



 (EN) **Molecular Detection Assay 2 - *Campylobacter***

2

## Product Instructions

# Molecular Detection Assay 2 - *Campylobacter*

### Product Description and Intended Use

The 3M™ Molecular Detection Assay 2 - *Campylobacter* is used with the 3M™ Molecular Detection System for the rapid and specific detection of *Campylobacter jejuni*, *Campylobacter lari* and *Campylobacter coli* in enriched foods and food process environmental samples.

The 3M Molecular Detection Assay uses loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations <sup>(1,2)</sup>.

The 3M Molecular Detection Assay 2 - *Campylobacter* 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 food or beverage. For example, 3M has not documented this product for testing pharmaceutical, cosmetics, clinical or veterinary samples. The 3M Molecular Detection Assay 2 - *Campylobacter* has not been evaluated with all possible food products, food processes, testing protocols or with all possible strains of bacteria.

**As with all test methods, the source, formulation and quality of enrichment medium can influence the results.** Factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may also influence results. 3M recommends evaluation of the method including enrichment medium, in the user's environment using a sufficient number of samples with particular foods and microbial challenges to ensure that the method meets the user's criteria.

3M has evaluated the 3M Molecular Detection Assay 2 - *Campylobacter* with the 3M™ *Campylobacter* Enrichment Broth and blood-free Bolton Enrichment Broth.

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 Molecular Detection Instrument.

3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

The 3M Molecular Detection Assay 2 - *Campylobacter* test kit contains 96 tests, described in Table 1.

**Table 1.** 3M Molecular Detection Assay Kit Components

Item	Identification	Quantity	Contents	Comments
3M™ Lysis Solution (LS)	Pink solution in clear tubes	96 (12 strips of 8 tubes)	580 µL of LS per tube	Racked and ready to use
3M™ Molecular Detection Assay 2 - <i>Campylobacter</i> Reagent Tubes	Purple tubes	96 (3 pouches; containing 4 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
Extra caps	Purple caps	96 (12 strips of 8 caps)		Ready to use
3M™ Reagent Control (RC)	Clear flip-top tubes	16 (2 pouches of 8 individual tubes)	Lyophilized control DNA, amplification and detection mix	Ready to use

The Negative Control (NC), not provided in the kit, is a sterile enrichment medium, e.g., 3M *Campylobacter* Enrichment Broth. Do not use water as a NC.

A quick start guide is available at [www.3M.com/foodsafety](http://www.3M.com/foodsafety)

### Safety

The user should read, understand and follow all safety information in the instructions for the 3M Molecular Detection System and the 3M Molecular Detection Assay 2 - *Campylobacter*. Retain the safety instructions for future reference.

**⚠ WARNING:** Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage.

**NOTICE:** Indicates a potentially hazardous situation which, if not avoided, could result in property damage.

**⚠ WARNING**

**Do not use the 3M Molecular Detection Assay 2 - *Campylobacter* in the diagnosis of conditions in humans or animals.**

**The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices<sup>(3)</sup>, ISO/IEC 17025<sup>(4)</sup>, or ISO 7218<sup>(5)</sup>.**

**To reduce the risks associated with a false-negative result leading to the release of contaminated product:**

- Follow the protocol and perform the tests exactly as stated in the product instructions.
- Store the 3M Molecular Detection Assay 2 - *Campylobacter* as indicated on the package and in the product instructions.
- Always use the 3M Molecular Detection Assay 2 - *Campylobacter* by the expiration date.
- Prepare 3M™ *Campylobacter* Enrichment Broth following product instructions
- Do not autoclave 3M *Campylobacter* Enrichment Broth
- Use the 3M Molecular Detection Assay 2 - *Campylobacter* with food and environmental samples that have been validated internally or by a third party.
- Use the 3M Molecular Detection Assay 2 - *Campylobacter* only with surfaces, sanitizers, protocols and bacterial strains that have been validated internally or by a third party.
- For an environmental sample containing Neutralizing Buffer with aryl sulfonate complex, perform a 1:2 dilution before testing (1 part sample into 1 part sterile enrichment broth). Another option is to transfer 10 µL of the neutralizing buffer enrichment into the 3M Lysis Solution tubes. 3M™ Sample Handling Products which include Neutralizing Buffer with aryl sulfonate complex: BPPFV10NB, RS96010NB, RS9604NB, SSL10NB, SSL10NB2G, HS10NB, HS10NB2G, and HS2410NB2G.

