HOW TO IMPLEMENT THE USE OF GENETICALLY MODIFIED MICROORGANISMS FOR ROUTINE QUALITY CONTROL OF METHODS IN LABS?

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INTRODUCTION

According to ISO/IEC 17025, testing laboratories should ensure they operate competently and are able to generate valid results. Implementation of Quality Control or Verification of methods requires the use of strains. Genetically Modified Microorganisms (GMM) (e.g., GFP tagged organisms) offer a less risky approach minimizing the impact of cross contamination events securing laboratory results.

BIOBALL®LUMINATE 2.0 is a range of microbiological reference material containing a precise number of viable bacterial cells, tagged with a Green Fluorescent Protein (GFP). The GFP, integrated in the chromosome, allows easy differentiation between accidental cross-contamination (linked to quality control artificial contaminations) and natural contaminants. The 2.0 range displays a high fluorescence and includes 5 different strains (Tab 1) with a target of 100 cfu adapted to various applications in the food industry.

Table 1: BIOBALL®LUMINATE 2.0 range & Protocols evaluated

Strain	Source	Reference #	Daily control	Method Verification ISO16140-3
Cronobacter sakazakii	From NCTC 11467 - WDCM 00214	423940	GENE-UP CRO (25g)	
				GENE-UP CRO (375g)
Escherichia coli O157:H7 (non-toxigenic stx-)	From NCTC 12900 - WDCM00014	423939	GENE-UP ECO (25g) 8h	GENE-UP ECO (25g) 8h & 24h
			VIDAS ECPT (25g) 24h	
			VIDAS ECPT (375g) 24h	
Listeria innocua	From NCTC 11288 - WDCM 00017	423942	GENE-UP LIS (25g)	
				VIDAS LPT (25g)
Listeria monocytogenes 4b	From NCTC 10527 - WDCM 00021	423941	GENE-UP LIS (25g)	VIDAS LPT (25g)
			GENE-UP LMO (25g)	VIDAS LMX (25g)
Salmonella Typhimurium	From NCTC 12023 - WDCM 00031	423938	GENE-UP SLM2 (25g)	
				VIDAS SPT (375g)

PURPOSE

Strains should be rightly qualified prior to routine use in the lab. To qualify & demonstrate the suitability of GMM, BIOBALL® LUMINATE 2.0 range is evaluated on pathogenic detection methods (Table 1), the most sensitive and suitable for each application. Such application includes (daily) positive control, verification of methods according to ISO 16140-3 and positive results confirmation.

DAILY CONTROL

The daily positive control or quality control is an experiment involving test repetition using a known positive working sample. The Food Analysis Laboratories need to have daily positive control to be confident that the method used in routine worked well and that a positive result obtained on a sample may be reported. This is their insurance to provide reliable test results.

To evaluate the use of BIOBALL® LUMINATE 2.0 for daily positive control, one BIOBALL® LUMINATE 2.0 was dissolved into 1 tube of 14-Day Re-Hydration Fluid 1,1 mL (Ref# 410386). After stirring, 200 µL (about 18 cfu) were pipetted out and used to inoculate bags of food samples. 5 bags were spiked and 1 bag used as a negative control (only necessary for the evaluation, not in routine) (Fig 1a). Upon positive results, GFP confirmation tests were run using plate method with UV check control at 365 nm. Successful evaluation is achieved when all daily control replicates are positive & their fluorescence confirmed.

Food samples & method protocols evaluated for daily positive controls are described in Table 1 & Fig 2.

To verify the precise count of BIOBALL® LUMINATE 2.0 used for each test, 200 µL aliquots were spread onto TSA plates in triplicates and incubated for 24h at 37°C.

Table 2 presents results obtained for daily control tests. All methods showed 5/5 confirmed positive at the targeted spiked level of about 18 cfu. This evaluation confirmed the suitability of BIOBALL® LUMINATE 2.0 range for daily control application.

