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AOAC Official Method 2018.01
Cronobacter species in Select Foods and Environmental Surfaces
3M™ Molecular Detection Assay (MDA) 2 – Cronobacter Method
First Action 2018

[Applicable to detection of Cronobacter species in powdered infant formula with probiotics (10 and 300 g), powdered infant cereal without probiotics (10 and 300 g), lactose powder (10 g), and environmental surface sponges (stainless steel).]

See Table 2018.01 for a summary of results of the collaborative study. See additional table in J. AOAC Int. 102, 108(2019) for detailed results of the collaborative study.

A. Principle

The 3M MDA 2 – Cronobacter method is used with the 3M MDS for the rapid and specific detection of Cronobacter species in select enriched food and food process environmental samples. The 3M MDA 2 – Cronobacter uses loop-mediated isothermal amplification (LAMP) of unique DNA target sequences with high specificity and sensitivity combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time, and negative results are displayed after the assay is completed. Samples are pre-enriched in buffered peptone water (BPW)-ISO formulation.

B. Apparatus and Reagents

Items B(b)–(g) are available as the 3M MDA 2 – Cronobacter kit from 3M Food Safety (St. Paul, MN, USA).

Items B(a)–(n) and (y) are available from 3M Food Safety.

(a) 3M Molecular Detection System (MDS100).
(b) 3M Molecular Detection Assay 2 – Cronobacter 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) BPW-ISO formulation.
(o) Micropipet.—Capable of 20 μL.
(p) Multichannel (8-channel) pipet.—Capable of 20 μL.
(q) Sterile filter pipet tips.—Capable of 20 μL.
(r) Filter Stomacher® bags.—Seward or equivalent.
(s) Stomacher.—Seward or equivalent.
(t) Thermometer.—Calibrated range to include 100 ± 1°C.
(u) Dry block heater unit.—Capable of maintaining 100 ± 1°C.
(v) Incubators.—Capable of maintaining 37 ± 1°C.
(w) Refrigerator.—Capable of maintaining 2–8°C for storing the 3M MDA components.
(x) Computer.—Compatible with the 3M Molecular Detection Instrument.
(y) 3M Hydrated Sponge Stick.

C. General Instructions

(a) Store the 3M MDA 2 – Cronobacter at 2–8°C. Do not freeze. Keep kit away from light during storage. After opening kit, check that foil pouch is undamaged. If 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 stability of lyophilized reagents. Store resealed pouches at 2–8°C for no longer than 60 days. Do not use 3M MDA 2 – Cronobacter past expiration date.

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

(c) The 3M MDA 2 – Cronobacter 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. The 3M MDA 2 – Cronobacter has not been evaluated with all possible food products, food processes, testing protocols, or with all possible strains of bacteria.

(d) As with all test methods, the source of enrichment medium can influence results. The 3M MDA 2 – Cronobacter has only been evaluated for use with the enrichment media specified in the Instructions for Use section.

D. Safety Precautions

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 organisms present in the sample. 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.

The user should not exceed the recommended temperature setting on the heater or the recommended heating time.

Use an appropriate, calibrated thermometer to verify the 3M Molecular Detection Heat Block Insert temperature (e.g., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer). The thermometer must be placed in the designated location in the 3M Molecular Detection Heat Block Insert.

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

To reduce the 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.

After use, the enrichment medium and the 3M MDA 2 – Cronobacter 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 solution (5250–6500 ppm) or DNA removal solution. When testing is complete, follow current industry standards for disposal of contaminated waste. Consult Safety Data Sheet for additional information and local regulations for disposal.
To reduce the risks associated with environmental contamination, follow current industry standards for disposal of contaminated waste.

### E. Sample Enrichment

**Food matrixes.—**

(a) Allow BPW-ISO to equilibrate to ambient laboratory temperature (20–25°C) for 10 g test portions or environmental samples or to 37°C for 300 g test portions.

(b) Enrich samples following a 1:9 enrichment ratio.

(1) For example, to 10 g test portions, a 90 mL volume of BPW-ISO is added.

(2) For 300 g powdered infant formula and powdered infant cereal with probiotics, 10 mg/L Vancomycin is required to be supplemented into 2700 mL BPW-ISO.

(c) Homogenize thoroughly by blending, stomaching, vortex mixing, or hand mixing for 2 ± 0.2 min, or until all lumps are completely dissolved and the enrichment suspension is homogeneous.

(d) **Incubation.**—(1) Incubate powdered infant formula and powdered infant cereal (10 g) for 18–20 h at 37 ±1°C.

(2) Incubate powdered infant formula nonprobiotic (300 g) for 18–24 h at 37 ± 1°C.

(3) Incubate powdered infant formula and powdered infant cereal with probiotics (300 g) for 22–24 h at 37 ± 1°C.

(4) Incubate lactose (10 g) for 18–24 h at 37 ±1°C.

Environmental samples.—

(a) Sample collection devices should be a sponge-hydrated with Dey-Engley Neutralizing Broth. It is recommended to sanitize the area after sampling.

(b) The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is at least 100 cm² (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 current sampling protocol or ISO 18593:2004 guidelines.

(c) Allow BPW-ISO to equilibrate to ambient laboratory temperature (20–25°C).