**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. Incubated enrichment media and equipment or surfaces that have come into contact with incubated enrichment media may contain pathogens at levels sufficient to cause risk to human health.
- 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 local/regional/national/ regulatory standards.
- 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 Molecular Detection Instrument.

**To reduce the risks associated with cross-contamination while preparing the assay:**

- Always wear gloves (to protect the user and prevent introduction of nucleases).

**To reduce the risks associated with exposure to hot liquids:**

- Do not exceed the recommended temperature setting on heater.
- Do not exceed 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.

**NOTICE**

**To reduce the risks associated with cross-contamination while preparing the assay:**

- Change gloves prior to reagent pellet hydration.
- Use of sterile, aerosol barrier (filtered), molecular biology grade pipette tips is recommended.
- Use a new pipette tip for each sample transfer.
- Use Good Laboratory Practices to transfer the sample from the enrichment to the lysis tube. To avoid pipettor contamination, the user may choose to add an intermediate transfer step. For example, the user can transfer each enriched sample into a sterile tube.
- Use a molecular biology workstation containing germicidal lamp where available.
- Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1-5% (v:v in water) household bleach solution or DNA removal solution.

**To reduce the risks associated with a false-positive result:**

- Never open reagent tubes post amplification.
- Always dispose of the contaminated tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.
- Never autoclave reagent tubes post amplification.

Consult the Safety Data Sheet for additional information and local regulations for disposal.

If you have questions about specific applications or procedures, please visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

**User Responsibility**

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety), or contact your local 3M representative or distributor for more information.

When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, laboratory technique and the sample itself may influence results.

It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.

As with any test method, results obtained from use of any 3M Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

To help customers evaluate the method for various food matrices, 3M has developed the 3M™ Molecular Detection Matrix Control kit. When needed, use the 3M Molecular Detection Matrix Control (MC) to determine if the matrix has the ability to impact the 3M Molecular Detection Assay 2 - *Campylobacter* results. Test several Samples, representative of the matrix, i.e. samples obtained from different origin, during any validation period when adopting the 3M method or when testing new or unknown matrices or matrices that have undergone raw material or process changes.

A matrix can be defined as a type of product with intrinsic properties such as composition and process. Differences between matrices may be as simple as the effects caused by differences in their processing or presentation for example, raw versus pasteurized; fresh versus dried, etc.

**Limitation of Warranties / Limited Remedy**

EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its authorized distributor will, at its option, replace or refund the purchase price of the product. These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery of any suspected defects in a product and return it to 3M. Please call Customer Service (1-800-328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods Authorization.

**Limitation of 3M Liability**

3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase price of the product alleged to be defective.

**Storage and Disposal**

Store the 3M Molecular Detection Assay 2 - *Campylobacter* at 2-8°C (35-47°F). Do not freeze. Keep 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 re-sealable pouch with the desiccant inside to maintain stability of the lyophilized reagents. Store resealed pouches at 2-8°C (35-47°F) for no longer than 90 days.

Do not use 3M Molecular Detection Assay 2 - *Campylobacter* past the expiration date. Expiration date and lot number are noted on the outside label of the box. After use, the enrichment medium and the 3M Molecular Detection Assay 2 - *Campylobacter* tubes can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Safety Data Sheet for additional information and local regulations for disposal.

