USDA

United States Department of Agriculture

Food Safety and
Inspection Service

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MAY 2 4 2016

Dr. Odilson Luiz Ribeiro e Silva
Under Secretary of the Office of International Affairs (SRI)
Ministry of Agriculture, Livestock and Food Supply (MAPA)
Esplanada dos Ministérios, Bloco D, Edifício Sede, 3° andar, Sala 300
70.043-900 Brasília, DF
Brazil

Dear Dr. Ribeiro e Silva,

The USDA Food Safety and Inspection Service (FSIS) conducted an on-site audit of Brazil's meat inspection system from November 9 through November 20, 2015. Enclosed is a copy of the final audit report. The comments received from the Government of Brazil are included as an attachment to the report.

FSIS is currently evaluating the updated laboratory testing methods for Shiga Toxin-Producing *Escherichia coli* (STEC) included with your comments, and will notify you of its decision concerning the importation of fresh (chilled or frozen) beef in a separate correspondence upon completion of its equivalence review. As part of the review process, FSIS will consider whether an additional on-site audit is necessary to verify your program's ability to implement the methods as described.

If you have any questions, please feel free to contact me directly at jane.doherty@fsis.usda.gov.

Sincerely,

Jane H. Doherty

Jane H. Doherty
International Coordination Executive
Office of International Coordination

Enclosure

FINAL REPORT OF AN AUDIT CONDUCTED IN

BRAZIL

November 9 – 20, 2015

EVALUATING THE FOOD SAFETY SYSTEM GOVERNING THE PRODUCTION OF MEAT PRODUCTS INTENDED FOR EXPORT TO THE UNITED STATES OF AMERICA

May 12, 2016

Food Safety and Inspection Service
United States Department of Agriculture

Executive Summary

This report describes the outcome of an on-site equivalence verification audit conducted by the United States Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) from November 9 - 20, 2015, to verify that Brazil's food safety system governing the production of meat continues to be equivalent to that of the United States. In addition to an assessment of programs governing products currently eligible for import, the audit also included an evaluation of the Central Competent Authority's (CCA) ability to implement newly instituted controls set forth with the intent to export fresh (chilled or frozen) beef products to the United States.

The audit identified the following operational (or procedural) findings within Brazil's meat inspection system:

- FSIS determined that the CCA needs to revisit its procedure entitled, *Investigation Procedures for International Notifications* (*306/2013)* as it relates to FSIS point-of-entry (POE) violation notifications. While FSIS requests a reply to these notifications within 30 calendar days, the auditors noted that the CCA's average response time is 109 calendar days. This finding is related to deficiencies identified during the last FSIS audit in September 2014.
- A portion of Brazil's inspection force was not familiar with procedures in the CCA's *Guidelines for Implementing the National Residue Control Plan (132/2012), which* govern the targeting of animals suspected of containing violative levels of chemical residues at ante-mortem. This is a repeat finding.
- FSIS determined that the CCA needs to improve its verification activities related to the safety of retort cooling water and retort maintenance.
- Although no direct product contamination was observed, deficiencies regarding construction and enforcement of sanitation performance standards (SPS) were identified at five of the eleven establishments audited.
- FSIS determined that the CCA needs to improve its slaughter verification activities. At one of the eight slaughter establishments audited, viscera did not routinely accompany carcasses railed-out for final veterinary dispositions. At another establishment, the design of a non-mobile stand used by the government for conducting zero-tolerance verification (contamination caused by feces, milk, or ingesta) did not permit adequate observation of carcass hindquarters.
- The CCA had not yet instituted a Shiga toxin-producing *Escherichia coli* (STEC) proficiency testing program at its government laboratories.
- Written STEC government laboratory testing procedures referenced the use of *E. coli* strain #EC 465-97, while the actual strain used was *American Type Culture Collection* (ATCC) #43888.
- No official procedure existed for the handling of inconclusive STEC sample results.

An analysis of these findings did not identify any systemic deficiencies representing an immediate threat to public health for those products that Brazil is currently eligible to export to the United States. However, the findings related to STEC testing will require submission of revised laboratory methods for equivalence review before FSIS can permit the import of fresh (chilled or frozen) beef products. As part of the equivalence review process, FSIS will consider whether an additional on-site audit is necessary in order to verify the CCA's ability to implement the revised methods once they are submitted.

During the audit exit meeting, the CCA committed to addressing the preliminary findings as presented. FSIS will evaluate the adequacy of the CCA's proposed corrective actions once received and base future equivalence verification activities on the information provided.

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I. INTRODUCTION

The Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) conducted an on-site audit of Brazil's food safety system from November 9 - 20, 2015. The audit began with an entrance meeting held on November 9, 2015 in Brasilia, Brazil, with the participation of representatives from the Central Competent Authority (CCA) – the Department of Inspection for Products of Animal Origin (DIPOA), and four FSIS auditors.

II. AUDIT OBJECTIVE, SCOPE, AND METHODOLOGY

The audit objective was to verify that Brazil's food safety system governing the production of meat continues to be equivalent to that of the United States.

In July 2015, USDA's Animal and Plant Health Inspection Service (APHIS) amended part 94 of its Code of Federal Regulations (9 CFR § 94) to permit the importation, under certain conditions, of fresh (chilled or frozen) beef from fourteen states in Brazil. In response to this change in animal disease status, Brazil requested that FSIS conduct an equivalence review of their written control program for *Escherichia coli* (*E. coli*) O157:H7 and six other Shiga toxin-producing *E. coli* (STEC), with the purpose of exporting fresh (chilled or frozen) beef products to the United States. Based on the written documentation received, FSIS made the preliminary determination that Brazil's written control program for STEC was equivalent. Consequently, the audit included an assessment of the CCA's ability to implement this program, in addition to an evaluation of programs governing the product categories for import from Brazil. Brazil is eligible to export beef and pork products to the United States within the following product categories: raw intact (pork only), raw non-intact (pork only), thermally processed/commercially sterile, not heat-treated shelf stable, heat-treated shelf stable, and fully-cooked not shelf stable.

FSIS used a risk-based procedure to determine the audit scope, which included an analysis of country performance within six equivalence components, production types and volumes, frequency of prior audit-related site visits, POE testing results, and specific oversight activities and testing capacities of government offices and laboratories. The review process included data collected by FSIS over a three-year timeframe in addition to information obtained directly from the CCA, through the foreign inspection system self-reporting tool (SRT), outlining the structure of the country's inspection system and identifying any significant changes that have occurred since the last audit.

The FSIS auditors were accompanied throughout the audit by representatives from the CCA or representatives from the state and local inspection offices. Determinations concerning program effectiveness focused on performance within the following six components upon which system equivalence is based: (1) Government Oversight (Organization and Administration), (2) Statutory Authority and Food Safety Regulations (Inspection System Operation and Product Standards), (3) Sanitation, (4) Hazard Analysis and Critical Control Points (HACCP) Systems, (5) Government Chemical Residues Testing Programs, and (6) Government Microbiological Testing Programs.

FSIS auditors reviewed administrative functions at CCA headquarters, one state office, and eleven local inspection offices, during which the auditors evaluated the implementation of those management control systems in place that ensure that the national system of inspection, verification, and enforcement was being implemented as intended.

A sample of 11 establishments was selected from 25 establishments certified to export to the United States. These also included five (5) establishments identified by Brazil as intending to export raw beef to the United States, in accordance with the equivalence determination and audit objectives.

During the establishment visits, auditors closely examined the extent to which industry and government interact to control hazards and prevent non-compliances that threaten food safety, with an emphasis on the CCA's ability to provide oversight through supervisory reviews conducted in accordance with 9 CFR 327.2, the FSIS regulation outlining equivalency requirements for foreign inspection systems.

Additionally, FSIS audited one microbiological laboratory to verify its ability to provide adequate technical support to the inspection system.

Central Competent Authority Visits		**#**	**Locations**
Central Competent Authority	Central	1	CCA (DIPOA) – Brasilia
	State Office	1	Inspection Service of Products of Animal Origin (SIPOA) Office – Porto Alegre
Laboratory		1	Government microbiology laboratory in Campinas (LANAGRO-SP)
Beef Slaughter and Processing Establishments (five of which were identified as intending to export raw beef to the United States)		7	Andradina, Barretos, Bataguassu, Campo Grande, Lins, Navirai, Palmeiras de Goiás
Beef Processing Establishments		3	Hulha Negra, Santo Antônio de Posse, Tres Rios
Pork Slaughter and Processing Establishment		1	Chapeco

The audit was undertaken under the specific provisions of United States' laws and regulations, in particular:

- The Federal Meat Inspection Act (21 United States Code [U.S.C.] 601, et seq.),
- The Humane Methods of Livestock Slaughter Act (7 U.S.C. 1901, et seq.), and
- The Federal Meat Inspection Regulations for Imported Products (9 CFR Part 327).

The audit standards applied during the review of Brazil's meat inspection system included: (1) All applicable legislation originally determined by FSIS as equivalent as part of the initial review process, and (2) any subsequent equivalence determinations that have been made by FSIS under provisions of the World Trade Organization's Sanitary/Phytosanitary Agreement.

FSIS has made the following equivalence determinations for Brazil's food safety system:

- Private laboratories analyze samples for *Salmonella*.
- Establishment employees collect the samples for *Salmonella*.
- The CCA suspends an establishment from the list of certified establishments after the establishment has failed three consecutive *Salmonella* sets.
- Establishment personnel take samples of food and non-food contact surfaces for *Listeria monocytogenes* in association with DIPOA's control program for RTE products.
- FSIS has made the preliminary determination that Brazil's control program for STEC is equivalent.

A detailed analysis of the CCA's continued ability to meet the original commitments related to these equivalence determinations is provided under section IX of this report, Government Microbiological Testing Programs.

III. BACKGROUND

Brazil is eligible to export beef and pork products to the United States within the following product categories: raw intact (pork only), raw non-intact (pork only, thermally processed/commercially sterile, not heat-treated shelf stable, heat-treated shelf stable, and fully cooked not shelf stable.

From October 1, 2012 to August 27, 2015, FSIS import inspectors performed 100% re-inspection for labeling and certification on 161,918,128 pounds of beef and pork products exported by Brazil to the United States. FSIS also performed re-inspection on 36,256,824 pounds at POE for additional types of inspection (TOI). Since the last FSIS audit in September 2014, the United States rejected a total of 90,687 pounds for the following food safety-related reasons: three (3) lots of canned product were rejected due to abnormal containers, one (1) lot of frozen cooked beef was rejected for abscesses, and one (1) lot of canned corned beef was rejected for violative levels for abamectin (an anthelminthic).

The audit included visits to the establishments implicated in these POE violations for which FSIS concluded that the Brazilian government had satisfactorily worked with food business operators to identify the root causes of the problems and institute appropriate corrective actions. Specific details regarding these follow-up activities are included in the subsequent sections of the audit report.

The FSIS final audit reports for Brazil's Food Safety System are available on the FSIS website at:
http://www.fsis.usda.gov/wps/portal/fsis/topics/international-affairs/importing-products/eligible-countries-products-foreign-establishments/foreign-audit-reports

IV. COMPONENT ONE: GOVERNMENT OVERSIGHT (ORGANIZATION AND ADMINISTRATION)

The first of the six equivalence components that the auditors reviewed was Government Oversight. FSIS import regulations require the foreign inspection system to be organized by the

national government in such a manner as to provide ultimate control and supervision over all official inspection activities; ensure the uniform enforcement of requisite laws; provide sufficient administrative technical support; and assign competent qualified inspection personnel at establishments where products are prepared for export to the United States.

DIPOA is under the Ministry of Agriculture, Livestock, and Supply (MAPA) and is comprised of several divisions, including the General Coordination for Inspection, the General Coordination for Special Programs, and the International Export and Import Programs Coordination Division, which are involved in the production of meat product destined for export to the United States. DIPOA ensures uniform implementation of regulatory requirements and is responsible for oversight of the official activities of inspection personnel at establishments eligible to export to the United States. There have not been any significant changes in the structure of DIPOA since the last FSIS audit.

At the state level, the State Inspection Service of Products of Animal Origin (SIPOA) represents DIPOA. SIPOA offices operate within the scope of the inspection operations coordinated by DIPOA and are responsible for the implementation and enforcement of inspection operations in the slaughterhouses, processing plants, and cold storage facilities within the state. This level of government also provides periodic supervisory reviews for the United States-eligible establishments. At the establishment level, the Federal Inspection Service (SIF) has responsibility to implement and enforce inspection laws at the establishments eligible to export meat products to the United States. All inspection personnel are employees of the government. The FSIS auditors verified this fact by reviewing on Brazil's internet system the *Transparency Portal for Federal Government Resources*. Through this portal the auditors were able to identify individuals assigned to United States-eligible establishments by name and confirm their federal employment and payment status.

The CCA's authority to enforce inspection laws is specified in Brazil's statute, *Regulations for the Inspection of Industrial Sanitation for Products of Animal Origin* (RIISPOA). The CCA has the legal authority and the responsibility to write, implement, and enforce requirements equivalent to those governing the system of meat inspection organized and maintained in the United States. To achieve these objectives, the CCA issues, distributes, and enforces a number of official circulars that are inspection-related guidelines and instructions to its inspection personnel.

- FSIS requires that foreign governments maintain a communication system to convey requirements related to exporting to the United States in a timely manner. Regarding POE violations, it is FSIS policy to request within its formal notification letter that foreign governments present the results of their investigations within thirty (30) calendar days of receipt. The previous FSIS audit (2014) identified a need for DIPOA to improve its ability to meet this timeframe, for which DIPOA had committed to better adhere to the procedures outlined in *Investigation Procedures for International Notifications (306/2013)*. Nevertheless, FSIS has continued to receive delayed responses from DIPOA after notifying that agency of POE violations. Consequently, the FSIS audit focused on DIPOA's ability to meet the established timeframes outlined in this procedural document:

- *Response to microbiological notifications* (10 calendar days for the food business operator's response to SIF, + 10 calendar days for the response of SIPOA to DIPOA, Total: 20 calendar days),
- *Response to physico-chemical notifications* (20 calendar days for the food business operator's response to SIF + 10 calendar days for the response of the SIPOA to DIPOA- Total: 30 calendar days), and
- *Response to notifications regarding the National Plan for Control of Residues in Products of Animal Origin* (20 calendar days for the food business operator's response to SIF + 10 calendar days for the response of SIPOA to DIPOA- Total: 30 calendar days).

The FSIS auditors noted that in the last two cases, the design of the procedure is not conducive to a thirty calendar day turnaround period as the food business operator/SIF/SIPOA are, in themselves, provided with thirty (30) calendar days to respond to DIPOA's request. This timeframe does not consider additional factors related to administrative activities which exist on either side of the response process, including a) initial distribution of the FSIS notification by the CCA, and b) final receipt and analysis of information by the CCA before responding to FSIS.

Furthermore, the FSIS auditors noted deficiencies related to implementation of this procedure as it relates to the five (5) food safety-related POE violations that occurred since the last FSIS audit. In all cases, the response times exceeded the timeframes outlined in the relevant procedure, for which average turnaround times are reported as follows:

- *Average time for FBO/SIF/SIPOA response to DIPOA*: 45 calendar days [30 calendar day target],
- *Average time for CCA analysis*: 61 calendar days [not considered in *Investigation Procedures for International Notifications (306/2013)*], and
- *Average Response time (total):* 109 calendar days [FSIS requests 30 calendar days].

The CCA's General Coordination Office for Laboratory Support (CGLA) conducts annual audits of its laboratories that perform analysis of products that are destined for export to the United States. The CGLA applies standard form, *Laboratory Audit Report* (RAL), to document its audit findings.

However, the audit noted that CGLA oversight of the microbiological testing program for STEC does not provide for:

1. Inter-laboratory testing at all National Plant and Animal Health Laboratory (LANAGRO) facilities conducting STEC testing in conjunction with intended export of raw beef for the United States.
2. Intra-laboratory proficiency testing for technicians at these laboratories.

Consequently, this procedure does not meet the following FSIS equivalence criterion related to government oversight of testing laboratories requiring that: *1.6.b.4 The CCA maintains oversight of laboratories conducting official testing in conjunction with export of product to*

the United States by ensuring that laboratories participate in appropriate proficiency testing schemes for food analysis.

While Brazil's inspection system has the legal authority and regulatory framework to impose requirements equivalent to those governing the system of meat inspection organized and maintained by the United States, the on-site audit identified procedural weaknesses and omissions in the delivery of certain aspects of the program. In response to this audit report, FSIS requests that DIPOA provide a detailed description of global changes instituted by the inspection system. These changes should be communicated within 60 calendar days of receipt of the audit report.

V. COMPONENT TWO: STATUTORY AUTHORITY AND FOOD SAFETY REGULATIONS (INSPECTION SYSTEM OPERATION AND PRODUCT STANDARDS)

The second of the six equivalence components that the FSIS auditors reviewed was Statutory Authority and Food Safety Regulations.

The inspection system must provide for humane handling and slaughter of livestock; ante-mortem inspection of animals; post-mortem inspection of carcasses and parts; controls over condemned materials; controls over establishment construction, facilities, and equipment; daily inspection; periodic supervisory visits to official establishments; and requirements for thermally processed/commercially sterile products.

The CCA's authority to enforce inspection laws is specified in Brazil's statute, RIISPOA. To achieve this objective, the CCA issues, distributes, and enforces a number of official circulars that provide inspection-related guidelines and instructions to its inspection personnel.

The FSIS auditors verified that an in-plant official veterinarian conducts ante-mortem inspection on the day of slaughter by reviewing the incoming registration and identification documents including *Animal Movement Permits*. In accordance with procedures outlined in the SRT, the official veterinarians observe all animals at rest and in motion from both sides in designated holding pens in order to determine whether they were fit for slaughter. Each establishment has a designated observation pen for further examination of suspect animals. The FSIS auditors observed and verified that all animals have access to water in all holding pens (including the pens used for suspect animals), and that if animals are held overnight, feed and water are provided. The implementation of ante-mortem inspection is in compliance with Brazil's RIISPOA, *Title VII, Chapter I, Ante-mortem Inspection*, which FSIS has determined to be equivalent. The FSIS auditors further verified through on-site record review, interviews, and observations that the CCA's requirements concerning ante-mortem and humane handling/slaughter of livestock were being met in all audited slaughter establishments, with the following exceptions:

- At one beef slaughter establishment, the FSIS auditors observed a deficiency in the walkway that livestock followed to cattle holding pens. An area of the walkway was cracked and presented a hole large enough in the surface that could potentially cause injury to the animal

during transit through this area. However, there was no evidence to indicate that injury to animals had actually occurred.

SIPOA officials interviewed at the Porto Alegre State (Rio Grande do Sul) Office were not familiar with the contents of the CCA's *Guidelines for Implementing the National Residue Control Plan (132/2012),* which directs field personnel to target animals at ante-mortem suspected of being treated with veterinary drugs. The reason for this lack of awareness was related to the fact that there are only processing (not slaughter) establishments certified for export to the United States in the State of Rio Grande do Sul. However, FSIS also learned that this State office was in the process of approving two new slaughter establishments for export to the United States in the region. Consequently, it is necessary that the CCA make changes throughout the various levels of its system (DIPOA/SIPOA/SIF) to ensure that inspectors assigned to certified establishments continue to meet FSIS standards related to the effective targeting of animals suspected of being treated with veterinary drugs. This is a repeat finding.

FSIS assessed post-mortem examinations through on-site record review, interviews, and observations of inspection activities in all audited slaughter establishments. The FSIS auditors observed and verified that proper presentation, identification, examination, and disposition of carcasses and parts are being implemented. Both in-plant veterinary and non-veterinary inspectors are adequately trained in performing their on-line post-mortem inspection duties. The FSIS auditors observed the performance of the inspection personnel examining the heads, viscera, and carcasses. The inspection personnel made the proper incision, observations, and palpation of required organs and lymph nodes, in accordance with Brazil's *Federal Inspection Service (RIISPOA), Title VII, Chapter III-Post-mortem Inspection*. The auditors noted that, for the most part, these requirements were being implemented with the following exception:

- At one pork slaughter establishment, the FSIS auditors noted that the viscera did not routinely accompany carcasses railed-out for final veterinary dispositions.

While on-site, the FSIS auditors were able to confirm that the appropriate APHIS requirements for the control of foot-and-mouth (FMD) disease were being met at all five establishments intending to export raw beef to the United States. Official government inspectors examined the coronary bands (hooves), lips, and snout of each individual animal slaughtered. The FSIS auditors also noted that establishment employees routinely measured the *p*H of each half-carcass after passing the maturation chamber. Verification of this activity was conducted by off-line government inspectors throughout the production day, and the inspectors made appropriate records of their inspections.

FSIS verified that documented periodic supervisory reviews are performed as required by 9 CFR 327.2(a) (2) (iv) (A). These reports were reviewed at the SIPOA office in Porto Alegre, in addition to local inspection offices at all audited establishments. In all locations, these reviews were conducted using a standard form, *Supervisory Report,* which consists of a detailed checklist with two main parts. The first part consists of sections for evaluating the adequacy of establishment food safety systems, including items related to inspection verification of sanitation performance standards (SPS), sanitation standard operating procedures (SSOP), HACCP, and microbiological controls (i.e., generic *E. coli*, *Salmonella*, and STEC). The second part consists

of questions for evaluating the knowledge, skills, and abilities of inspection personnel to conduct assigned responsibilities at establishments certified for export to the United States.

Within Brazil's inspection system, the principal documents governing the export of thermally processed, commercially sterile (TPCS) products include:

- Articles 377 to 392 of RIISPOA,
- Production Control of Preserved Food in Establishments Approved for Export to the United States of America (28/1978),
- Inspection Guidelines for the Production of Low-acid Canned Foods, Beef Jerky, and Cooked or Frozen Foods (362/2013),
- Procedures for Incubation of Samples of Meat Products Subject to Commercial Sterilization (285/2005), and
- Technical Regulations of Identity and Quality for Corned Beef (83/2003).

Brazil's *Production Control of Preserved Food in Establishments Approved for Export to the United States of America (28/1978),* requires that all the thermal process applications be submitted to the state offices for technical analysis to ensure that the performance standard of a 12-log reduction (12D) for *Clostridium botulinum* is met. This review also ensures that the process schedules submitted by exporting establishments have sufficiently addressed the aspects for commercial sterility of the product. Commercial sterility is further ensured through the implementation of *Procedures for Incubation of Samples of Meat Products Subject to Commercial Sterilization (285/2005),* which instructs local SIF inspection officials to collect samples at a rate of 1/1000 for incubation.

The audit scope included verification of the corrective actions for two establishments involved in POE violations for TPCS product, in which a total of three shipments presented a fraction of abnormal containers (e.g., "leakers and swellers"). The audit confirmed the results of the CCA's prior investigations, whereby it was communicated to FSIS that these violations resulted from the improper closure of the containers rather than under-processing. Specific on-site verification activities conducted by the FSIS auditors included review of:

- Process schedules for products exported to the United States.
- Incubation records, which continuously demonstrated the absence of abnormal containers or other defects related to under-processing.
- Retort heat-distribution tests, for which no concerns were identified.
- Installation of new equipment purchased by establishments to address potential improper closure of cans and pouches.
- Production records, indicating that all implicated lots of product had been appropriately precluded from export to the United States.

However, although not directly related to the presence of abnormal containers identified at POE, the following deficiencies related to the inspection program's ability to meet the FSIS regulatory objectives outlined in 9 CFR § 318 for TPCS product were identified at one of the audited establishments:

- *Retort cooling water*. The FSIS auditors noted inconsistencies in establishment records documenting *p*H values for chlorinated retort cooling water. A review of establishment records for a given day indicated that, while measurements obtained through the use of test kits on the production floor identified *p*H values around 8.0, the result of in-house laboratory testing was 7.43. The measurement of *p*H is important because chlorine is a much better bactericide at lower *p*H levels. The auditors also noted that microbiological testing of the cooling water did not include facultative anaerobes. As the introduction of facultative anaerobes from unclean water though micro-leaks is a food safety concern, the lack of microbiological testing in association with the inconsistencies identified in *p*H measurement rendered it difficult to ascertain the safety of the cooling water at this establishment. Consequently, FSIS has identified the need for DIPOA/SIPOA to improve activities related to the verification of the suitability of retort cooling water at thermal processing facilities.

- *Retort maintenance*. The auditors also noted the presence of thin metal condensate tubing in each retort that originated from below the bottom basket brackets and exited near the top. Although no bent tubing was observed at the time of the audit, the design raised concerns about the frailty of the setup. The inability for damaged tubing to indicate that excess condensate is collecting when the bottom of the retort fills with water creates the potential for under-processing. The auditors further noted that an ongoing review of the integrity of this tubing was not conducted, either within the context of the establishment's food-safety system or of government verification activities.

In response to this audit report, FSIS requests that DIPOA provide a detailed description of the corrective actions that exporting establishments are taking to address these findings, as well as global changes instituted by the inspection system to verify retort cooling water and maintenance. These changes should be communicated within 60 calendar days of receipt of the audit report.

VI. COMPONENT THREE: SANITATION

The third of the six equivalence components that the FSIS auditors reviewed was Sanitation. To be considered equivalent to FSIS' program, the CCA must provide general requirements for sanitation, sanitary handling of products, and SSOP. The CCA has compiled specific sanitation requirements related to United States export *Verification Procedures for the Self-inspection Programs (175/2005).*

The FSIS auditors reviewed sanitation plans and records related to the design and implementation of sanitation programs at all of the audited establishments. In one of the audited establishments, the FSIS auditors verified the actual pre-operational inspection by shadowing and observing the in-plant inspector conducting pre-operational sanitation verification of slaughter and processing areas. The in-plant inspection personnel's hands-on verification procedures began after the establishment personnel conducted their pre-operational sanitation and determined that the facility is ready for pre-operational sanitation verification activities. The in-plant inspection personnel conducted this activity in accordance with the CCA's established procedures.

The FSIS auditors followed the off-line inspector and observed in-plant inspection verification of operational sanitation procedures at all of audited establishments. These verification activities include direct observation of operations and review of the establishments' associated records.

Some of the establishments included in the audit scope were visited during the previous FSIS audit. This situation presented the FSIS auditors with an opportunity to verify the status of corrective actions at those locations. While the auditors determined that the CCA and food business operators had worked together to resolve these identified concerns in an expedited manner, the auditors found deficiencies related to enforcement of SPS at five of the visited establishments that had not been included in the 2014 FSIS audit:

- In one establishment, numerous sections of walls in product transit areas and box freezers were in a state of disrepair.
- In one establishment, numerous cooler doors presented deteriorated rubber seals.
- In one establishment, overhead structures in the fabrication department were not adequately maintained.
- In three establishments, heavy condensate was observed on cooling units above boxed product in a cooler.
- In two establishments, conveyor belts used for transporting packaged raw beef were inappropriately maintained.

Consequently, these deficiencies represent a need for improvement in the government's routine enforcement of sanitation standards.