Table 2: Daily positive control results when using BIOBALL® LUMINATE 2.0 range

Strain	Test method	True inoculation in CFU (Target 18CFU)	Presumptive positive	Confirmed GFP positive (agar)	Pass/ fail
Cronobacter sakazakii	GENE-UP CRO (25g)	19	5/5	5/5	Pass
Escherichia coli O157:H7	GENE-UP ECO (25g) 8h	19	5/5	5/5	Pass
	VIDAS ECPT (25g) 24h	19	5/5	5/5	Pass
	VIDAS ECPT (375g) 24h	19	5/5	5/5	Pass
Listeria innocua	GENE-UP LIS (25g)	19	5/5	5/5	Pass
Listeria monocytogenes 4b	GENE-UP LIS (25g)	19	5/5	5/5	Pass
	GENE-UP LMO (25g)	19	5/5	5/5	Pass
S. Typhimurium	GENE-UP SLM2 (25g)	16	5/5	5/5	Pass

FIGURES

Fig 1 BIOBALL® preparation (a/ for daily control; b/ for method verification ISO 16140-3)

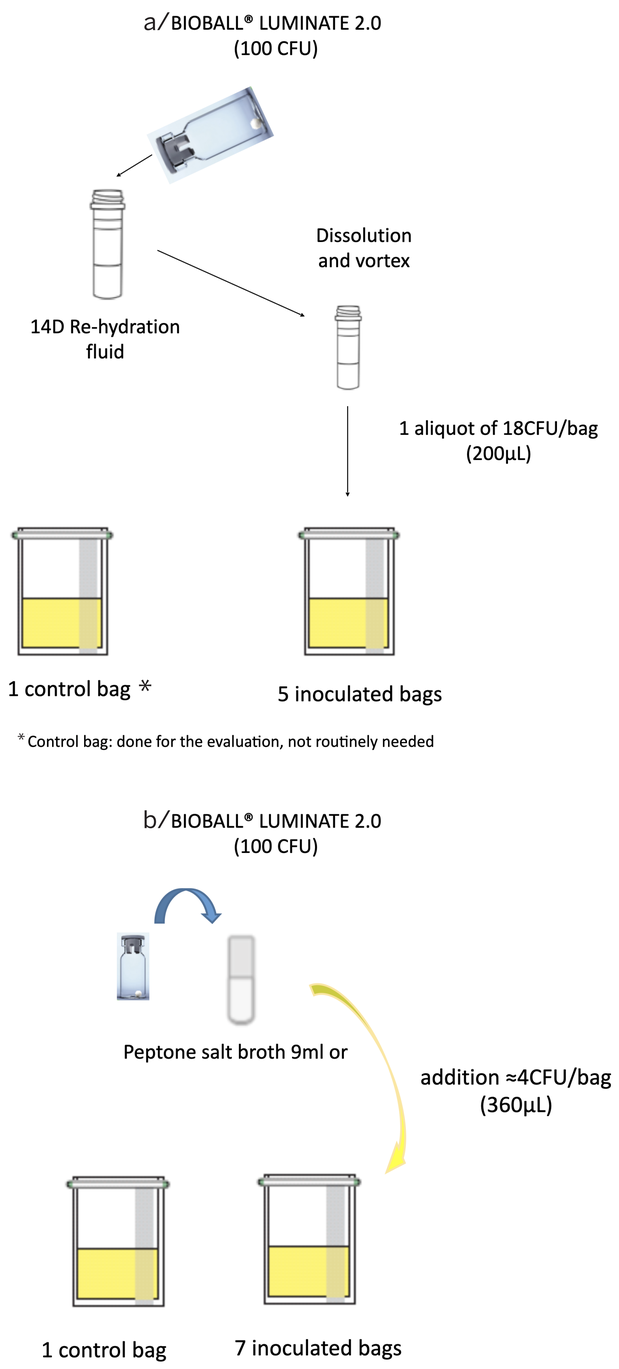


Fig 2 Sample matrices & Methods description

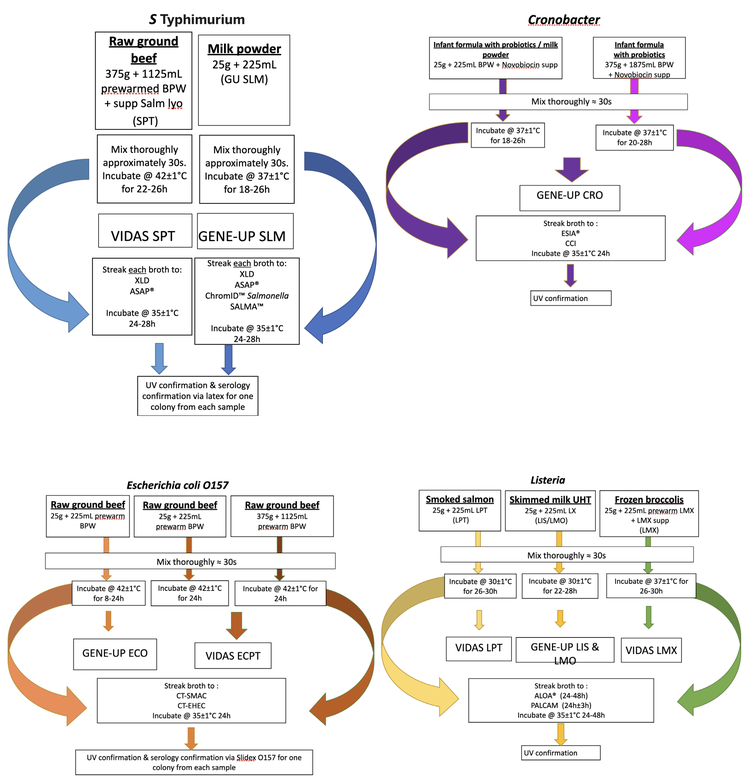
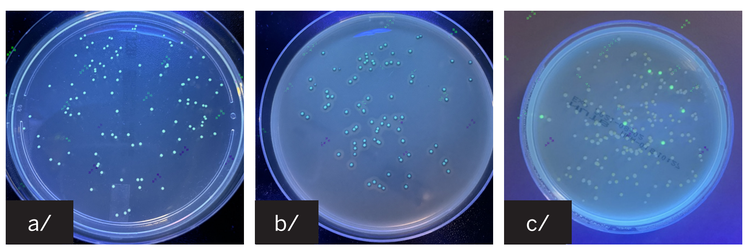


Fig 3: Illustration of BIOBALL® LUMINATE 2.0 strains onto agar plate under UV light (365 nm) (a/ L. monocytogenes on TSA, b/ L. innocua on ALOA®, c/ C. sakazakii among environmental flora)



METHOD VERIFICATION ISO 16140-3

For ISO 16140-3, protocol 3 was applied. To do so, the recommended spiking level is between 3-5 cfu. An inoculation of about 4 cfu was targeted: 1 BIOBALL® LUMINATE 2.0 was dissolved into 1 tube of peptone salt 9 mL. After dissolution & stirring, a volume of 360 µL (about 4 cfu) of the initial suspension was used for artificial contamination. (Fig 1b)

To verify the precise count of BIOBALL® LUMINATE 2.0 used for each test, 1 mL was spread onto 3 TSA plates and incubated for 24h at 37°C.

For each method (Table 1 & Fig 2), 7 bags were spiked and 1 used as negative control. Method verification was considered successful when 6 out of 7 samples were tested true positive (presumed positive confirmed by UV check on agar plate).