## Instructions for Use

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

Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1-5% (v:v in water) household bleach solution or DNA removal solution.

The user should complete the 3M Molecular Detection System operator qualification (OQ) training, as described in the “Installation Qualification (IQ) / Operational Qualification (OQ) Protocols and Instructions for 3M Molecular Detection System” document<sup>(6)</sup>.

### Media Preparation

Prepare 3M™ *Campylobacter* Enrichment Broth (CE250) following product instructions. **Do not autoclave medium before use.** Use the prepared medium within 24 hours of preparation. Store prepared broth at 2-8°C<sup>(7)</sup> protected from light if it will not be immediately used after preparation. Ensure media is tempered to 20-30°C before use.

### Sample Collection

**3M *Campylobacter* Enrichment Broth should not be used for bird rinsing or transport media.** Collect and transport samples following your established sample collection procedures.

### Sample Enrichment

Table 2 present guidance for general enrichment protocols for food and environmental samples.

It is the user’s responsibility to validate alternate sampling protocols or dilution ratios to ensure this test method meets the user’s criteria.

### Sample Preparation

#### a. Carcass rinses and raw poultry part rinses

1. Rinse one eviscerated raw poultry carcass with 400 mL of buffered peptone water (BPW) for one minute. If rinsing raw poultry parts, rinse 1.8 to 2 Kg (4 lb ± 10%) of bird parts with 400 mL of BPW<sup>(1,8)</sup>.
2. For carcass and raw poultry parts, allow excess liquid to drip before rinsing the sample to avoid transferring excess processing liquid in the sample bag<sup>(8)</sup>.
3. For poultry that have been treated with Cetylpyridinium chloride (CPC), it is necessary to add 5 mL per L of Polysorbate 80 (IUPAC: Polyoxyethylene (20) sorbitan monooleate; CAS 9005-65-6) to the prepared 3M *Campylobacter* Enrichment Broth. Polysorbate 80 can be added to water before sterilizing to facilitate dissolution or it can be added directly to sterile water before preparing 3M *Campylobacter* Enrichment Broth.
4. Aseptically transfer 30 mL of rinse to a sterile bag and add 30 mL of 3M *Campylobacter* Enrichment Broth.

#### b. Carcass sponge

1. Sponges should be hydrated before taking the sample with up to 25 mL of BPW before taking the sample<sup>(1)</sup>. If transporting the samples ensure that the bag is roll down and kept at 2-8°C.
2. Swab poultry carcass or collect sample with sponge.
3. Place swab into a sterile bag and add 25 mL of 3M *Campylobacter* Enrichment Broth. Ensure that the swab or sponge is covered by the enrichment media.

#### c. Raw poultry products

1. Aseptically weight 325 ± 32.5 g of sample and place into a sterile bag. Add 1625 ± 32.5 mL of BPW to raw poultry product. To disperse clumps, mix thoroughly by brief hand massaging.
2. After mixing, add 30 mL of the raw poultry product mixture to a sterile bag and then add 30 mL of 3M *Campylobacter* Enrichment Broth and mix thoroughly.

#### d. Raw and ready-to-eat meat

1. Aseptically weight 25 g of sample and place into a sterile bag. A filter bag is recommended to facilitate sampling.
2. Add 225 mL of 3M *Campylobacter* Enrichment Broth.
3. Massage by hand to break up clumps, avoid creating bubbles when mixing. Do not process the bag by stomaching or blending.

#### e. Primary production boot swabs

1. Collect sample with boot swabs or socks following your established sampling collection procedures.
2. Place ONE sock into a sterile bag and add 100 mL of 3M *Campylobacter* Enrichment Broth.

**f. Drag swab**

1. Collect sample with pre-moistened swab device following your established sampling collection procedures.
2. Place swab into a sterile bag and add 100 mL of 3M *Campylobacter* Enrichment Broth.

