**Section 1422.APPENDIX A Initial Efficacy Test Procedures**

All PIMW treatment units must demonstrate that the infectious potential has been eliminated by using an Initial Efficacy Test in this Appendix.

a) This Option 1 is for a treatment unit that compromises the integrity of the container of test microorganisms (e.g., grinding followed by chemical disinfection).

1) The purpose of this Phase 1 is to determine the dilution of each test microorganism from the treatment unit for each challenge load (Types A through C) identified in Appendix A, Table C.

A) Prepare and sterilize by autoclaving, two challenge loads of Type A as identified in Appendix A, Table C. Reserve one challenge load for Phase 2.

B) Process each test microorganism in separate runs through the treatment unit. Prior to each run, the number of viable test microorganisms in each container must be determined using applicable manufacturer's recommendations and Standard Methods for the Examination of Water and Wastewater (see 35 Ill. Adm. Code 1420.103).

C) Process the PIMW within 30 minutes after introducing the container of test microorganisms into the treatment unit.

D) Process the container of test microorganisms and challenge loads together without the physical or chemical agents designed to kill the test microorganisms. For example, in treatment units that use a chemical disinfectant, an equal volume of liquid (e.g., sterile saline solution (0.9%, volume/volume), phosphate buffer solution, tap water) must be substituted in place of the chemical disinfectant.

E) Take a minimum of five representative grab samples from the processed residue of each challenge load in compliance with Test Methods for Evaluating Solid Waste, Physical/Chemical Methods (SW-846) (see 35 Ill. Adm. Code 1420.103). Determine the number of viable test microorganisms in each grab sample using applicable manufacturer's recommendations and Standard Methods for the Examination of Water and Wastewater (see 35 Ill. Adm. Code 1420.103).

F) Calculate the effect of dilution for the treatment unit as follows:

SA = Log NoA - Log N1A; where Log N1A ≥ 6

where: SA is the log of the number of viable test microorganisms (CFU/gram of waste solids and PFU/gram of waste solids) that were not recovered after processing challenge load Type A.

NoA is the number of viable test microorganisms (CFU/gram of waste solids and PFU/gram of waste solids) introduced into the treatment unit for challenge load Type A.

N1A is the number of viable test microorganisms (CFU/gram of waste solids and PFU/gram of waste solids) remaining in the processed residue for challenge load Type A.

If Log N1A is less than 6, then the number of viable test microorganisms introduced into the treatment unit must be increased and steps (A) through (F) in Phase 1 must be repeated until Log N1A is ≥ 6. NoA is the inoculum size for challenge load Type A in Phase 2 below.

G) Repeat steps (A) through (F) in Phase 1 for challenge loads of PIMW for Types B and C identified in Appendix A, Table C to determine the effect of dilution (SB and SC, respectively).

2) The purpose of this Phase 2 is to determine the log kill of each test microorganism in each challenge load (Types A through C) identified in Appendix A, Table C.

A) Using the inoculum size (NoA) determined in Phase 1 above, repeat Phase 1 steps (A) through (E) under the same operating parameters, except that the physical and chemical agents designed to kill the test microorganisms must be used.

B) Calculate the effectiveness of the treatment unit by subtracting the log of viable cells after treatment from the log of viable cells introduced into the treatment unit as the inoculum, as follows:

LA = Log NoA - SA - Log N2A ≥ 6

where: LA is the log kill of the test microorganisms (CFU/gram of waste solids and PFU/gram of waste solids) after treatment in the challenge load Type A.

NoA is the number of viable test microorganisms (CFU/gram of waste solids and PFU/gram of waste solids) introduced into the treatment unit as the inoculum for challenge load Type A as determined in Phase 1 above.

SA is the log of the number of viable test microorganisms (CFU/gram of waste solids and PFU/gram of waste solids) that were not recovered after processing the challenge load Type A in Phase 1 above.

N2A is the number of viable test microorganisms (CFU/gram of waste solids and PFU/gram of waste solids) remaining in the treated residue for challenge load Type A.

C) Repeat the steps in subsections (a)(2)(A) and (B) in Phase 2 for challenge loads Types B and C identified in Appendix A, Table C to determine the effectiveness of the treatment unit (LB and LC, respectively).

b) This Option 2 is for a treatment unit that maintains the integrity of the container of test microorganisms (e.g., autoclaves).

1) Place one microbiological indicator assay containing one of the test microorganisms at numbers greater than 1,000,000 in a sealed container that remains intact during treatment. The inside diameter of the container must be no larger than required to contain the assay vials. The vials must only contain the test microorganisms.

2) Place the container of test microorganisms within a Type A challenge load as identified in Appendix A, Table C.

3) Calculate the effectiveness of the treatment unit by subtracting the log of viable cells after treatment from the log of viable cells introduced into the treatment unit as the inoculum, as follows:

LA = Log No - Log N2A ≥ 6

where: LA is the log kill of the test microorganisms (CFU and PFU) after treatment in challenge load Type A.

No is the number of viable test microorganisms (CFU and PFU) introduced into the treatment unit as the inoculum.

N2A is the number of viable test microorganisms (CFU and PFU) remaining after treatment in challenge load Type A.

4) Repeat steps (b)(1) through (3) in this option for challenge loads Types B and C identified in Appendix A, Table C to determine the effectiveness of the treatment unit (LB and LC, respectively).

c) This Option 3 is for a treatment unit that uses thermal treatment and maintains the integrity of the container of indicator microorganism spores (e.g., autoclaves and incinerators).

1) Place one microbiological indicator assay containing at least 1,000,000 spores of one of the indicator microorganisms listed in Appendix A, Table B in a sealed container that remains intact during treatment. The inside diameter of the container must be no larger than required to contain the assay vials. The vial must contain only the indicator microorganism vial.

2) Place the container of indicator microorganisms within a Type A challenge load as identified in Appendix A, Table C.

3) Calculate the effectiveness of the treatment unit by subtracting the log of viable cells after treatment from the log of viable cells introduced into the treatment unit as the inoculum, as follows:

LA = Log No - Log N2A ≥ 6

where: LA is the log kill of the viable indicator microorganisms (CFU) after treatment in challenge load Type A.

No is the number of viable indicator microorganisms (CFU) introduced into the treatment unit as the inoculum.

N2A is the number of viable indicator microorganisms (CFU) remaining after treatment in challenge load Type A.

4) Repeat steps (b)(1) through (3) in this option for challenge loads Types B and C identified in Appendix A, Table C to determine the effectiveness of the treatment unit (LB and LC, respectively).

(Source: Amended at 43 Ill. Reg. 10072, effective August 30, 2019)