AOAC Official Method 2016.07
Detection of *Listeria* Species in Select Foods and Environmental Surfaces
3M Molecular Detection Assay (MDA) 2–*Listeria* Method
First Action 2016

{Applicable to the detection of *Listeria* species in hot dogs (25 and 125 g), salmon (25 g), deli turkey (25 and 125 g), cottage cheese (25 g), vanilla ice cream (25 g), queso fresco (25 g), spinach (25 g), melon (whole), raw chicken leg pieces (25 g), raw chicken fillet (25 g); and concrete [3M Hydrated Sponge Stick with Dey–Engley (D/E) broth; 225 and 100 mL], stainless steel (3M Hydrated Sponge Stick with D/E broth; 225 mL), and plastic (3M Enviro Swab with Letheen; 10 mL) environmental samples.}

See Tables **2016.07A** and **2016.07B** for summary of results of the interlaboratory study supporting acceptance of the method.

A. Principle

The 3M Molecular Detection Assay (MDA) 2–*Listeria* method is used with the 3M Molecular Detection System (MDS) for the rapid and specific detection of *Listeria* in enriched food and food-process environmental samples. 3M MDA 2–*Listeria* uses loop-mediated isothermal amplification of unique DNA target sequences with high specificity and sensitivity, combined with bioluminescence, to detect amplification. Presumptive positive results are reported in real time, whereas negative results are displayed upon assay completion. Samples are pre-enriched in demi-Fraser (DF) broth with ferric ammonium citrate (FAC).

B. Apparatus and Reagents

Items (**a**)–(**n**), (**z**), and (**aa**) are available from 3M Food Safety. Items (**b**)–(**g**) are available as part of the 3M MDA 2–*Listeria* kit from 3M Food Safety.

(a) *3M MDS.*—Model MDS100.

(b) *3M MDA 2–Listeria reagent tubes.*—Twelve strips of eight tubes.

(c) *Lysis solution (LS) tubes.*—Twelve strips of eight tubes.

(d) *Extra caps.*—Twelve strips of eight caps.

(e) *Reagent control (RC).*—Eight reagent tubes.

(f) *Quick Start Guide.*

(g) *3M Molecular Detection Speed Loader Tray.*

(h) *3M Molecular Detection Chill Block Insert.*

(i) *3M Molecular Detection Heat Block Insert.*

(j) *3M Molecular Detection Cap/Decap Tool for reagent tubes.*

(k) *3M Molecular Detection Cap/Decap Tool for lysis tubes.*

(l) *Empty lysis tube rack.*

(m) *Empty reagent tube rack.*

(n) *DF broth.*

(o) *FAC.*—American Chemical Society grade, 5% sterilized (MP Biomedicals or equivalent).

(p) *Disposable pipet.*—Capable of 20 µL.

(q) *Multichannel (eight-channel) pipet.*—Capable of 20 µL.

(r) *Sterile filter pipet tips.*—Capable of 20 µL.

(s) *Filter Stomacher bags.*—Seward or equivalent.

(t) *Stomacher.*—Seward or equivalent.

(u) *Thermometer.*—Calibrated range to include 100 ± 1°C.

(v) *Dry block heater unit.*—Capable of maintaining 100 ± 1°C.

(w) *Incubators.*—Capable of maintaining 37 ± 1°C.

(x) *Refrigerator.*—Capable of maintaining 2–8°C. For storing 3M MDA components.

(y) *Computer.*—Compatible with the 3M Molecular Detection Instrument.

(z) *3M Enviro Swab.*—Hydrated with Letheen (3M Food Safety, Australia).

(aa) *3M Hydrated Sponge Stick with 10 mL D/E broth.*

C. General Instructions

(a) Store 3M MDA 2–*Listeria* at 2–8°C. Do not freeze. Keep the kit away from light during storage. After opening the kit, check that the foil pouch is undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should always be stored in the resealable pouch with the desiccant inside to maintain the stability of the lyophilized reagents. Store the resealed pouches at 2–8°C for no longer than 60 days. Do not use 3M MDA 2–*Listeria* past the expiration date.

(b) *Safety precautions*.—Follow all instructions carefully. Failure to do so may lead to inaccurate results.

(*1*) 3M MDA 2–*Listeria* is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than the food and beverage industries. For example, 3M has not documented this product for testing drinking water, pharmaceutical, cosmetics, clinical, or veterinary samples. 3M MDA 2–*Listeria* has not been evaluated with all possible food products, food processes, or testing protocols or with all possible strains of bacteria.