(d) Enrich samples by adding a 90 mL volume BPW-ISO to a sampling sponge.

(e) Homogenize thoroughly by stomaching or hand mixing for 2 ± 0.2 min. Incubate at 37 ± 1°C for 18–24 h.

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**Table 2018.01. Summary of results for detection of Cronobacter in powdered infant formula with probiotics**

<table>
<thead>
<tr>
<th>Method</th>
<th>Inoculation level</th>
<th>3M MDA 2 – Cronobacter</th>
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<th>95% CI</th>
<th>LPOD&lt;sub&gt;s&lt;/sub&gt;</th>
<th>95% CI</th>
<th>LPOD&lt;sub&gt;r&lt;/sub&gt;</th>
<th>95% CI</th>
<th>LPOD&lt;sub&gt;L&lt;/sub&gt;</th>
<th>95% CI</th>
<th>LPOD&lt;sub&gt;R&lt;/sub&gt;</th>
<th>95% CI</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>Candidate presumptive positive/total No. of samples analyzed</td>
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<td>88/168</td>
<td>168/168</td>
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<td>0.51 (0.46, 0.52)</td>
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<td>Candidate confirmed positive/total No. of samples analyzed</td>
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<td>0.00 (0.00, 0.15)</td>
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<td>0.51 (0.46, 0.52)</td>
<td>0.00 (0.00, 0.15)</td>
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<td>0.00 (0.00, 0.21)</td>
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<tr>
<td>dLPOD (candidate presumptive vs. candidate confirmed)&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.01 (–0.10, 0.12)</td>
<td>0.00 (–0.02, 0.02)</td>
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<sup>a</sup> Results include 95% confidence intervals.

<sup>b</sup> P value = Homogeneity test of laboratory PODs.

<sup>c</sup> A confidence interval for dLPOD that does not contain the value 0 indicates a statistically significant difference between the two methods.
J. 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 place them in a dry double block heater for 30 s at 100 ± 1°C.

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

(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 desired LS tube number—Select the number of individual LS tubes or 8-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 enriched sample to LS tubes. Transfer enriched sample into individual LS tube first. Transfer each enriched sample into individual LS tube first. Transfer the NC last.

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

(5) Agitate the enrichment bag before collecting the sample from the filtered side when working with viscous samples.

(6) Transfer 20 μL sample into a LS tube (see Figure 2018.01A).

(7) Place reagent tubes in an empty rack.

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

(a) Use the 3M Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip one strip at a time.

(b) One LS tube is required for each sample and the NC sample.

(c) Transfer enriched sample to LS tubes. Transfer each enriched sample into individual LS tube first. Transfer the NC last.

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

(e) Place 20 μL sample into a LS tube (see Figure 2018.01A).

(f) Place reagent tubes in an empty rack.

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

(a) One 3M MDA 2 – Cronobacter reagent tube is required for each sample and the NC.

(1) Reagent tubes strips can be cut to desired tube number. Select the number of individual reagent tubes or 8-tube strips needed.

(2) Place reagent tubes in an empty rack.
(3) Avoid disturbing the reagent pellets from the bottom of the tubes.
(b) Select one RC tube and place in rack.
(c) To avoid cross-contamination, decap one reagent tubes strip at a time and use a new pipet tip for each transfer step.
(d) Transfer lysate to reagent tubes and RC tube. Transfer each sample lysate into individual reagent tubes first followed by the NC. Hydrate the RC tube last.
(e) Use the 3M Molecular Detection Cap/Decap Tool-Reagent to decap the reagent tubes one reagent tubes strip at a time. Discard cap.
(f) Transfer 20 μL sample lysate from the upper half of the liquid (avoid precipitate) in the LS tube into corresponding reagent tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down five times.
(1) Repeat step I(e)/(f) until each individual sample lysate has been added to a corresponding reagent tube in the strip.
(2) Cover the reagent tubes with the provided extra cap and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.
(4) Repeat I(e)/(f)–(3) as needed for the number of samples to be tested.
(5) When all sample lysates have been transferred, repeat I(e)/(f)–(3) to transfer 20 μL NC lysate into a reagent tube.
(6) Transfer 20 μL NC lysate into an RC tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down five times.
(f) Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray. See Figure 2018.01B. Close and latch the 3M Molecular Detection Speed Loader Tray lid.
(g) Review and confirm the configured run in the 3M Molecular Detection Software.

(h) Click the “Start” button in the software and select instrument for use. The selected instrument’s lid automatically opens.
(i) 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 60 min, although positives may be detected sooner.
(j) 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 in a 1–5% (v/v in water) household bleach (5250–6500 ppm) solution for 1 h and away from the assay preparation area.

Note: To minimize the risk of false positives from cross-contamination, never open reagent tubes containing amplified DNA. This includes RC, reagent, and matrix control tubes. Always dispose of sealed reagent tubes by soaking in a 1–5% (v/v in water) household bleach (5250–6500 ppm) solution for 1 h and away from the assay preparation area.

Reference: [J. AOAC Int. 102, 108(2019)]
DOI: https://doi.org/10.5740/jaoacint.18-0233

Posted: October 2018, February 2019