EN ISO 16140-2 VALIDATION STUDY OF A RAPID METHOD FOR THE DETECTION OF *SALMONELLA* SPP. IN RAW MATERIALS FOR CHOCOLATE INDUSTRIES USING GENE-UP® PCR AND NEW EGENE-UP® EASYPREP SOLUTION

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INTRODUCTION

The chocolate industry is particularly concerned by *Salmonella* spp. contaminations in raw materials derived from cocoa beans. *Salmonella* is ubiquitous and present in the environment and can persist in cocoa products along the process of cocoa beans transformation.

Salmonella infection is characterized by acute gastroenteritis. Symptoms include diarrhea, fever, abdominal cramps, and vomiting lasting 4-7 days in most people.

When present in cocoa products, *Salmonella* strains are subjected to a high level of stress and can be injured, making complex the detection by conventional and rapid methods. Moreover, the need of a rapid method in one shift is important due to specific manufacturing constraints.

For this reason, bioMérieux has developed a new protocol to reduce significantly the time-to-result from 24 hours for current GENE-UP® SLM2 alternative method to 12 hours for 375g raw materials for chocolate industries.

PURPOSE

An ISO 16140-2 validation study has been conducted by ADRIA Développement expert laboratory in order to propose to our customers a « one-day » GENE-UP® protocol for the detection of *Salmonella* spp. in 375g portions of raw materials for chocolate industries. The alternative method was compared to ISO 6579-1 reference method for the detection of *Salmonella* spp.

METHOD

Workflow of the EGENE-UP® EASYPREP protocol for chocolate industry:

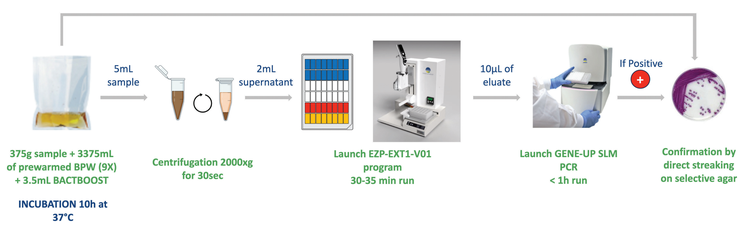

Figure 1. EGENE-UP® EASYPREP workflow for the detection of Salmonella spp. in raw materials for chocolate industries

The enrichment is performed in 9X prewarmed **BPW + BACTBOOST™** supplement to optimize growth of injured Salmonella. After **10h of incubation at 37°C**, 5mL of sample are centrifugated for 30 sec at 2000g and 2mL of supernatant are transferred in **EGENE-UP® EASYPREP** plate containing NUCLISENS® reagents. The run of DNA extraction is then launched on EGENE-UP® EASYPREP instrument. The principle is based on a DNA extraction using silica beads to bind DNA; after different washing steps and elution where the DNA is released from silica beads, we obtain a pure and concentrated DNA eluate. Then, 10µL of eluate are transferred in GENE-UP SLM PCR and the PCR run is launched on the GENE-UP® instrument. Positive results are confirmed by direct streaking on selective agar.

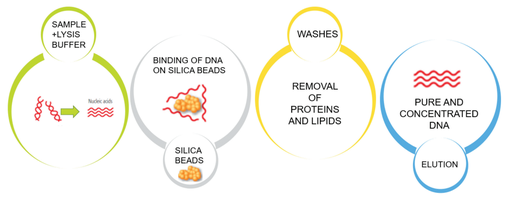

Figure 2. Principle of EGENE-UP EASYPREP DNA extraction

EN ISO 16140-2 validation study:

The AFNOR validation study consisted of a sensitivity study on a minimum of 30 positive and 30 negative samples of raw products of chocolate industries. The scope of validation was divided in 3 types of products as **Cocoa based raw materials, Milk based products & other ingredients used in chocolate factories.** The samples were artificially contaminated by seeding, following ISO 16140-2 requirements.

A RLOD study was also performed on Cocoa powder.

