

Supporting STEC control in Uruguay's beef industry: reference material and proficiency testing

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Introduction

To address regulatory requirements set by DILAVE (División de Laboratorios Veterinarios), which operates under the Ministry of Livestock, Agriculture, and Fisheries (MGAP), for controlling STEC (Shiga Toxin-Producing *Escherichia coli*) in bovine meat intended for both domestic consumption and export in Uruguay, a Reference Material was developed for detecting specific genes associated with this pathogen. Additionally, a Proficiency Test was established to assess laboratories' ability to accurately detect the *stx* and *eae* genes from STEC and identify the corresponding serogroups.

These initiatives are particularly crucial, as beef is Uruguay's most significant export product. By standardizing detection methods and improving the reliability of testing, these efforts strengthen quality control within the beef industry, ensuring that Uruguay's meat exports comply with international safety standards. This, in addition to supporting the improvement of the quality of food safety controls, helps maintain the competitiveness of Uruguay's beef products in the global market, fosters trust among international consumers, and reinforces the country's reputation as a leading beef exporter.

Objectives

01

To develop a Reference Material (RM) that serves as a positive control for the detection of the *stx* and *eae* genes of STEC, as well as for those associated with the seven most relevant serogroups (the Big Seven): O103, O121, O111, O45, O145, O26, and O157:H7.

02

To develop a Proficiency Test (PT) to validate laboratories' ability to accurately detect *stx* and *eae* genes of STEC and identify the associated serogroups.

Development and Application

MR Production (Obj. 1)

As the National Metrology Institute, we have expertise in the production of Certified Reference Materials and Reference Materials, following the ISO/IEC 17034 guidelines. We worked with the Microbiology Department of LATU to produce an initial pilot batch of the STEC Reference Material. Initially, a pilot batch was produced, consisting of 70 polypropylene vials containing approximately 700 µL of genetic material.



STEC Reference Material

Validation

Three main studies were conducted for the batch study and validation:



Homogeneity Study: This study was also used for value assignment. The variation of all parameters of interest was evaluated between separate units of the material as well as within each unit. To assess this, duplicate measurements were performed for each unit. To determine the number of units to be analyzed in the homogeneity and stability studies for this qualitative Reference Material—where results can only be "Detectable" or "Not Detectable"—a binomial distribution was used. Initially, the probability of rejecting the batch was set, which then determined the number of units required to ensure that, with this probability,

if any result did not match the expected value, the batch would be rejected. For the batch to be considered acceptable, all results obtained from each sample had to match the assigned value.



Stability Study: This study included both long-term and short-term stability assessments. Long-term stability was evaluated at 1.5, 3, 6, 12, 18, 22, and 25 months. Short-term stability was assessed to anticipate potential issues during the transportation of the refrigerated sample (-20°C). For this, three samples were analyzed after being exposed to 3±2°C for three days.



Open-Container Study: This study aimed to simulate the conditions of material reuse by the end user. The same samples were analyzed after being opened on five different occasions.

PT Development (Obj. 2)

The objective of developing this Proficiency Testing (PT) is to provide participating laboratories with an effective tool to verify their technical competence in the detection of STEC (*stx/eae* genes and serogroups O26, O45, O103, O111, O121, O145, and O157:H7) in meat products using their preferred methodologies. All rounds followed the guidelines of the ISO/IEC 17043 standard. This PT also serves as a tool to:

- Enhance the reliability of test results in laboratories.
- Identify issues in the applied methodologies and initiate corrective actions.
- Obtain national and international recognition for test results.
- Support laboratories in their accreditation process.

Two samples were sent to the laboratories (one from each prepared batch – Batch 1 and 2), each consisting of a vial with the inoculum and a jar containing 25 g of frozen cooked ground meat.

For each sample, they were required to report the detection or non-detection of the

eae and *stx* genes and the correct identification of the serogroups.