(*2*) As with all test methods, the source of enrichment medium can influence the results. 3M MDA 2–*Listeria* has only been evaluated for use with the enrichment media specified in *Apparatus and Reagents* section.

(*3*) The 3M Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy any organisms present in the sample. *Caution*: Samples that have not been properly heat-treated during the assay lysis step may be considered a potential biohazard and should not be inserted into the 3M MDS instrument.

(*4*) The user should read, understand, and follow all safety information in the instructions for the 3M MDS and 3M MDA 2–*Listeria*. Retain the safety instructions for future reference.

(*5*) To reduce risks associated with exposure to chemicals and biohazards, perform pathogen testing in a properly equipped laboratory under the control of trained personnel. Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples. Avoid contact with the contents of the enrichment media and reagent tubes after amplification. Dispose of enriched samples according to current industry standards.

(*6*) *Listeria monocytogenes* is of particular concern for pregnant women, the aged, and the infirm. It is recommended that these concerned groups avoid handling this organism. After use, the enrichment medium and the 3M MDA 2–*Listeria* tubes can potentially contain pathogenic materials. Periodically decontaminate laboratory benches and equipment (pipets, cap/decap tools, etc.) with a 1–5% (v/v, in water) household bleach or DNA-removal solution. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information and local regulations for disposal.

(*7*) To reduce the risks associated with environmental contamination, follow current industry standards for the disposal of contaminated waste.

Table 2016.07A. Summary of results for the detection of *Listeria* in deli turkey (125 g)

Method[a]	3M MDA 2–*Listeria*		
Inoculation level	Uninoculated	Low	High
Candidate-presumptive positive/total number of samples analyzed	0/132	68/132	132/132
POD_{CP}[b]	0.00 (0.00, 0.03)	0.52 (0.43, 0.60)	1.00 (0.97, 1.00)
s_r	0.00 (0.00, 0.16)	0.51 (0.45, 0.52)	0.00 (0.00, 0.16)
s_L[c]	0.00 (0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P-value[d]	1.0000	0.8762	1.0000
Candidate-confirmed positive/total number of samples analyzed	0/132	66/132	132/132
POD_{CC}[e]	0.00 (0.00, 0.03)	0.50 (0.41, 0.59)	1.00 (0.97, 1.00)
s_r	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P-value	1.0000	0.9123	1.0000
Candidate-presumptive positive that confirmed positive/total number of samples analyzed	0/132	66/132	132/132
POD_C[f]	0.00 (0.00, 0.03)	0.50 (0.41, 0.59)	1.00 (0.97, 1.00)
s_r	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P-value	1.0000	0.9123	1.0000
Positive reference samples/total number of samples analyzed	0/132	60/132	132/132
POD_R[g]	0.00 (0.00, 0.03)	0.45 (0.37, 0.54)	1.00 (0.97, 1.00)
s_r	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.00 (0.00, 0.11)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P-value	1.0000	0.9829	1.0000
$dLPOD_{C\ vs\ R}$[h,i]	0.00 (–0.03, 0.03)	0.04 (–0.08, 0.17)	0.00 (–0.03, 0.03)
$dLPOD_{CP\ vs\ CC}$[i,j]	0.00 (–0.03, 0.03)	0.02 (–0.11, 0.14)	0.00 (–0.03, 0.03)

[a] Results include 95% confidence intervals.
[b] CP = Candidate-presumptive.
[c] Among-laboratory SD.
[d] *P*-value for the homogeneity test of laboratory PODs.
[e] CC = Candidate-confirmed.
[f] C = Candidate result.
[g] R = Reference method.
[h] C vs R = Candidate versus reference.
[i] A confidence interval for dLPOD that does not contain the value 0 indicates a statistically significant difference between the two methods.
[j] CP vs CC = Candidate-presumptive versus candidate-confirmed.

D. Sample Enrichment

(a) *Foods*.—(*1*) Allow the DF broth enrichment medium (which includes FAC) to equilibrate to ambient laboratory temperature (20–25°C).

(*2*) Aseptically combine the enrichment medium and sample according to Table **2016.07C**. For all meat and highly particulate samples, the use of filter bags is recommended.

(*3*) Homogenize thoroughly by stomaching or hand mixing for 2 ± 0.2 min. Incubate at 37 ± 1°C according to Table **2016.07C**.

(b) *Environmental samples*.—(*1*) Sample-collection devices can be a sponge hydrated with a neutralizing solution to inactivate the effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge. Neutralizing solution can be D/E neutralizing broth or Letheen broth. It is recommended to sanitize the area after sampling.

