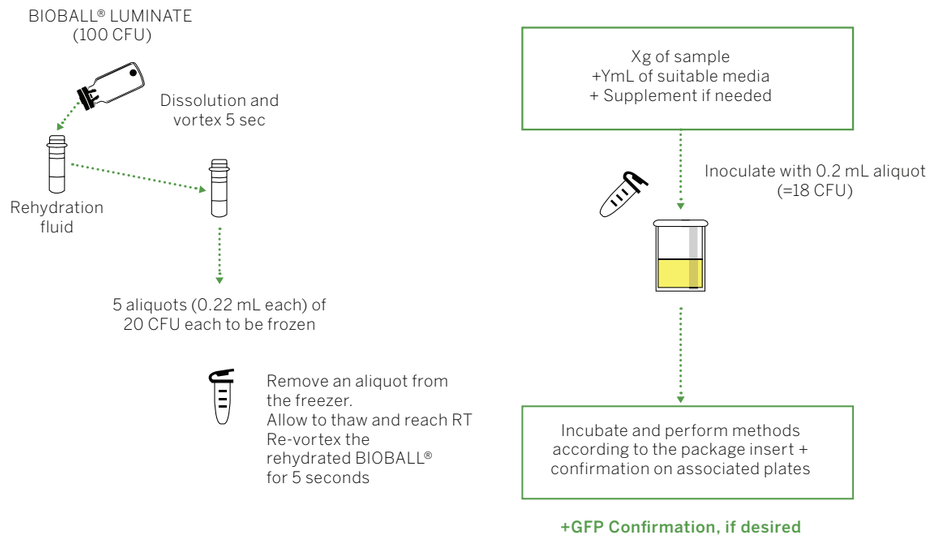
Preparation of the sample:

- ➜ In a blender bag with filter, place Xg of the selected food product + mL of the suitable media and, if necessary, the supplement (according to the protocol of the tested method) + one aliquot of BIOBALL® LUMINATE 2.0.
- ➜ Follow the manufacturer or reference method instructions.
- ➜ The daily positive control protocol, described in this document, was successfully evaluated on the most representative bioMérieux validated rapid methods. A higher inoculum level can also be used, according to your laboratory requirements and your methods. A lower inoculum level (e.g., 0.16 mL aliquots of BIOBALL® (6 shots of 14 CFU)) will require a verification before use.

5.3. GFP confirmation of your positive QC (if needed)

It should not be necessary to confirm a positive result for your positive (daily) control, if your food sample was negative prior to the seeding with the BIOBALL® LUMINATE 2.0. If desired, confirm your positive results according to instructions "GFP CONFIRMATION TECHNIQUES" (e.g., VERIFLOW® GFP confirmation)