Table 3: Method verification ISO 16140-3 results with BIOBALL® LUMINATE 2.0 range

Strains	Test method	Real count in CFU (Target 3-5 CFU)	Positive screening	Confirmed GFP positive (agar)	Method verification Pass/fail
Cronobacter sakazakii	GENE-UP CRO (25g)	4.9	7/7	7/7	Pass
	GENE-UP CRO (375g)	3.8	7/7	7/7	Pass
Escherichia coli O157:H7	GENE-UP ECO (25g) 8h & 24h	4.6	7/7	7/7	Pass
	VIDAS ECPT (25g) 24h	3.8	7/7	7/7	Pass
	VIDAS ECPT (375g) 24h	3.8	6/7	3/7*	Pass
Listeria innocua	VIDAS LPT (25g)	2.5	7/7	7/7	Pass
Listeria monocytogenes 4b	VIDAS LPT (25g)	2.6	7/7	7/7	Pass
	VIDAS LMX (25g)	2.6	7/7	7/7	Pass
S. Typhimurium	VIDAS SPT (375g)	2,8	7/7	7/7	Pass

*tested only on CT-SMAC agar

All methods evaluated with BIOBALL® LUMINATE 2.0 strains (Table 3) show consistent results within the targeted inoculation range with minimum 6/7 positive replicates. This confirms the suitability of such reference material for lab to perform method verification according to ISO 16140-3.

GFP CONFIRMATION

To demonstrate the ability to differentiate a natural contamination from a cross-contamination coming from the GFP tagged control strain, two techniques were evaluated: fluorescence confirmation under UV light on agar plate & VERIFLOW® GFP confirmation.

VERIFLOW® GFP is a molecular-based assay for detecting GFP coding sequence of BIOBALL® LUMINATE 2.0 strains used for rapid confirmation on the VERIFLOW® platform. From an enriched culture (Fig 2), 500 µL sample were used for VERIFLOW® confirmation. Additional enrichment methods were also included in this assessment (Table 4).

Compatible agar plates assessed for fluorescence confirmation under UV light at 365 nm are also given.

Table 4: Confirmation methods evaluation

Strain	VERIFLOW® GFP confirmation		Fluorescence confirmation under UV light (365 nm)
	Enrichment broth	Isolation plate	Isolation plate
Cronobacter sakazakii	BPW+novobiocine CSB	ESIA®	CCI, ESIA®, COS, TSA
Escherichia coli O157:H7	BPW+VCC, mTSB+novobiocine, mTSB+acriflavine, BPW+acriflavine	-	CT-SMAC, ChromID®EHEC, COS, TSA
Listeria innocua & *Listeria monocytogenes*	LPT broth LMX broth	-	ALOA®, COS, TSA
S. Typhimurium	BPW+supplement, SX2, MKTTn, RVS M Broth, NFDM* + 0.01% brilliant green solution, mTSB	SALMA®	ASAP®, SALMA®, ChromID®SLM, Bismuth Sufite, Hektoen, XLD, COS, TSA

*Non-Fat dry milk

On all the enrichment methods tested, VERIFLOW® GFP confirmation was successful. For all the agar plates, a distinct and high fluorescence of the tagged colonies under UV light was observed (Fig 3).
NB: PALCAM agar presents a limitation due to a natural fluorescence of the agar.

STUDY HIGHLIGHT

BIOBALL® LUMINATE 2.0 are rightly qualified for pathogenic detection methods QC and verification

- After artificial contamination of food samples with BIOBALL® LUMINATE 2.0 strains, 100% of the presumptive positive results obtained with VIDAS®, GENE-UP® and ISO methods were confirmed
- These results confirm the suitability of BIOBALL® LUMINATE 2.0 for (daily) quality controls and verification of methods (ISO 16140-3)

Presence of BIOBALL® LUMINATE 2.0 can be easily confirmed

- Detection of fluorescence under UV light on agar plates and with VERIFLOW® GFP were successfully evaluated
- These 2 techniques allow the easy confirmation of the presence of BIOBALL® LUMINATE 2.0 and thus a false positive result in case of cross-contamination

Food testing labs can replace their corresponding in-house collection strains with these ready to use GMM to minimize the imp act of false positives and without additional qualification when testing bioMérieux methods.

- Caution: before routine use for food application, labs should perform their own evaluation prior to use other methods than bioMérieux validated ones such as GENE-UP® or VIDAS®

ACKNOWLEDGEMENT

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