

E. coli testing; Pathogen Reduction Standards

§ 310.25 Contamination with microorganisms; process control verification criteria and testing; pathogen reduction standards.

(a) Criteria for verifying process control; *E. coli* testing.

(1) Each official establishment that slaughters livestock must test for *Escherichia coli* Biotype 1 (*E.coli*) Establishments that slaughter more than one type of livestock or both livestock and poultry shall test the type of livestock or poultry slaughtered in the greatest number. The establishment shall:

(i) Collect samples in accordance with the sampling techniques, methodology, and frequency requirements in paragraph (a) (2) of this section;

(ii) Obtain analytic results in accordance with paragraph (a)(3) of this section; and

(iii) Maintain records of such analytic results in accordance with paragraph (a)(4) of this section.

(2) Sampling requirements.

(i) *Written procedures.*

Each establishment shall prepare written specimen collection procedures which shall identify employees designated to collect samples, and shall address location(s) of sampling, how sampling randomness is achieved, and handling of the sample to ensure sample integrity. The written procedure shall be made available to FSIS upon request.

(ii) *Sample collection.*

The establishment must collect samples from all chilled livestock carcasses, except those boned before chilling (hot-boned), which must be sampled after the final wash. Samples must be collected in the following manner;

(A) For cattle, establishments must sponge or excise tissue from the flank, brisket and rump, except for hide-on calves, in which case establishments must take samples by sponging from inside the flank, inside the brisket, and inside the rump.

(B) For sheep, goat, horse, mule, or other equine carcasses, establishments must sponge from the flank, brisket and rump, except for hide-on carcasses, in which case establishments must take samples by sponging from inside the flank, inside the brisket, and inside the rump.

(C) For swine carcasses, establishments must sponge or excise tissue from the ham, belly and jowl areas.

(iii) *Sampling frequency.*

Slaughter establishments, except very low volume establishments as defined in paragraph (a)(2)(v) of this section, must take samples at a frequency proportional to the volume of production at the following rates:

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(A) Cattle, sheep, goats, horses, mules, and other equines: 1 test per 300 carcasses, but, a minimum of one sample during each week of operation. Swine: 1 test per 1,000 carcasses, but a minimum of one sample during each week of operation.

(iv) *Sampling frequency alternatives.*

An establishment operating under a validated HACCP plan in accordance with § 417.2(b) of this chapter may substitute an alternative frequency for the frequency of sampling required under paragraph (a)(2)(iii) of this section if,

(A) The alternative is an integral part of the establishment's verification procedures for its HACCP plan and,

(B) FSIS does not determine, and notify the establishment in writing, that the alternative frequency is inadequate to verify the effectiveness of the establishment's processing controls.

(v) *Sampling in very low volume establishments.*

(A) Very low volume establishments annually slaughter no more than 6,000 cattle, 6,000 sheep, 6,000 goats, 6,000 horses, mules or other equines, 20,000 swine, or a combination of livestock not exceeding 6,000 cattle and 20,000 total of all livestock. Very low volume establishments that collect samples by sponging shall collect at least one sample per week, starting the first full week of operation after June 1 of each year, and continue sampling at a minimum of once each week the establishment operates until June 1 of the following year or until 13 samples have been collected, whichever comes first.

Very low volume establishments collecting samples by excising tissue from carcasses shall collect one sample per week, starting the first full week of operation after June 1 of each year, and continue sampling at a minimum of once each week the establishment operates until one series of 13 tests meets the criteria set forth in paragraph (a)(5)(i) of this section.

(B) Upon the establishment's meeting requirements of paragraph (a)(2)(v)(A) of this section, weekly sampling and testing is optional, unless changes are made in establishment facilities, equipment, personnel or procedures that may affect the adequacy of existing process control measures, as determined by the establishment or FSIS. FSIS determinations that changes have been made requiring resumption of weekly testing shall be provided to the establishment in writing.

(3) *Analysis of samples.*

Laboratories may use any quantitative method for analysis of *E. coli* that is approved as an AOAC Official Method of the AOAC International (formerly the Association of Official Analytical Chemists) or approved and published by a scientific body and based on the results of a collaborative trial conducted in accordance with an internationally recognized protocol on collaborative trials and compared against the three tube Most Probable Number (MPN) method and agreeing with the 95 percent upper and lower confidence limit of the appropriate MPN index.

(4) *Recording of test results.* The establishment shall maintain accurate records of all test results, in terms of CFU/cm² of surface area sponged or excised. Results shall be recorded onto a process control chart or table showing at least the most recent 13 test results, by type of livestock

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slaughtered. Records shall be retained at the establishment for a period of 12 months and shall be made available to FSIS upon request.

(5) *Criteria for evaluation of test results.*

(i) An establishment excising samples from carcasses is operating within the criteria when the most recent *E.coli* test result does not exceed the upper limit (M), and the number of samples, if any, testing positive at levels above (m) is three or fewer out of the most recent 13 samples (n) taken, as follows:

TABLE 1—EVALUATION OF *E. COLI* TEST RESULTS

Type of Livestock	Lower limits of marginal range (m)	Upper limit of marginal range (M)	Number of samples tested (n)	Maximum number permitted in marginal range (c)
Cattle.....	Negative*.....	100	13	3
.....	CFU/cm ²	13	3
Swine.....	10		
.....	CFU/cm ²	10,000		
		CFU/cm ²		
			

***Negative is defined by the sensitivity of the method used in the baseline study with a limit of sensitivity of at least 5 cfu/cm² carcass surface area.**

(ii) Establishments sponging carcasses shall evaluate *E. coli* test results using statistical process control techniques.