Annex 1: Flow diagram of (Daily) quality control protocol



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 local competent 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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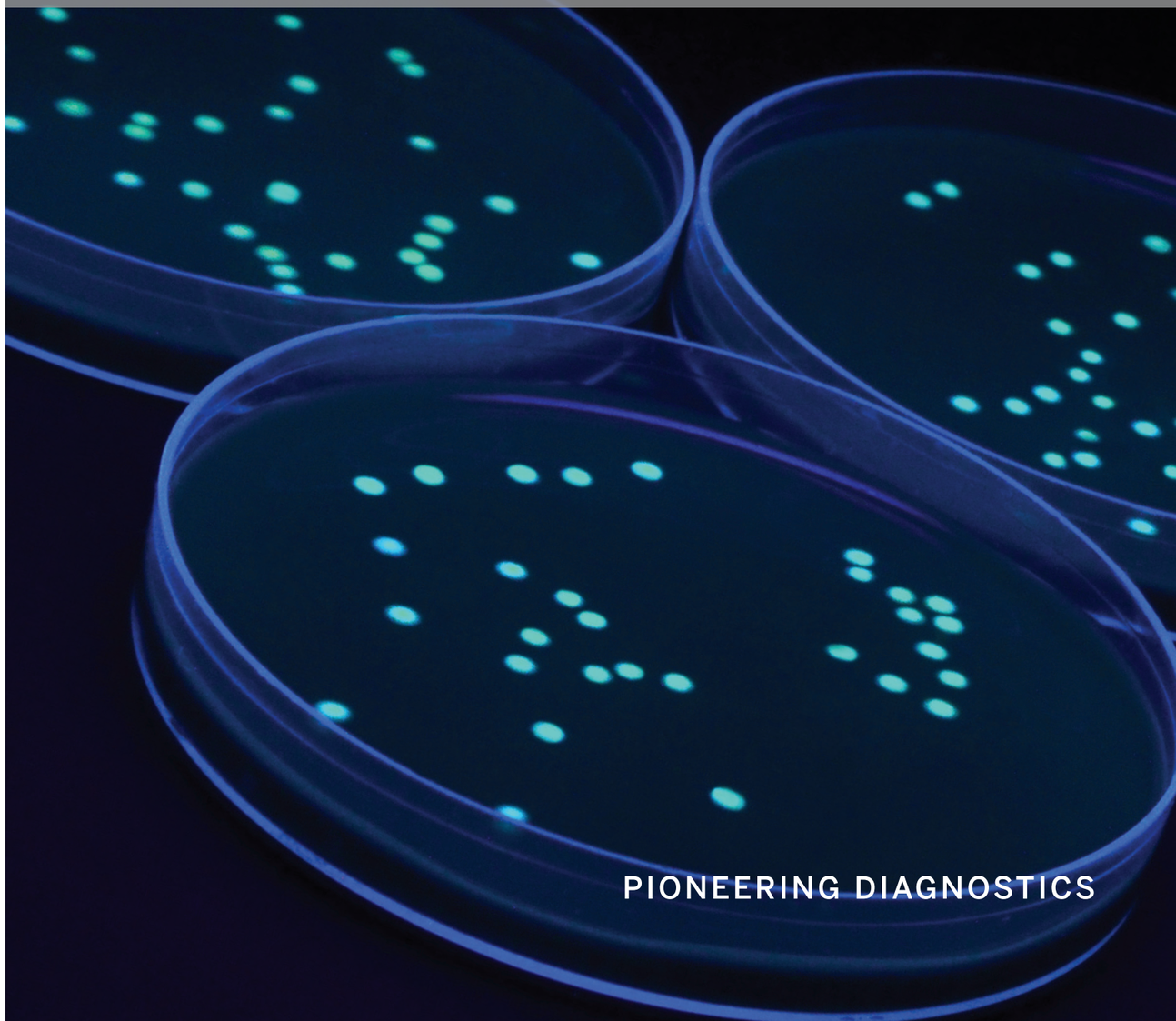
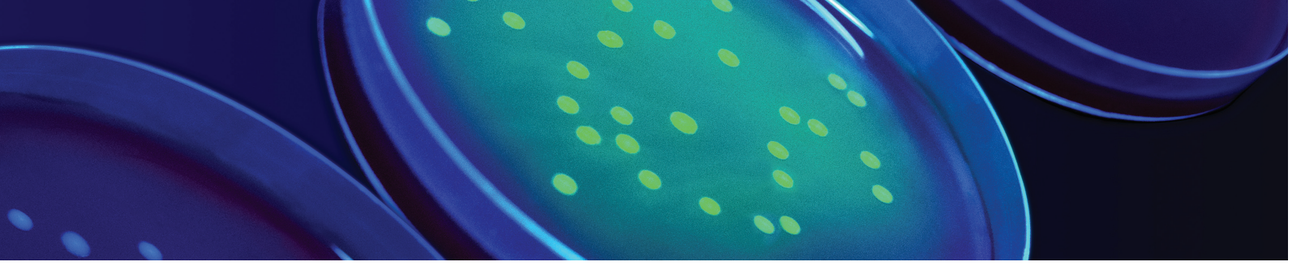
BIOBALL® LUMINATE 2.0

PROTOCOL
For (Daily) Positive Quality
Control of Qualitative Methods





1. Context of daily positive quality control

The (daily) positive control or quality control is an experiment that involves the repetition of the test using a working sample known to produce a positive result. The Food Analysis Laboratories need to have daily or regular positive control to be sure that the method used routinely worked well and that the positive results obtained on a food may be approved.

The Green Fluorescent Protein (GFP) BIOBALL® LUMINATE 2.0 corresponds to an accurate and precise number of lyophilized bacteria into a small water-soluble ball where the green fluorescent protein gene sequence has been incorporated into the chromosome. This allows strain to be easily distinguished from non-modified food contaminants.