VII. COMPONENT FOUR: HAZARD ANALYSIS AND CRITICAL CONTROL POINT (HACCP) SYSTEMS

The fourth of the six equivalence components that the FSIS auditors reviewed was HACCP. The inspection system must require that each official establishment develop, implement, and maintain a HACCP plan and verify the effectiveness of processes and process controls.

Brazil's meat inspection system has codified FSIS HACCP regulatory requirements prescribed in 9 CFR Part 417 in its *Procedures for* the *Verification of Establishment Food Safety Control Programs (175/2005)*, which addresses the evaluation of written HACCP programs, monitoring, verification, corrective actions, recordkeeping, and hands-on verification inspection.

The FSIS auditors verified through record review and observation that the in-plant inspection personnel at certified establishments conducted daily verification of HACCP plans, for which verification results are entered on *Form 01: Hazard Analysis and Critical Control Points*. The in-plant inspection personnel verification of HACCP plans includes verification of critical control points (CCP) for all production shifts.

At eight slaughter establishments audited, the FSIS auditors conducted an on-site review of the zero tolerance (feces, ingesta, and milk) CCP records generated during the past year. In addition, the FSIS auditors reviewed the in-plant inspection's associated zero tolerance verification records (*Form 02: HACCP Monitoring*) at these locations. All establishments audited were

conducting 100 % monitoring of carcasses for this CCP. The review of the establishment's corrective actions in response to the few observed deviations from the zero tolerance critical limit indicated that all four parts of the corrective actions were correctly addressed, in accordance with section 14.2.V of *Procedures for the Verification of Establishment Food Safety Control Programs (175/2005).* Furthermore, the FSIS auditors confirmed that the physical CCP monitoring location for government verification was before the final wash in all establishments audited.

- However, at one establishment, an FSIS auditor noted that verification of this CCP could be affected by the use of a non-mobile stand that did not permit observation of the entirety of the carcass. As designed, the government inspector's line-of-sight did not extend beyond the lumbar area and consequently did not provide for adequate observation of the carcass hindquarter.

Concerning the subset of five slaughter establishments intending to export raw beef to the United States, the FSIS auditors noted that food business operators had addressed contamination of carcasses with STEC (*E. coli* O157:H7, O26, O45, O103, O111, 0121, and O145) within the context of their HACCP system. In addition to 100 % monitoring of the zero tolerance CCP, additional control points typically employed by establishments included: chlorinated live animal washes; post-stun washing of the perianal region; and sanitizing of utensils between each carcass during bleeding, dehorning, skinning, and removal of udders. The auditors' review of microbiological testing results for carcasses (generic *E. coli*) and beef trimmings (STEC) further supported the conclusions reached in their hazard analyses.

In addition to establishment controls, the FSIS auditors also noted that the CCA routinely verifies that establishments employ sanitary dressing procedures that prevent visible contamination and is conducting a baseline study to determine contamination levels in beef trim and other components. The CCA intends to publish the results of this study in June 2016 and to make additional modifications to its inspection system based on this outcome. These modifications are to include issuing guidance regarding the possible use of carcass interventions as well as the measures to take to address high-event periods (HEP).

At establishments producing frozen cooked beef and beef jerky, the auditors reviewed the HACCP programs for these processes with a special emphasis on lethality for *Salmonella* and other relevant pathogens. For frozen cooked beef, the auditors observed that all establishments had a CCP in place in order to meet Brazilian *Ordinance No. 711, Technical Standards for Slaughter Establishments and Equipment (1995),* which requires a minimum internal temperature of 71ºC (159.8ºF) for cooked meat products. In the three audited facilities that were producing beef jerky, the establishments had adopted the recommendations included in the *FSIS Compliance Guideline for Meat and Poultry Jerky* and included appropriate measures to address lethality: relative humidity within the cooking cycle, cooking temperature, and water activity. The auditors also reviewed the validation documents at these establishments, which indicated that the actual lethality achieved by these processes exceeded the minimum five-log reduction for *Salmonella* prescribed in the aforementioned FSIS guidelines.

The FSIS auditors verified that establishments approved for export to the United States have reviewed their specified risk material (SRM) control programs in accordance with DIPOA's *Circulars 622/2014* and *463/2004* to include: brain, skull, eyes, trigeminal ganglion, spinal cord, spinal ganglia roots, spinal column (excluding the caudal vertebrae, the transversal processes of the thoracic and lumbar vertebrae and sacral wings) of bovines 30 months of age and older, and the tonsils and the distal portion of the ileum for bovines of all ages. In response to the 2014 audit findings, the FSIS auditors found that establishments were now removing lingual tonsils in addition to palatine tonsils within their prescribed measures for SRM control in beef slaughter establishments.

The audit indicated that all establishments continued to implement controls for avermectins in accordance with the following issuances:

- *Evaluating Food Business Operator Reassessment and Validation of HACCP Plans (17/2010,*
- *Criteria for Evaluating Food Business Operator Reassessment and Validation of HACCP Plans (18/2010),*
- *Guidelines for Validating Critical Limits of the HACCP Plans and the Control Points of the Pre-requisite Programs (21/2010),*
- *Official Program for Avermectin Analysis (22/2010),*
- *Use of Letters of Guarantee to Control Ivermectin in Cattle (127/2010),*
- *Ivermectin Analyses in Final Product (139/2010),* and
- *Review of Ivermectin in Final Product (198/2010).*

The FSIS auditors' on-site assessment of these controls was based on the following elements outlined in the *FSIS Compliance Guide for Residue Prevention (2013):*

1. *Confirmation of producer history.* All audited establishments maintained lists to identify previous violators to ensure that animals received from these individuals would not be used in association with United States export.

2. *The purchase of animals that are free of chemical residues.* All audited slaughter establishments required letters of guarantee at animal receipt (as a CCP) attesting that withdrawal times had been respected. In addition, each establishment maintained a list of prohibited compounds. All audited establishments maintained outreach programs with suppliers. All slaughter establishments conducted ivermectin testing on each lot of animals received, in accordance with established frequencies outlined in the Brazilian sampling tables, as per *Official Program for Avermectin Analysis (22/2010).*

   For the establishment implicated in the POE violation for abamectin, the FSIS auditors confirmed that an on-farm investigation had been conducted in accordance with established protocols. In addition, FSIS noted that establishments conducted ongoing farm visits (audits and outreach) even in the absence of violative results.

   In accordance with *Ivermectin Analyses in Final Product (139/2010)* and *Review of Ivermectin in Final Product (198/2010),* all establishments audited were subjecting finished

product to High Performance Liquid Chromatography/Ultra-Performance Liquid Chromatography (HPLC/UPLC) avermectin testing, during which product is held until results are received (i.e., hold and test). This testing included:

a) Government-mandated testing at approved laboratories (observed at all audited establishments), and
b) Company internal testing (observed at some audited establishments, which may also be accompanied by testing of livers from slaughtered animals).

3. *Animal identification.* All of the audited slaughter establishments maintained records sufficient to conduct accurate traceback and trace forward activities. During the audit, establishments demonstrated the ability to segregate product lots that exceed established maximum residue levels (MRL) from United States export. In the case of the POE violation related to abamectin, the FSIS auditors were able to confirm that all remaining product had been appropriately segregated and precluded from export to the United States.

4. *Notification of violative results to suppliers.* All establishments presented procedures to notify suppliers of violative samples. These procedures included the use of either tracked emails or registered mail. Educational outreach materials are also routinely included in these communications. In the case of the POE violation related to abamectin, the FSIS auditors confirmed that the notification procedures had been appropriately followed.

The audit results show that the CCA verifies that operators of official establishments implement the CCA's requirement to develop, implement, and maintain HACCP programs for each processing category. The FSIS auditors' analysis determined that the CCA continues to demonstrate the ability to effectively implement and verify its regulatory requirements for those products that Brazil is currently eligible to export to the United States. However, before FSIS can permit the import of fresh (chilled or frozen) beef, the CCA will need to submit corrective actions to address the lack of a STEC proficiency testing program as well as the findings related to the laboratory STEC testing method outlined within *Component 6: Government Microbiological Testing Programs*. As part of its equivalence review process, FSIS will consider whether an additional on-site audit is necessary in order to verify the CCA's ability to implement these corrective actions once they are submitted. Once eligibility for fresh beef is granted, FSIS will place Brazil into a higher frequency of STEC testing at POE in order to evaluate over time whether to maintain this heightened verification on an annual basis, until publication of the afore-mentioned national baseline and any related changes in the CCA's inspection system are instituted.

VIII. COMPONENT FIVE: GOVERNMENT CHEMICAL RESIDUE CONTROL PROGRAMS

The FSIS auditors reviewed Government Chemical Residue Control Programs as the fifth of the six equivalence components. The FSIS criteria for this component include the design and implementation of a program managed by the CCA that carries out effective regulatory activities to prevent chemical residue contamination of food products. To be considered equivalent to FSIS' residue control program, the CCA's program needs to include random

sampling of internal organs, fat, and muscle from carcasses for chemical residues identified by the exporting countries and FSIS as potential contaminants. In addition, the CCA needs to identify the laws, regulations, or other decrees that serve as the legal authority for the implementation of the program; provide a description of its residue sampling and testing plan and the process used to design the plan; describe the actual operation of its residue plan and actions taken to deal with unsafe residues as they occur; and provide oversight of laboratory capabilities and analytical methodologies to ensure the validity and reliability of test data.

The Brazilian *National Plan for Control of Residues in Products of Animal Origin* (PNCRC) was established by Ministerial Decree # 51, on May 6, 1986, and by Ministerial Decree # 527, of August 15, 1995. The PNCRC has the control and surveillance of products as its basic regulatory function. Its actions are aimed at understanding and preventing the violation of safety standards or MRLs for allowed substances and the occurrence of residues and chemicals banned for use in the country at all levels. For this purpose, samples are collected from slaughtered animals and industrialized food products destined for human consumption originating from the establishments under federal inspection.

Within the PNCRC, subprograms that are of particular interest to FSIS include:

1. *Subprogram for Monitoring*: aims at generating information on the frequency, level, and distribution of residues in the country, over time. The types of residues to be researched are selected based on potential risk and availability of analytical methodology appropriate to the goals of the monitoring being performed. The number of samples, the MRL, the methodology analysis, the matrices and the drugs being analyzed, and the official and accredited laboratories are included in the annual schedule. This subprogram does not require that product be held until sample results are received (except in response to follow-up testing, as described below).

2. *Subprogram for Exploration*: developed in special situations (e.g., in relation to United States export) to generate information about the frequency and levels where substance residues occur in Brazil. To control avermectins in the exploratory subprogram, DIPOA determines eligibility for export based on the MRL established by the United States Food and Drug Administration (FDA) for avermectins, which includes 650 ppb (parts-per-billion) in muscle for ivermectin and 10 ppb abamectin. Under this subprogram, samples are held until test results are received.

While on-site, the FSIS auditors were able to verify that the appropriate follow-up procedures were performed in conjunction with all violative samples identified through implementation of the PNCRC since the last FSIS audit, which included:

1. Investigation of the farm involved in the violation. These investigations included an on-site visit, document review, and interviews. In some cases, the investigation extended to neighboring properties or other farms associated with the violative lot.
2. Development of a corrective action plan (including preventive measures) by the SIF establishment.

3. Collection of samples of the next batches of animals/production from the farm involved in the infringement directed to slaughter/processing until the farm reached five (5) consecutive conforming lots. Products from these lots were retained in the SIF establishment until the results of analysis were known. The CCA maintained records to demonstrate that the samples had been collected and tested accordingly.
4. Withholding of *Animal Movement Permits* from the farms in question for a period of 6 months (for illegal drugs), or throughout the withdrawal period (for authorized drugs).

Since the last FSIS audit, a single lot of Brazilian product was rejected for violative residue levels (abamectin) for which specific follow-up activities conducted by FSIS at the implicated establishment have already been described under *Component Four: HACCP Systems*.

While Brazil's inspection system has the legal authority and regulatory framework to impose residue testing requirements equivalent to those governing the system of meat inspection organized and maintained by the United States, findings that may impact DIPOA's effective implementation of the PNCRC have been described in previous sections of the report. These include weaknesses related to the timely propagation of information related to POE violation notifications throughout its inspection system, and targeting of animals suspected of violative drug residues at ante-mortem. Brazil will need to correct these weaknesses before it can be found eligilble to export fresh beef to the United States.

IX. COMPONENT SIX: GOVERNMENT MICROBIOLOGICAL TESTING PROGRAMS

The last of the six equivalence components that the FSIS auditors reviewed was Microbiological Testing Programs. This component pertains to the microbiological testing programs organized and administered by the CCA to verify that products destined for export to the United States are safe, wholesome, and meet all equivalence criteria.

The evaluation of this component included verification of *Procedures for* the *Verification of Establishment Food Safety Control Programs (175/2005)*, previously submitted by the CCA as support for the responses provided in the SRT. This circular describes the official inspection methodology for a continuous and systematic assessment of inspection activities during routine verifications of microbiological tests, including *Enterobacteriaceae, Salmonella* spp., generic *E .coli,* and *Listeria monocytogenes (Lm)* in RTE products. Although there is no explicit requirement within Brazil's inspection system for product to be held in association with government testing, the auditors noted that this was a common practice at the establishments audited.

The CCA has a *Salmonella* testing program for chilled livestock (cattle and swine) carcass sampling that is consistent with the FSIS *Salmonella* performance standards in 9 CFR 310.25(b). The CCA requires that one *Salmonella* set be scheduled per year that consists of 82 samples from beef (55 samples from swine) carcasses with one positive sample considered acceptable from beef (up to six in swine), and two positive samples considered a set failure. An establishment failing its first *Salmonella* set must take immediate corrective action and reassess its HACCP plan, after which a second set of samples is collected. If the establishment fails to meet the

performance standard on the second sample set, then the HACCP plan is audited by the Brazilian inspection service, and another sample set is collected. If an establishment fails three consecutive sample sets, it is removed from the list of establishments eligible to export to the United States. The suspension would remain in effect until the establishment achieves the performance standard set based on number of samples tested (n) and maximum number of positives to achieve standard (c). The CCA's *Salmonella* performance standards for bovine ($n = 82$, $c \leq 1$) and swine ($n = 55$, $c \leq 6$) are the same as FSIS' standards.

As indicated previously, Brazil's equivalence determination for *Salmonella* requires the following activities:

- Establishment employees collect the samples, and
- Private laboratories analyze the samples.

In order to ensure that the food safety measures and objectives associated with this equivalence determination continue to be met, the FSIS auditors verified the following aspects related to the implementation of this program, for which no concerns were identified:

- DIPOA schedules each sample series. The state inspection offices (e.g., SIPOA) are responsible for informing local inspection personnel at SIF establishments when sampling is to begin/end and for monitoring of the results.
- SIF inspection personnel randomly select carcasses on the morning the sample is to be collected, with no prior notification to the establishment.
- SIF inspection personnel observe the collection of each sample taken by establishment personnel, as well as measures related to sample integrity and security (i.e., application of security seals to the mailing container).
- Private laboratories must be approved by DIPOA and are audited twice per year by CGLA. Approved laboratories use FSIS Microbiological Laboratory Guidebook (MLG) methods for *Salmonella* analysis.

The CCA conducts verification activities that monitor each establishment's generic *E. coli* testing program in chilled livestock carcasses. The testing program is outlined in *Microbiological Tests on Livestock Carcasses (835/2006)* and *Interpretation of Generic E. coli Results (1058/2008),* determined equivalent by FSIS. While on-site, the FSIS auditors verified that the responsible individuals presented the skills to implement this type of testing on an ongoing basis. Similarly, both the establishment and inspection personnel are familiar with the upper and lower control limits, as well as the correct actions to be taken when the upper limits are exceeded. However, no such loss of process control was identified in the on-site documents reviewed.

The CCA has a verification-testing program in place to test for *Lm* and *Salmonella* species in RTE products that are eligible to be exported to the United States. Furthermore, the CCA requires that establishments exporting RTE products to the United States have a program in place to meet FSIS equivalence criteria for control of *Lm*. In addition to product testing, establishments are required to take five samples (three food-contact surfaces and two non-food contact surfaces) per production line per week. All samples are collected under observation by

inspection personnel and sent in a secured package to a CGLA-approved laboratory for analysis. Sample sponges are collected using a 30x30 cm template, and analyzed using the FSIS MLG method 8.09: *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Samples*.

Within its *Internal Standard No. 2 (2013)*, DIPOA stipulates a zero tolerance policy for *E. coli* O157:H7, O26, O45, O103, O111, 0121, and O145 in raw bovine products exported to the United States. This document further outlines requirements for establishment sampling and testing for each lot of boneless manufacturing meat used as raw ground beef components or non-intact products intended for export to the United States. Section 2.5.3 instructs DIPOA officials to verify the sample collection and submission procedures (for which associated training records were reviewed by the FSIS auditors) and section 2.5.4 directs in-plant officials to verify the HACCP plans, control system for eligible and ineligible product and pre-shipment HACCP records. Inspection officials regularly review establishment test results and conduct independent N-60 government verification testing at a frequency of at least once per month. Supervisory reviews routinely include aspects of processing that can contribute to microbial contamination, e.g., hygienic dressing, HACCP, and SSOPs. DIPOA implements an enforcement strategy that includes immediate corrective actions, followed by HACCP reassessment, review of HACCP and SSOP records and other results from the days before and after the positive result to identify any trends and additional verification for STEC. None of the audited establishments presented positive test results within their sampling and testing history (including government verification testing).

Within its SRT submission, DIPOA indicated that it had adopted the following FSIS methods for STEC testing:

a. MLG 5.09: *Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges*, and
b. MLG 5B.05: *Detection and Isolation of non-O157 Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products and Carcass and Environmental Sponges.*

However, the audit of the government laboratory revealed the following deviations from the MLG methods:

- The written laboratory methods referenced the use of *E. coli* strain #EC 465-97, while the actual strain used ATCC43888.
- The written procedures did not address handling of inconclusive STEC sample results. Specific types of inconclusive results outlined in the FSIS MLG methods, but not addressed in the homologous Brazilian laboratory procedures included:

a. *FSIS MLG 5.09, section 5.7, item (e)*: isolates, or any additional presumptive positive colony picks from mRBA that are ultimately determined to be BAX® Real-time polymerase chain reaction (PCR) negative or indeterminate for Shiga toxin (stx) or intimin (eae) genes,

b. *FSIS MLG 5.09, section 5.7, item (g*): isolates that are flagellar antigen (H7) negative and found to be Shiga toxin and gene negative (by Enterohemorrhagic *E. Coli* [EHEC] test and genetic test for Shiga toxin genes), and
c. *FSIS MLG 5.B.05, section 5.B.8*: confirmatory tests which are PCR positive but biochemically negative.

The deviations related to STEC testing warrant submission of the revised methods for equivalence review before FSIS can permit the importation of fresh (chilled or frozen) beef products from Brazil.

X. CONCLUSION AND NEXT STEPS

An exit meeting was held on November 20, 2015, at DIPOA headquarters in Brasilia, Brazil. At this meeting, the preliminary findings from the audit were presented by the FSIS auditors. The CCA understood and accepted the findings.

The audit identified the following operational (or procedural) findings within Brazil's meat inspection system:

- FSIS determined that the CCA needs to revisit its procedure entitled, *Investigation Procedures for International Notifications* (*306/2013)* as it relates to FSIS point-of-entry (POE) violation notifications. While FSIS requests a reply to these notifications within 30 calendar days, the auditors noted that the CCA's average response time is 109 calendar days. This finding is related to deficiencies identified during the last FSIS audit in September 2014.
- A portion of Brazil's inspection force was not familiar with procedures in the CCA's *Guidelines for Implementing the National Residue Control Plan (132/2012), which* govern the targeting of animals suspected of containing violative levels of chemical residues at ante-mortem. This is a repeat finding.
- FSIS determined that the CCA needs to improve its verification activities related to the safety of retort cooling water and retort maintenance.
- Deficiencies regarding construction and enforcement of sanitation performance standards (SPS) were identified at five of the eleven establishments audited. However, no direct product contamination was observed.
- FSIS determined that the CCA needs to improve its slaughter verification activities. At one of the eight slaughter establishments audited, viscera did not routinely accompany carcasses railed-out for final veterinary dispositions. At another establishment, the design of a non-mobile stand used by the government for conducting zero-tolerance verification (contamination caused by feces, milk, or ingesta) did not permit adequate observation of carcass hindquarters.
- The CCA had not yet instituted a Shiga toxin-producing *Escherichia coli* (STEC) proficiency testing program at its government laboratories.
- Written STEC government laboratory testing procedures referenced the use of *E. coli* strain #EC 465-97, while the actual strain used was *American Type Culture Collection* (ATCC) #43888.
- No official procedure existed for the handling of inconclusive STEC sample results.

An analysis of these findings did not identify any systemic deficiencies representing an immediate threat to public health for those products that Brazil is currently eligible to export to the United States. However, the findings related to STEC testing will require submission of revised laboratory methods for equivalence review before FSIS can permit the import of fresh (chilled or frozen) beef products. As part of the equivalence review process, FSIS will consider whether an additional on-site audit is necessary in order to verify the CCA's ability to implement the revised methods once they are submitted.

During the audit exit meeting, the CCA committed to addressing the preliminary findings as presented. FSIS will evaluate the adequacy of the CCA's proposed corrective actions once received and base future equivalence verification activities on the information provided.

APPENDICES

Appendix A: Individual Foreign Establishment Audit Checklists

United States Department of Agriculture
Food Safety and Inspection Service

Foreign Establishment Audit Checklist

1. ESTABLISHMENT NAME AND LOCATION	2. AUDIT DATE	3. ESTABLISHMENT NO.	4. NAME OF COUNTRY
Ferreira International Ltda. Tres Rios (Rio de Janeiro) Brazil	11/16/2015	SIF-0013	Brazil
	5. NAME OF AUDITOR(S) Kenneth E. Witek – SPA, CSO		6. TYPE OF AUDIT [X] ON-SITE AUDIT [ ] DOCUMENT AUDIT

Place an X in the Audit Results block to indicate noncompliance with requirements. Use O if not applicable.

Part A - Sanitation Standard Operating Procedures (SSOP) Basic Requirements	Audit Results
7. Written SSOP	
8. Records documenting implementation.	
9. Signed and dated SSOP, by on-site or overall authority.	
Sanitation Standard Operating Procedures (SSOP) Ongoing Requirements	
10. Implementation of SSOP's, including monitoring of implementation.	
11. Maintenance and evaluation of the effectiveness of SSOP's.	
12. Corrective action when the SSOP's have failed to prevent direct product contamination or adulteration.	
13. Daily records document item 10, 11 and 12 above.	
Part B - Hazard Analysis and Critical Control Point (HACCP) Systems - Basic Requirements	
14. Developed and implemented a written HACCP plan .	
15. Contents of the HACCP list the food safety hazards, critical control points, critical limits, procedures, corrective actions.	
16. Records documenting implementation and monitoring of the HACCP plan.	
17. The HACCP plan is signed and dated by the responsible establishment individual.	
Hazard Analysis and Critical Control Point (HACCP) Systems - Ongoing Requirements	
18. Monitoring of HACCP plan.	
19. Verification and validation of HACCP plan.	
20. Corrective action written in HACCP plan.	
21. Reassessed adequacy of the HACCP plan.	
22. Records documenting: the written HACCP plan, monitoring of the critical control points, dates and times of specific event occurrences.	
Part C - Economic / Wholesomeness	
23. Labeling - Product Standards	
24. Labeling - Net Weights	
25. General Labeling	
26. Fin. Prod. Standards/Boneless (Defects/AQL/Pork Skins/Moisture)	
Part D - Sampling Generic *E. coli* Testing	
27. Written Procedures	O
28. Sample Collection/Analysis	O
29. Records	O
Salmonella Performance Standards - Basic Requirements	
30. Corrective Actions	O
31. Reassessment	O
32. Written Assurance	O

Part D - Continued Economic Sampling	Audit Results
33. Scheduled Sample	
34. Species Testing	
35. Residue	
Part E - Other Requirements	
36. Export	
37. Import	O
38. Establishment Grounds and Pest Control	
39. Establishment Construction/Maintenance	
40. Light	
41. Ventilation	
42. Plumbing and Sewage	
43. Water Supply	
44. Dressing Rooms/Lavatories	
45. Equipment and Utensils	
46. Sanitary Operations	
47. Employee Hygiene	
48. Condemned Product Control	
Part F - Inspection Requirements	
49. Government Staffing	
50. Daily Inspection Coverage	
51. Enforcement	
52. Humane Handling	O
53. Animal Identification	O
54. Ante Mortem Inspection	O
55. Post Mortem Inspection	O
Part G - Other Regulatory Oversight Requirements	
56. European Community Directives	O
57. Monthly Review	
58.	
59.	

60. Observation of the Establishment Ferreira International Ltda., Est. SIF-0013, Processing, 11/16/2015

There were no significant findings to report after consideration of the nature, degree and extent of all observations.

61. NAME OF AUDITOR	62. AUDITOR SIGNATURE AND DATE
Kenneth E. Witek – SPA, CSO	11/16/2015

United States Department of Agriculture
Food Safety and Inspection Service

Foreign Establishment Audit Checklist

1. ESTABLISHMENT NAME AND LOCATION	2. AUDIT DATE	3. ESTABLISHMENT NO.	4. NAME OF COUNTRY
Pampeano Aliminetos S/A Estação Santo Antonio Km 32 Vila Bordon Hulha Negra	11/12/2015	SIF226	Brazil
	5. NAME OF AUDITOR(S)		6. TYPE OF AUDIT
	A. Lauro and J. Filus		[X] ON-SITE AUDIT [ ] DOCUMENT AUDIT

Place an X in the Audit Results block to indicate noncompliance with requirements. Use O if not applicable.

