VERMONT AGENCY OF AGRICULTURE, FOOD & MARKETS
FOOD SAFETY CONSUMER PROTECTION DIVISION
Meat Inspection Service
MONTPELIER, VT
Anson Tebbetts, Secretary

MIS DIRECTIVE **Adopted from FSIS Directive 10240.6**	10240.6	04/15/24 FOR USE BEGINNING

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

I. PURPOSE

A. This new directive provides instructions to Enforcement, Investigations, and Analysis Officers (EIAOs) and Office 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. VT 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. VT Directive 10,300.1, *Intensified Verification Testing (IVT) Protocol for Sampling of Product, Food Contact Surfaces and Environmental Surfaces for Listeria Monocytogenes*;

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

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

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

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

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

C. Per 6 V.S.A. § 3305 (8), the federal meat inspection regulations and federal poultry inspection regulations of the U.S. Department of Agriculture, Title 9, Code of Federal Regulations, Chapter 3, 9 CFR §§ 300.1 et seq., together with any amendments, supplements, or revisions thereto, are adopted, for the State meat inspection program to operate in an 'equal to' status.

II. BACKGROUND

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

B. When the laboratory finds more than one *Lm* positive sample in an establishment, it uses GenomeTrakr to perform WGS. This information is shared with FSIS. It can be used 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. The *Listeria* WGS report and can be used by the EIAOs during performance of a PHRE.
2. Harborage is the persistence of harmful bacteria in a processing environment over time. Potential harborage is reported 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 compares all isolates from the establishment within the past five years.

3. Cross-contamination is the transfer of harmful bacteria among food, FCS, or NFCS environmental surfaces. Potential cross-contamination is reported 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 receives the SRA number from the lab, and can upload 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).

III. ACTIONS IN RESPONSE TO WGS RESULTS FOR 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 VT Directive 10,240.5, VT Directive 10,300.1, and VT 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 RTE product results, IPP follow instructions in VT Directive 10,240.3, *Ready-to-Eat Sampling Programs*.

B. EIAOs and the Chief 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, 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 the Chief are to be aware that typically, 7-14 days after the confirmed positive, results of *Listeria* WGS may be available.

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 is reported, EIAOs and the Chief is to review the results and establishment history 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 EIAO and Chief will have to decided whether there is potential harborage, cross-contamination, or both;

2. FSIS may determine that the isolate is "potentially related to a clinical isolate(s)" uploaded to the NCBI database within the last two years and may provide further context as to whether the isolate is of interest to FSIS.

   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 if the isolate is involved in an active illness investigation.

E. EIAOs and the Chief are to share the *Listeria* WGS results 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 results being sent, the Chief is to share how the findings in the *Listeria* WGS results, 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 results, the EIAO and Chief 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 Chief is to also share the *Listeria* WGS findings with the Vermont Department of Health or U.S. Food and Drug Administration Regional Office as appropriate as described in VT Directive 5730.1.

G. If the Chief recommend an enforcement action associated with the R*Lm* or IVT sampling, the Chief may include the findings of the WGS in the enforcement letter (VT Directive 5100.1). If potential harborage or cross-contamination is reported or if a potentially related clinical isolate of interest is reported, VTMIS may determine based on other findings (*e.g.,* compliance history and FSAfindings) 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 VT Directive 5100.1 and VT Directive 5100.3. See E.2. of this section for a summary of guidance to establishments regarding corrective actions in response to *Lm* positives and findings of harborage and cross-contamination.

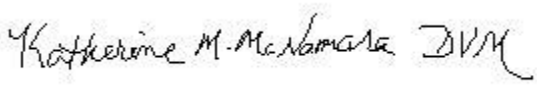
2. When verifying corrective actions, EIAOs are to be aware that repetitive positives indicate previous corrective actions were ineffective and retraining is not sufficient on its own.

3. If the positive sample type from the prior sampling event (Product, R*Lm*, or IVT) is a product or FCS sample, VTMIS 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 (VT Directive 5100.4, VT Directive 10,300.1 and VT Directive 5100.1).

4. Depending on the findings of any follow-up verification activities, including sampling, noncompliance may be documented. As indicated in VT Directive 5100.3, the Chief may also recommend an enforcement action when an establishment has multiple, recurring noncompliances; implements ineffective corrective actions; receives multiple adulterant positive results from VTMIS

testing; or ships adulterated product. For more information on incorporating WGS information in an enforcement action, refer to G. of this section.

Questions can be referred to the meat inspection office at 802-828-2426.



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