(6) *Failure to meet criteria.* Test results that do not meet the criteria described in paragraph (a)(5) of this section are an indication that the establishment may not be maintaining process controls sufficient to prevent fecal contamination. FSIS shall take further action as appropriate to ensure that all applicable provisions of the law are being met.

(7) *Failure to test and record.* Inspection shall be suspended in accordance with rules of practice that will be adopted for such proceedings upon a finding by FSIS that one or more provisions of paragraphs (a) (1)–(4) of this section have not been complied with and written notice of same has been provided to the establishment.

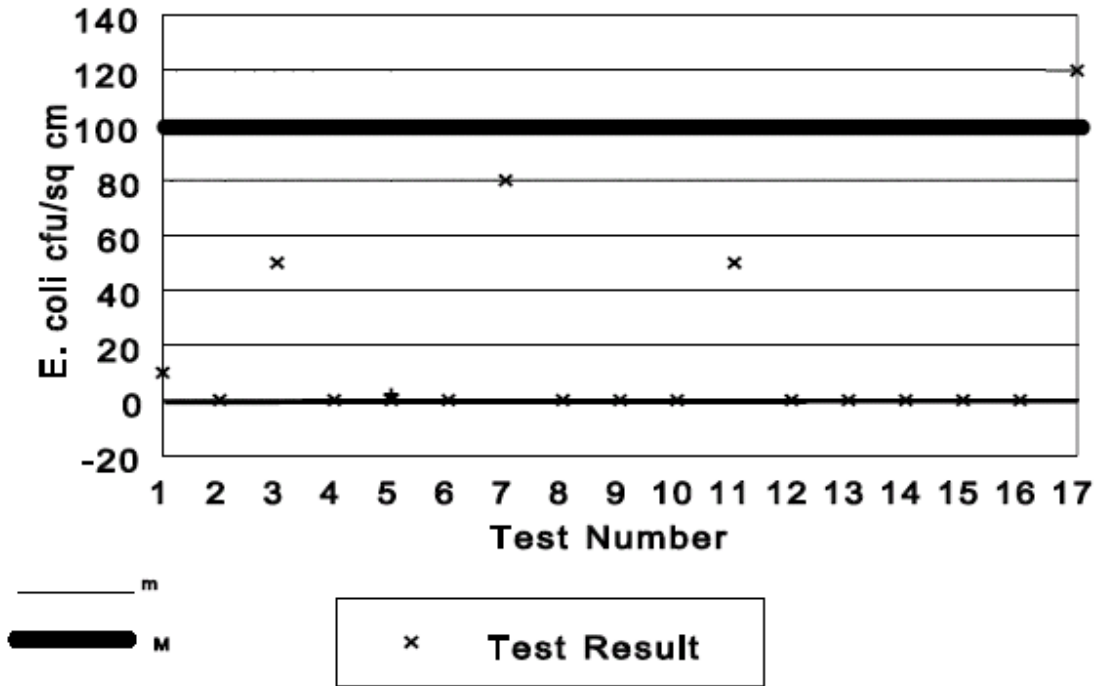
Since *E. coli* sampling test results that do not meet the criteria described, it is an indication that the establishment may not be maintaining process controls sufficient to prevent fecal contamination. Establishments are expected to react to the test results by determining where and why the process is out of control and taking action to correct the problems.

If an establishments process is out of control government inspectors are justified in increasing their frequency of verifying zero tolerance requirements and if problems are identified during the verification they must document the problems and require the establishment to take appropriate corrective actions.

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E. Coli Control Chart

Figure 4



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Cattle Excision Test Results

Test #	Date	Test Result (cfu/cm ²)	Result unacceptable?	Result marginal?	Number marginal or unacceptable in last 13	Pass/Fail?
1	10-07	10	No	Yes	1	Pass
2	10-07	Negative	No	No	1	Pass
3	10-08	50	No	Yes	2	Pass
4	10-08	Negative	No	No	2	Pass
5	10-09	Negative	No	No	2	Pass
6	10-09	Negative	No	No	2	Pass
7	10-10	80	No	Yes	3	Pass
8	10-10	Negative	No	No	3	Pass
9	10-11	Negative	No	No	3	Pass
10	10-11	Negative	No	No	3	Pass
11	10-14	50	No	Yes	4	Fail
12	10-14	Negative	No	No	4	Fail
13	10-15	Negative	No	No	4	Fail
14	10-15	Negative	No	No	3	Pass
15	10-16	Negative	No	No	3	Pass
16	10-16	Negative	No	No	2	Pass
17	10-17	120	Yes	No	3	Fail

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Government inspectors verify that the establishment has developed a written *E. coli* testing procedure and that the procedure includes all of the pertinent information. There are some questions that may be useful in the verification.

1. Is the establishment collecting samples from the species that are slaughtered in the greatest number?
2. Is the establishment collecting their samples at the required location in the process?
3. Is the establishment using the sponge or excising tissue method?
4. If the establishment is using the sponge method, do they have a statistical process control chart established?
5. Is the establishment using the correct frequency for sample collection?
6. Does the procedure include a random sample selection and is the establishment following that procedure?
7. Is the laboratory using an AOAC Official Method?
8. Does the establishment have a recordkeeping system that includes either a chart or table to track the latest 13 samples?
9. Does the charting system include the little m and big M criteria?