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Development of a candidate for Reference Material from genomic DNA of *Salmonella spp.*

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INTRODUCTION

Salmonellosis is a human foodborne disease produced by members of *Salmonella genus*. [1]. Cell culture has been the gold standard method for its identification [2], however, it can take from one to two weeks to get a conclusive result. There are several commercial PCR assays to identify *Salmonella spp*., which are based on their own internal positive controls but sometimes there is no a consensus among the different responses [3], due to the lack of a common reference to make them comparable. In that sense, it is needed to develop a reference material in terms of genomic DNA, to guarantee traceability and comparability of measurement results related to the identity and content of *Salmonella sp*p. This work presents the results of the production of a candidate to reference material, based on the genomic DNA content of *Salmonella Enteritidis* ATCC 13076.

MATERIALS AND METHODS

With the aim of producing and characterizing a reference material based on a *Salmonella* genomic DNA, the growth kinetics in selective medium and the DNA extraction method were optimized. In addition, a droplet digital PCR assay (ddPCR) was validated and the main uncertainty sources were identified.

ddPCR method development

Sequences selection

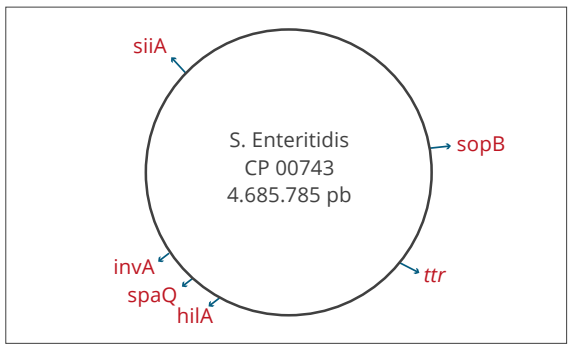

Figure 1. S. *Enteritidis* genome scheme. The genes for the PCR method are shown. They were selected / designed based on bibliography and BLAST analysis.

ddPCR validation method

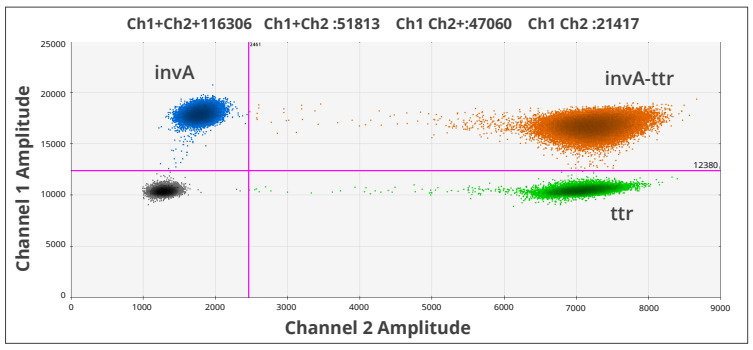

Figure 2. ddPCR method. Three genes were selected to assign the value of the property: invA-ttr run in duplex reaction (2D plot) and *hilA* in simplex form (data not shown).

Method performance

Parameter	Description
Linear range	8 – 8000 cp /µL[(a)]
Detection limit	2 cp / µL
Quantification limit	8 cp / µL
Precision	18 – 3%

Table 1. Validation results. (a) The linear range was calculated in the reaction mix. The precision was measured as RSD.

Cell culture and DNA extraction

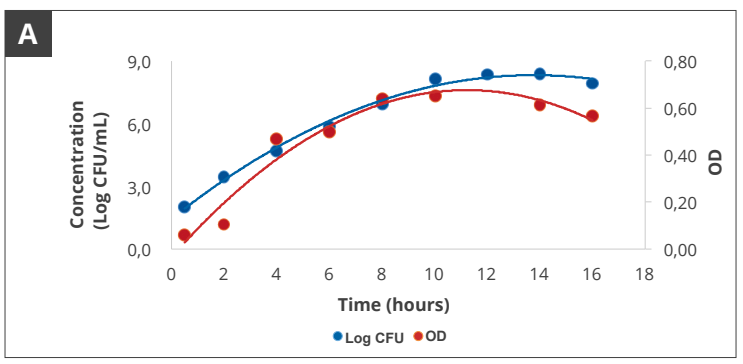

Figure 3. S. *Enteritidis* cell growth kinetics, 200 mL RV broth. Measurements on UFC and optical density vs time. (A). It was selected 10 hours – begin of the stationary phase – to extract DNA by modified CTAB method. (B) Lanes 1 and 2 DNA extracted, lane 3 negative control and lane 4 DNA base pairs ladder 100 pb.