In order to give flexibility to the user, we added the possibility to perform the standard GENE-UP® SLM workflow after 22h of incubation, on the same enrichment protocol.

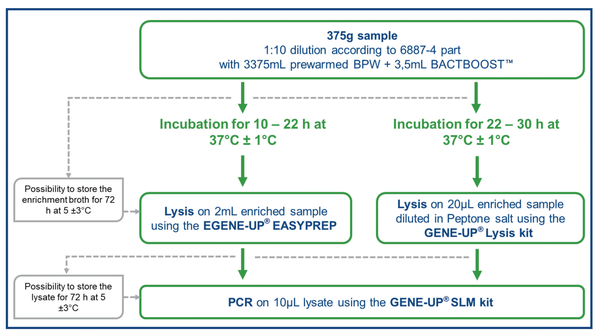

Figure 3. Protocols validated during the EN ISO 16140-2 study

RESULTS

Sensitivity study:

Enrichment protocol	PA	NA	PD	ND	PPND	PPNA	SE alt %	SE ref %	RT %	FPR %	Analysis of discordant results		
											N+	(ND+PPND)-PD	AL
10 h EGENE-UP® EASYPREP	21	29	5	5	0	0	83,9	83,9	83,3	0,0	31	0	3
22 h EGENE-UP® EASYPREP	21	28	6	5	0	0	84,4	81,3	81,7	0,0	32	-1	3
22 h GENE-UP® Lysis kit	21	28	6	5	0	0	84,4	81,3	81,7	0,0	32	-1	3

Figure 4. Calculation of the relative trueness (RT), the sensitivity (SE) and the false positive ratio (FPR)

During the sensitivity study, we obtained 5 samples in negative deviations whatever the protocol used and 5 samples in positive deviation at 10h enrichment; 1 additional positive deviation was observed after 22h incubation time. None of the samples in negative deviation was confirmed positive using the cultural confirmation procedure.
The three protocols tested **meet the acceptance limit of ISO 16140-2** for unpaired samples.

The protocols are also compatible with a storage of enrichment broth or lysate at 5± 3°C for 72h. From 10h incubation time, a positive result can be confirmed by direct streaking on the following selective agars: CHROMID® *Salmonella*, XLD, ASAP™ or SALMA®.

Relative Level of Detection:

Enrichment protocol	Matrix / Strain pair	AL	RLOD	Level of detection at 50% (CFU/sample size)	
				Reference Method	Alternative Method
10 h EGENE-UP® EASYPREP	Cocoa powder *S. Typhimurium*	2,5	2,0	0.3 [0.2 – 0.6]	1.0 [0.6 – 1.9]
22 h EGENE-UP® EASYPREP	Cocoa powder *S. Typhimurium*	2,5	1,9	0.3 [0.2 – 0.6]	0.8 [0.4 – 1.3]
22 h GENE-UP® Lysis kit	Cocoa powder *S. Typhimurium*	2,5	1,9	0.3 [0.2 – 0.6]	0.8 [0.4 – 1.3]

Figure 5. Calculation of the relative level of detection and LoD50% according to Wilrich & Wilrich.

The RLOD of the three protocols **meet the acceptance limit of ISO 16140-2** for unpaired samples.

Inclusivity study:

The EGENE-UP® EASYPREP lysis protocol has been applied using the specific enrichment protocol with the shortest incubation time (BPW + BACTBOOST™, 10 h incubation time) on **100 *Salmonella* strains. All gave expected positive results** by PCR.

STUDY HIGHLIGHTS

- The EGENE-UP® EASYPREP protocol for Raw material of chocolate industries allows a detection of *Salmonella* spp. in less than 12H.
- The alternative method meets the requirements of ISO 16140-2 and is certified NF VALIDATION BIO 12/38-06/16.

ISO 16140-2:2016 Microbiology of the food chain Method validation Part2: Protocol for the validation of alternative (proprietary) methods against a reference method; ISO6579-1:2017 Microbiology of the food chain Horizontal method for the detection, enumeration and serotyping of Salmonella