Warning: Should you select to use neutralizing buffer (NB) that contains aryl sulfonate complex as the hydrating solution for the sponge, it is required to perform a 1:2 dilution (one part sample into one part sterile enrichment broth) of the enriched environmental

Table 2016.07B. Summary of the results for the detection of *Listeria* in raw chicken breast fillet (25 g)

Method[a]	3M MDA 2–*Listeria*		
Inoculation level	Uninoculated	Low	High
Candidate-presumptive positive/total number of samples analyzed	2/132	88/132	131/132
POD_{CP}[b]	0.02 (0.00, 0.06)	0.67 (0.58, 0.75)	0.99 (0.96, 1.00)
s_r	0.12 (0.11, 0.16)	0.48 (0.42, 0.52)	0.09 (0.08, 0.16)
s_L[c]	0.00 (0.00, 0.05)	0.00 (0.00, 0.18)	0.00 (0.00, 0.04)
s_R	0.12 (0.11, 0.14)	0.48 (0.43, 0.52)	0.09 (0.08, 0.10)
P-value[d]	0.5190	0.6044	0.4338
Candidate-confirmed positive/total number of samples analyzed	1/132	86/132	132/132
POD_{CC}[e]	0.01 (0.00, 0.04)	0.65 (0.57, 0.73)	1.00 (0.97, 1.00)
s_r	0.09 (0.08, 0.16)	0.48 (0.43, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.04)	0.00 (0.00, 0.18)	0.00 (0.00, 0.16)
s_R	0.09 (0.08, 0.10)	0.48 (0.43, 0.52)	0.00 (0.00, 0.23)
P-value	0.4338	0.5632	1.0000
Candidate-presumptive positive that confirmed positive/total number of samples analyzed	1/132	85/132	131/132
POD_C[f]	0.01 (0.00, 0.04)	0.64 (0.56, 0.73)	0.99 (0.96, 1.00)
s_r	0.09 (0.08, 0.16)	0.48 (0.43, 0.52)	0.09 (0.08, 0.16)
s_L	0.00 (0.00, 0.04)	0.00 (0.00, 0.18)	0.00 (0.00, 0.04)
s_R	0.09 (0.08, 0.10)	0.49 (0.43, 0.52)	0.09 (0.08, 0.10)
P-value	0.4338	0.6228	0.4338
Positive reference samples/total number of samples analyzed	0/132	64/132	132/132
POD_R[g]	0.00 (0.00, 0.03)	0.48 (0.40, 0.57)	1.00 (0.97, 1.00)
s_r	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P-value	1.0000	0.9192	1.0000
$dLPOD_{C\ vs\ R}$[h,i]	0.01 (–0.02, 0.04)	0.16 (0.04, 0.28)	–0.01 (–0.04, 0.02)
$dLPOD_{CP\ vs\ CC}$[i,j]	0.01 (–0.03, 0.05)	0.02 (–0.10, 0.13)	–0.01 (–0.04, 0.02)

[a] Results include 95% confidence intervals.

[b] CP = Candidate-presumptive.

[c] Among-laboratory SD.

[d] *P*-value for the homogeneity test of laboratory PODs.

[e] CC = Candidate-confirmed.

[f] C = Candidate result.

[g] R = Reference method.

[h] C vs R = Candidate versus reference.

[i] A confidence interval for dLPOD that does not contain the value 0 indicates a statistically significant difference between the two methods.

[j] CP vs CC = Candidate-presumptive versus candidate-confirmed.

sample before testing to reduce the risks associated with a false-negative result leading to the release of contaminated product. Another option is to transfer 10 µL NB enrichment into the LS tubes.

(*2*) The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is at least 100 cm^2 (10 × 10 cm or 4 × 4 in.). When sampling with a sponge, cover the entire area going in two directions (left to right, then up and down) or collect environmental samples following your current sampling protocol or according to guidelines from the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (8th Ed., 1998, Revision A, http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm), U.S. Department of Agriculture-Food Safety and Inspection Service *Microbiology Laboratory Guidebook*, or ISO 18593.

(*3*) Allow the DF broth enrichment medium (which includes FAC) to equilibrate to ambient laboratory temperature (20–25°C).

(*4*) Aseptically combine the enrichment medium and sample according to Table **2016.07C**.