Part A - Sanitation Standard Operating Procedures (SSOP) Basic Requirements	Audit Results
7. Written SSOP	
8. Records documenting implementation.	
9. Signed and dated SSOP, by on-site or overall authority.	
Sanitation Standard Operating Procedures (SSOP) Ongoing Requirements	
10. Implementation of SSOP's, including monitoring of implementation.	
11. Maintenance and evaluation of the effectiveness of SSOP's.	
12. Corrective action when the SSOP's have failed to prevent direct product contamination or adulteration.	
13. Daily records document item 10, 11 and 12 above.	
Part B - Hazard Analysis and Critical Control Point (HACCP) Systems - Basic Requirements	
14. Developed and implemented a written HACCP plan .	
15. Contents of the HACCP list the food safety hazards, critical control points, critical limits, procedures, corrective actions.	
16. Records documenting implementation and monitoring of the HACCP plan.	
17. The HACCP plan is signed and dated by the responsible establishment individual.	
Hazard Analysis and Critical Control Point (HACCP) Systems - Ongoing Requirements	
18. Monitoring of HACCP plan.	
19. Verification and validation of HACCP plan.	
20. Corrective action written in HACCP plan.	
21. Reassessed adequacy of the HACCP plan.	
22. Records documenting: the written HACCP plan, monitoring of the critical control points, dates and times of specific event occurrences.	
Part C - Economic / Wholesomeness	
23. Labeling - Product Standards	
24. Labeling - Net Weights	
25. General Labeling	
26. Fin. Prod. Standards/Boneless (Defects/AQL/Pork Skins/Moisture)	
Part D - Sampling Generic *E. coli* Testing	
27. Written Procedures	O
28. Sample Collection/Analysis	O
29. Records	O
Salmonella Performance Standards - Basic Requirements	
30. Corrective Actions	O
31. Reassessment	O
32. Written Assurance	O

Part D - Continued Economic Sampling	Audit Results
33. Scheduled Sample	
34. Species Testing	
35. Residue	
Part E - Other Requirements	
36. Export	
37. Import	
38. Establishment Grounds and Pest Control	
39. Establishment Construction/Maintenance	
40. Light	
41. Ventilation	
42. Plumbing and Sewage	
43. Water Supply	
44. Dressing Rooms/Lavatories	
45. Equipment and Utensils	
46. Sanitary Operations	
47. Employee Hygiene	
48. Condemned Product Control	
Part F - Inspection Requirements	
49. Government Staffing	
50. Daily Inspection Coverage	
51. Enforcement	
52. Humane Handling	O
53. Animal Identification	O
54. Ante Mortem Inspection	O
55. Post Mortem Inspection	O
Part G - Other Regulatory Oversight Requirements	
56. European Community Directives	O
57. Monthly Review	
58.	
59.	

60. Observation of the Establishment

There were no significant findings to report after consideration of the nature, degree, and extent of all observations.

61. NAME OF AUDITOR	62. AUDITOR SIGNATURE AND DATE
A. Lauro and J. Filus	Alexmar F. Lauro 11/12/2015

United States Department of Agriculture
Food Safety and Inspection Service

Foreign Establishment Audit Checklist

1. ESTABLISHMENT NAME AND LOCATION	2. AUDIT DATE	3. ESTABLISHMENT NO.	4. NAME OF COUNTRY
JBS S/A Parque Industrial S/N° Lins	11/10/2015	SIF337	Brazil
	5. NAME OF AUDITOR(S)		6. TYPE OF AUDIT
	A. Lauro and J. Filus		[X] ON-SITE AUDIT [ ] DOCUMENT AUDIT

Place an X in the Audit Results block to indicate noncompliance with requirements. Use O if not applicable.

Part A - Sanitation Standard Operating Procedures (SSOP) Basic Requirements	Audit Results
7. Written SSOP	
8. Records documenting implementation.	
9. Signed and dated SSOP, by on-site or overall authority.	
Sanitation Standard Operating Procedures (SSOP) Ongoing Requirements	
10. Implementation of SSOP's, including monitoring of implementation.	
11. Maintenance and evaluation of the effectiveness of SSOP's.	
12. Corrective action when the SSOP's have failed to prevent direct product contamination or adulteration.	
13. Daily records document item 10, 11 and 12 above.	
Part B - Hazard Analysis and Critical Control Point (HACCP) Systems - Basic Requirements	
14. Developed and implemented a written HACCP plan .	
15. Contents of the HACCP list the food safety hazards, critical control points, critical limits, procedures, corrective actions.	
16. Records documenting implementation and monitoring of the HACCP plan.	
17. The HACCP plan is signed and dated by the responsible establishment individual.	
Hazard Analysis and Critical Control Point (HACCP) Systems - Ongoing Requirements	
18. Monitoring of HACCP plan.	
19. Verification and validation of HACCP plan.	
20. Corrective action written in HACCP plan.	
21. Reassessed adequacy of the HACCP plan.	
22. Records documenting: the written HACCP plan, monitoring of the critical control points, dates and times of specific event occurrences.	
Part C - Economic / Wholesomeness	
23. Labeling - Product Standards	
24. Labeling - Net Weights	
25. General Labeling	
26. Fin. Prod. Standards/Boneless (Defects/AQL/Pork Skins/Moisture)	
Part D - Sampling Generic *E. coli* Testing	
27. Written Procedures	
28. Sample Collection/Analysis	
29. Records	
Salmonella Performance Standards - Basic Requirements	
30. Corrective Actions	
31. Reassessment	
32. Written Assurance	

Part D - Continued Economic Sampling	Audit Results
33. Scheduled Sample	
34. Species Testing	
35. Residue	
Part E - Other Requirements	
36. Export	
37. Import	
38. Establishment Grounds and Pest Control	
39. Establishment Construction/Maintenance	
40. Light	
41. Ventilation	
42. Plumbing and Sewage	
43. Water Supply	X
44. Dressing Rooms/Lavatories	
45. Equipment and Utensils	X
46. Sanitary Operations	
47. Employee Hygiene	
48. Condemned Product Control	
Part F - Inspection Requirements	
49. Government Staffing	
50. Daily Inspection Coverage	
51. Enforcement	X
52. Humane Handling	
53. Animal Identification	
54. Ante Mortem Inspection	
55. Post Mortem Inspection	
Part G - Other Regulatory Oversight Requirements	
56. European Community Directives	O
57. Monthly Review	
58.	
59.	

60. Observation of the Establishment

43/51. The FSIS auditors noted inconsistencies in establishment records documenting pH values for chlorinated retort cooling water. The measurement of pH is important because chlorine is a much better bactericide at lower pH levels (regardless of the amount of free available chlorine). On a given day, a review of establishment records indicated that while measurements obtained through the use of "kits" on the production floor identified pH values around 8.0, the result of laboratory testing was 7.43. Microbiological testing of the cooling water did not include testing for facultative anaerobes. Facultative anaerobes introduced post process from unclean water via micro-leaks, are a potential food safety concern. The lack of microbiological testing in association with the inconsistencies identified in PH rendered it difficult to ascertain the safety of the cooling water at this establishment. Consequently, FSIS has identified the need for DIPOA/SIPOA to improve its activities related to the verification of the suitability of retort cooling water at thermal processing facilities.

45/51. The FSIS auditors noted the presence of thin metal condensate tubing in each retort that originated from below the bottom basket brackets and exited near the top. Although no bent tubing was observed at the time of the audit, the current design raised concerns about the frailty of the setup. The inability for damaged tubing to transfer steam when the bottom of the retort fills with condensate creates the potential for under-processing. The auditors further noted that an ongoing review of the integrity of this tubing was not conducted, either within the context of the establishment's food-safety system or government verification activities.

United States Department of Agriculture
Food Safety and Inspection Service

Foreign Establishment Audit Checklist

1. ESTABLISHMENT NAME AND LOCATION	2. AUDIT DATE	3. ESTABLISHMENT NO.	4. NAME OF COUNTRY
JBS S/A Av. José Batista Sobrinho S/N° Andradina São Paulo	11/17/15	SIF 385	Brazil
	5. NAME OF AUDITOR(S) Juan F. Rodriguez, DVM		6. TYPE OF AUDIT [X] ON-SITE AUDIT [ ] DOCUMENT AUDIT

Place an X in the Audit Results block to indicate noncompliance with requirements. Use O if not applicable.

Part A - Sanitation Standard Operating Procedures (SSOP) Basic Requirements	Audit Results
7. Written SSOP	
8. Records documenting implementation.	
9. Signed and dated SSOP, by on-site or overall authority.	
Sanitation Standard Operating Procedures (SSOP) Ongoing Requirements	
10. Implementation of SSOP's, including monitoring of implementation.	
11. Maintenance and evaluation of the effectiveness of SSOP's.	
12. Corrective action when the SSOP's have failed to prevent direct product contamination or adulteration.	
13. Daily records document item 10, 11 and 12 above.	
Part B - Hazard Analysis and Critical Control Point (HACCP) Systems - Basic Requirements	
14. Developed and implemented a written HACCP plan .	
15. Contents of the HACCP list the food safety hazards, critical control points, critical limits, procedures, corrective actions.	
16. Records documenting implementation and monitoring of the HACCP plan.	
17. The HACCP plan is signed and dated by the responsible establishment individual.	
Hazard Analysis and Critical Control Point (HACCP) Systems - Ongoing Requirements	
18. Monitoring of HACCP plan.	
19. Verification and validation of HACCP plan.	
20. Corrective action written in HACCP plan.	
21. Reassessed adequacy of the HACCP plan.	
22. Records documenting: the written HACCP plan, monitoring of the critical control points, dates and times of specific event occurrences.	
Part C - Economic / Wholesomeness	
23. Labeling - Product Standards	
24. Labeling - Net Weights	
25. General Labeling	
26. Fin. Prod. Standards/Boneless (Defects/AQL/Pork Skins/Moisture)	
Part D - Sampling Generic *E. coli* Testing	
27. Written Procedures	O
28. Sample Collection/Analysis	O
29. Records	O
Salmonella Performance Standards - Basic Requirements	
30. Corrective Actions	
31. Reassessment	
32. Written Assurance	

Part D - Continued Economic Sampling	Audit Results
33. Scheduled Sample	
34. Species Testing	
35. Residue	
Part E - Other Requirements	
36. Export	
37. Import	
38. Establishment Grounds and Pest Control	
39. Establishment Construction/Maintenance	
40. Light	
41. Ventilation	
42. Plumbing and Sewage	
43. Water Supply	
44. Dressing Rooms/Lavatories	
45. Equipment and Utensils	
46. Sanitary Operations	X
47. Employee Hygiene	
48. Condemned Product Control	
Part F - Inspection Requirements	
49. Government Staffing	
50. Daily Inspection Coverage	
51. Enforcement	X
52. Humane Handling	
53. Animal Identification	
54. Ante Mortem Inspection	
55. Post Mortem Inspection	
Part G - Other Regulatory Oversight Requirements	
56. European Community Directives	
57. Monthly Review	
58.	
59.	

60. Observation of the Establishment

November 17, 2015 | SIF 385 | JBS S/A, Andradina, São Paulo | (S/P) | Brazil

There were no significant findings to report after consideration of the nature, degree, and extent of all observations.

Species slaughtered and processed: Bovine

61. NAME OF AUDITOR	62. AUDITOR SIGNATURE AND DATE
FOR Juan F. Rodriguez. DVM	Carmen A. Lamo 11/17/2015

United States Department of Agriculture
Food Safety and Inspection Service

Foreign Establishment Audit Checklist

1. ESTABLISHMENT NAME AND LOCATION	2. AUDIT DATE	3. ESTABLISHMENT NO.	4. NAME OF COUNTRY
Minerva Foods SA Barretos, SP Brazil	11/18/2015	SIF-0421	Brazil
	5. NAME OF AUDITOR(S) Kenneth E. Witek – SPA, CSO		6. TYPE OF AUDIT [X] ON-SITE AUDIT [ ] DOCUMENT AUDIT

Place an X in the Audit Results block to indicate noncompliance with requirements. Use O if not applicable.

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23. Labeling - Product Standards	
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26. Fin. Prod. Standards/Boneless (Defects/AQL/Pork Skins/Moisture)	
Part D - Sampling Generic *E. coli* Testing	
27. Written Procedures	
28. Sample Collection/Analysis	
29. Records	
Salmonella Performance Standards - Basic Requirements	
30. Corrective Actions	
31. Reassessment	
32. Written Assurance	

Part D - Continued Economic Sampling	Audit Results
33. Scheduled Sample	
34. Species Testing	
35. Residue	X
Part E - Other Requirements	
36. Export	
37. Import	O
38. Establishment Grounds and Pest Control	
39. Establishment Construction/Maintenance	X
40. Light	
41. Ventilation	
42. Plumbing and Sewage	
43. Water Supply	
44. Dressing Rooms/Lavatories	
45. Equipment and Utensils	X
46. Sanitary Operations	
47. Employee Hygiene	
48. Condemned Product Control	
Part F - Inspection Requirements	
49. Government Staffing	
50. Daily Inspection Coverage	
51. Enforcement	X
52. Humane Handling	X
53. Animal Identification	
54. Ante Mortem Inspection	
55. Post Mortem Inspection	
Part G - Other Regulatory Oversight Requirements	
56. European Community Directives	O
57. Monthly Review	
58.	
59.	

60. Observation of the Establishment Minerva Foods SA, Est. SIF-0421, Bovine Slaughter/Processing/Canning, 11/18/2015

35/51 Residue Sampling Program:
The CCA has not issued instructions to in-plant inspectors and establishments that require the establishment to hold or maintain control over any livestock carcass selected for directed monitoring residue sampling until the CCA's laboratory test results are reported. This establishment does not hold the selected carcass pending the test results.

39/51 Establishment Construction/Maintenance: Walls and Floors:
In carcass coolers throughout the facility it was observed that there the rubber seals that outline the doors and contact the door jamb surface have become deteriorated. The rubber has become cracked, dislodged from the door and peeling off in pieces small pieces. Carcasses make incidental contact with these areas of the door due to the observation of blood resides on one door. This deficiencies observed creates an insanitary condition which could result in the contamination of product (no direct product contamination observed), and surfaces that could not be readily cleaned.

A review of establishment and inspection verification records provided no evidence that these deficiencies were previously identified.
[Regulatory reference: 9CFR 416.2(b), 9CFR 327(a)(2)(i)(D), 416.17, and Section 3.c. of Brazilian Regulation No. 175/2005/CGPE/DIPOA)]

45/51 Equipment:
The FSIS auditor observed that in the Fabrication Department a white fiber interlocking conveyor belt that carries bagged raw beef product to boxing was inappropriately maintained. The belt was severely frayed and cracked at both edges of the belt, with numerous holes in the belts surface. In addition, the stainless steel channel side of the conveyor belt that feed the previous mentioned belt was severely cracked with a jagged hole at the end of the channel's side. This equipment makes incidental contact with the bagged product. These deficiencies observed creates surfaces that could not be readily cleaned creating an insanitary condition, which could result in the contamination of product (no direct product contamination observed).

A review of establishment and inspection verification records provided no evidence that these deficiencies were previously identified. Immediate corrective actions were taken by the establishment and verified by SIF with inspectors additional measure to prevent the reoccurrence will be provide to inspection personnel.
[Regulatory reference: 9CFR 416.3(a), 416.4 (d), 327(a)(2)(i)(D), 416.17]

52/51 Humane Handling: Establishment Construction:
The FSIS auditor observed deficiency in the walkway that routes livestock received to cattle holding pens. Observation included that a concrete slab that is positioned over a drainage hole in the middle of the runway between two pens was flat with the surrounding surface. However, an area of the slab was cracked and presented a hole large enough in the surface, creating a condition that could cause injury to the animal if its foot became lodged or unsteadied during transit through this area. A review of establishment and inspection verification documents provided no evidence that this deficiency was previously identified. An area of the walkway was cracked and presented a hole large enough in the surface that could cause injury to the animal during transit through this area.
[Regulatory reference: 9CFR 416.2(b), 9CFR 327(a)(2)(i)(D), 416.17]

61. NAME OF AUDITOR	62. AUDITOR SIGNATURE AND DATE
Kenneth E. Witek – SPA, CSO	11/18/2015

United States Department of Agriculture
Food Safety and Inspection Service

Foreign Establishment Audit Checklist

1. ESTABLISHMENT NAME AND LOCATION	2. AUDIT DATE	3. ESTABLISHMENT NO.	4. NAME OF COUNTRY
Minerva Foods Palmeiras de Goiás, GO Brazil	11/10/2015	SIF-0431	Brazil
	5. NAME OF AUDITOR(S) Kenneth E. Witek – SPA, CSO		6. TYPE OF AUDIT [X] ON-SITE AUDIT [ ] DOCUMENT AUDIT

Place an X in the Audit Results block to indicate noncompliance with requirements. Use O if not applicable.

Part A - Sanitation Standard Operating Procedures (SSOP) Basic Requirements	Audit Results
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Sanitation Standard Operating Procedures (SSOP) Ongoing Requirements	
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12. Corrective action when the SSOP's have failed to prevent direct product contamination or adulteration.	
13. Daily records document item 10, 11 and 12 above.	
Part B - Hazard Analysis and Critical Control Point (HACCP) Systems - Basic Requirements	
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20. Corrective action written in HACCP plan.	
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22. Records documenting: the written HACCP plan, monitoring of the critical control points, dates and times of specific event occurrences.	
Part C - Economic / Wholesomeness	
23. Labeling - Product Standards	
24. Labeling - Net Weights	
25. General Labeling	
26. Fin. Prod. Standards/Boneless (Defects/AQL/Pork Skins/Moisture)	
Part D - Sampling Generic *E. coli* Testing	
27. Written Procedures	
28. Sample Collection/Analysis	
29. Records	
Salmonella Performance Standards - Basic Requirements	
30. Corrective Actions	
31. Reassessment	
32. Written Assurance	

Part D - Continued Economic Sampling	Audit Results
33. Scheduled Sample	
34. Species Testing	
35. Residue	X
Part E - Other Requirements	
36. Export	
37. Import	O
38. Establishment Grounds and Pest Control	
39. Establishment Construction/Maintenance	X
40. Light	
41. Ventilation	X
42. Plumbing and Sewage	
43. Water Supply	
44. Dressing Rooms/Lavatories	
45. Equipment and Utensils	X
46. Sanitary Operations	
47. Employee Hygiene	
48. Condemned Product Control	
Part F - Inspection Requirements	
49. Government Staffing	
50. Daily Inspection Coverage	
51. Enforcement	X
52. Humane Handling	
53. Animal Identification	
54. Ante Mortem Inspection	
55. Post Mortem Inspection	X
Part G - Other Regulatory Oversight Requirements	
56. European Community Directives	O
57. Monthly Review	
58.	
59.	

60. Observation of the Establishment Minerva Foods, Est. SIF-0431, Bovine Slaughter/Processing, 11/10/2015

35/51 Residue Sampling Program:
The CCA has not issued instructions to in-plant inspectors and establishments that require the establishment to hold or maintain control over any livestock carcass selected for directed monitoring residue sampling until the CCA's laboratory test results are reported. This establishment does not hold the selected carcass pending the test results.

39/51 Establishment Construction/Maintenance:

1. Walls and Floors:

In the boxed product freezers and along traffic hall areas throughout the facility it was observed that there were numerous sections of walls that were in a state of disrepair in which walls of concrete were deteriorated, and metal not smooth fastened and sealed to the wall but pulled apart from the wall. Concrete coving in these areas was also crumbling and not providing a smooth sealed transition to the walls and floor. Floors in the traffic hall areas of these freezers and cooler were also in a deteriorated state by evidence of cracking and the upper surface of the concrete dislodges in several areas. These deficiencies observed creates surfaces that could not be readily cleaned.

2. Overhead Structures:

Various deficiencies in the maintenance of overhead structures were observed by the FSIS auditor in the establishments Fabrication Department. Observations included rust and flacking paint on rail support beams, leading to the Fabrication Department, caulking that was not smooth, dislodging and area where gapes and hole caused by overhead structures and equipment were not properly sealed thereby creating insanitary conditions, which could result in the contamination of product (no direct product contamination observed).

A review of establishment and inspection verification records provided no evidence that these deficiencies were previously identified.
[Regulatory reference: 9CFR 416.2(b), 9CFR 327(a)(2)(i)(D), 416.17, and Section 3.c. of Brazilian Regulation No. 175/2005/CGPE/DIPOA)]

41/51 Ventilation:
The FSIS auditor observed that in a boxed product cooler heavy condensate was forming on a cooling unit above boxed product. In addition another unit's refrigerant pipe of its's cooling unit was being defrosted directly over boxed product with no way of preventing it from dripping onto the boxed product creating an insanitary condition which could result in the contamination of product.

In addition, the FSIS auditor observed that in several boxed product freezers an extensive amount of frozen condensate had formed above boxed product near the cooling units and other areas of the freezers. Supervisory review reports had documented this same deficiency approximately 6-months previous. The establishment is still in the process of correcting these issues but has not implemented a permanent corrective action. However, they have failed to identify the condensation problems now in the boxed product cooler.
[Regulatory reference: 9CFR 416.2(d), 9CFR 327(a)(2)(i)(D), 416.17]

45/51 Equipment:
The FSIS auditor observed that in the Fabrication Department a white vinyl conveyor belt that carries bagged raw beef product to the freeze tunnel was inappropriately maintained. The belt was severely frayed and cracked at both edges in addition to numerous holes and torn areas in the belts surface. This deficiency observed creates surfaces that could not be readily cleaned creating an insanitary condition, which could result in the contamination of product (no direct product contamination observed as the equipment was not deployed for use yet at this point in the production day).

A review of establishment and inspection verification records provided no evidence that these deficiencies were previously identified. Immediate corrective actions were taken by the establishment and verified by SIF with inspectors additional measure to prevent the re-occurrence will be provide to inspection personnel.
[Regulatory reference: 9CFR 416.3(a), 416.4 (d), 327(a)(2)(i)(D), 416.17]

55/51 Post-mortem Inspection - Inspection Zero-Tolerance Verification:
The FSIS auditor observed that the SIF inspector that performs the carcass zero-tolerance verification for contamination caused by feces, and ingesta was positioned on a low platform prior to the carcass wash process. The design of this platform does not permit the inspector to evaluate the ability of the establishment to meet the zero-tolerance standard. The SIF inspector's eye level only reaches to the kidney area of the carcass, as the inspector was not able to observe the full hindquarter of the carcass.
[Regulatory reference: 9CFR 327(a)(2)(i)(C) & (D), 416.17, and 417.8]

61. NAME OF AUDITOR	62. AUDITOR SIGNATURE AND DATE
Kenneth E. Witek – SPA, CSO	Kenneth E. Witek 11/10/2015

United States Department of Agriculture
Food Safety and Inspection Service

Foreign Establishment Audit Checklist

1. ESTABLISHMENT NAME AND LOCATION	2. AUDIT DATE	3. ESTABLISHMENT NO.	4. NAME OF COUNTRY
Meat Snack Partners do Brasil Ltda ROD. SP 340 km142 s/n° - Rural Santo Antonio de Posse	11/17/2015	SIF1690	Brazil
	5. NAME OF AUDITOR(S)		6. TYPE OF AUDIT
	Alexander L. Lauro, DVM		[X] ON-SITE AUDIT [ ] DOCUMENT AUDIT

Place an X in the Audit Results block to indicate noncompliance with requirements. Use O if not applicable.

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12. Corrective action when the SSOP's have failed to prevent direct product contamination or adulteration.	
13. Daily records document item 10, 11 and 12 above.	
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22. Records documenting: the written HACCP plan, monitoring of the critical control points, dates and times of specific event occurrences.	
Part C - Economic / Wholesomeness	
23. Labeling - Product Standards	
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25. General Labeling	
26. Fin. Prod. Standards/Boneless (Defects/AQL/Pork Skins/Moisture)	
Part D - Sampling Generic *E. coli* Testing	
27. Written Procedures	O
28. Sample Collection/Analysis	O
29. Records	O
Salmonella Performance Standards - Basic Requirements	
30. Corrective Actions	O
31. Reassessment	O
32. Written Assurance	O

Part D - Continued Economic Sampling	Audit Results
33. Scheduled Sample	
34. Species Testing	
35. Residue	
Part E - Other Requirements	
36. Export	
37. Import	
38. Establishment Grounds and Pest Control	
39. Establishment Construction/Maintenance	
40. Light	
41. Ventilation	
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44. Dressing Rooms/Lavatories	
45. Equipment and Utensils	
46. Sanitary Operations	
47. Employee Hygiene	
48. Condemned Product Control	
Part F - Inspection Requirements	
49. Government Staffing	
50. Daily Inspection Coverage	
51. Enforcement	
52. Humane Handling	O
53. Animal Identification	O
54. Ante Mortem Inspection	O
55. Post Mortem Inspection	O
Part G - Other Regulatory Oversight Requirements	
56. European Community Directives	O
57. Monthly Review	
58.	
59.	

60. Observation of the Establishment

There were no significant findings to report after consideration of the nature, degree, and extent of all observations.

61. NAME OF AUDITOR

Alexander L. Lauro, DVM

62. AUDITOR SIGNATURE AND DATE

Alexander L. Lauro 11/6/2015

United States Department of Agriculture
Food Safety and Inspection Service

Foreign Establishment Audit Checklist

1. ESTABLISHMENT NAME AND LOCATION	2. AUDIT DATE	3. ESTABLISHMENT NO.	4. NAME OF COUNTRY
JBS S/A Naviraí Mato Grosso do Sul	11/13/15	SIF 3181	Brazil
	5. NAME OF AUDITOR(S)		6. TYPE OF AUDIT
	Juan F. Rodriguez, DVM		[X] ON-SITE AUDIT [ ] DOCUMENT AUDIT

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Part D - Continued Economic Sampling	Audit Results
33. Scheduled Sample	
34. Species Testing	O
35. Residue	
Part E - Other Requirements	
36. Export	
37. Import	O
38. Establishment Grounds and Pest Control	
39. Establishment Construction/Maintenance	X
40. Light	
41. Ventilation	X
42. Plumbing and Sewage	
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53. Animal Identification	
54. Ante Mortem Inspection	
55. Post Mortem Inspection	
Part G - Other Regulatory Oversight Requirements	
56. European Community Directives	O
57. Monthly Review	
58.	
59.	

60. Observation of the Establishment

39. In the boxed product freezers and along traffic halls throughout the facility it was observed that there were numerous sections of floors were in a state of disrepair as evidenced by cracking of the floor as well as areas where the upper surface of concrete floors became dislodged. Floor to wall junctions in several areas of the carcass coolers were in need of repair and did not present a smooth sealed transition to from the walls to the floor. These deficiencies observed creates surfaces that could not be readily cleaned. A review of establishment and inspection verification documents revealed that these deficiencies had been identified and repairs had been programmed to begin in March 2016.

41. The FSIS auditor observed that in several boxed product freezers an extensive amount of frozen condensate had formed above boxed product near the cooling units and other areas of the freezers. The in-plant inspection team immediately took control of the areas and documented the deficiencies in a non-compliance report. No direct product contamination was observed.

Species slaughtered and processed: Bovine

61. NAME OF AUDITOR for
Juan F. Rodriguez. DVM

62. AUDITOR SIGNATURE AND DATE
11/13/2015

United States Department of Agriculture
Food Safety and Inspection Service

Foreign Establishment Audit Checklist

1. ESTABLISHMENT NAME AND LOCATION	2. AUDIT DATE	3. ESTABLISHMENT NO.	4. NAME OF COUNTRY
Cooperativa Central Oeste Catarinese Chapeco, Santa Catarina Brazil	11/12/2015	SIF-3548	Brazil
	5. NAME OF AUDITOR(S) Kenneth E. Witek – SPA, CSO		6. TYPE OF AUDIT [X] ON-SITE AUDIT [ ] DOCUMENT AUDIT

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33. Scheduled Sample	
34. Species Testing	
35. Residue	X
Part E - Other Requirements	
36. Export	
37. Import	O
38. Establishment Grounds and Pest Control	
39. Establishment Construction/Maintenance	X
40. Light	
41. Ventilation	
42. Plumbing and Sewage	
43. Water Supply	
44. Dressing Rooms/Lavatories	
45. Equipment and Utensils	X
46. Sanitary Operations	
47. Employee Hygiene	
48. Condemned Product Control	
Part F - Inspection Requirements	
49. Government Staffing	
50. Daily Inspection Coverage	
51. Enforcement	X
52. Humane Handling	
53. Animal Identification	
54. Ante Mortem Inspection	
55. Post Mortem Inspection	X
Part G - Other Regulatory Oversight Requirements	
56. European Community Directives	O
57. Monthly Review	
58.	
59.	

