Interpretation Criteria for PFGE and MLVA

To determine the likelihood that isolates are related and may have originated from a common source, molecular or genetic tests are performed. The molecular/genetic characterization is an important component of investigations: isolates with matching genetic fingerprints or profiles are more likely to have originated from a common source, or have an epidemiological relationship, than those with different profiles. If pathogens are identified in a food sample, their genetic profiles are compared to those isolated from the human cases from the outbreak investigation. Determining if the isolates "match" provides critical evidence for an outbreak investigation. Laboratory methods used for genetic profiling, and the criteria for interpreting the results, are based on procedures of the PulseNet Canada network. Pulsed-field gel electrophoresis (PFGE) is the current gold standard method for comparing genetic profiles of most foodborne bacterial pathogens. Additional methods may be applied, such as Multiple Loci VNTR Analysis (MLVA) particularly for *E. coli* O157:H7.

The following Tables provide details on the strength of the evidence for these test results. These criteria are used to assess matches among human cases, and to assess matches between food isolates and human cases.

Strength of Microbiological Evidence: Interpretation Criteria for Determining Isolate Matches by PFGE – *E. coli* O157:H7, non-O157 VTEC, *Listeria monocytogenes*, *Salmonella, Shigella, Vibrio*.

Criteria	Nature of PFGE Evidence	Weight
A. Does the organism show suitable diversity by PFGE	**Based on historical data, the organism shows suitable diversity by; historic sporadic cases show diverse PFGE patterns** **Testing by other appropriate laboratory methods demonstrate significant characteristics that link two or more isolates with a high degree of confidence.** **Little or no historical data exists for this organism.** **Based on historical data, the organism shows little diversity by PFGE; a large proportion of sporadic cases have indistinguishable or highly similar PFGE patterns.**	**Strong** **Weak**
B. Are the PFGE patterns indistinguishable by 2 enzymes.	**Isolates are indistinguishable by two enzymes.** **Isolates have indistinguishable 1st enzyme and distinguishable 2nd enzyme patterns; differences are considered minor (high % similarity)** **Isolates have distinguishable 1st and 2nd enzyme PFGE patterns, differences are considered minor (high % similarity)** **Clinical and food isolates do not match (e.g. by multiple bands, lower % similarity)**	**Strong** **Weak**
C. What is the historic frequency of the PFGE pattern combination?	**The PFGE pattern, or pattern combination, is new.** **Based on historic pattern frequency, the PFGE pattern is not common.** **Based on historic pattern frequency, the PFGE pattern is common.**	**Strong** **Weak**

Strength of Microbiological Evidence: Interpretation Criteria for Determining Isolate Matches by MLVA – *E. coli* O157:H7

MLVA is routinely used for *E. coli* O157:H7 characterization as a supplemental method to the primary test (PFGE). Together, PFGE and MLVA provide optimal discrimination for *E. coli* O157:H7; MLVA adds additional information that contributes to the weight of evidence. Interpretation criteria, applied in conjunction with the interpretation of PFGE results, are described in the Table below.

Criteria	Nature of MLVA Evidence – *E. coli* O157:H7	Weight
A. Do the isolates differ at locus VNTR 34?	The isolates have the same number of repeats at locus VNTR 34.	Strong
	The isolates have different numbers of repeats at locus VNTR 34.	Weak
	Strains with differences in locus VNTR 34 are unlikely to be related	
B. Are the isolates the same at the other seven loci?	Isolates have the same number of repeats at all loci; OR, isolates have 3 or fewer repeat differences at a maximum of 1 locus, OR, isolates have a maximum of 1 repeat difference at up to 3 loci (not including VNTR 34).	Strong
	Isolates differ by more than 3 repeats at a single locus, or by greater than one repeat at three or more loci.	Weak

Additional Tests:

For other foodborne pathogens, including foodborne viruses and parasites, standardized genetic profiling tests either may not be routinely used via the PulseNet Canada network, or methods may not exist. Laboratory tests and their interpretation are determined for each situation in consultation with stakeholders.

While PFGE and MLVA are the gold standard subtyping methods for foodborne bacterial pathogens, newer methods are being developed for genetic profiling, including whole genome seqeuncing. This research and development is done to ensure that the best technbology and most current science are available for foodborne disease investigations and with the application of validated interpretation criteria and assesmsent of weight of evidence. When new tests are applied during outbreak investagations for this purpose, they are applied in parallel to the primary tests and are carefully interpreted on a case-by-case basis.