Table 2016.07C. Enrichment protocols using DF broth at 37 ± 1°C according to AOAC PTM Certificate No. 111501[a]

Sample matrix	Sample size	Enrichment broth volume, mL	Enrichment time, h
Beef hot dogs, queso fresco, vanilla ice cream, 4% milk fat cottage cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, and cold smoked salmon	25 g	225	24–30
Raw chicken	25 g	475	28–32
Deli turkey	125 g	1125	24–30
Cantaloupe[b]	Whole melon	Enough volume to allow the melon to float	26–30
Environmental samples			
Stainless steel	1 Sponge	225	24–30
Sealed concrete	1 Sponge	100	24–30
Plastic[c]	1 Swab	10	24–30

[a] All samples for the AOAC validation were homogenized by stomaching unless otherwise noted.

[b] Homogenize sample by hand-mixing.

[c] Homogenize sample by mixing on a vortex mixer.

(*5*) Homogenize thoroughly by mixing on a vortex mixer or stomaching for 2 ± 0.2 min. Incubate at 37 ± 1°C for 24–30 h.

E. Preparation of the 3M Molecular Detection Speed Loader Tray

(a) Wet a cloth or paper towel with a 1–5% (v/v, in water) household bleach solution and wipe the 3M Molecular Detection Speed Loader Tray.

(b) Rinse the 3M Molecular Detection Speed Loader Tray with water.

(c) Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.

(d) Ensure the 3M Molecular Detection Speed Loader Tray is dry before use.

F. Specific Instructions for Validated Methods

AOAC INTERNATIONAL Performance Tested Method℠ *(PTM) 111501*.—In AOAC PTM studies, 3M MDA 2–*Listeria* was found to be an effective method for the detection of *Listeria* species. The matrixes tested in this study are shown in Table **2016.07C**. The LOD for the 3M MDA 2–*Listeria* method is 1–5 CFU per validated test portion size in Table **2016.07C**.

G. Preparation of the 3M Molecular Detection Heat Block Insert

Place the 3M Molecular Detection Heat Block Insert into the dry double-block heater unit. Turn on the dry block heater unit and set the temperature to allow the 3M Molecular Detection Heat Block Insert to reach and maintain a temperature of 100 ± 1°C.

Note: Depending on the heater unit, allow approximately 30 min for the 3M Molecular Detection Heat Block Insert to reach temperature. Using an appropriate calibrated thermometer (e.g., a partial-immersion or digital thermocouple thermometer, but not a total-immersion thermometer) placed in the designated location, verify that the 3M Molecular Detection Heat Block Insert is at 100 ± 1°C.

H. Preparation of the 3M Molecular Detection Instrument

(a) Launch the 3M Molecular Detection Software and log in.

(b) Turn on the 3M Molecular Detection Instrument.

(c) Create or edit a run with data for each sample. Refer to the 3M MDS User Manual for details.

Note: The 3M Molecular Detection Instrument must reach and maintain a temperature of 60°C before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 min and is indicated by an orange light on the instrument's status bar. When the instrument is ready to start a run, the light on the status bar will turn green.

I. Lysis

(a) Allow the LS tubes to warm up by setting the rack at room temperature (20–25°C) overnight (16–18 h). Alternatives to equilibrate the LS tubes to room temperature are to set the LS tubes on the laboratory bench for at least 2 h, incubate the LS tubes in a 37 ± 1°C incubator for 1 h, or to place the LS tubes in a dry double-block heater for 30 s at 100 ± 1°C.

(b) Invert the capped tubes to mix the content. Proceed to the next step within 4 h.

(c) Remove the enrichment broth from the incubator.

(d) One LS tube is required for each sample and the negative control (NC; sterile enrichment medium) sample.

(*1*) LS tube strips can be cut to the desired LS tube number. Select the number of individual LS tubes or eight-tube strips needed. Place the LS tubes in an empty rack.

(*2*) To avoid cross-contamination, decap one LS tube strip at a time and use a new pipet tip for each transfer step.

(*3*) Transfer the enriched sample to the LS tubes specifically in the following order: Transfer each enriched sample into individual LS tube first. Then transfer the NC last.

(*4*) Use the 3M Molecular Detection Cap/Decap Tool for lysis tubes to decap one LS tube strip one strip at a time.

(*5*) Discard the LS tube cap. If lysate will be retained for a retest, place the caps in a clean container for reapplication after lysis.

(*6*) Transfer 20 µL sample into an LS tube.