60. Observation of the Establishment Cooperativa Central Oeste Catarinese, Est. SIF-3548, Swine Slaughter/Processing, 11/12/2015

35/51 Residue Sampling Program:
The CCA has not issued instructions to in-plant inspectors and establishments that require the establishment to hold or maintain control over any livestock carcass selected for directed monitoring residue sampling until the CCA's laboratory test results are reported. This establishment does not hold the selected carcass pending the test results.

39/51 Establishment Construction/Maintenance:

1. Walls and Floors:

In the slaughter floor area, fabrication room, and along traffic hall areas throughout the facility it was observed that there were numerous sections of walls where metal plating covering sections of walls, doors, and door way casings were not smooth fastened and sealed to the wall but pulled apart from the wall. In addition, metal coving in the fabrication room was not providing a smooth sealed transition to the walls and floor. These deficiencies observed creates surfaces that could not be readily cleaned.

2. Overhead Structures:

Carcass rails and rail switch piston leading from the slaughter floor area to the first cooler. Observations included rust, oxidation and flacking paint (switch piston) thereby creating insanitary conditions, which could result in the contamination of product (no direct product contamination observed).

A review of establishment, inspection verification records in addition to supervisory reviews provided no evidence that these deficiencies were previously identified.
[Regulatory reference: 9CFR 416.2(b), 9CFR 327(a)(2)(i)(D), 416.17, and Section 3.c. of Brazilian Regulation No. 175/2005/CGPE/DIPOA)]

45/51 Equipment:

1. Fabrication and Packaging Area:

A stainless steel tray that moves fabricated product along an overhead conveyor system was pitted having a rough surface, and in the packaging area had a white fiber vat that had jagged edges. This deficiency observed creates surfaces that could not be readily cleaned creating an insanitary condition which could result in the contamination of product. No direct product contamination was observed as these observations were observed during pre-operational verification of inspection personnel. Immediate corrective actions were taken by the establishment and verified by SIF with inspectors. Additional measure to prevent the reoccurrence will be provided to inspection personnel.

2. Slaughter Floor Area:

The final carcass shower had jagged holes torn into the stainless steel casement of the unit. Metal employee platforms used during slaughter dressing procedures were in a deteriorated state. The top plating of the numerous platforms was separated and in some areas falling through. These platforms come in close proximity of the carcass and when the carcasses pass by there is the possibility of residue from the platform's separated surface to splashing and adulterate the carcass due to the vibration caused during normal operations.

A review of establishment and inspection verification records provided no evidence that these deficiencies were previously identified.
[Regulatory reference: 9CFR 416.3(a), 416.4 (d), 327(a)(2)(i)(D), 416.17]

55/51 Post-mortem Inspection – Rail-out - Pathology Final Disposition:
The FSIS auditor observed that the viscera did not routinely accompany carcasses railed-out for final veterinary dispositions. The SIF veterinarian responsible for final disposition of carcasses railed out for pathological conditions was not requiring the establishment to hold and present the viscera for the carcasses railed out for pathological conditions so that an adequate disposition could be conducted the veterinarian. In one instance, the veterinarian condemned a carcass due for pathological conditions but was not able to confirm that the viscera was not allowed to pass for human consumption since it was not held with the carcass that was held by the veterinarian.
[Regulatory reference: 9CFR 327(a)(2), 416.17, and 417.8]

61. NAME OF AUDITOR	62. AUDITOR SIGNATURE AND DATE
Kenneth E. Witek – SPA, CSO	11/12/2015

United States Department of Agriculture
Food Safety and Inspection Service

Foreign Establishment Audit Checklist

1. ESTABLISHMENT NAME AND LOCATION	2. AUDIT DATE	3. ESTABLISHMENT NO.	4. NAME OF COUNTRY
Marfrig Alimentos S.A. Rod. BR 267 Km 35 Distrito Industrial Bataguassu São Paulo	11/16/15	SIF 4238	Brazil
	5. NAME OF AUDITOR(S)		6. TYPE OF AUDIT
	Juan F. Rodriguez, DVM		[X] ON-SITE AUDIT [ ] DOCUMENT AUDIT

Place an X in the Audit Results block to indicate noncompliance with requirements. Use O if not applicable.

Part A - Sanitation Standard Operating Procedures (SSOP) Basic Requirements	Audit Results
7. Written SSOP	
8. Records documenting implementation.	
9. Signed and dated SSOP, by on-site or overall authority.	
Sanitation Standard Operating Procedures (SSOP) Ongoing Requirements	
10. Implementation of SSOP's, including monitoring of implementation.	
11. Maintenance and evaluation of the effectiveness of SSOP's.	
12. Corrective action when the SSOP's have failed to prevent direct product contamination or adulteration.	
13. Daily records document item 10, 11 and 12 above.	
Part B - Hazard Analysis and Critical Control Point (HACCP) Systems - Basic Requirements	
14. Developed and implemented a written HACCP plan .	
15. Contents of the HACCP list the food safety hazards, critical control points, critical limits, procedures, corrective actions.	
16. Records documenting implementation and monitoring of the HACCP plan.	
17. The HACCP plan is signed and dated by the responsible establishment individual.	
Hazard Analysis and Critical Control Point (HACCP) Systems - Ongoing Requirements	
18. Monitoring of HACCP plan.	
19. Verification and validation of HACCP plan.	
20. Corrective action written in HACCP plan.	
21. Reassessed adequacy of the HACCP plan.	
22. Records documenting: the written HACCP plan, monitoring of the critical control points, dates and times of specific event occurrences.	
Part C - Economic / Wholesomeness	
23. Labeling - Product Standards	
24. Labeling - Net Weights	
25. General Labeling	
26. Fin. Prod. Standards/Boneless (Defects/AQL/Pork Skins/Moisture)	
Part D - Sampling Generic *E. coli* Testing	
27. Written Procedures	
28. Sample Collection/Analysis	
29. Records	
Salmonella Performance Standards - Basic Requirements	
30. Corrective Actions	
31. Reassessment	
32. Written Assurance	

Part D - Continued Economic Sampling	Audit Results
33. Scheduled Sample	
34. Species Testing	O
35. Residue	
Part E - Other Requirements	
36. Export	
37. Import	
38. Establishment Grounds and Pest Control	
39. Establishment Construction/Maintenance	X
40. Light	
41. Ventilation	X
42. Plumbing and Sewage	
43. Water Supply	
44. Dressing Rooms/Lavatories	
45. Equipment and Utensils	
46. Sanitary Operations	
47. Employee Hygiene	
48. Condemned Product Control	
Part F - Inspection Requirements	
49. Government Staffing	
50. Daily Inspection Coverage	
51. Enforcement	
52. Humane Handling	
53. Animal Identification	
54. Ante Mortem Inspection	
55. Post Mortem Inspection	
Part G - Other Regulatory Oversight Requirements	
56. European Community Directives	
57. Monthly Review	
58.	
59.	

60. Observation of the Establishment

39. While conducting a walk-thru of the facility, the FSIS auditor observed that the floor at the entrance to the boxed product freezer, located adjacent to the shipping dock the floor, was in a state of disrepair. The upper surface of the concrete floor was cracked, becoming dislodged in some areas.

41. The FSIS auditor observed that in two of the boxed product freezers there was a light accumulation of frozen condensate on boxed products. No direct product contamination was observed. The observation was discussed with local and CCA inspection officials after the walk-thru of the facility.

61. NAME OF AUDITOR	62. AUDITOR SIGNATURE AND DATE
Juan F. Rodriguez. DVM for	11/16/2015

United States Department of Agriculture
Food Safety and Inspection Service

Foreign Establishment Audit Checklist

1. ESTABLISHMENT NAME AND LOCATION	2. AUDIT DATE	3. ESTABLISHMENT NO.	4. NAME OF COUNTRY
JBS S/A RDV BR 060 Sn Km 359.8 Margen Direita, Zona Rural Campo Grande Matto Grosso do Sul	11/11/15	SIF 4400	Brazil
	5. NAME OF AUDITOR(S)		6. TYPE OF AUDIT
	Juan F. Rodriguez, DVM		[X] ON-SITE AUDIT [ ] DOCUMENT AUDIT

Place an X in the Audit Results block to indicate noncompliance with requirements. Use O if not applicable.

Part A - Sanitation Standard Operating Procedures (SSOP) Basic Requirements	Audit Results	Part D - Continued Economic Sampling	Audit Results
7. Written SSOP		33. Scheduled Sample	
8. Records documenting implementation.		34. Species Testing	O
9. Signed and dated SSOP, by on-site or overall authority.		35. Residue	
Sanitation Standard Operating Procedures (SSOP) Ongoing Requirements		**Part E - Other Requirements**	
10. Implementation of SSOP's, including monitoring of implementation.		36. Export	
11. Maintenance and evaluation of the effectiveness of SSOP's.		37. Import	O
12. Corrective action when the SSOP's have failed to prevent direct product contamination or adulteration.		38. Establishment Grounds and Pest Control	
13. Daily records document item 10, 11 and 12 above.		39. Establishment Construction/Maintenance	
Part B - Hazard Analysis and Critical Control Point (HACCP) Systems - Basic Requirements		40. Light	
14. Developed and implemented a written HACCP plan .		41. Ventilation	
15. Contents of the HACCP list the food safety hazards, critical control points, critical limits, procedures, corrective actions.		42. Plumbing and Sewage	
16. Records documenting implementation and monitoring of the HACCP plan.		43. Water Supply	
17. The HACCP plan is signed and dated by the responsible establishment individual.		44. Dressing Rooms/Lavatories	
Hazard Analysis and Critical Control Point (HACCP) Systems - Ongoing Requirements		45. Equipment and Utensils	
18. Monitoring of HACCP plan.		46. Sanitary Operations	
19. Verification and validation of HACCP plan.		47. Employee Hygiene	
20. Corrective action written in HACCP plan.		48. Condemned Product Control	
21. Reassessed adequacy of the HACCP plan.		**Part F - Inspection Requirements**	
22. Records documenting: the written HACCP plan, monitoring of the critical control points, dates and times of specific event occurrences.		49. Government Staffing	
Part C - Economic / Wholesomeness		50. Daily Inspection Coverage	
23. Labeling - Product Standards		51. Enforcement	
24. Labeling - Net Weights		52. Humane Handling	
25. General Labeling		53. Animal Identification	
26. Fin. Prod. Standards/Boneless (Defects/AQL/Pork Skins/Moisture)		54. Ante Mortem Inspection	
Part D - Sampling Generic *E. coli* Testing		55. Post Mortem Inspection	
27. Written Procedures		**Part G - Other Regulatory Oversight Requirements**	
28. Sample Collection/Analysis		56. European Community Directives	O
29. Records		57. Monthly Review	
Salmonella Performance Standards - Basic Requirements		58.	
30. Corrective Actions		59.	
31. Reassessment			
32. Written Assurance			

60. Observation of the Establishment

November 11, 2015 | SIF 4400 | JBS S/A, Campo Grande, Matto Grosso do Sul | (S/P/CS) | Brazil

There were no significant findings to report after consideration of the nature, degree, and extent of all observations.

Species slaughtered and processed: Bovine

61. NAME OF AUDITOR	62. AUDITOR SIGNATURE AND DATE
Juan F. Rodriguez. DVM	11/11/2015

Appendix B: Foreign Country Response to the Draft Final Audit Report

MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO
Esplanada dos Ministérios, bloco D, sala 348 , Brasília/DF, CEP 70043900
Telefone: 61 3218-2927 e Fax - http://www.agricultura.gov.br

21000.007797/2016-11

Ofício nº 410/2016/CGSF/DNNT-SRI/SRI/GM/MAPA

Brasília, 02 de maio de 2016.

Ao Senhor
Ministro Conselheiro de Agricultura Clay Hamilton
Serviço Exterior de Agricultura – FAS
Embaixada dos Estados Unidos da América
SES 801, Lote 03
70403-900 - Brasília - DF

C/C:
Ao Senhor
Chefe da DPB Braz Baracuhy
Divisão de Produtos de Base
Ministério das Relações Exteriores (Palácio Itamaraty)
Esplanada dos Ministérios - Bloco H
70170-900 - Brasília - DF

	Findings	"Achados" (Column 2 is the Portuguese translation of Column 1)	Actions	Deadlines
1	FSIS determined that the CCA needs to revisit its procedure entitled, *Investigation Procedures for International Notifications* (*306/2013)* as it relates to FSIS point-of-entry (POE) violation notifications. While FSIS requests a reply to these notifications within 30 calendar days, the auditors noted that the CCA's average response time is 109 calendar days. This finding is related to deficiencies identified during the last FSIS audit in September 2014.	O FSIS constatou que a Autoridade Competente Central (CCA) precisa reavaliar seu procedimento intitulado, *Procedimentos para o Tratamento de Notificações Internacionais* (*Memo 306/2013)* em relação às notificações de violações no Ponto de Entrada (POE) do FSIS. Embora o FSIS requeira uma resposta a essas notificações dentro de 30 dias corridos, os auditores observaram que o tempo médio de resposta é de 109 dias corridos. Este achado refere-se às deficiências identificadas durante a auditoria anterior do FSIS realizada em setembro de 2014.	Investigation procedures were supplemented and brought up to date by Memo 306/2013, and with regard to POEVs by Circular n° 086/2015/CGI/DIPOA dated November 5, 2015. DIPOA, through CGI/DIPOA, is managing the deadlines of the investigations so as to avoid them exceeding 30 days; and when there is a need to extend the deadline, FSIS/USDA is consulted before expiry.	Completed on November 5, 2015. We point out that CASE FILE 2016-BR-SIF385-01 was neither solved within the regular deadline nor within the extended deadline because of excess demand of the Central Authorities. A further extension was not requested because we felt it would be unsuitable to do so.
2	A portion of Brazil's inspection force was not familiar with procedures in the CCA's *Guidelines for Implementing the National Residue Control Plan (132/2012), which* govern the targeting of animals suspected of containing violative levels of chemical residues at ante-mortem. This is a repeat finding.	Uma parte dos fiscais brasileiros não conhecia os procedimentos da Autoridade Competente Central (CCA) Diretrizes para a Implementação do Plano Nacional de Controle de Resíduos (132/2012), que determina o direcionamento para análise, durante a inspeção ante mortem, de animais suspeitos de conter níveis violadores de resíduos químicos. Este é um achado repetido.	We detected that Official Letter (Ofício) SDA/MAPA n° 132/2012 dated April 18, 2012, was for some reason no longer on the SIGSIF (Management Information System of the Federal Inspection Service - *Sistema de Informações Gerenciais do Serviço de Inspeção Federal*) bulletin board, which is the internal communication tool available to officials of the Federal Inspection Service (SIF). The *Ofício* has once again been made available to all officials and was formally forwarded by digital case file n° 21000.018172/2016-85.	Completed on November 20, 2015.
3	FSIS determined that the CCA needs to improve its verification activities related to the safety of retort cooling water and retort maintenance.	O FSIS constatou que a Autoridade Competente Central (CCA) precisa melhorar suas atividades de verificação relacionadas à segurança da água de resfriamento das retortas e à manutenção das retortas.	Memorandum-Circular n° 34/2016/CGI/DIPOA, dated April 22, 2016, ordered reinforcement actions for monitoring and official verification of the maintenance conditions of the retorts/autoclaves and of the quality of cooling water used in them.	Completed on April 22, 2016.
4	Deficiencies regarding construction	Deficiências de construção e na	The individual findings of	Completed on April 22, 2016.

	and enforcement of sanitation performance standards (SPS) were identified at five of the eleven establishments audited. However, no direct product contamination was observed.	execução dos Padrões de Desempenho Sanitário (SPS) foram identificadas em cinco dos onze estabelecimentos auditados. Entretanto, não foi observada contaminação direta do produto.	establishments were verified and we found that they were sporadic situations. Corrective and preventive actions were taken by the establishments involved, and have been assessed as satisfactory by the local Official Service.	
5	5 FSIS determined that the CCA needs to improve its slaughter verification activities. At one of the eight slaughter establishments audited, viscera did not routinely accompany carcasses railed out for final veterinary dispositions. At another establishment, the design of a non-mobile stand used by the government for conducting zero tolerance verification (contamination caused by feces, milk, or ingesta) did not permit adequate observation of carcass hindquarters.	O FSIS constatou que a Autoridade Competente Central (CCA) precisa melhorar suas atividades de verificação de abate. Em um dos oito estabelecimentos de abate auditoria, como rotina, as vísceras não acompanhavam as carcaças desviadas para o DIF. Em outro estabelecimento, o design de uma plataforma fixa utilizada pelo governo para realizar a verificação da tolerância zero (contaminação fecal, por leite ou conteúdo gastrointestinal) não permitia a adequada observação dos traseiros.	1. The failure found in *post mortem* activities at a hog slaughtering establishment was immediately corrected as per a document presented in the action plan of establishment inspected by SIF 3548. 2. CGI/DIPOA issued Circular n° 094/2015/CGI/DIPOA, dated November 13, 2015, containing specific instructions for officials working at all slaughter plants inspected by the SIF in Brazil in order to avoid this failure. 3. The failure identified in relation to the design of a platform was deemed a sporadic finding, and corrective actions were taken by SIF 431. The procedure was changed in order to ensure that the official can carry out verification of fecal, milk or gastric content contamination after the CCP, for both forequarters and hindquarters, so as to enable an unimpeded view.	1. Completed on November 12, 2015. 2. Completed on November 13, 2015. 3. Completed on November 10, 2015.
6	The CCA had not yet instituted a Shiga toxin-producing *Escherichia coli* (STEC) proficiency testing program at its government laboratories.	A Autoridade Competente Central (CCA) ainda não havia instituído um programa de ensaio de proficiência para Escherichia coli produtora de toxina Shiga (STEC) em seus laboratórios oficiais.	Proficiency tests for E. coli O157:H7, for the LANAGROS in Pernambuco, São Paulo and Minas Gerais have already been purchased.	We are awaiting the delivery of the tests, which we expect to occur in June 2016.
7	Written STEC government laboratory testing procedures referenced the use of *E. coli* strain #EC 465-97, while the actual strain used was *American Type Culture Collection* (ATCC) #43888.	Os procedimentos escritos das análises laboratoriais do governo para STEC faziam referência ao uso da cepa *E. coli* #EC 465-97 de E. coli , enquanto a cepa realmente utilizada era a *American Type Culture Collection* (ATCC) #43888.	Met through MET MIC/LANAGRO/SP (ATTACHED)	Completed on March 31, 2016
8	No official procedure existed for the handling of inconclusive STEC sample results.	Não havia um procedimento oficial para como lidar com os resultados de amostras STEC inconclusivas.	1. DIPOA/SDA Within the establishment, the local Official Service will receive the positive or negative results, for which the procedures to be adopted have been laid down in Circular n° 506/2015/CGPE/DIPOA, dated June 15, 2015. 2. Met through MET MIC/LANAGRO/SP (ATTACHED)	1. Completed on June 15, 2015. 2.Completed on March 31, 2016

MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY – MAPA
Secretariat of Animal and Plant Health - SDA
Department Of Inspection Of Animal Products - DIPOA
General Coordination of the Brazilian System for the Inspection of Animal Products—CGI
Esplanada dos Ministérios, Bloco D, Anexo Ala A, 4º andar, Sala 422, Brasília/DF – CEP 70.043-900.
Tel: (61) 3218-2719 e-mail: cgi.dipoa@agricultura.gov.br

Circular nº 086/2015/CGI/DIPOA/SDA Brasilia, November 5, 2015

From: The Coordinator-General of CGI/DIPOA and the Coordinator-General of CGPE/DIPOA.

To: Heads of SIPOA/SISA/SIFISA.

Subject: Bovines. Pigs. USA. Violations. Point of entry violations (POEV).

Dear Heads of Department,

The US health authorities have a warning system for every violation detected in product exported to the USA.

Once a POEV is detected, a "case file" is opened, containing the information necessary to notify the health authority of origin and to begin the investigation in order to determine the possible causes of the violation. This investigation is standardized by the Food Safety and Inspection Service (FSIS/USDA), in Directive 5100.1.

We have detected a need to standardize the report to be used in investigations, and their on-going process is laid down by Memorandum 306/2013/GAB/DIPOA.

This report must only be used in violations notified by the FSIS, in accordance with the types of violation and the categories of products involved, as you can see in the table below:

Report templates	Product
Meat tool	Raw material
Meat tool	Fresh Beef
Thermally Processed, commercially sterile tool	Commercially sterile canned products
RTE Products Tools	Frozen cooked beef
RTE Products Tools	*Beef Jerky*

The investigation report tools can be found at:

MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY – MAPA
Secretariat of Animal and Plant Health - SDA
Department Of Inspection Of Animal Products - DIPOA
General Coordination of the Brazilian System for the Inspection of Animal Products—CGI
Esplanada dos Ministérios, Bloco D, Anexo Ala A, 4º andar, Sala 422, Brasília/DF – CEP 70.043-900.
Tel: (61) 3218-2719 e-mail: cgi.dipoa@agricultura.gov.br

http://www.fsis.usda.gov/wps/portal/fsis/topics/regulations/directives/5000series/!ut/p/a1/04_Sj9CPykssy0xPLMnMz0vMAfGjzOINAg3MDC2dDbz83RzdDDz9jN3CLPzcDQ1MDIEKIlEUWBqCFIQF-ns7OxtY-BkTqR8HcDQgpNLCAuMinydfdP1owoSSzJ0M_PS8vUjUjKLUpNLMstSi_UjTIEW6RanFmUCOeH6UagGGhgCIdDAYBMPLz9jA38TdAVYfAxRgNtLBbmhEVU-HgaZno6KAJkIDlI!/?1dmy&current=true&urile=wcm%3apath%3a%2Ffsis-content%2Finternet%2Fmain%2Ftopics%2Fregulatory-compliance%2Ffood-safety-assessments

Another important point is that the assessment of the inspection in the establishment involved, and the filling out of the reports indicated, must be carried out by an outside team, and not that of the local Federal Inspection Service (SIF).

Yours faithfully,

MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY.
Secretariat of Animal and Plant Health
Department of Inspection of Animal Products - DIPOA
General Coordination of Inspection - CGI

Circular Memorandum no 15/2014/CGI/DIPOA/SDA Brasilia, April 3, 2014

To Official Veterinarians in the States of Brazil

copied: Heads of SISA/SIFISA/SIPOAs

Subject: SDA/MAPA Official Letter no 132/2012 - Guidance for Procedures concerning Brazil's National Plan for Control of Residues and Contaminants—PNCRC/MAPA

Dear Superintendent,

We are enclosing a copy of SDA/MAPA Official Letter no 132/2012 - Guidance for Procedures concerning Brazil's National Plan for Control of Residues and Contaminants - PNCRC/MAPA;

2. ALL those involved in the Federal Inspection Service must be informed so that the contents may be complied with immediately.

3. Possible queries should be sent to cgi.dipoa@agricultura.com.br, and will be replied to appropriately.

Yours faithfully,

Published SKJSII- cm 0.1 April 2014.

Esplanada dos Ministérios, Bloco B - 4° Andar - Sala 422 A - 70.043-900 - Brasília / DF - Tel; (61) 321B - 2719 - Fax: (61) 3225 [redacted]4605

MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY
Secretariat of Animal and Plant Health
Cabinet

Official Letter SDA/MAPA n° 132 /2012

Guidance for Procedures in the National Plan for Control of Residues and Contaminants - PNCRC/MAPA.

Brasília, April 18, 2012.

To: Federal Superintendents of Agriculture - SFA

Subject: Temporary ban on the issuing of Animal Movement Permits (*Guia de Trânsito Animal*—GTAs) and other procedures, in cases of violation produced by non-compliant PNCRC results, suspicion of misuse of veterinary products, and accusation of use of banned or clandestine veterinary products.

Remarks:

I - This procedure updates and revokes Official Letter CRC7SDA n° 24/2011;
EI - This procedure is guidance and does not replace the standards laid down in Normative Instruction no. 55/2010, Ordinance 396/2009, or any other applicable provision that underpins the actions of the investigation sub-program;
III - For cases involving banned substances (items 2 and 3), after CRC assesses the investigation on the farm, SDA, as proposed by CRC, will forward the case file to the SFA Superintendent in the **State** where the farm is located, and will officially ask it to send it to the local Federal Prosecution Office and the Regional Federal Police Superintendent's Office as per art. 11 of Ordinance 396 dated 23/11/2008;
IV - Where substances of the Stilbene group are identified (Hexestrol, Dienestrol and Diethylstilbestrol) the measures laid down in art. 5 of Normative Instruction 55 dated 01/12/2011 are to be adopted.

See below **the guidance** for **each case:**

1**) REGARDING VIOLATIONS CONCERNING PERMITTED VETERINARY PRODUCTS:**

a) After a Violation Notification has been issued, put in place a temporary block on the issuing of GTAs—Animal Movement Permits for the exit of animals of the same category as the sampled batch of animals, as well as animals of the same species in subsequent categories, during the "withdrawal period of the veterinary product that was used", when it is identified during an investigation, or for the "longest" withdrawal period among the veterinary products registered at MAPA containing the same active substance as in the violation, when it is impossible to identify the product during the investigation;
b) When appropriate the SFA notifies the Official Veterinary Service in the State, requesting a ban on the issuing of GTAs for the duration and in the same way as laid down in item 1a)

MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY
Secretariat of Animal and Plant Health
Cabinet

c) Investigation of the farm that was involved, based on the Violation Notice issued, as set forth in Ordinance 396/2009;
d) After the period of the ban on animal movements laid down in item 1.a), samples for analysis of the next lots of animals sent for slaughter must be collected until *a total* of 5 consecutive compliant results are obtained, in accordance with Ordinance n° 396/2009.