The protocol described in this document is recommended for (daily) positive quality control of detection methods using BIOBALL® LUMINATE 2.0 (100 CFU). A verification of these protocols was carried out on the most representative bioMérieux validated detection methods.

2. GFP Strains (100 CFU) and reagent

REFERENCE 100 cfu/10 vials	MICROORGANISM	BIOBALL® STRAIN (derived from)	CORRESPONDING STRAIN
423938	*Salmonella* Typhimurium	NCTC 12023	WDCM 00031
423939	*Escherichia coli* O157:H7	NCTC 12900	WDCM00014
423940	*Cronobacter sakazakii*	NCTC 11467	WDCM 00214
423941**	*Listeria monocytogenes* 4b	NCTC 10527	WDCM 00021
423942**	*Listeria innocua*	NCTC 11288	WDCM 00017

➔ Storage of BIOBALL® LUMINATE 2.0 (100 CFU) between -18°C and -33°C

➔ CFU mean of between 85 and 115 CFU with a standard deviation ≤15% of the mean

➔ BIOBALL® 14-Day Rehydration fluid (1.1 mL) - Reference 410386 (storage between 2°C and 8°C)

***contact us for availability*

3. Food matrices

➔ *Salmonella* spp., *Listeria* spp. or *Cronobacter* spp.: UHT skimmed milk or milk powder

➔ *E. coli* O157:H7: Raw meat of beef.

The selected food sample must be tested and confirmed negative for the targeted microorganism, prior to the inoculation with the BIOBALL® LUMINATE 2.0 control strain.

Other matrices may be used after checking that they are suitable for this positive control use.

4. Handling requirements

The use of BIOBALL® LUMINATE 2.0 at a precise concentration of 100 CFU makes it possible to limit handling and risks of cross-contamination of your samples.

Nevertheless, all precautions must be taken to avoid these possible contaminations during your positive control tests.

It is recommended to dedicate a particular space, material and time, to the preparation of suspensions / aliquots, to sample seeding and its analysis.

5. Protocol

5.1. BIOBALL® LUMINATE 2.0 preparation

5.1.1. Rehydration using BIOBALL® 14-day Rehydration fluid (bioMérieux REF: 410386)

➔ Label the BIOBALL® 14-Day Rehydration tube with the spare label included in the box of BIOBALL® LUMINATE 2.0 (100 CFU)

➔ Remove the cap from BIOBALL® 14-Day Rehydration Fluid.

➔ Remove the stopper from the glass BIOBALL® vial and tip the BIOBALL® into the 14-Day Rehydration Fluid.

➔ Replace the cap and wait 3 minutes for the BIOBALL® to fully dissolve.

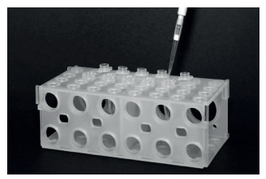
Note: 14-Day Rehydration Fluid needs to be at room temperature when the BIOBALL® is added.

➔ Vortex for 5 seconds.

5.1.2. Aliquot preparation

➔ Draw 0.2 mL aliquots of BIOBALL® (5 shots of ~18 CFU each shot).

Note 1: Aliquots can be used for up to 2 hours after rehydration if stored at 15-25°C or up to 8 hours after rehydration if stored at 2°C to 8°C.
Note 2: The aliquots must be frozen below -18°C within 2 hours of rehydration if used over 7 to 14 days (depending on the strain - Refer to section 5.1.4). For the preparation of aliquots intended for freezing, transfer 0.22 mL to be able to easily take 0.2 mL during the quality control.



5.1.3. Use of a frozen aliquot

➔ Remove an aliquot from the freezer; allow to thaw and reach room temperature.

➔ Re-vortex the rehydrated BIOBALL® for 5 seconds before each use.

➔ The aliquots can be used immediately after thawing at 15°C-25°C and up to 2 hours after thawing at 2°C to 8°C.

5.1.4. Stability of aliquots at -18°C

In 14-Day Re-hydration fluid	Reference	Stability
Salmonella Typhimurium	423938	14 Days
Escherichia coli O157:H7	423939	14 Days
Cronobacter sakazakii	423940	14 Days
Listeria monocytogenes 4b	423941	Work in progress
Listeria innocua	423942	Work in progress