PT sample

Results

Accuracy percentage of the gen results based on the round years:

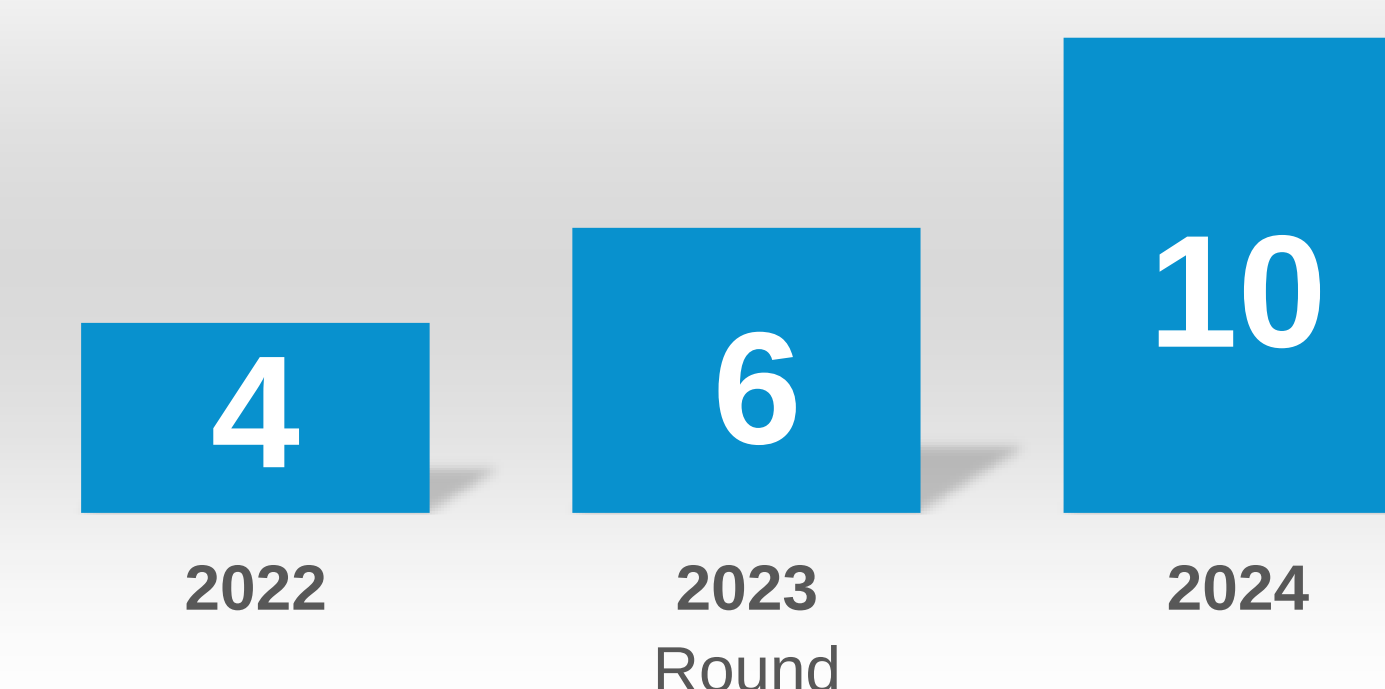
	<i>eae</i> gen	<i>stx</i> gen	Number of samples
2022	98%	88%	56
2023	97%	97%	62
2024	100%	100%	54

Accuracy percentage in the serogroups results:

	2023	2024
O26	85%	-
O45	40%	92%
O111	-	92%

Number of accredited laboratories participating in the PT:

Accredited laboratories



Discussion

Discussion

Since the development of the Reference Material in late 2020, five batches have been produced. This process has not only fulfilled DILAVE's request but has also identified and addressed a national need. The increased demand for the material is evident not only in higher purchases by the same laboratory but also in a growing number of companies acquiring it.

The implementation of the first PT round enabled LATU to obtain accreditation for this test under ISO/IEC 17043, allowing its inclusion in the annual schedule. This also highlighted the significant need for PT and resulted in a remarkable improvement in laboratory results. At the national level, incorporating this round led to a more than twofold increase in the number of accredited laboratories within two years, strengthening the country's quality infrastructure.

Conclusion

The production of reference materials and the implementation of the PT have been essential for improving the meat industry's quality while providing laboratories with a national alternative to costly and challenging imports. This experience has also enabled the development of two new materials under study: fat and moisture in canola and moisture in powdered milk, which will benefit Uruguay's dairy and cereal industries. Additionally, work is underway to develop RM for *Salmonella* and *Listeria*, addressing further needs in the food industry.

References

1. ISO 17034:2016 General requirements for the competence of reference material producers
2. ISO/IEC 17043:2010 Conformity assessment — General requirements for the competence of proficiency testing providers
3. ISO 13528:2022 Statistical methods for use in proficiency testing by interlaboratory comparison
4. ISO Guide 35:2017 Reference materials — Guidance for characterization and assessment of homogeneity and stability

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