2) FOR VIOLATIONS INVOLVING BANNED OR CLANDESTINE VETERINARY PRODUCTS:

a) After issuing a Violation Notice, impose a temporary ban on issuing Animal Movement Permits—GTAs, for the outward movement of animals of the same species as the sampled category and for animals of the same species in subsequent categories, lasting six (6) months.
b) When appropriate the SFA notifies the Official Veterinary Service in the State, requesting a ban on the issuing of GTAs for the duration and in the same way as laid down in item 2.a);
Investigation of the farm that was involved, based on the Violation Notice issued, as set forth in Ordinance 396/2000;
d) After the period of the ban on animal movements laid down in item 2.a), samples for analysis of the next lots of animals sent for slaughter must be collected until a total of 5 consecutive compliant results are obtained, in accordance with Ordinance n° 396/2009;

3) FOR WELL-FOUNDED SUSPICIONS OR ACCUSATIONS OF THE USE, OR IDENTIFICATION OF BANNED OR CLANDESTINEVETERINARY PRODUCTS:

a) When identified, immediate seizure, after issuing of notice of confiscation, and recall of products, as per Decree n° 5053/2004;
b) As a preventivehealth measure, temporary ban on issuingof GTAs —Animal MovementPermits for animals ofthe same category as the sampled lot, and for animals of the same species in subsequent categories, for a period of "6 (six) months";
c) When appropriate the SFA notifies the Official Veterinary Service in the State, requesting a ban on the issuing of GTAs for the duration and in the same way as laid down in item 3.b;
d) Notify CRC/SDA of the occurrence using the form in Appendix 1, in order to issue the Violation Notice;
e) Investigation of the farm that was involved, based on the Violation Notice issued, as set forth in Ordinance no. 396/2009;
f) After the period of the ban on animal movements laid down in item 3.b),
there being an analytical method validated for the analysis of the substance(s) that is/are the focus of the suspicion/accusation in the laboratories of the Official Network of MAPA Laboratories, samples must be taken for analysis of the next lots of animals, should they be
sent to slaughter, until a number of 5 consecutive compliant results is obtained, pursuant to Ordinance No 396/2009.
If no analytical method has been validated, then after the period established in item 3.b), the ban on issuing GTAs must be lifted and the animals authorized for slaughter.

MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO
MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY
Secretariat of Animal and Plant Health
Cabinet

4) FOR SLAUGHTER OF ANIMALS AT AN ESTABLISHMENT INSPECTED BY SIF, WHEN THERE IS SUSPICION OF USE OF BANNED OR CLANDESTINE VETERINARY PRODUCTS OR IDENTIFICATION OF INAPPROPRIATE USE OF PERMITTED VETERINARY PRODUCTS:

a) When suspect animals are identified, if they have been slaughtered the local Federal Inspection Service SIF will hold all products deriving from the lot of slaughtered animals, in compliance with Decree 30,691/1952;
b) As a preventive health measure, temporary ban on issuing of Animal Movement Permits - GTAs for the exit of animals of the same category as the lot of animals sampled, as well as of animals of the same species in subsequent categories, "until appropriate official actions show whether or not there has been a violation";
c) CRC/SDA must be notified of the occurrence using the form, as per Appendix II so that the correct Notification of Suspicion of Violation may be issued and sent to DFIP/SDA for appropriate investigation of the farm involved, as set forth in Ordinance 396/2009;
d) After investigation of the farm, as laid down in item 4.c), provided NO violation is shown to have occurred, the ban on issuing Animal Movement Permits GTAs on the farm may be lifted and the sequestered products being held in the local SIF may be released for consumption;
e) After investigation of the farm, as laid down in item 4.c), once the occurrence of violations or misuse is shown, follow the same procedures and steps as set forth in items 1, 2 or 3 of this Official Letter, as may be the case.

Yours.

Enio Antorrá Kfarques Pereira
Secretary for Animal and Plant Health

FEDERAL PUBLIC SERVICE
MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY - MAPA
FEDERAL SUPERINTENDENCE OF AGRICULTURE - SFA/ (UF)

APPENDIX I - NOTICE OF VIOLATION

OFFICIAL NOTICE OF ACCUSATION OF USE / SUSPECTED USE OF PRODUTO BANNED OR CLANDESTINE VETERINARY PRODUCT.

N" / ___ /20____
(State)

National Residues and Contaminants Control Plan – PNCRC/MAPA

1. PROPERTY INFORMATION

1.1 NAME	
1.2 ADDRESS	
1.3 – CEP (Postal code)	
1.4 MUNICIPALITY / STATE	
1.5 GEOREFERENCE	
OFFICIAL SERVICE CODES. (Fill out at least one of the three codes below)	
1.6 OFFICIAL SERVICE CODE	
1.7 STATE ENROLLMENT	
1.8 NIRF (Farm's Inland Revenue	

2. OWNER DATA

21 NAME	
2.2 ADDRESS	
2.3 CEP	
2A MUNICIPALITY /	
2.5 CPF or CNPJ	Type: ( ) CPF) CNPJ Number:
2.5 BUSINESS	PHONE) MOBILE ( )

3. DETAILS OF ACCUSATION / SUSPICION

3.1 SPECIES 3.2 PRODUCT FOUND;

FEDERAL PUBLIC SERVICE
MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY - MAPA
FEDERAL SUPERINTENDENCE OF AGRICULTURE - SFA/ (UF)

3.3 ACTIVE SUBSTANCE (WHEN SHOWN ON VIAL)	

Relevant remarks (if needed):

PLACE AND DATE: **TIME:**

STAMP, SIGNATURE AND NUMBER OF THE FEDERAL AGRICULTURE

WITNESS 1
Legible name:
I.D.:

WITNESS 2
Legible name:
I.D.:

Official notice of accusation of use / suspected use of banned or clandestine veterinary product issued in 2 copies. One: file at SFA; the other: send to Coordination for Residues and Contaminants - CRC/SDA.

FEDERAL PUBLIC SERVICE
MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY - MAPA
FEDERAL SUPERINTENDENCE OF AGRICULTURE - SFA/ (UF)

APPENDIX II - NOTICE OF SUSPECTED VIOLATION

OFFICIAL NOTICE OF SUSPECTED MISUSE OF PERMITTED VETERINARY PRODUCT OR USE OF BANNED OR CLANDESTINE VETERINARY PRODUCT.

N° _____ / ____ / 20 __
(State)

National Residues and Contaminants Control Plan – PNCRC/MAPA

1, FARM INFORMATION

1.1 NAME	
1.2 ADDRESS	
1.3 – CEP (Postal code)	
1.4 MUNICIPALITY / STATE	
1.5 GEOREFERENCING	
OFFICIAL SERVICE CODES (Fill out at least one of the three codes below)	
1.6 OFFICIAL SERVICE CODE	
1.7 INSC.ESTADUAL	
1.8 NIRF (Farm's Inland Revenue	

2. OWNER DATA

2.1 NAME
2.2 ADDRESS
2 3 CEP
2.4 MUNICIPALITY / STATE
2.5 CPF or CNPJ Type: ( ) CPF ( ) CNPJ Number:
2.5 BUSINESS PHONE ( ) MOBILE ( )

3. DETAILS OF

3 1 SPECIES

FEDERAL PUBLIC SERVICE
MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY - MAPA
FEDERAL SUPERINTENDENCE OF AGRICULTURE - SFA/ (UF)

3.2 PRODUCT SUSPECTED OR FOUND:	
3.3 ACTIVE SUBSTANCE (WHEN SHOWN ON VIAL. HEALTH BULLETIN OR SHOWN IN INVESTIGATION)	
Relevant remarks (when necessary):	

PLACE AND DATE: **TIME:**

STAMP SIGNATURE AND NUMBER OF THE FEDERAL AGRICULTURE

WITNESS 1

Legible name.

I.D.:

WITNESS 2

Legible name.

I.D.:

Official notice of accusation of use / suspicion of use of banned or clandestine veterinary product issued in 2 copies. One: file at SFA; the other: send to Coordination for Residues and Contaminants – CRC/SDA.

MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY
GENERAL COORDINATION FOR THE BRAZILIAN SYSTEM FOR INSPECTION OF ANIMAL PRODUCTS -SDA - CGI
Ministry of Agriculture, Livestock and Food Supply, Anexo Ala A, 4° Andar, Sala 422 – Bairro Zona Cívico-Administrativa - DF, CEP 70043900
Tel: (61) 3218-2719 - http://www.agricultura.gov.br

Memorandum-Circular n° 34/2016/CGI/DIPOA/SDA/GM/MAPA

Brasilia, April 22, 2016.

To:
Dear Heads of SIPOA/SISA/SIFISA

Subject: **Bovines. Pigs. United States. Procedures for Official Monitoring and Verification of autoclaves/retorts and cooling water.**

Given the findings of the Report of the United States Veterinary Mission held from November 9 to 20, 2015 and based upon the sole paragraph of art. 51 of Decree n° 30,691, enacted 29 March 1952, and art. 20 of Decree n° 8,701, enacted 31 March 2016, the General Coordination determines that:

I - establishments with autoclaves/retorts must reinforce monitoring of the "maintenance" of this equipment, as an element of control, so as to ensure perfect operation of the equipment, and that this must also cover the internal piping;

II- establishments that have autoclaves/retorts must include in their self-control programs the monitoring of the cooling water of this equipment, carrying out periodical laboratory analyses including—but not restricted to—pH, free residual chlorine and facultative anaerobic micro-organisms;

III -the Federal Inspection Service (SIF) working in these establishments must include autoclaves/retorts in their inspection plans so as to include them in the official inspection element headed "maintenance"; and

IV SIFs working in such establishments must include cooling water for this equipment in the set of water points already checked officially for quality, including periodical laboratory analyses that include—and are not limited to—pH, free residual chlorine and facultative anaerobic micro-organisms (microbiological codes M04 and M09).

Definition of the frequency of monitoring of the maintenance and of the cooling water for the machinery mentioned lies with the establishment by means of its self-control programs.
However, it must prove that it has effective control over the standard of quality of the cooling water for the autoclaves/retorts within the frequencies that are defined.

Definition of the frequency of monitoring of the maintenance and of the cooling water for the machinery mentioned lies with the local SIF in its inspection plan. Official verification must be more frequent than company monitoring.

The SIF must officially notify the establishment of the content of this Memorandum-Circular to be complied with.

Yours faithfully,

This document signed electronically by **RAFAEL OLIVIERI FILIPPUTTI, General Coordinator of the Brazilian Animal Product Inspection System,** on 22/04/2016, at 14:59, official Brasilia time, using the digital certificate issued in ICP-Brasil, based on art. 10, paragraph 2, of Provisional Measure n 2.200-2, dated 24 August 2001.
Serial no. of Certificate: 938859315566328915896313238162933894 92

The authenticity of this document may be verified at http://sistemas.agricultura.gov.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, give verification code **0300633** and CRC code **C58C1C70.**

Reference: Case file n° 21000.018074/2016/-48 SEI n° 0300633

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MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY – MAPA
Secretariat of Animal and Plant Health - SDA
Department Of Inspection Of Animal Products - DIPOA
General Coordination of the Brazilian System for the Inspection of Animal Products—CGI
Esplanada dos Ministérios, Bloco D, Anexo Ala A, 4º andar, Sala 422, Brasília/DF – CEP 70.043-900.
Tel: (61) 3218-2719 e-mail: cgi.dipoa@agricultura.gov.br

Circular nº 094/2015/CGI/DIPOA/SDA Brasilia, Friday, November 13, 2015

From: The General Coordinator of CGI/DIPOA

To: Heads of SIPOA/SISA/SIFISA.

Subject: Pigs. Post-mortem inspection. Final Inspection Department (DIF - Depto de Inspeção Final).

Dear Heads of Department,

We are asking the local Federal Inspection Service (SIFs) in establishments to make sure that the establishments possess the structural and operational means to transport the viscera from pig carcasses railed out to the Final Inspection Department (DIF) and ensure that post mortem inspection services can be carried out properly.

If any deficiency is identified (for example because of a lack of material means for the transport—lack of cart or tray—or lack of suitable flow, or lack of synchronization between carcass and viscera, or because the DIF is too small), the SIF, pursuant to art. 46 or 102, item 2, of Decree 30,691 published 29 March 1952, must demand that the establishment carry out the appropriate short-and long-term changes.

Viscera or other parts of carcasses railed out to the DIF must not be condemned before they have been inspected by the Official Veterinarian responsible for post mortem inspection.

Yours faithfully,

MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY
Secretariat of Agricultural Defense - SDA
Department for Inspection of Animal Origin Products - DIPOA
General Coordination of Special Programs - CGPE

Circular nº /2015/CGPEDIPOA

Brasília, 2015.

From: Coordinator of CGPE/DIPOA/SDA

To Federal Superintendents of Agriculture

For the Heads of SIPOA/SISA/SIFISA and Vigiagro

Subject: United States of America USA. Guidelines for the monitoring of Verotoxigenic Escherichia coli (STEC) in batches of bovine meat in nature headed for exportation. Supplements the Newsletters 540/2006 and 835/2006/CGPE/DIPOA.

1. Bearing in mind the progress of negotiations for the start of bilateral trade of bovine meat in nature between Brazil and the United States of America, this Coordination informs that the national establishments wishing to export this type of product to the USA must meet the specific requirements for qualification for this market and also undertake monitoring of the presence of Verotoxigenic Escherichia coli (E.coli STEC) serogroups O157: H7, O26, O45, O103, O111, O121 and O145 in 100% of batches for exportation, since this pathogen is considered of high risk for public health.

2. The official check analyses will be performed at least on a monthly frequency.

3. The Guidelines for the monitoring of Verotoxigenic Escherichia coli (STEC) in batches of bovine meat in nature headed for exportation are attached.

4. This memorandum supplements Newsletter no. 540/2006/CGPE/DIPOA and 835/2006/CGPE/DIPOA, remaining unchanged the microbiological testing of E. coli (generic) to be performed on bovine carcasses at the establishments members of raw product suppliers lists to the US.

Sincerely.

MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY
Secretariat of Agricultural Defense - SDA
Department for Inspection of Animal Origin Products - DIPOA
General Coordination of Special Programs - CGPE

Guidelines for the monitoring of Verotoxigenic Escherichia coli (STEC) in batches of bovine meat in nature headed for exportation to the USA.

1. National establishments wishing to export bovine meat in nature to the USA must meet the specific requirements for qualification for this market and also undertake monitoring of the presence of Verotoxigenic Escherichia coli (E.coli STEC) serogroups O157: H7, O26, O45, O103, O111, O121 and O145 in 100% of batches for exportation, since this pathogen is considered of high risk for public health.

2. Analyses can be carried out in laboratories of companies or laboratories accredited by MAPA belonging to the National Network of Agricultural Laboratories possessing methods planned by FSIS / USDA (Food Safety and Inspection Service) of the USA in its scope. All procedures for sample collection, analytical methodology and actions to be taken in case of deviations should be included in facilities self-control programs.

3. In addition to E. coli STEC research, enabled establishments should also review self-control programs in the following respects:

a) The HACCP plan shall foresee the possibility of E. coli STEC in meat products in nature;
b) The third party receiving program should be amended so that, in the case of raw material for the preparation of meat products in nature to be exported to the US, it is provided that suppliers also perform these controls;
c) Hygiene procedures are more stringent especially when E. coli STEC is detected;
d) Self-control programs should rely on verification tools to confirm the effectiveness of the established hygiene procedures.
e) Verification of self-control programs to be effective;
f) The results of laboratory tests for E. coli STEC research should be included in the pre-shipment report.

4. It should be noted that an establishment that receives or manufactures meat products in nature from raw materials derived from another establishment should not, in their self-control programs, conclude that the likelihood of E. coli STEC is low simply by receiving previously inspected products. The inspection seal is a guarantee that the products have been produced in accordance with the appropriate health procedures and not to the levels of pathogens were eliminated or reduced to undetectable levels.

5. Self-control programs should also include a definition of the batch, which shall consist of the following:

a) Represent a defined production unit clearly identified and completely distinct from other units;
b) To be produced within a given time interval, in the same line, or processing unit, without flow interruption or other alterations (such as the use of different

sources of raw material) which may cause a portion of the batch to differ significantly from another;

c) To be complete;
d) Include reworking;
e) Be accessible for inspection and testing and
f) Be traceable from origin to distribution.

6. In addition, the constitution of batches also must obligatorily possess a scientific basis allowing the correlating of its size with the sampling program for E. coli STEC developed and used by the establishment.

7. Analysis for E. coli STEC comprises three steps:

First stage - consists of “PCR Screening Test" to detect the potentially positive result;

Second stage - consists of isolation and reaction with antigen for detection of the presumably positive;

Third stage - consists of serological or genetic determination for the detection of confirmed positive serogroup.

8. Under these circumstances, the product can only be sent to another establishment after a negative result in “PCR Screening Test".

9. Establishments that choose to use only the "Screening Test" and find a positive result, without performing the confirmation of E. coli STEC through the following stages, should consider and treat the product as positive. A second “Screening Test" should not be performed because it is not a conclusive test.

10. The product with a potentially positive result on the "Screening Test" can only be exported after the performance of additional tests (second and third stages described above) confirmed to be negative for the seven serogroups E. coli STEC tested.

11. However, the product with a potentially positive result on the "Screening Test” and headed for a treatment to inactivate E. coli STEC, is exempt from the second and third stages for confirmation of serogroup.

12. Local FI should check the records associated with the analysis of E. coli STEC performed by the establishment and observe if corrective measures are being performed when necessary. If the establishment detects a positive result and do not adopt the actions provided in the HACCP plan, the local FI shall issue a Non Conformity Report (NCR).

13. The official verification carried out by the local FI should give special emphasis to actions adopted by the establishment for the prevention of occurrence of fecal contamination on carcasses due to operational failures at every stage of slaughter.

14. Establishments authorized to export to the USA that receive batches positive for E. coli STEC as raw materials for the development of heat treated products and their subsequent inactivation, shall provide this possibility in their self-control programs. All records

required with respect to the receipt, identification, segregation, storage and heat treatment of the batches should be kept to ensure the destruction of the pathogen, thus allowing effective traceability of that raw material and products made from the same.

15. Local FI will collect, as official verification, samples of batches of bovine meat in nature with minimum monthly periodicity which will be forwarded to other laboratories in the National Network of Agricultural Laboratories defined by CGAL and released by DIPOA, following the provisions of the Internal Standard DIPOA that will approve the procedures for the collection and analysis of Verotoxigenic Escherichia coli.

16. In the event that the analysis by the official verification detects a product with positive result for E. coli STEC and the establishment has also tested the same batch, FI should compare the results, adopting the following procedures, according to the case:

a) When local FI detects a positive product, the establishment also detects the presence of E. coli STEC and the product is separated into its industrial facilities without being used, there is no need to adopt any fiscal action. The federal inspection should check if the establishment has taken appropriate corrective and preventive actions.
b) When local FI detects a positive product for E. coli STEC and the result of analysis performed by the establishment is negative, the federal inspection shall:

b.1) Check if the product is in the custody of the establishment, properly identified and segregated. If not, the establishment must immediately initiate recall procedures for proper subsequent disposal. If the establishment has received the raw material from third parties for production of batches, the slaughterhouse of origin of the raw material should be communicated about the result so that it can adopt the necessary procedures aimed at preventing its recurrence.

b.2) Check if the establishment:

- Identified and eliminated the cause of the deviation (in the case of a slaughterhouse);
- Restored the sanitary conditions of the product. Heat treatment should be sufficient to ensure complete destruction of the pathogen. Treatments for the reduction of 6.5D of Salmonella or more severe are indicated, since this pathogen has heat resistance comparable to that of E. coli STEC. Another possible option is the use of the product for sterilization by heat or rendering plant. If the final destination of positive batches is outside of the premises, there must be records that the product was received by the receiving establishment.
- Adopted procedures in order to prevent recurrence of deviation and;
- Adopted actions preventing the product with presence of E. coli STEC from reaching consumers.

b.3) Issue a report of non-compliance (RNC), which should indicate the failure in the verification procedures of the establishment. In this case the local FI should

check the history of establishment for violations with regard to E. coli STEC to verify if the deviation was random or indicated a systemic problem, whereupon the relevant actions should be taken which include but are not limited to review of procedures, section interdiction and even interruption of international health certification.

17. Local FI must register on the sheet (Annex I) and forward the results of official collections in digital form monthly to SIPOA / SISA / SIFISA as well as fiscal actions taken in case of deviations, the results of E. coli STEC analyses performed by the company as well as corrective and preventive actions taken by it.

MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY
Secretariat of Agricultural Defense - SDA
Department for Inspection of Animal Origin Products - DIPOA
General Coordination of Special Programs - CGPE

Annex I

Month of reference (mm/yyyy)							
State	SFI	Collection date (dd/mm/yyyy)	Batch	Product	Company Result	Verification summary FI	Adopted actions (FI and/or company)

Detection, isolation and identification of *Escherichia coli* O157:H7 (MLG 5.09 and MLG 5A.04)

Approved by:	Amaury dos Santos	Person in charge of the Unit
Reviewed by:	Virna Clemente	Alternate person in charge
	Yuri Fernandes Feltrin	Alternate person in charge

1.0 Purpose

To establish an analytical methodology for the detection of *Escherichia coli* O157:H7 in raw meat products in accordance with FSIS/USDA/USA methods MLG 5.09 and MLG 5A.04.

2.0 Scope

This applies to personnel of the Microbiological Foodstuffs and Water Testing Unit who take part in microbiological assays.

3.0 Description and definitions

***Escherichia coli* O157:H7**: an enterohemorrhagic serotype of *E. coli* that causes outbreaks of hemorrhagic colitis and food borne hemolytic uremic syndrome, the main reservoir of which is cattle, and to a lesser extent, other ruminants.

***Enterohemorrhagic E. coli* (EHEC—i.e. STECs)**: Strains of *E. coli* that cause diseases, including Hemolytic uremic syndrome, similar to those caused by *Shigella dysenteriae*, above all in children and the elderly. EHECs produce one or more Shiga toxins.

4.0 Procedures

Traceability of stages of this assay methodology is guaranteed by filling out FORM MIC/069 – Meat, FORM MIC/063 – Detection of STEC, and FORM MIC/071 – identification of *E. coli* O157:H7.

4.1 Safety precautions

E. coli O157:H7 is a human pathogen with a low infectious dose (ingestion of 100 cells can cause disease). The use of gloves and eye protection is mandatory for all post-enrichment viable culture work. Work surfaces must be disinfected prior to and immediately after use. A class II biosafety cabinet is recommended for activities with potential for producing aerosols of pathogens.

4.2 Quality control

- Unless otherwise stated, all measurements cited in this method have a tolerance of ± 2 %;
- All media and E-buffer must be pre-warmed to 18ºC-35ºC prior to use;
- A strain of *E. coli* O157:H7 is used in this procedure as a positive control.
- After the stationary phase, inoculate the *E. coli* O157:H7 culture using a loop in 25g matrix free of the target analyte and add 75 mL of Modified Tryptone Soya Broth (mTSB) – dilution 1:4. Incubate the inoculated enrichment broth at 42ºC ± 1ºC overnight along with the samples being tested;
- *E. coli* ATCC strain 25922, or equivalent, may be used as the optional negative control for the latex agglutination assay;
- Prepare at least one "blank" (incubated but uninoculated pre-enrichment broth) in the PCR screen test, to provide a sterility control for the process;
- In the absence of positive test samples, controls must be terminated at the same point as the samples analyzed.

4.3 Sample preparation and primary enrichment

- Weigh and prepare the sample following the guidelines in IT MIC/007 – "*Preparação de amostras, suspensões iniciais e diluições decimais*—Preparation of samples, initial suspensions, and decimal dilutions";

- For beef trim/trim components, raw ground beef and raw beef/pork blends, prepare in a sterile bag a single sample in enrichment broth with a 1:4 dilution (one portion of product in three portions of medium), e.g. 325 g ± 32.5 g of sample with 975 mL ± 19.5 mL of mTSB broth). Pummel, blend or hand massage until pellets are dispersed;
- Incubate the bags and contents at 42 ± 1ºC for 15-24 hours for mTSB broth or for 15-22 hours for mTSB+n broth. Include a positive control and an uninoculated medium for each group of samples tested;
- Use the enriched cultures from the bags to proceed to the screening test as described below. The enrichment culture may be analyzed immediately upon removal from the incubator without waiting for tempering to room temperature.

4.4 BAX® System real-time PCR *E. coli* O157:H7 Screening test

4.4.1 General remarks

- In this procedure, use inputs and instruments that are part of the real-time BAX® System *E. coli* O157:H7 detection assay (DuPont, kit nº D14203648).
- Always wear talcum-free gloves;
- Enrichment broths must be prepared using deionized water compatible with PCR analysis;
- Clean the work area and all materials and supplies prior to and/or after use, using 1% sodium hypochlorite followed by 70% ethanol and rinse with deionized water;
- Turn on the UV light for 30 minutes prior to and/or after using clean air equipment. Tools and other equipment must be turned over if necessary to ensure complete exposure to UV light;
- To prevent cross-contamination between samples during transfers, set aside the right number of microtubes prior to use;
- Use a pincer to withdraw the microtubes and caps from the bags. Once the bag has been opened, store the unused caps in a zip-lock bag;
- The use of filtered tips is recommended for all pipettes;
- DO NOT OPEN TUBES AFTER AMPLIFICATION.

4.4.2 Preparation of the equipment

- Turn on the block heaters and set temperatures to 37 ± 2ºC and 95 ± 3ºC prior to the sample addition stage (consult IU MIC/026 – Dry Block Heater). Wait the required time until the temperatures have stabilized;
- Make sure the cooling blocks have been cooled overnight or chilled to 2-8ºC;
- Use the BAX® System software, as per instructions in IU MIC/025 – BAX® System Q7, to create the rack file and fill out the sample position guide-sheet accompanying the assay kit;
- Boot up the BAX® System Q7 prior to lysis of samples, following instructions in IU MIC/025 – BAX® System Q7 and selecting run the whole process.

4.4.3 Sample lysis

- While the equipment is warming up, prepare the lysis reaction as follows:
  - Set aside the appropriate number of strip microtubes for the lysis reaction and arrange them on the microtube rack in accordance with the file created. Use the guide sheet to determine the arrangement of the samples in the rack;

- Prepare the lysis reagent by adding 150µL protease to 12mL lysis buffer. Label the vial with the prepared lysis reagent with the date of preparation. The lysis reagent may be used for up to two weeks if stored at 2-8ºC. If necessary, smaller volumes of lysis reagent may be prepared, maintaining the ratio of 12.5µL protease to 1mL lysis buffer.

  N.b.: the lysis reagent in the sealed microtubes is valid for two weeks if refrigerated at 2-8ºC;
- Transfer 200µL of lysis reagent into each microtube;
- Make sure all tubes contain the same volume of lysis reagent;
- With a tip for each sample transfer a **20µL** aliquot of each enriched sample to the corresponding microtubes, including the blank. **Do not shake or mix the enrichment prior to transferring the samples to the tubes**. If the samples are shaken after enrichment leave them to rest for at least 10 minutes before transferring the aliquots to the microtubes. Be careful to pipette the aliquot from the center of the liquid to prevent the suspension of particles of the sample interfering in the result;
- After the aliquots have been transferred, place the caps on the vials making sure they are properly sealed;
- Place the rack with the microtubes on the pre-warmed block heater at 37ºC ± 2ºC and warm for 20 minutes;
- Transfer the microtubes to the pre-warmed block heater at 95ºC ± 3ºC and warm for 10 minutes;
- After lysis is complete, place the cooled metal block (2-8ºC) on the white plastic block. Note: minimize the time between removing the metal blocks from the refrigerator to their use in order to keep them as cold as possible. The time to the end of use of the block must be less than 30 minutes after removal from the refrigerator;
- Place the tubes on the colling block and allow to cool for at least 5 minutes. Meanwhile prepare to transfer the lysate to the PCR tubes.

