UNITED STATES DEPARTMENT OF AGRICULTURE
FOOD SAFETY AND INSPECTION SERVICE
WASHINGTON, DC

FSIS DIRECTIVE	10,240.6	1/19/24

USE OF WHOLE GENOME SEQUENCING RESULTS FOR FSIS READY-TO-EAT SAMPLING PROGRAMS

I. PURPOSE

A. This new directive provides instructions to Enforcement, Investigations, and Analysis Officers (EIAOs) and District Office (DO) personnel on use of whole genome sequencing (WGS) results, including findings of harborage and cross-contamination, when responding to *Listeria monocytogenes* (*Lm*) positive results following instructions in these directives:

1. FSIS Directive 10,240.5, *Verification Procedures for Enforcement, Investigations, and Analysis Officers for the Listeria monocytogenes Regulation and Routine Risk-Based Listeria monocytogenes Sampling Program*;

2. FSIS Directive 10,300.1, *Intensified Verification Testing (IVT) Protocol for Sampling of Product, Food Contact Surfaces and Environmental Surfaces for Listeria Monocytogenes*;

3. FSIS Directive 5730.1, *Responsibilities in Dual Jurisdiction Establishments*;

4. FSIS Directive 5100.1, *Food Safety Assessment Methodology*;

5. FSIS Directive 5100.3, *Administrative Enforcement Action Decision-Making and Methodology*; and

6. FSIS Directive 5100.4, *Public Health Risk Evaluation Methodology*

B. This directive also provides instructions to EIAOs and DO personnel for how to obtain reports containing WGS results, including findings of harborage and cross-contamination, and how EIAOs and DO personnel can understand the information in the reports.

II. BACKGROUND

A. Ready-to-eat (RTE) sampling under the RTEPROD, Routine Risk-Based *Lm* (RLm), and IVT programs is one of the many activities FSIS conducts to verify the adequacy of an establishment's food safety system, including *Lm* control measures.

B. When FSIS finds more than one *Lm* positive sample in an establishment, it uses WGS information to determine if there is potential for harborage or cross-contamination when the isolates are closely related (i.e., if the first four fields of the allele code match). See WGS FAQs in IPP Help for more information.

1. FSIS reports findings of harborage and cross-contamination in the *Listeria* WGS report that is sent by e-mail to the DO Biological Information Transfer and E-mail System (BITES) Distribution list, the Public Health Risk Evaluation (PHRE) report in the Public Health Information System (PHIS) (under the Further Characterization of Positive Samples for an Establishment tab) that EIAOs access during performance of a PHRE, and the Quarterly Establishment Information Letters that are sent by e-mail to establishments.

2. Harborage is the persistence of harmful bacteria in a processing environment over time. FSIS reports potential harborage in the reports described in 1. above when two or more closely related *Lm* isolates are collected from the same establishment over multiple days, weeks, months, or years from product, food contact surface (FCS), or non-food contact surface (NFCS) environmental samples.

NOTE: To determine harborage, the PHRE report in PHIS and the Quarterly Establishment Information Letters compare all isolates from the establishment within the past five years while the *Listeria* WGS report compares all isolates FSIS has ever collected from the establishment with WGS available.

3. Cross-contamination is the transfer of harmful bacteria among food, FCS, or NFCS environmental surfaces. FSIS reports potential cross-contamination in the reports described in 1. above when two or more closely related *Lm* isolates are collected from the same establishment on the same day from product, FCS, or NFCS environmental samples.

C. FSIS also uploads the sequences from the *Lm* isolates to the National Center for Biotechnology Information (NCBI) database and determines whether there are closely related clinical isolates (human listeriosis). FSIS reports findings of closely related clinical isolates in the *Listeria* WGS report. This information cannot be found in the PHRE report in PHIS or the Quarterly Establishment Information Letters.

III. FSIS ACTIONS IN RESPONSE TO WGS RESULTS FOR FSIS RTE SAMPLING PROGRAMS

A. Immediately after a product, FCS, or NFCS environmental sample is confirmed positive during R*Lm* or IVT sampling, EIAOs are to follow instructions in FSIS Directive 10,240.5, FSIS Directive 10,300.1, and FSIS Directive 5100.1, to verify immediate corrective actions, such as product disposition, to prevent adulterated product from entering commerce (for positive product and FCS samples), determine whether to recommend a noncompliance record, and if noncompliance exists, what regulations to cite.

NOTE: In response to RTEPROD results, IPP follow instructions in FSIS Directive 10,240.3, *FSIS Ready-to-Eat Sampling Programs*.

B. EIAOs and DO personnel are not to wait for the harborage or cross-contamination results or other *Listeria* WGS results prior to verifying the establishment's immediate corrective actions. In addition, as described in G. below, FSIS's findings of harborage or cross-contamination can be used in conjunction with compliance history and FSA findings to further demonstrate an establishment's failure to control *Lm* in the post-lethality environment through its sanitation and *Lm* control programs and to support food safety concerns leading to enforcement action.

C. EIAOs and DO personnel are to be aware that typically, 7-14 days after the confirmed positive, a *Listeria* WGS report will be sent in e-mail to the BITES Distribution list.