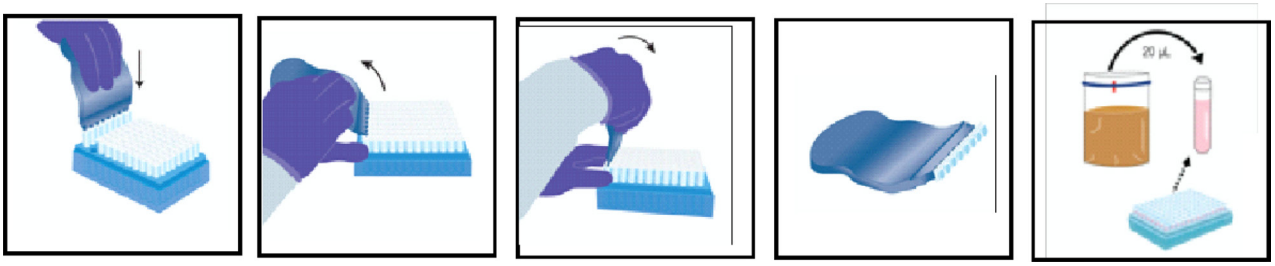

Figure 2016.07A.

Figure 2016.07B.

(e) Repeat step (**d**)(*2*) until each individual sample has been added to a corresponding LS tube in the strip as illustrated in Figure **2016.07A**.

(f) Repeat steps (**d**)(*1*)–(*6*) as needed for the number of samples to be tested. When all samples have been transferred, then transfer 20 µL NC into an LS tube. Do not recap the tubes.

(g) Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100 ± 1°C. Place the rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ± 1 min. During heating, the LS solution will change from pink (cool) to yellow (hot).

(h) Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert for a minimum of 5 min and a maximum of 10 min. The 3M Molecular Chill Block Insert, used at ambient temperature (20–25°C) without the Molecular Detection Chill Block Tray, should sit directly on the laboratory bench. When cool, the color of the LS will revert to pink.

(i) Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.

J. Amplification

(a) *One reagent tube is required for each sample and NC.*—(*1*) Reagent tubes strips can be cut to the desired tube number. Select the number of individual reagent tubes or eight-tube strips needed.

(*2*) Place the reagent tubes in an empty rack.

(*3*) Avoid disturbing the reagent pellets in the bottom of the tubes.

(b) Select one RC tube and place it in the rack.

(c) To avoid cross-contamination, decap one reagent tube strip at a time and use a new pipet tip for each transfer step.

(d) Transfer lysate to the reagent tubes and RC tube specifically in the following order. Transfer each sample lysate into individual reagent tubes first followed by the NC. Hydrate the RC tube last.

(*1*) Use the 3M Molecular Detection Cap/Decap Tool for reagent tubes to decap the reagent tubes one tube strip at a time. Discard the cap.

(*2*) Transfer 20 µL sample lysate from the upper half of the liquid (to avoid precipitate) in the LS tube into corresponding reagent tube. Dispense the lysate at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.

(*3*) Repeat step (**d**)(*2*) until the individual sample lysate has been added to a corresponding reagent tube in the strip.

(*4*) Cover the reagent tubes with the extra cap provided and use the rounded side of the 3M Molecular Detection Cap/Decap Tool for reagent tubes to apply pressure in a back-and-forth motion, ensuring that the cap is tightly applied.

(*5*) Repeat steps (**d**)(*1*)–(*4*) as needed for the number of samples to be tested.

(*6*) When all sample lysates have been transferred, repeat steps (**d**)(*1*)–(*4*) to transfer 20 µL NC lysate into a reagent tube.

(*7*) Transfer 20 µL NC lysate into an RC tube. Dispense the lysate at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.

(e) Load the capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray (*see* Figure **2016.07B**). Close and latch the 3M Molecular Detection Speed Loader Tray lid.

(f) Review and confirm the configured run in the 3M Molecular Detection Software.

(g) Click the Start button in the software and select the instrument for use. The selected instrument's lid will automatically open.

(h) Place the 3M Molecular Detection Speed Loader Tray into the 3M MDS instrument and close the lid to start the assay. Results are provided within 75 min, although positives may be detected sooner.

(i) After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from the 3M Molecular Detection Instrument and dispose of the tubes by soaking them in a 1–5% (v/v, in water) household bleach solution for 1 h away from the assay-preparation area.

Note: To minimize the risk of false-positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes the RC, reagent, and matrix control tubes. Always dispose of sealed reagent tubes by soaking them in a 1–5% (v/v in water) household bleach solution for 1 h away from the assay-preparation area.

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