Note: *Sealed lysates may be stored for up to 7 days at 2-8ºC or for up to 14 days at -20ºC prior to hydration of the PCR tablets.* ***Do not interrupt the protocol before the end of lysis.*** *Open lysates may be stored for up to one week at -20ºC for later analysis.*

4.4.4 Hydration of PCR pelletss

Do not hydrate PCR pellets with lysate until the thermocycler reaches the right temperature and the signal that the equipment is ready lights up*;*

- Select a cooling block for PCR. Remember that you must finish using the cooling block within 30 minutes of removing it from refrigeration;
- Place a rack of PCR tubes in the clip;
- Withdraw the right number of PCR tubes from the packaging in the refrigerator and reseal the bag tightly;
- Place the PCR tubes in the stand. Check to see if each tube contains a pellet at the bottom. If the pellets are stuck to the top or to the walls of the tubes, lightly tap the tubes against the bench or a flat surface, to shift them to the bottoms of the tubes. Mark the top of each row of tubes to maintain orientation when placing them in the equipment;
- Make sure the equipment has reached the right temperature and is indicating to continue;
- Remove the caps of the first row of tubes with the correct tool (Figure 1). Note: the pellets may come out if too much force is used when removing the caps of the PCR tubes. Check to see if each PCR tube contains a whole white pellet. If the pellets have shrunk or are pinkish, discard the tubes and replace them by new ones before proceeding;
- Dispense **30µL** of the lysed sample into the PCR tube by tilting the pipette 45º and resting the tip against the tube wall to avoid capturing the pellet. Use filtered tips. The tip must not be washed after sample transfer. Use the guide form to accompany this stage;

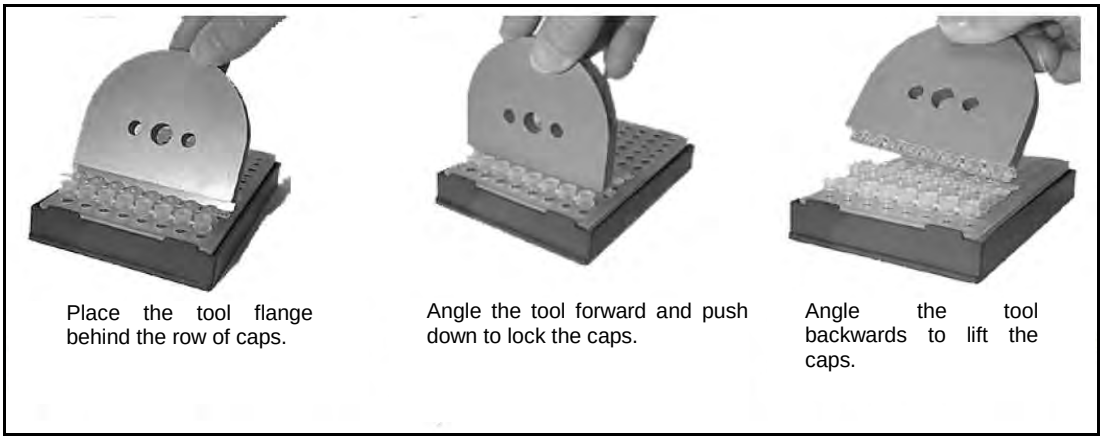


Figure 1. Removal of PCR tube caps.

- Place new optical caps on the row of tubes and press them down firmly as shown in Figure 2. Make sure they are accurately and firmly placed on the tubes;

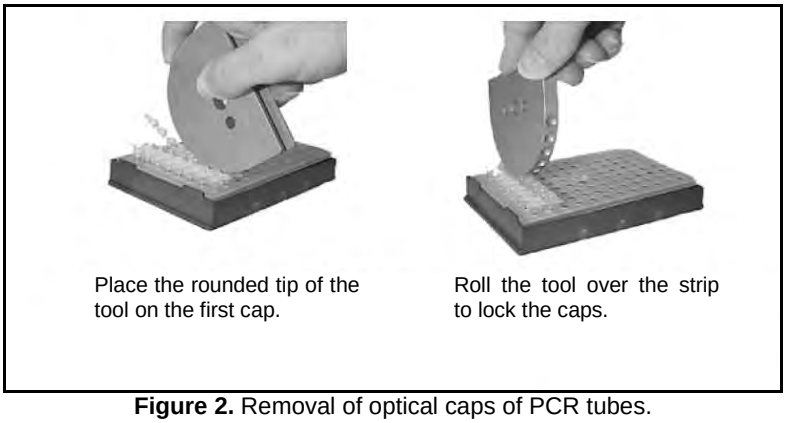


Figure 2. Removal of optical caps of PCR tubes.

- Remove the caps of the first row of tubes and repeat this stage until all the tubes have been sealed again;
- N.B.: the pellets must be moistened and sealed again within10 minutes of loosening the caps of the PCR tubes;
- Load the metal rack with PCR tubes into the BAX® System Q7 equipment immediately after hydration of the pellets and continue as in item 4.4.5.

Note: *Samples must be kept on the cooling block until loaded into the equipment*.

- Mark the lysis tubes for orientation, store the rack of lysates at 2-8ºC until the results are ready and checked;
- The cooling blocks must be washed with 1% sodium hypochlorite, rinsed in deionized water and left to dry before being placed back into the refrigerator.

4.4.5 DNA amplification and detection

- Load the rack and follow the complete process following the instructions given in IU MIC/025 – BAX® System Q7;
- To remove samples after completion of the analysis, follow the guidance given in Q7 in IU MIC/025 – BAX® System Q7;
- Clean the racks and other items that are routinely placed in the PCR instrument using 1% sodium hypochlorite, rinse with deionized water or 70% ethanol and leave to dry after each use.

4.4.6 Viewing results

- To view and print the results follow the instructions given in IU MIC/025 – BAX® System Q7;
- For **positive** samples (Figure 3), the two *E. coli* targets make a sigmoid curve and Ct score between 0 and 40. The Ct score usually fluctuates between 20 and 40;
- For **negative samples** (Figure 4), neither *E. coli* target makes a sigmoid curve and the IPC is positive;
- If only one target is present (Figure 5), the sample is deemed **negative**;

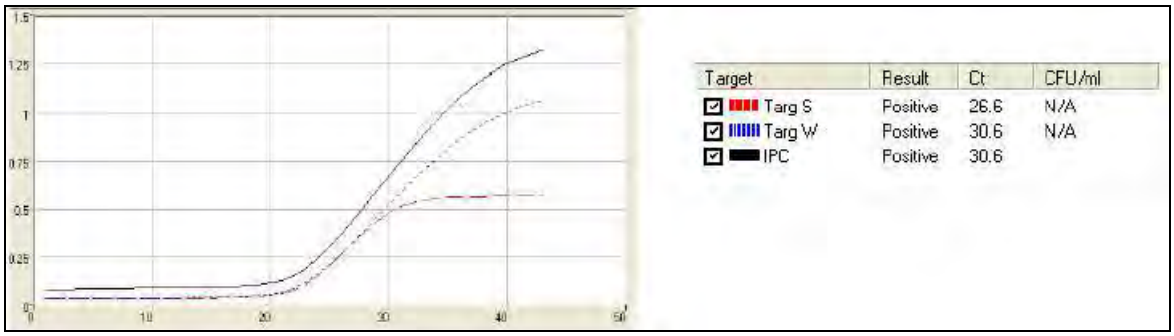


Figure 3. Positive result for *E. coli* O157:H7 (both targets positive).

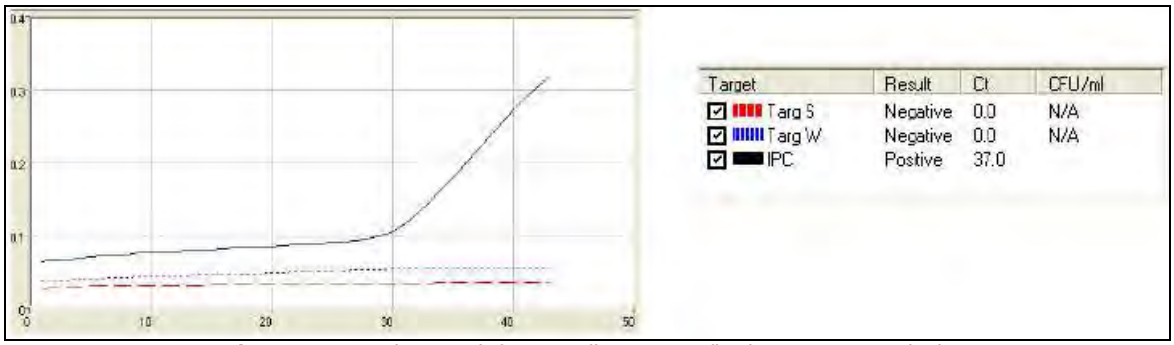


Figure 4. Negative result for *E. coli* O157:H7 (both targets negative).

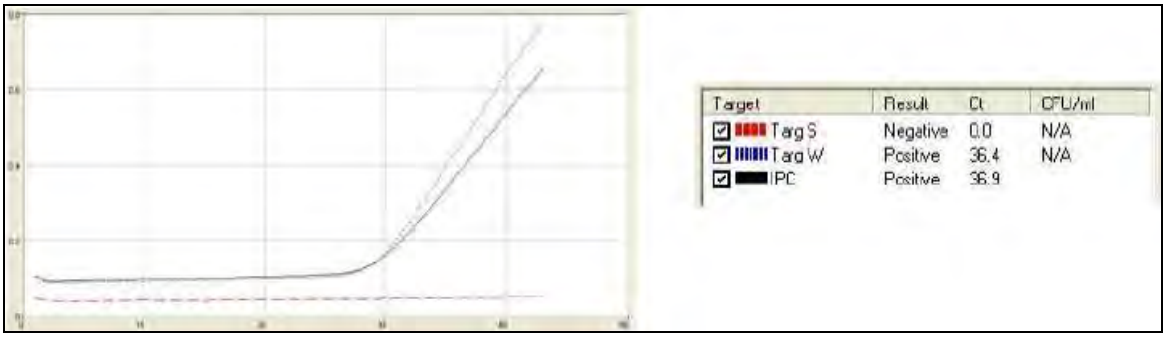


Figure 5. Negative result for *E. coli* O157:H7 (one target negative, one target positive).

- Print the results, attach them to FORM MIC/063 – *Detecção de STEC*—"STEC detection", and record them in the appropriate field of FORM MIC/069 – *Carne* ("Meat");
- The person responsible for checking the data recorded on FORM MIC/063 – STEC detection, must sign the sheet containing the printed BAX® results after checking them.

4.4.7 Interpretation of the results

- Negative samples from the BAX® PCR screening test must be reported as negative for *E. coli* O157:H7 and finalized;
- Positive, inconclusive or invalid samples must be confirmed by the traditional method (item 4.5) or the laboratory may review the cause and perform a correction. Based on the findings the laboratory may:

- Repeat the BAX® analysis from the rack loading step;
- Prepare new BAX® tubes and repeat the analysis.

- In analytical runs where the positive control tests BAX®-negative, indeterminate or has a signal-error result, the entire batch of samples is affected and a review of the cause and a correction shall be performed. Based on the findings the laboratory may:
  - Repeat the BAX® analysis from the rack loading step;
  - Prepare new BAX® tubes and repeat the analysis.
  - Analyze the samples by the traditional method (item 4.5).
- If reanalysis is unsuccessful then prepare fresh analytical portions from the sample reserve or discard the sample.

4.5 Isolation

Begin the isolation procedure from the enrichment cultures for the positive screen test samples and for the controls.

Note: Steps *a* to *h* must be carried out in a sequence that is convenient for the analysts.

a. Remove mRBA plates from the refrigerator (2-8°C), allowing 4 plates for each screen-positive culture and 2 plates for the *E. coli* O157:H7 control. Make sure the plate is dry before inoculation. If necessary dry the plates (for example, for up to 30 minutes in a laminar flow hood, without the lid removed) prior to use. Dried unused pl ates must be identified as "dry", bagged, and returned to the refrigerator (2-8°C);

b. Remove the E-buffer from the refrigerator (2-8°C) and decant 7 mL of it into each suspect culture and each control in sterile tubes or flasks. Maintain at room temperature until it reaches 18ºC at least. Return the stock of E-buffer to 2-8ºC;

c. For each positive control and screen-positive culture, keep in order and label the 50 mL conical centrifuge tubes so that the positive control is the last. Maintain this order for subsequent steps;

d. For each positive control and screen-positive culture, identify 6 sterile 1.5 mL micro centrifuge tubes and one 50 mL conical centrifuge tube. For each set of tubes, label one and add 0.9 mL of E-buffer in three of the tubes;

e. If necessary, prepare an immunomagnetic bead suspension for *E. coli* O157:H7, as per manufacturer's recommendations. Be sure to include the positive control in the total number of cultures. Use the bead suspension immediately or hold at 2-8ºC.

f. Briefly vortex the bead solution (for 2 to 3 seconds). Add the volume recommended by the manufacturer of the immunomagnetic capture beads to each one of the labeled microcentrifuge tubes (in step *d*): one for the control and one for each suspect culture. Use immediately or hold these tubes at 2-8ºC;

g. Place a sterile 40µm cell strainer for each of the 50 mL conical centrifuge tubes of step *d*. Pipet 5 mL ± 1 mL from each control and enrichment culture into the respective cell strainer and collect at least 1.0 mL of the filtrate;

h. Do not proceed with more than the number of tubes that the OctoMACS® magnetic separator will hold. Transfer 1.0 mL of the filtrate (step *g*) to the corresponding microcentrifuge tube containing the immunomagnetic bead suspension (step *f*) and place it in the clips of the tube agitator. Rotate the tubes for 10 to 15 minutes at 18-30ºC;

i. Attach the OctoMACS® magnet to the multistand;

j. Place a recipient of disinfectant solution below the OctoMACS® magnet so that it will collect the filtrate passing through the columns;

k. Label and place the appropriate number of Large Cell Separation columns in the OctoMACS® magnet. Insert columns from the front making sure the column tips do not touch any surfaces. Leave the plungers in the bags to maintain sterility;

l. Transfer at least 0.5 mL E-Buffer to the top of each column and let the buffer run through;

m. Resuspend, then transfer each culture and control from step *h* to its corresponding column;

n. After a culture or control has drained through, wash the column by applying 1.0 mL of E Buffer and allowing it to drain. Repeat 3 more times for a total of 4 washes;

o. After the last wash, remove the column from the OctoMACS® magnet and insert the tip into an empty labeled microcentrifuge tube (from step *d*). Apply 1.0 ml of E Buffer to the column and using the plunger supplied with the column, **immediately** flush out the beads into the tube. Use a smooth steady motion to avoid splattering. Cap the tubes. Repeat the procedure for each column. If the OctoMACS® magnet is to be used for a second set of cultures, it must be decontaminated as in step z. Repeat steps *i to s* for the additional cultures;

p. Prepare a 1:10 dilution of each treated bead suspension by adding 0.1 mL of the bead suspension to a labeled microcentrifuge tube containing 0.9mL E-buffer. Make a 1:100 dilution by adding 0.1 mL of the 1:10 dilution to a labeled microcentrifuge tube containing 0.9 mL E Buffer;

q. Vortex briefly to maintain beads in suspension and plate 0.1 mL from each tube (1:10 and 1:100 dilutions) onto a labeled mRBA plate. Use a hockey-stick or spreader to spread the beads on the plate, being careful not to spread the beads against the edge of the plate;

r. As soon as there is no visible moisture on the agar surface, invert plates and incubate for 20 to 24 hours at 35 ± 2 ºC;

s. **Acid treatment**: for each sample, transfer 450 µL of the undiluted bead solution (eluant from the MACS column) to an empty labeled microcentrifuge tube. Add 25 µL of 1N hydrochloric acid (HCl)solution to this bead suspension and vortex briefly. This will bring the pH to 2.0 - 2.5 using E-buffer.

t. Place the microcentrifuge tubes containing the acid-treated suspension on an agitator and rotate the tubes for one hour at 18-30ºC;

u. After 1 hour, dilutent the suspension by adding 475 µL of E-buffer;

v. Vortex briefly to maintain beads in suspension and plate 0.1 mL of the neutralized suspension onto a labeled mRBA plate. Use a hockey-stick or spreader to spread the beads on the plate, being careful not to spread the beads against the edge of the plate;

w. Add 0.1 mL of the suspension to a labeled tube containing 0.9 mL of E buffer and vortex briefly. This shall represent a 1:10 dilution of the acid-treated cell suspension. Plate 0.1 ml of the diluted suspension onto an appropriately labeled mRBA plate;

x. As soon as there is no visible moisture on the agar surface, invert plates and incubate for 20 to 24 hours at 35 ± 2 ºC;

y. *Optional*: streak *E. coli* ATCC strain 25922 or equivalent to TSA with 5% sheep's blood agar for use as a latex negative control;

z. Decontaminate the OctoMACS® magnet by applying 2% Lysol IC® disinfectant (or equivalent) directly to its surface. After approximately 10 minutes, rinse with deionized or tap water. Allow to air-dry or dry the unit with paper towels.

4.6 Identification and Confirmation

- After incubation, *E. coli* O157:H7 colonies are black or gray in Rainbow agar. When *E. coli* O157:H7 colonies are surrounded by pink or magenta colonies, they may take on a bluish hue. Mark typical colonies and perform a latex agglutination assay for O157, following manufacturer's instructions. Samples with non-typical colonies on mRBA or typical colonies that are latex agglutination negative for O157 can be reported as negative for *E. coli* O157:H7. Streak all latex positive colonies up to a total of 5 per sample (one per sub-sample if possible) onto trypticase soy agar plates with 5% of sheep's blood (SBA) or tryptone soy agar (TSA). Incubate these plates for 16 to 24 hours at 35ºC ± 2ºC;
- If the SBA or TSA plates appear pure and uncontaminated, perform the following confirmatory tests:
  - **Biochemical confirmation**: inoculate Vitek® 2 system GN cards.
  - **O157 and H7 confirmation**: to confirm presence or absence of O157 and H7 antigens, use an *E. coli* O157:H7 latex test agglutination kit (RIM® *E. coli* O157:H7 Latex Test Kit). Use growth from the SBA or TSA plate. Genetic testing (for example PCR) may be necessary for inconclusive results;

- **Shiga toxin/toxin genes confirmation**: the presence of Shiga toxin(s) in a culture isolate(s) is confirmed by toxin assay, for example the Meridian Premier® EHEC kit. When Shiga toxins are not demonstrated, detection of one or more toxin producing genes by PCR is used for confirmation.

  Alternatively, the toxin gene PCR assay, for example the BAX® System Real-Time PCR Assay STEC screening (*stx*, *eae*) may be used in lieu of the toxin assay.

- To perform confirmation of O157 and H7 antigens by serological agglutination and Shiga toxin gene(s) by BAX® System Real-Time PCR Assay STEC screening (*stx*, *eae*), use the following procedure:
  - After SBA or TSA incubation, perform the *E. coli* O157:H7 latex test agglutination kit agglutination test on the SBA or TSA plate colonies;
  - To confirm agglutination-positive colonies by BAX® Real-time PCR Assay STEC Screening (*stx*, *eae*) , prepare a template by suspending an agglutination-positive colony from the SBA or TSA plates in 50 µL of Molecular Grade Water and by adding 5 µL of this suspension to the BAX® lysis buffer;
  - Continue with the BAX® system protocol. The lysate will then be used for the PCR assay;
  - In addition, perform biochemical identification (Vitek® 2) on agglutination-positive colonies from the incubated SBA or TSA;
  - If the isolate is serologically positive for "O157", BAX® real-time PCR-positive for the *stx*/*eae* gene and biochemically identified as *E. coli*, the sample is positive.

- The following definitions are used for reporting *E. coli* O157:H7:
  - **Potential positive**: a sample causes a positive reaction with the screen test;
  - **Presumptive positive**: a sample that has typical colonies, observed on modified Rainbow agar, and reacts specifically with O157 antiserum;
  - **Confirmed positive**: a biochemically identified *E. coli* isolate that is serologically or genetically determined as being "O157" and that meets at least one of the following criteria:
    - Positive for Shiga toxin (ST) production;
    - Positive for Shiga toxin gene(s) (*stx*);
    - Genetically determined as "H7".

- If the laboratory has an isolate or any colony picks from mRBA determined as BAX® Real-time PCR negative or indeterminate for *stx*/*eae*, then the isolate must be sent to Lanagro-MG for further Shiga toxin gene and H7 PCR testing.

- If a laboratory performs a toxin assay and the isolate fails to express Shiga toxin (toxin-negative result), but PCR detects the genes necessary to express Shiga toxin, it is then considered to be *E. coli* O157:H7 confirmed positive;
- If the isolate is H7 negative in latex and also Shiga toxin and gene negative (EHEC test and genetic test for Shiga toxin genes), additional PCR tests for H7 gene(s) are performed. Send the strains to LANAGRO MG. If the H7 PCR test is positive, the isolate is considered *E. coli* O157:H7 confirmed positive;
- If the isolate is *E. coli* O157 presumptive positive, but additional tests show it to be H7 negative (by latex agglutination and PCR) and is Shiga toxin negative (by EHEC test and genetic test for Shiga toxin genes), then the isolate must be reported as *E. coli* O157:H7 negative.

4.7 Issuing the results

- The results must be issued and notified as laid down in DIPOA/SDA Internal Norm nº 01, published 17 June, 2015.
- Issue the results as laid down by IT MIC/008 – *Análise microbiológica de amostras* ("Microbiological analysis of samples").

5.0 Bibliography

DuPont. Dupont™ Bax® System. Q7 Instrument. User Guide. 2013.
Downes, F. P; Ito, K. (Eds.). Compendium of methods for the microbiological examination of foods. 4º ed. American Public Health Association, 2001.

United States Department of Agriculture. Food Safety and Inspection Service. Office of Public Health Science. MLG 5.09. Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges. Available at: http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook Acesso em 06.11.2015.

United States Department of Agriculture. Food Safety and Inspection Service. Office of Public Health Science. MLG 5A.04. FSIS Procedure for the Use of *Escherichia coli* O157:H7 Screening Tests for Meat Products and Carcass and Environmental Sponges. Available at: http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-andprocedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook Retrieved 06.11.2015.

6.0 Appendices

- Not applicable.

7.0 Additional documents

- FORM MIC/063 – STEC detection;
- FORM MIC/069 – Meat;
- FORM MIC/071 – Identification of *E. coli* O157:H7;
- IT MIC/007 – *Preparo de amostras, suspensões iniciais e diluições decimais* ("Preparation of samples, initial suspensions, and decimal dilutions");
- IT MIC/008 – *Análise microbiológica de amostras* ("Microbiological analysis of samples");
- IU MIC/025 – BAX® System Q7;
- IU MIC/026 – Dry Block Heater;
- DIPOA/SDA Internal Norm Nº 01 published June 17, 2015

8.0 History of changes

ITEM	SUMMARY OF CHANGES
	•

9.0 Critical analysis

Evidence of critical analysis	

Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) (MLG 5B.05)

Approved by:	Amaury dos Santos	Person in charge of the Unit
Reviewed by:	Yuri Fernandes Feltrin	Alternate person in charge
	Virna Clemente	Alternate person in charge

1.0 Purpose

To establish an analytical methodology for routine monitoring of the presence of non-O157 *Escherichia coli* STEC in meat products, in accordance with USDA/FSIS method MLG 5B.05.

2.0 Scope

This applies to personnel of the Microbiological Foodstuffs and Water Testing Unit who take part in microbiological assays.

3.0 Description and definitions

- **Shiga-toxin producing *Escherichia coli* (STEC):** important enteric pathogens that cause diarrhea with or without visible bleeding and hemolytic uremic syndrome. STEC are unique among diarrhea-causing *Escherichia coli* in that they produce Shiga toxins type 1 and 2, which are factors of virulence responsible for bloody diarrhea and hemolytic uremic syndrome;
- **Non-O157 Shiga-toxin producing *Escherichia coli* (STEC):** STEC belonging to serogroups other than O157.

4.0 Procedures

Traceability of stages of this assay methodology is guaranteed by filling out FORM MIC/069 – "Meat", FORM MIC/063 – "Detection of STEC", and FORM MIC/070 – "identification of non-O157 STEC".

4.1 Safety precautions

Non-O157 serotypes of STEC are human pathogens with a low infectious dose, therefore it is important when handling them to take special precautions such as:

- Mandatory use of gloves and eye protection for all post-enrichment viable culture work;
- Work surfaces must be disinfected prior to and immediately after use;
- A class II biosafety cabinet must be used for activities with potential for producing aerosols.

4.2 Quality control

4.2.1 General

Unless otherwise stated, weight and volume ranges and minutes have a tolerance of ± 2 %.

All media, plates and buffers shall be pre-warmed to 18ºC-35ºC prior to use.

The following control strains of non-O157 STEC obtained from reference culture collection centers must be used when so indicated in the method:

- *E. coli* O26, which shall be *stx* positive and *eae* positive;
- *E. coli* O45, which shall be *stx* positive and *eae* positive;
- *E. coli* O103, which shall be *stx* positive and *eae* positive;
- *E. coli* O111, which shall be *stx* positive and *eae* positive;
- *E. coli* O121, which shall be *stx* positive and *eae* positive;

- *E. coli* O145, which shall be *stx* positive and *eae* positive.

Note: *In the absence of positive test samples, controls must be terminated at the same point as the samples analyzed.*

4.2.2 Sample enrichment controls

Include with each sample batch a positive growth control (*E. coli* O157:H7 strain 465-97 or other reference strain that is *stx*-, *eae*+) inoculated into a meat matrix free of the target analyte, and an uninoculated pre-enrichment media (mTSB) control.

4.2.3 Bax® Real-time PCR controls

- **stx/eae screen PCR:**
  - 20 µL enrichment from an *E. coli* O157:H7 strain (growth control);
  - DNA template (5 µL) from a cocktail of top six STEC cultures (PCR positive control);
  - Uninoculated mTSB medium (20 µL).

- **Serogroup-specific screen PCR (Panel 1 and Panel 2):**
  - DNA template (5 µL) from a cocktail of top six STEC cultures (PCR positive control);
  - Uninoculated mTSB medium (20 µL).

- **Optional *stx/eae* presumptive PCR / *stx/eae* confirmatory PCR:**
  - DNA template (5 µL) from a cocktail of top six STEC cultures (PCR positive control).

- **Optional serogroup-specific presumptive PCR (Panel 1 and Panel 2) / Serogroup-specific confirmatory PCR (Panel 1 and Panel 2):**
  - DNA template (5 µL) from a cocktail of top six STEC cultures (PCR positive control).