Material processing

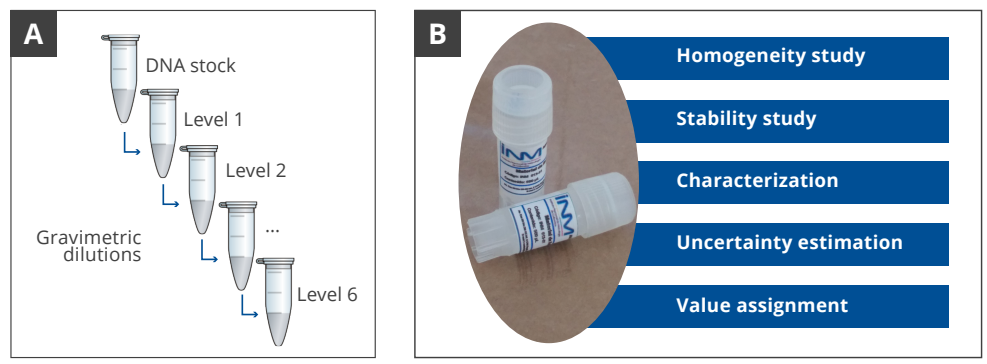

Figure 4. Gravimetric dilutions to prepare six concentration levels in the range 10 cp/µL– 200.000 cp/µL. (A). Material characterization by ddPCR (B).

RM characterization

Stability study

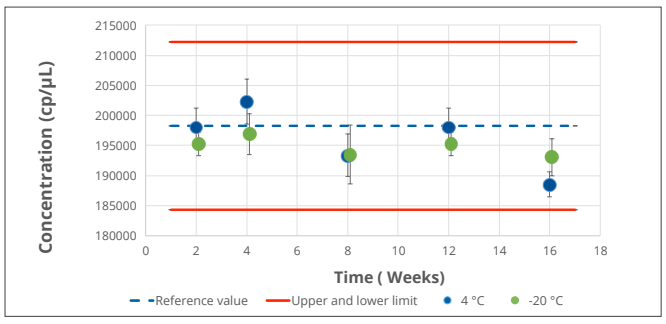

Figure 5. Stability study during 16 weeks for level No 1. Two temperatures were evaluated: 4°C and -20 °C.

Uncertainty sources

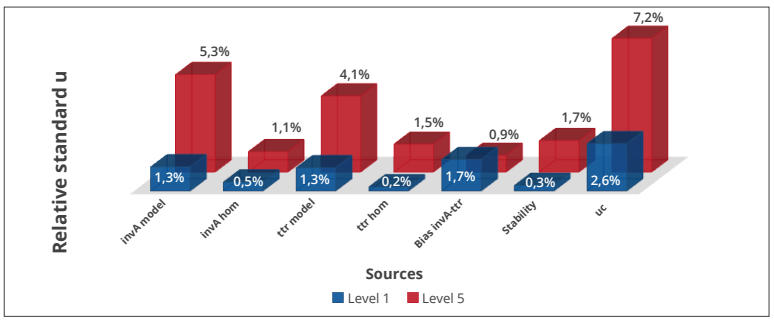

Figure 6. Uncertainty budget for levels 1 and 5. At higher concentrations the main component is associated with the bias between *invA* and *ttr* sequences, while for lower ones, the mathematical model is the main source.

Reference values

Level	Reference value ± u (cp/ µL)	u_c (%)
1	198264 ± 4957	2.6
2	112974 ± 2485	2.2
3	11159 ± 279	2.5
4	1042 ± 32	3.1
5	96 ± 7	7.2
6	9 ± 2	24.3

Table 2. Reference values and combined uncertainties obtained for each level.

CONCLUSIONS

A candidate to reference material based on *Salmonella* genomic DNA was produced, the higher concentration levels are stable up to four months at both 4°C and -20°C, thus these can be used as proficiency assay items. For lower ones, the stability is shorter. For this reason, we will continue studying how to improve the stability for low concentration levels. The uncertainty sources varied with concentration level, being the most important the bias between the sequences used for the higher and the mathematical model for the lower ones. This material is the first one produced in Colombia and it would be useful for method validation, quality control in the identification/quantification of *Salmonella spp*., and assessment of method performance in endpoint and real-time PCR assays.

AKNOWLEDGEMENTS

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REFERENCES

[1] P. Grimont and F.-X. Weill, "Antigenic formulae of the Salmonella servovars," WHO Collab. Cent. Ref. Res. *Salmonella*, pp. 1–167, 2008.
[2] ISO, ISO 6579-1:2017 *Microbiology of the food chain- Horizontal method for the detection, enumeration and serotyping of Salmonella*-Part 1: *Detection of Salmonella spp*. 2017.
[3] P. Y. Cheung, C. W. Chan, W. Wong, T. L. Cheung, and K. M. Kam, "Evaluation of two real-time polymerase chain reaction pathogen detection kits for *Salmonella spp*. in food," Lett. Appl. Microbiol., vol. 39, no. 6, pp.

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