NOTE: For more information on FSIS WGS methods see the Microbiological Laboratory Guidebook (MLG) Chapter 42, *WGS Sequencing of Bacterial Isolates.*

D. After the *Listeria* WGS report is sent, EIAOs and DO personnel are to review the report and are to be aware of the following:

1. In cases where the establishment has had multiple positives during the same or different sampling event, the report will indicate whether there is potential harborage, cross-contamination, or both;

2. The report will also indicate whether the FSIS isolate is "potentially related to a clinical isolate(s)" uploaded to the NCBI database within the last two years and will provide further context as to whether the isolate is of interest to FSIS during subsequent verification or enforcement actions.

    a. A closely related clinical isolate means the product, FCS, or NFCS environmental isolate collected at this establishment is capable of causing human illness, but FSIS can't determine if the establishment was the cause of the illness without epidemiological information connecting the isolates;

    b. Subject matter experts will typically determine an isolate is of interest when there are no other isolates from other sources in the same NCBI cluster, other isolates that are in the cluster have a potential relationship to the FSIS isolate, or the FSIS isolate and clinical isolates are determined to be more closely related than other isolates from other sources in the NCBI cluster; and

    c. Subject matter experts will typically determine an isolate is not of interest when there are many other non-clinical isolates from other sources in the same NCBI cluster indicating this is a common *Lm* subtype and FSIS does not believe there is any relationship to the FSIS isolate at the time of reporting. FSIS may provide more information in the report if the isolate is involved in an active illness investigation.

E. EIAOs and DO personnel are to share the *Listeria* WGS report with the establishment management and are to document the discussion in a Memorandum of Interview.

1. If an enforcement action was issued prior to the *Listeria* WGS report being sent, the DO is to share how the findings in the *Listeria* WGS report, including any findings of potential harborage, cross-contamination, or potentially related clinical isolates of interest, may further support the enforcement action.

2. When sharing the *Listeria* WGS report, the EIAO and DO personnel are to make the establishment management aware of the recommendations in the FSIS' Guideline Controlling *Listeria monocytogenes* in Post-lethality Exposed Ready-to-eat Meat and Poultry Products related to considering findings of harborage and cross-contamination when taking corrective actions. The guideline recommends establishments conduct the following actions, which should be escalated in the event of consecutive positives and potential harborage (page 78):

    a. Determine *Listeria* trends (page 122), perform a comprehensive investigation into the source of positives (page 122), and conduct intensified sampling as described below (pages 117 and 123);

    b. Provide employee training with a focus on what equipment to clean and how to clean it (page 82) to address harborage and on employee product handling hygiene practices to address cross-contamination (pages 74 and 81);

    c. Conduct intensified sanitation, including increasing the frequency of cleaning and sanitizing,

breaking down equipment, repairing or replacing broken equipment, and constructing new walls to separate raw and RTE areas, if needed (pages 78 and 117) and;

d. Perform intensified sampling to find sources of contamination that should include collection of FCS, NFCS environmental, and product samples. At least 3-5 samples per site of prior positives should be collected per sampling event until negatives are found (pages 117 and 123).

F. If the establishment is a DJE, the DO is to also share the *Listeria* WGS report findings with the U.S. Food and Drug Administration Regional Office as described in FSIS Directive 5730.1.

G. If DO personnel recommend an enforcement action associated with the R*Lm* or IVT sampling, the DO personnel may include the findings in the WGS report in the enforcement letter (FSIS Directive 5100.1). If potential harborage or cross-contamination is reported or if a potentially related clinical isolate of interest is reported, FSIS may determine based on other findings (*e.g.,* compliance history and FSA findings) that the establishment's food safety system (*i.e.,* either the HACCP plan, prerequisite program, or Sanitation Standard Operating Procedure depending on where the *Lm* control measures are included) is inadequate to control *Lm* in the post-lethality environment, the Sanitation Standard Operating Procedure (Sanitation SOP) is not properly implemented or maintained, or the establishment has not maintained sanitary conditions to prevent *Lm* product adulteration (9 CFR 500). The enforcement letter identifying harborage or cross-contamination should include the WGS findings in addition to the specific compliance history.

H. In addition to immediate actions to prevent adulterated product from entering commerce, EIAOs are to verify the establishment takes further planned actions to meet the other corrective action requirements per 9 CFR 417.3(a), 9 CFR 417.3(b), or 9 CFR 416.15.

1. EIAOs are to consider findings of harborage and cross-contamination as part of any follow-up verification activities as described in FSIS Directive 5100.1 and FSIS Directive 5100.3. See E.2. of this section for a summary of FSIS guidance to establishments regarding corrective actions in response to *Lm* positives and findings of harborage and cross-contamination.

2. When verifying corrective actions, EIAOs are to be aware that repetitive positives indicate previous corrective actions were ineffective and retraining is not sufficient on its own.

3. If the positive sample type from the prior sampling event (RTEPROD, R*Lm*, or IVT) is a product or FCS sample, FSIS will conduct a PHRE and may recommend to verify the establishment's corrective actions by conducting IVT sampling and may also conduct an additional FSA (FSIS Directive 5100.4, FSIS Directive 10,300.1 and FSIS Directive 5100.1).

NOTE: EIAOs are instructed in FSIS Directive 5100.4 to access the Further Characterization of Positive Samples for an Establishment tab of the PHRE for an Establishment report in PHIS to assess WGS results for indications of harborage or cross-contamination during the performance of a PHRE.

4. Depending on the findings of any follow-up verification activities, including sampling, noncompliance may be documented. As indicated in FSIS Directive 5100.3, DO personnel may also recommend an enforcement action when an establishment has multiple, recurring noncompliances; implements ineffective corrective actions; receives multiple adulterant positive results from FSIS testing; or ships adulterated product. For more information on incorporating WGS information in an enforcement action, refer to G. of this section.

III. QUESTIONS

Refer questions regarding this Directive to your supervisor or as needed to the Office of Policy and Program Development through askFSIS or by telephone at 1-800-233-3935. When submitting a question, complete the web form and select Sampling for the Inquiry Type.

NOTE: Refer to FSIS Directive 5620.1, *Using askFSIS*, for additional information on submitting questions.

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