- **To prepare DNA template:**
  - Inoculate the six STEC cultures on SBA (tryptone soya agar with 5% sheep blood) and incubate at 35 ± 2ºC for 16-24 h;
  - Suspend the colonies in PCR certified water until reaching a concentration of 10^9 CFU/mL;
  - In one tube, 1.0 mL from each suspension shall be added to 4.0 mL of PCR certified water to create a 10 mL suspension containing 10^8 CFU/mL of each strain;
  - One hundred microliter (100 µL) aliquots of the suspension are then transferred to PCR tubes or microcentrifuge tubes and heated at 95-99ºC for 10 minutes on a thermocycler or heating block;
  - The tubes shall be centrifuged at 10,000 x *g* for 3 minutes to pellet cellular debris;
  - The supernatant (DNA template) shall be used as the PCR positive control for all PCR assays. This control can be prepared as a batch, transferred to smaller volume tubes, and stored at ≤ - 20°C for 1 year.

4.2.4 Immunomagnetic separation (IMS) plating controls

- Streak an isolate from the serogroup(s) of interest (based on serogroup-specific PCR results) onto mRBA and incubate along with the samples that have been treated with the IMS procedure.

4.3 Sample preparation and primary enrichment

- Weigh and prepare the sample following the guidelines in IT MIC/007 – "*Preparação de amostras, suspensões iniciais e diluições decimais*—Preparation of samples, initial suspensions, and decimal dilutions";
- For raw beef, beef trims and trim components, place 325 g ± 32.5 g of the sample into sterile bags. Add 975 mL ± 19.5 mL of mTSB to the sample to provide a 1:4 dilution (one portion of product to three portions of broth). Homogenize in a Stomacher (peristaltic homogenizer) or hand massage until well mixed;
- Incubate bags and contents at 42 ± 1ºC for 15-24 hours. Each group of samples should include a positive control enrichment (*E. coli* O157:H7) and an uninoculated enrichment medium control;

4.4 Screening procedure using BAX® Real-time PCR

Following incubation perform the rapid screen as described in the following items using 20 µL of mTSB sample enrichment.

4.4.1 General remarks

- In this procedure, use inputs and instruments that are part of the real-time BAX® System *E. coli* O157:H7 detection assay (DuPont, STEC Screening kit nº D14642964: *stx*/*eae*), D14642970 (STEC Panel 1: *E. coli* O26, O111, O121) and D14642987 (STEC Panel 2: *E. coli* O45, O103, O145)];
- Always wear non-talcum gloves;
- Enrichment broths must be prepared using deionized water compatible with PCR analysis;
- Clean the work area and all materials and supplies prior to and/or after use, using 1% sodium hypochlorite followed by 70% ethanol;
- Turn on the UV light for 30 minutes prior to and/or after using the PCR cabinet. Tools and other equipment in the cabinet must be turned over if necessary to ensure complete exposure to UV light;
- To prevent cross-contamination between samples during transfers, set aside the right number of microtubes prior to use;
- Use a pincer to withdraw the microtubes and caps from the bags;
- The use of filtered tips is recommended for all pipettes;
- DO NOT OPEN TUBES AFTER AMPLIFICATION.

4.4.2 Preparation of the equipment

- Turn on the block heaters and set temperatures to 37 ± 2ºC and 95 ± 3ºC (consult IU MIC/026 – Dry Block Heater);
- Make sure the cooling blocks have been cooled overnight or chilled to 2-8ºC;
- Use the BAX® System software, as per instructions in IU MIC/025 – BAX® System Q7, to create the rack file and fill out the sample position guide-sheet;
- Boot up the BAX® System Q7 prior to lysis of samples, following instructions in IU MIC/025 – "BAX® System Q7" and select run the whole process.

4.4.3 Sample lysis

While the equipment is warming up, prepare the lysis reaction as follows:

- Set aside the appropriate number of strip microtubes for the lysis reaction and arrange them on the microtube rack in accordance with the file created. Use the guide sheet accompanying the microtubes to determine the arrangement of the samples in the rack;
- Prepare the lysis reagent by adding 150µL protease to 12mL lysis buffer. Label the vial with the prepared lysis reagent with the date of preparation. The lysis reagent may be used for up to two weeks if stored at 2-8ºC. If necessary, smaller volumes of lysis reagent may be prepared, maintaining the ratio of 12.5µL protease to 1mL lysis buffer.

  Note: *The lysis reagent in the sealed microtubes is valid for two weeks if refrigerated at 2-8ºC;*
- Transfer 200µL of lysis reagent into each microtube;
- With a tip for each sample transfer a **20µL** aliquot of each enriched sample to the corresponding microtubes, including the blank. **Do not shake or stir the enrichment prior to transferring the samples to the tubes**. If the samples are shaken after enrichment leave them to settle for at least 10 minutes before transferring the aliquots to the microtubes;
- After the aliquots have been transferred, place the caps on the vials making sure they are properly sealed;
- Place the rack with the microtubes on the pre-warmed block heater at 37ºC and warm for 20 minutes;
- Transfer the microtubes to the pre-warmed block heater at 95ºC and warm for 10 minutes;
- After lysis is complete, place the cooled metal block (2-8ºC) on the white plastic block.

  Note*: minimize the time between removing the metal blocks from the refrigerator to their use in order to keep them as cold as possible. The time to the end of use of the block must be less than 30 minutes after removal from the refrigerator;*
- Place the tubes on the cooling block and allow to cool for at least 5 minutes. Refrigerate the samples until the next step of the process. Meanwhile prepare to transfer the lysate to the PCR tubes.

Note: *Sealed lysates may be stored for up to 7 days at 2-8ºC or for up to 14 days at -20ºC prior to hydration of the PCR tablets.* ***Do not interrupt the protocol before the end of lysis.*** *Open lysates may be stored for up to one week at -20ºC for later analysis.*

4.4.4 Hydration of PCR tablets

- **Do not hydrate PCR tablets with lysate until the thermocycler reaches the right temperature and the signal that the equipment is ready lights up**;
- Select a cooling block and use within 30 minutes of removal from refrigeration;
- Place a rack of PCR tubes in the clip;
- Withdraw the right number of PCR tubes from the packaging in the refrigerator and reseal the bag tightly;
- Place one PCR tube per sample in the stand. Check to see if each tube contains a pellet at the bottom. If the pellets are stuck to the top or to the walls of the tubes, lightly tap the tubes against the bench or a flat surface, to shift them to the bottoms of the tubes. Mark the top of each row of tubes to maintain orientation when placing them in the equipment;
- Remove the caps of the first row of tubes with the correct tool (Figure 1). N.b.: the pellets may come out if too much force is used when removing the tops of the PCR tubes. Check to see if each PCR tube contains a whole white pellet. If the pellets have shrunk or are pinkish, discard the tubes and replace them by new ones before proceeding;
- Dispense **30µL** of the lysed sample into the PCR tube by tilting the pipette 45º and resting the tip against the tube wall to avoid capturing the pellet. Use filtered tips. The tip must not

be washed after sample transfer. Use the guide form to accompany this step. Make sure all wells are filled with the same volume;

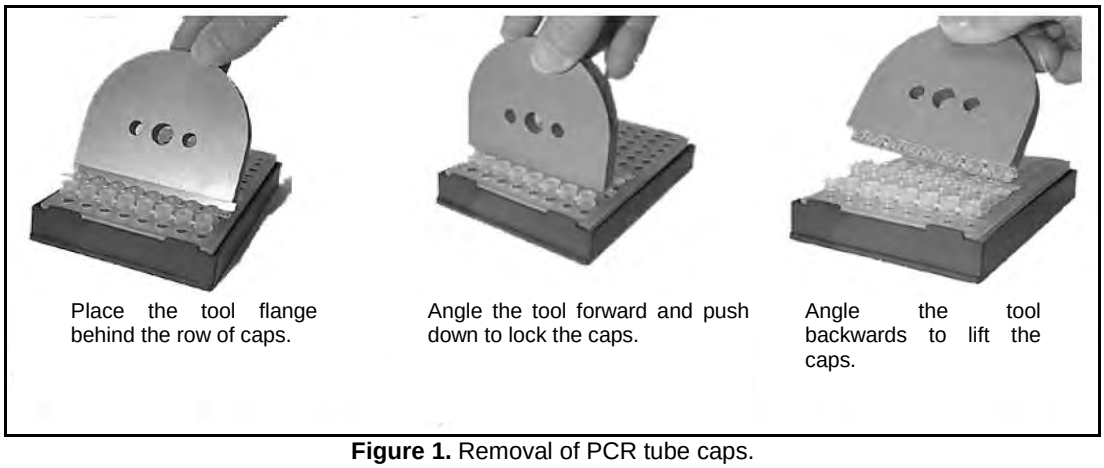


Figure 1. Removal of PCR tube caps.

- Place new optical caps on the row of tubes and press them down firmly as shown in Figure 2. Make sure they are accurately and firmly placed on the tubes;

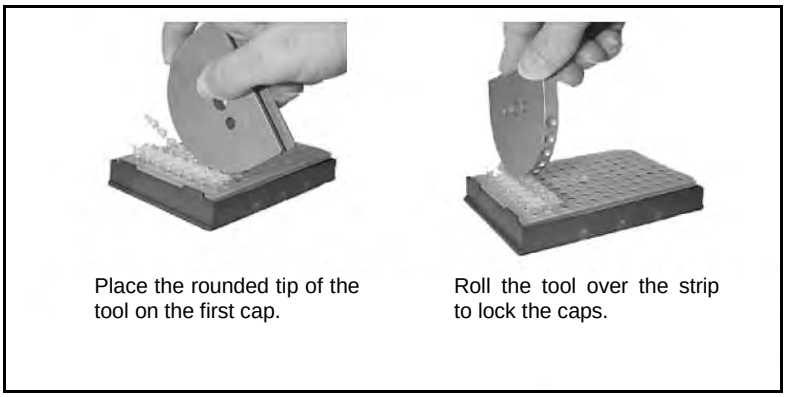


Figure 2. Removal of optical caps of PCR tubes.

- Remove the caps of the first row of tubes and repeat this stage until all the tubes have been sealed again;
- *Note*: the pellets must be moistened and sealed within10 minutes of loosening the caps of the PCR tubes;

- Load the metal rack with PCR tubes into the BAX® System Q7 equipment **immediately** after hydration of the pellets and continue as in item 4.4.5;

  Note: *Samples must be kept on the cooling block until loaded into the equipment*.

- Mark the lysis tubes for orientation, store the rack of lysates at 2-8ºC until the results are ready and checked;
- Lysates may be stored at 2-8ºC for up to 7 days, or at -20ºC for up to 14 days;
- The cooling blocks must be washed with 1% sodium hypochlorite, rinsed in deionized water and left to dry before being placed back into the refrigerator.

4.4.5 DNA amplification and detection

- When the equipment reaches the ideal temperature, load the rack and follow the complete process following the instructions given in IU MIC/025 – "BAX® System Q7";
- To remove samples after completion of the analysis, follow the guidance given in Q7 in IU MIC/025 – "BAX® System Q7";
- Clean the racks and other items that are routinely placed in the PCR instrument using 1% sodium hypochlorite, rinse with deionized water or 70% ethanol and leave to dry after each use.

4.4.6 Viewing results

- To view and print the results follow the instructions given in IU MIC/025 – "BAX® System Q7".

4.4.6.1 Screen PCR

- For **positive** samples (Figure 3), the *stx* and *eae* targets make a sigmoid curve and show a Ct score between 0 and 43. The Ct score usually fluctuates between 15 and 43;
- For **negative samples** (Figure 4), neither of the targets is present and the IPC is positive;
- If only one target—*stx* or *eae*—is present (Figure 5), the sample is deemed **negative**.

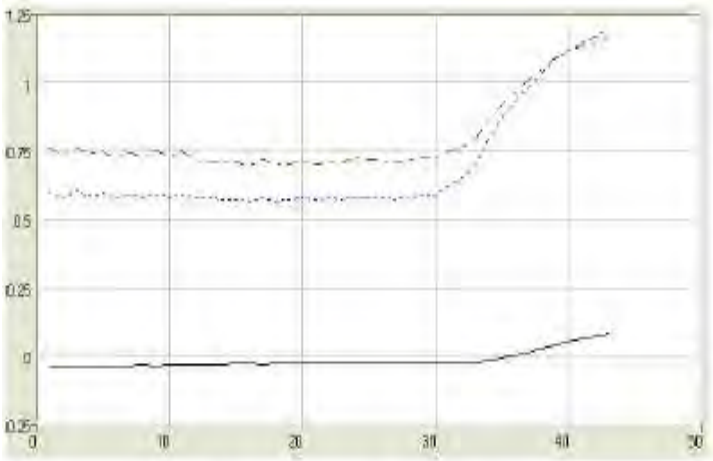


Figure 3. STEC positive (*stx*+/*eae*+).

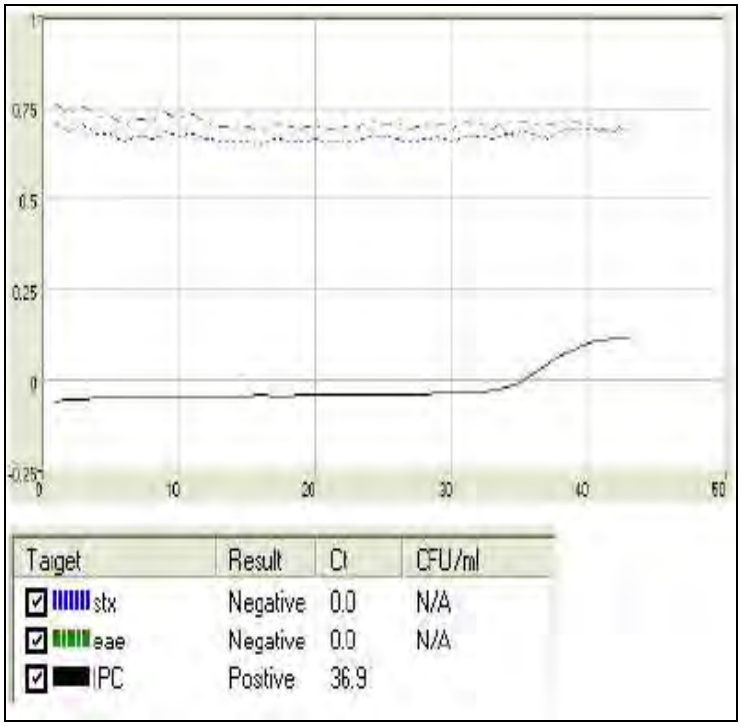


Figure 4. STEC negative (*stx-/eae-*).

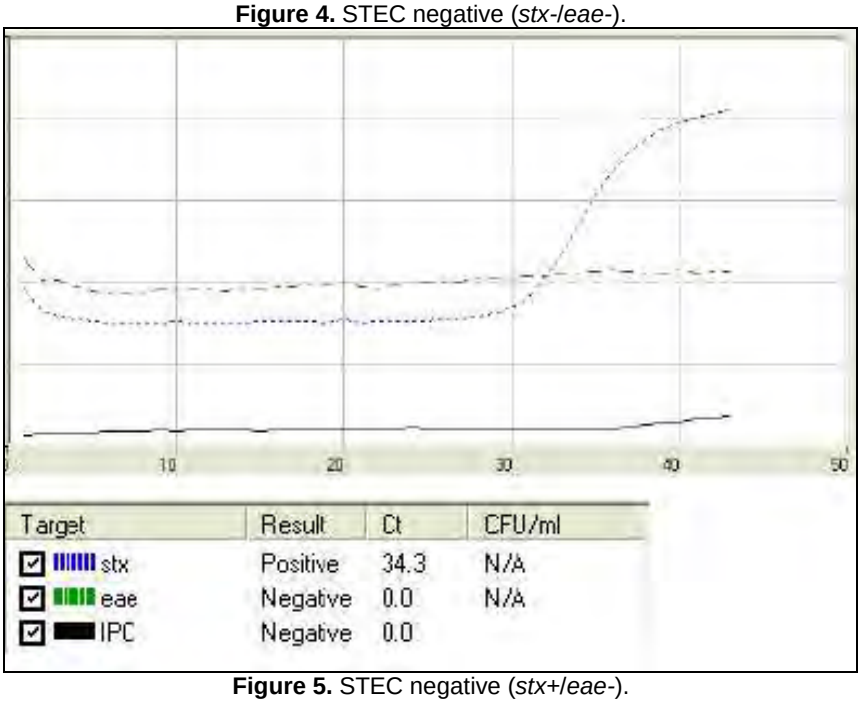


Figure 5. STEC negative (*stx+/eae-*).

4.4.6.2 Panel 1

- For **positive** samples (Figure 6), one or more E. coli targets of Panel 1 make a sigmoid curve and Ct score between 0 and 43. The Ct score usually fluctuates between 15 and 43;
- For **negative samples** (Figure 7), none of the three targets makes a sigmoid curve and the IPC is positive;

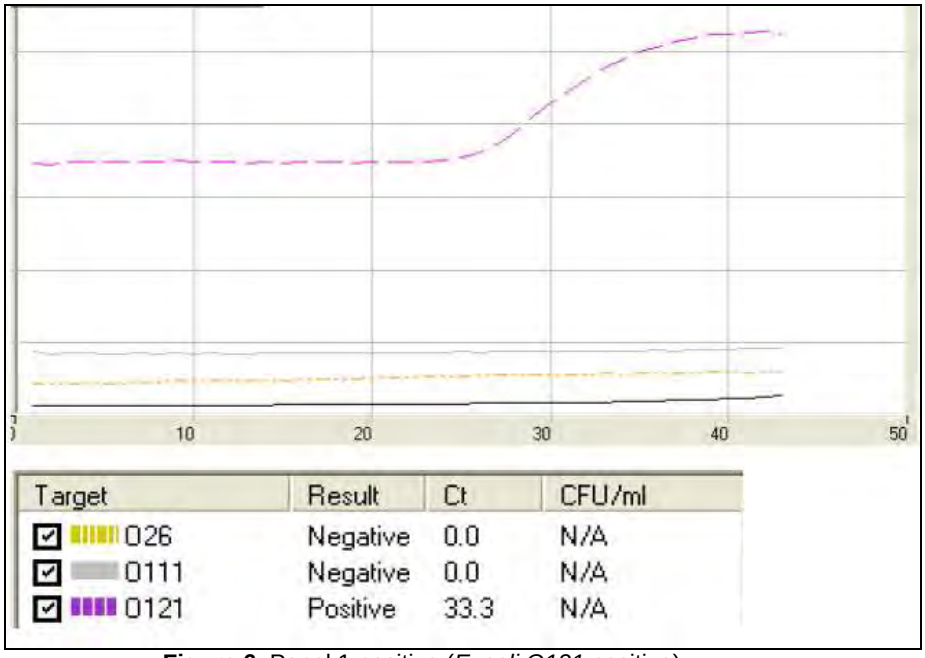


Figure 6. Panel 1 positive (*E. coli O121* positive).

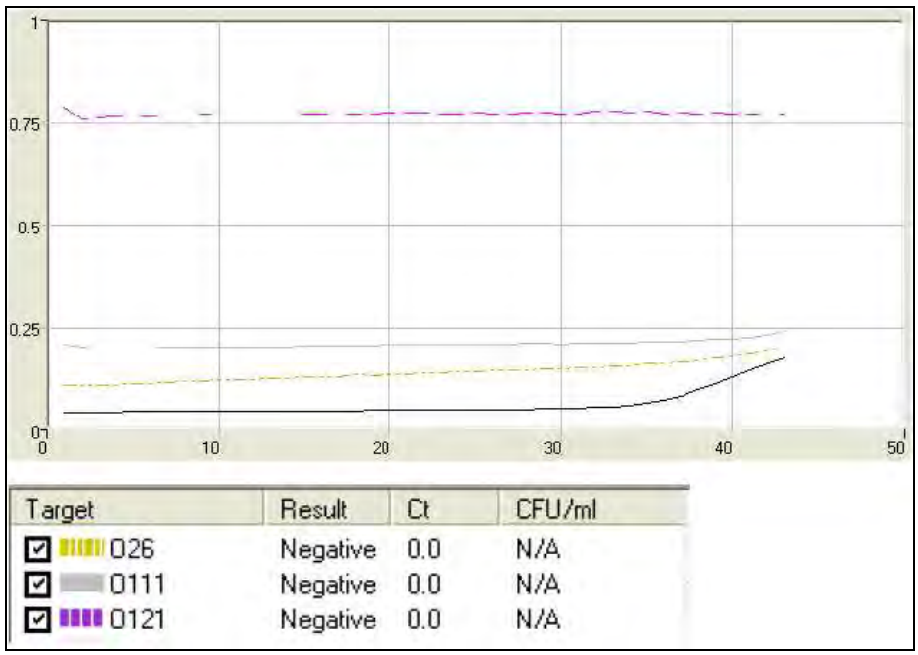


Figure 7. Panel 1 negative.

4.4.6.3 Panel 2

- For **positive** samples (Figure 8), one or more E. coli targets of Panel 2 make a sigmoid curve and Ct score between 0 and 43. The Ct score usually fluctuates between 15 and 43;
- For **negative samples** (Figure 9), none of the three targets makes a sigmoid curve and the IPC is positive;

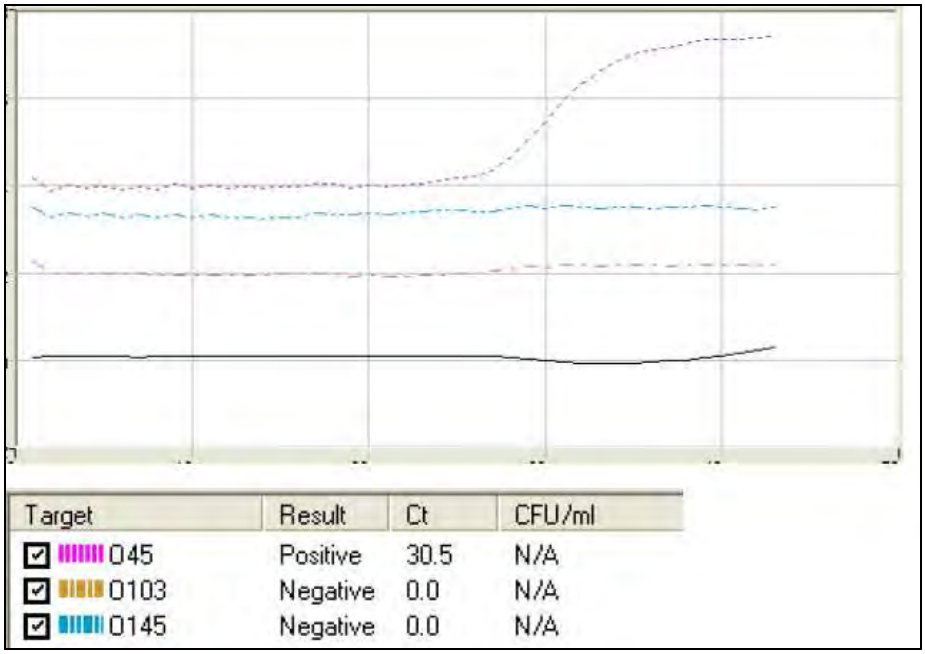


Figure 8. Panel 2 positive (*E. coli* O45 positive).

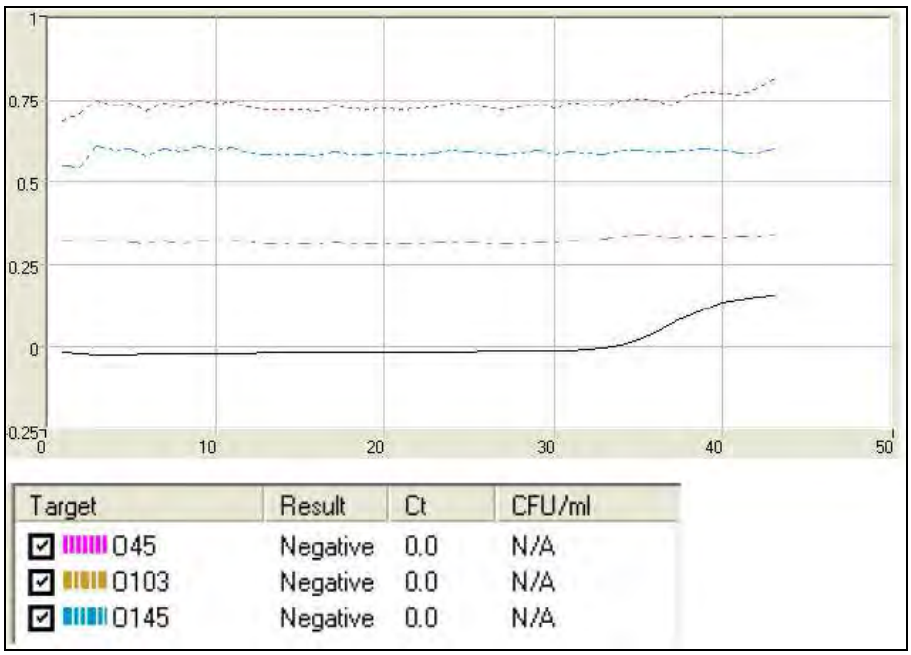


Figure 9. Panel 2 negative.

- Print the results, attach them to FORM MIC/063 – *Detecção de STEC*—"STEC detection", and record them in the appropriate field of FORM MIC/069 – *Carne* ("Meat");
- The person responsible for checking the data recorded on FORM MIC/063 – "STEC detection", must sign the sheet containing the printed BAX® results.

4.4.7 Interpretation of the results

- Samples that test negative for the BAX® STEC screening PCR (*stx*, *eae*) shall be reported as negative. Samples that test positive must be analyzed by using the positive lysate in the Panel 1 (O26, O111, O121) and Panel 2 (O45, O103, O145) tests. Samples must remain chilled at 2-8°C until loaded into the instrument. Remaining lysate must be sealed and stored for additional testing. These lysates may be stored at 2-8ºC for up to 7 days, or at -20ºC ± 3°C for up to 14 days.

***Note**: For Panel 1 and Panel 2 results, each well must be clicked individually (on the computer) and the result for each individual O-group must be recorded.*

- Samples that test positive for the STEC screening PCR (*stx*, *eae*) but negative for both Panel 1 and Panel 2 shall be reported as negative. If any of the O-groups from Panel 1 or Panel 2 are positive, perform isolation as described below;
- Samples with an indeterminate result or that have an invalid result for the STEC screening PCR (*stx*, *eae*) must be tested again using STEC screening PCR and Panels 1 and 2 assays using either the same lysate or preparing new lysate tubes;
- Samples that are STEC screening PCR (*stx*, *eae*) positive but indeterminate or have an invalid result on one or both Panel 1 and Panel 2 assays, proceed to indeterminate serogroup isolation;

- Alternatively, the laboratory may review the cause and perform a correction. Based on the findings the laboratory may:
  - Repeat the BAX® analysis from the rack loading step; or
  - Prepare new BAX® tubes and repeat the analysis.

- In analytical runs where the positive control tests BAX®-negative, indeterminate or has a signal-error result, the entire batch of samples is affected and a review of the cause and a correction shall be performed. Based on the findings the laboratory may:
  - Repeat the BAX® analysis from the rack loading stage;
  - Prepare new BAX® tubes and repeat the analysis;
  - Analyze the samples by the traditional method (item 4.5).

If reanalysis is unsuccessful then use an alternative screening method, perform an isolation analysis, prepare fresh analytical portions from the sample reserve or discard the sample.

4.5 Isolation

Samples that are potentially positive by PCR screen results shall be plated onto mRBA following IMS. In the isolation procedure, IMS beads shall be used for the specific serogroup identified by the serogroup PCR reaction as below:

- anti-O26 for positive reactions to O26;
- anti-O45 for positive reactions to O45;
- anti-O103 for positive reactions to O103;
- anti-O121 for positive reactions to O121;
- anti-O111 for positive reactions to O111;
- anti-O145 for positive reactions to O145.

4.6 Immunomagnetic separation and Culture plating

- Remove mRBA plates from 2-8°C storage, allowing 4 plates for each screen-positive culture and one plate for each serogroup control strain. Make sure the plate is dry before inoculation. If necessary dry the plates (e.g.for up to 30 minutes in a laminar flow hood, with the lid removed) prior to use. Dried plates that are not used should be labeled as "dried", placed in bags and returned to 2-8°C;
- For each screen-positive culture, label 1 sterile 50mL conical centrifuge tube and 6 sterile microcentrifuge tubes. For 3 of the tubes, add 0.9 mL E-buffer and label one tube as 1:10, one as 1:100 and one tube as acid 1:10;
- **Sample preparation from enrichment**: for each serogroup that the sample is positive, transfer approximately 2-5 mL from the enrichment broth through a 40 µm sterile cell strainer adapted to a 50 mL conical centrifuge tube;
- **Binding of paramagnetic antibody beads to specific serogroup**: transfer 50.0 µL (or volume recommended by the manufacturer) of appropriate immunomagnetic capture beads determined by the serogroup PCR screen results (O26, O45, O103, O111, O121 or O145) to a sterile labeled microcentrifuge tube. Next, add 1.0 mL of enrichment filtrate to the tube;
- Place the microcentrifuge tubes containing enrichments and capture beads on LabQuake® Agitator or similar equipment. Rotate tubes for 15 minutes at 18-30ºC (or time recommended by the manufacturer);
- For each sample, place one Large Cell Separation Column onto the OctoMACS® Separation Magnet. Fill the tray below the separation magnet with disinfectant. Prime each separation column with at least 0.5 ml of E-Buffer and allow the liquid to pass completely through the column before adding the sample;
- **Binding of beads to magnetic columns**: once the liquid has passed through the column, add the 1.0 mL of enrichment plus the IMS beads to each appropriately labeled column and allow the liquid to pass completely through;
- **Wash steps (4X)**: add 1.0 mL E-Buffer to the top of each column and let the buffer run through. Repeat 3 more times for a total of 4 washes;

- **Elution step**: After the last wash, remove the column from the OctoMACS® magnet and insert the tip into an empty labeled microcentrifuge tube. Apply 1.0 ml of E Buffer to the column and using the plunger supplied with the column, immediately flush out the beads into the tube. Use a smooth steady motion to avoid splattering. Cap the tubes. Repeat the procedure for each column;
- Prepare a 1:10 dilution of each treated bead suspension by adding 0.1 mL of the bead suspension to a labeled tube containing 0.9mL E-buffer. Make a 1:100 dilution by adding 0.1 mL of the 1:10 dilution to a labeled tube containing 0.9 mL E Buffer;
- Vortex briefly to maintain beads in suspension and plate 0.1 mL from each tube (1:10 and 1:100 dilutions) onto a labeled mRBA plate. Use a hockey-stick or spreader to spread the beads on the plate, being careful not to spread the beads against the edge of the plate;
- As soon as there is no visible moisture on the agar surface, invert plates and incubate for 20 to 24 hours at 35 ± 2 ºC;
- **Acid treatment**: for each sample, transfer 450 µL of the undiluted bead solution (eluant from the MACS column) to an empty labeled microcentrifuge tube. Add 25 µL of 1N hydrochloric acid (HCl)solution to this bead suspension and vortex briefly. This will bring the pH to 2.0 - 2.5 using E-buffer;
- Place the microcentrifuge tubes containing the acid-treated suspension on an agitator and rotate the tubes for 1 hour at 18-30ºC;
- After 1 hour, dilutent the suspension by adding 475 µL of E-buffer;
- Vortex briefly to maintain beads in suspension and plate 0.1 mL of the neutralized suspension onto a labeled mRBA plate. Use a hockey-stick or spreader to spread the beads on the plate, being careful not to spread the beads against the edge of the plate;
- Add 0.1 mL of the suspension to a labeled tube containing 0.9 mL of E buffer and vortex briefly. This represents a 1:10 dilutions of the acid-treated cell suspension. Plate 0.1 ml of the diluted suspension onto an appropriately labeled mRBA plate;
- As soon as there is no visible moisture on the agar surface, invert plates and incubate for 20 to 24 hours at 35 ± 2 ºC.

4.7 Identification and Confirmation

- Following incubation of mRBA, plates will be examined for colonies that agglutinate with latex agglutination reagents specific for the serogroup of interest. Colony colors from representative strains of each serogroup on mRBA plates are listed in Appendix A. However, the coloration of colonies may vary based on proximity to other competitor colonies or medium discoloration due to competitor colony growth. Since the morphologies of the targeted STEC colonies may vary widely among strains and serogroups, test at least 1 colony from each identified morphology found on the mRBA plate;
- Samples that have no growth or only contain agglutination negative colonies on mRBA are negative for non-O157 STEC;
- Any sample with agglutination positive colonies for the serogroup of interest is a presumptive positive for non-O157 STEC. Agglutination positive colonies shall be streaked onto tryptone soya agar with 5% sheep blood (SBA) or tryptone soya agar (TSA) for confirmation on the following day;
- Following a restreak of presumptive colonies and 16-24 hour incubation of SBA or TSA, agglutination-positive colonies shall be confirmed with BAX® Real-time PCR and biochemical identification.
- The confirmatory BAX® shall include the Screening assay (*stx* and *eae*) and the Panel which includes the serogroup for which the colony had a positive agglutination reaction (i.e. Panel 1 for O26, O111 and O121; and Panel 2 for O45, O103 and O145). If no colony isolated from mRBA confirms by PCR and VITEK® 2, the sample is negative for non-O157 STEC.

- If the laboratory finds insufficient results to allow identification of the strain, for example if the sample is PCR positive, but biochemically negative, this must be considered non-O157 STEC positive.

4.7.1 Serological agglutination and Confirmation PCR Procedure

- Use a needle or loop to transfer a portion of an isolated colony from the mRBA plate to a serological agglutination reagent. Follow manufacturer's instructions on procedure and interpretation;
- **Control reactions**: a reference strain from the serogroup of interest plated on mRBA shall be used as the positive control culture;
- Transfer the remainder of an agglutination positive colony from the mRBA plate onto SBA or TSA for further biochemical and genetic confirmation. Streak up to 5 agglutination positive colonies onto SBA plates. Incubate plates at 35 ± 2°C for 16 - 24 hours;
- Following incubation, perform the agglutination test again on colonies from the SBA or TSA plate;
- To confirm agglutination-positive colonies using BAX® real-time PCR, suspend the colony from the SBA or TSA plate in 50 µL of Molecular Grade Water and add 5 µL of this suspension to BAX® lysis buffer;
- Continue with the BAX® System protocol from the "Perform Lysis" step. The lysate will then be used for the STEC screening assay and the appropriate Panel.
  Note: *each PCR assay shall include a positive control as described in item 4.2;*
- Additionally, perform biochemical identification (Vitek® 2) on agglutination-positive colonies from the incubated SBA or TSA. A positive isolate shall be biochemically identified as *E. coli*;
- If the isolate is agglutination positive for one of the 6 STEC serogroups, BAX® real-time PCR positive for *stx*, *eae* and for genes of the six serogroups, and biochemically identified as *E. coli*, the sample is positive for non-O157 STEC;
- If the isolate and any additional colony picks from mRBA are ultimately determined to be BAX® real-time PCR negative for *stx*, *eae* and top six serogroup genes, the sample is negative for non-O157 STEC.

4.8 Issuing the results

- Issue the results as laid down by IT MIC/008 – *Análise microbiológica de amostras* ("Microbiological analysis of samples").
- The results must be issued and notified as laid down in DIPOA/SDA Internal Norm nº 01, published 17 June, 2015.

5.0 Bibliography

DuPont. Dupont™ Bax® System. Q7 Instrument. User Guide. 2013.

HUNT, J. M. Shiga toxin-producing *Escherichia coli* (STEC). Clinics in Laboratory Medicine, v. 30, n. 1. pp. 21-45, 2010.

United States Department of Agriculture. Food Safety and Inspection Service. Office of Public Health Science. MLG 5B.05. Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat Products and Carcass and Environmental Sponges.

United States Department of Agriculture. Food Safety and Inspection Service. Office of Public Health Science. MLG 5B Appendix 2.0. Morphology of Representative Strains from Six non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) Grown on Modified Rainbow Agar. Available at: http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiologylaboratoryguidebook/microbiologylaboratory-guidebook. Accessed on 11/4/2015.

6.0 Appendices

- Appendix A: Morfologia de cepas representativas de seis *Escherichia coli* produtoras de toxina Shiga (STEC) não O157 em ágar Rainbow Modificado. ("Morphology of six Shiga toxin-producing strains of non-O157 E. coli in modified Rainbow Agar").

7.0 Additional documents

- FORM MIC/063 – STEC detection;
- FORM MIC/069 – Meat;
- FORM MIC/070 – Identificação de STEC não O157; ("Identification of non-O157 STEC");
- IT MIC/007 – *Preparo de amostras, suspensões iniciais e diluições decimais* ("Preparation of samples, initial suspensions, and decimal dilutions");
- IT MIC/008 – *Análise microbiológica de amostras* ("Microbiological analysis of samples");
- IU MIC/025 – BAX® System Q7;
- IU MIC/026 – Dry Block Heater;
- DIPOA/SDA Internal Norm Nº 01 published June 17, 2015

8.0 History of changes

ITEM	SUMMARY OF CHANGES

9.0 Critical analysis

Evidence of critical analysis	

Appendix A

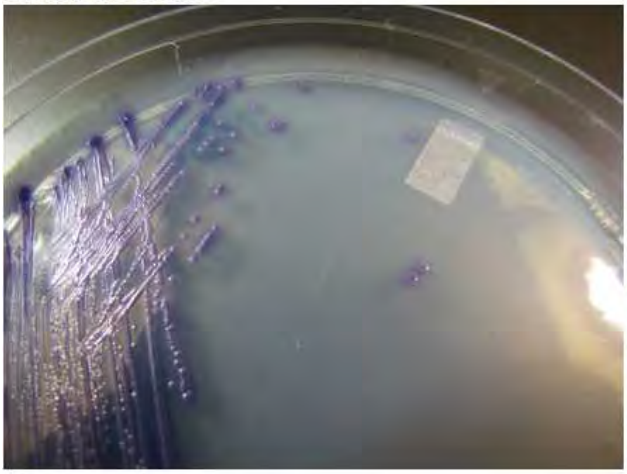
Morfologia de cepas representativas de seis *Escherichia coli* produtoras de toxina Shiga (STEC) não O157 em ágar Rainbow Modificado. ("Morphology of six Shiga toxin-producing strains of non-O157 E. coli in modified Rainbow Agar").

***Note 1**: Morphology or phenotypes of non-157 STEC cultures plated on modified Rainbow Agar may vary among strains of the same serotype. Additionally, the coloration of colonies of the same strain may vary when target colonies grow in proximity to competing organisms. Analysts should not rely on coloration alone when choosing colonies in mRBA for serological agglutination assays.*

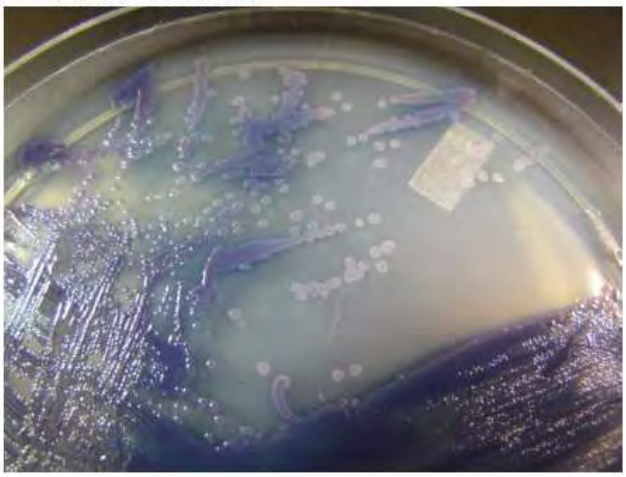
***Note 2**: The following photographs show the range of colors among strains representing each of the six non-O157 STECs grown in pure culture on modified Rainbow Agar.*

SEROGROUP O26

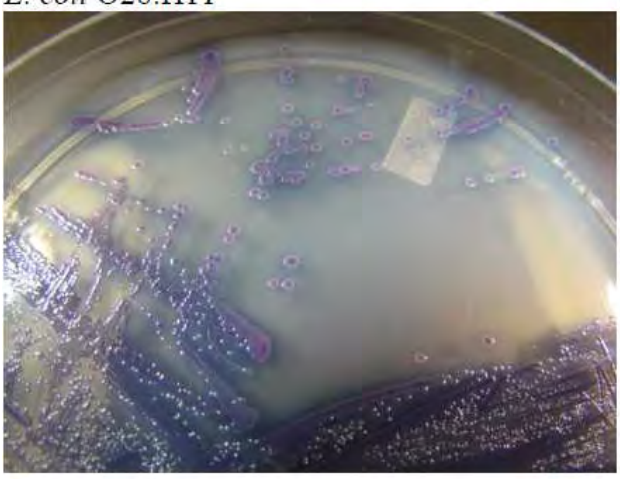
E. coli O26:NM



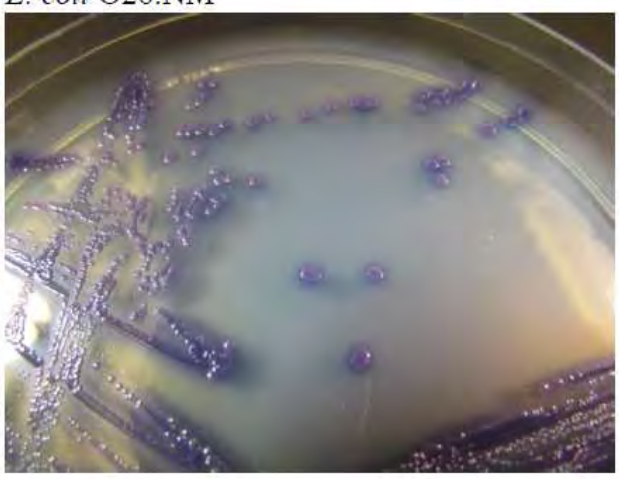
E. coli O26:H11



E. coli O26:H11

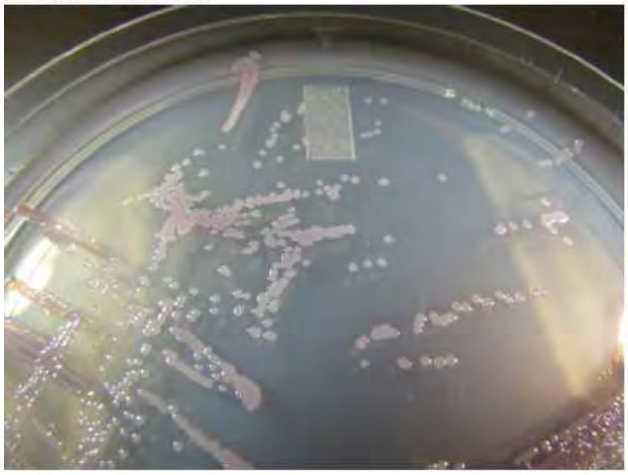


E. coli O26:NM



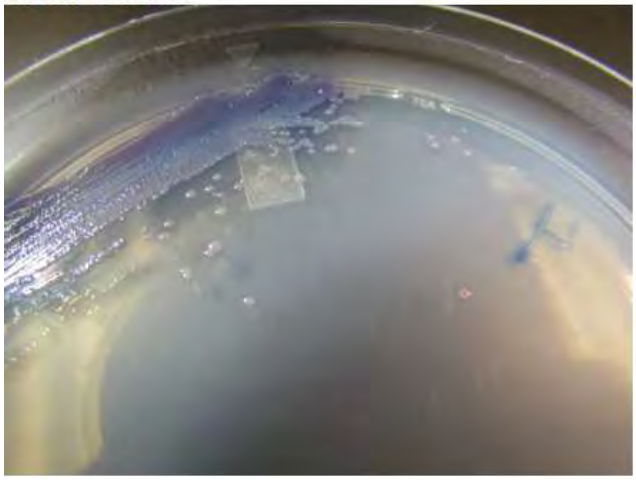
SEROGROUP O45

E coli O45:NM



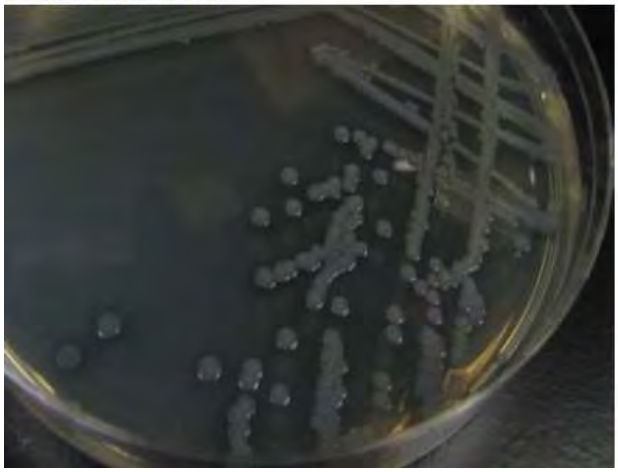
E coli O45:H2

E coli O45:H2

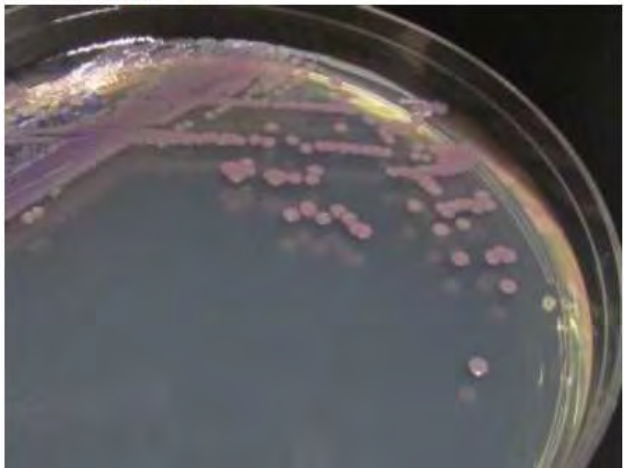


SEROGROUP O103

E. coli O103:H25

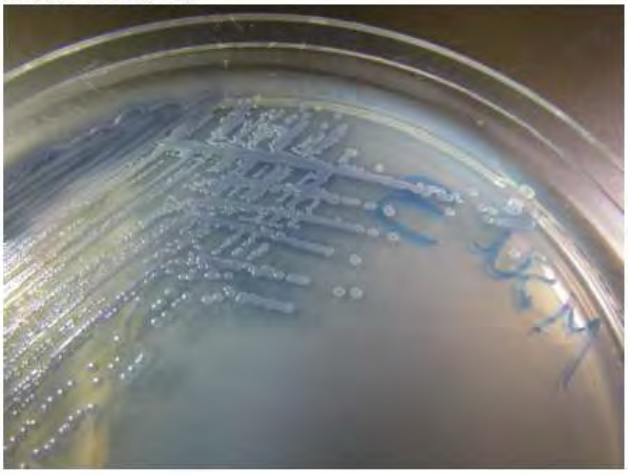


E. coli O103:H2

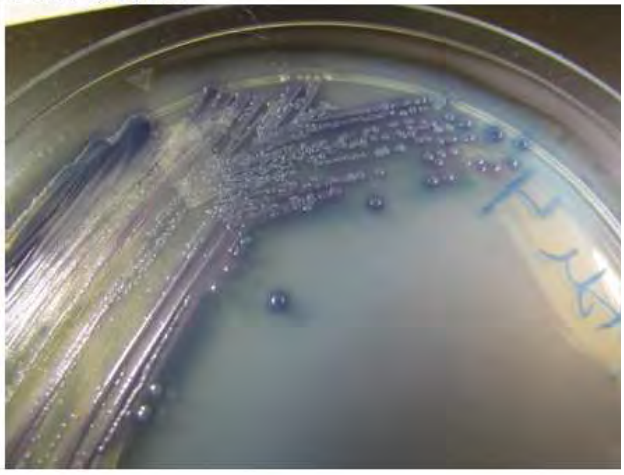


SEROGROUP O111

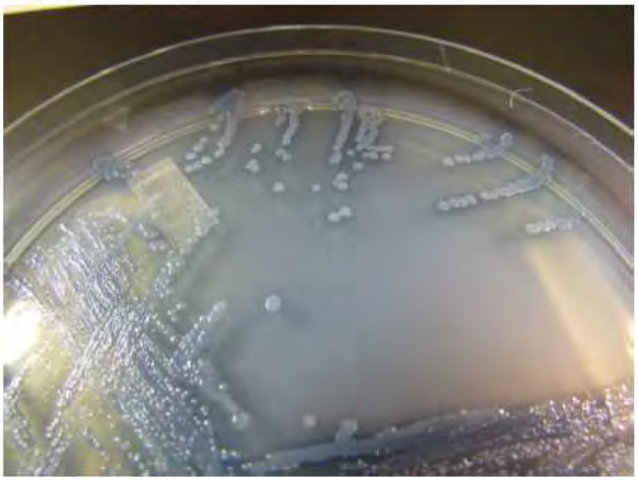
E coli O111:H-



E coli O111:H-

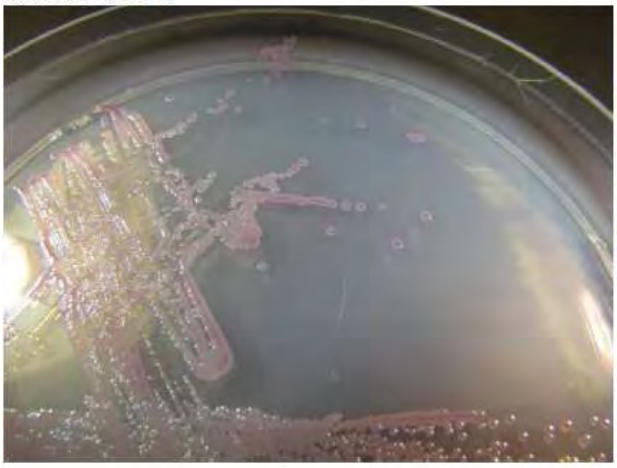


E coli O111:H8

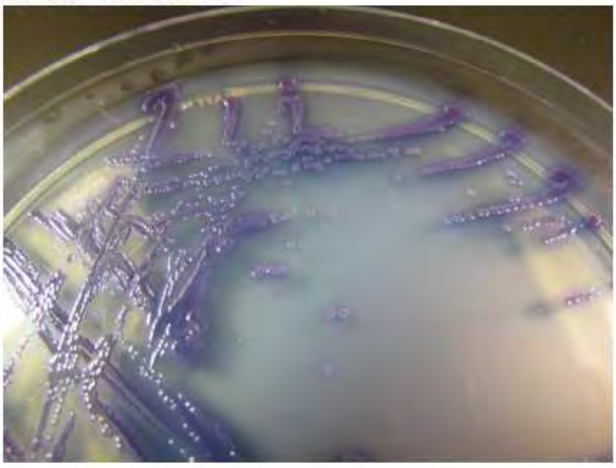


SEROGROUP O121

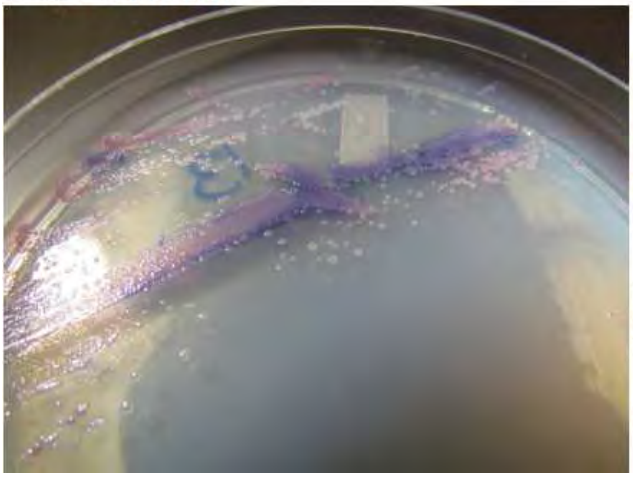
E. coli O121



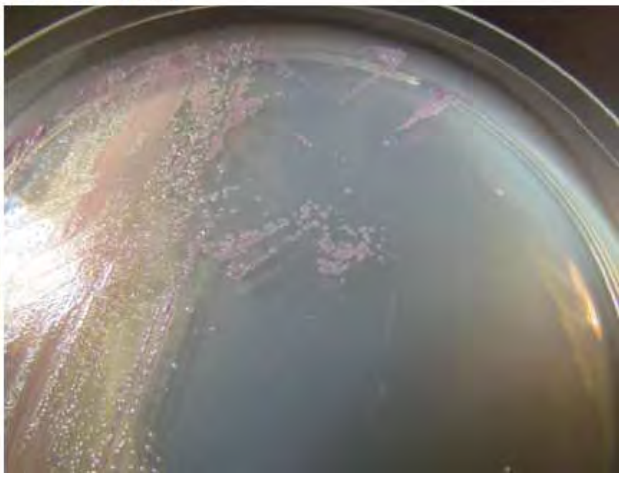
E. coli O121:H19



E. coli O121:H19

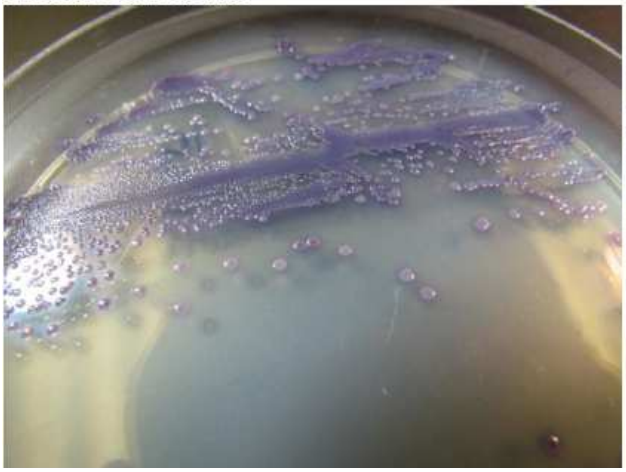


E. coli O121



SEROGROUP O145

E. coli O145:NM



E. coli O145:NM

Source: United States Department of Agriculture. Food Safety and Inspection Service, Office of Public Health Science. MLG 5B Appendix 2.0. Morphology of Representative Strains from Six non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) Grown on Modified Rainbow Agar