**Incubation of Enrichment**

1. Roll down the bag to minimize headspace and prevent exposure of enrichment to air. Gently massage the bag for about 10 ± 2 seconds. **Do not process the bag by stomaching or blending and avoid creating bubbles when mixing.**
2. Incubate the bag aerobically at 41.5 ± 1°C, refer to table 2 for the appropriate incubation time.

**WARNING:** Should you select to use neutralizing buffer that contains aryl sulfonate complex as the hydrating solution for the sponge, it is required to perform a 1:2 dilution (1 part sample into 1 part sterile enrichment broth) of the enriched environmental sample before testing in order to reduce the risks associated with a false-negative result leading to the release of contaminated product. Another option is to transfer 10 µL of the Neutralizing Buffer enrichment into the 3M Lysis Solution tubes.

It is the user’s responsibility to validate alternate sampling protocols or dilution ratios to ensure this test method meets the user’s criteria.

**Table 2.** General Enrichment Protocols.

Sample Matrix	Sample Size	3M <i>Campylobacter</i> Enrichment Broth (mL) <sup>(b)</sup>	Enrichment Temperature (± 1°C)	Enrichment Time (hours)	Sample Analysis Volume (µL) <sup>(c)</sup>
<ul style="list-style-type: none"> <li>• Carcass rinses<sup>(a)</sup></li> <li>• Bird part rinses<sup>(a)</sup></li> </ul>	30 mL of rinsate in BPW	30	41.5	22-26	20
<ul style="list-style-type: none"> <li>• Carcass sponge<sup>(a)</sup></li> </ul>	1 sponge pre-moistened with up to 25 mL of BPW	25	41.5	22-26	20
<ul style="list-style-type: none"> <li>• Raw meat</li> <li>• Ready-to-eat meat</li> </ul>	25 g	225	41.5	24-28	20
<ul style="list-style-type: none"> <li>• Boots swabs from primary production</li> </ul>	1 boot swab	100	41.5	22-26	20
<ul style="list-style-type: none"> <li>• Drag swab from primary production</li> </ul>	1 pre-moistened device	100	41.5	22-26	20

- (a) If birds are treated with Cetylpyridinium chloride (CPC), it is necessary to add 5 mL per L of (Polysorbate 80; IUPAC: Polyoxyethylene (20) sorbitan monooleate CAS 9005-65-6) to the prepared 3M *Campylobacter* Enrichment Broth. Polysorbate 80 can be added to water before sterilizing or it can be added to sterile water before preparing 3M *Campylobacter* Enrichment Broth.
- (b) 3M *Campylobacter* Enrichment Broth should be used within 24 h of preparation. Medium should be at ambient temperature (25-30°C) before use.
- (c) Before collecting enrichment sample for analysis, gently massage the bottom of bag. **After collecting the sample, roll down the bag to prevent exposure of the enrichment to air.** Additional sample may be required for re-testing or confirmatory steps.

**Specific Instructions for Validated Methods**

**AOAC® Performance Tested<sup>SM</sup> (PTM) Certificate #111803**



In AOAC Research Institute PTM<sup>SM</sup> studies, the 3M Molecular Detection Assay 2 - *Campylobacter* was found to be an effective method for the detection of *Campylobacter*. The matrices tested in the study are shown in Table 3.

**Table 3.** Enrichment Protocols According to AOAC PTM<sup>SM</sup> Certificate #111803.

Sample Matrix	Sample Size	3M <i>Campylobacter</i> Enrichment Broth (mL) <sup>(c)</sup>	Enrichment Temperature ( $\pm 1^\circ\text{C}$ )	Enrichment Time (hours)	Sample Analysis Volume ( $\mu\text{L}$ ) <sup>(d)</sup>
Whole carcass rinsed in 400 mL of BPW <sup>(a) (b)</sup>	30 mL of rinsate in BPW	30	41.5	22-26	20
Bird part (1.8 to 2 Kg) rinsed in 400 mL of BPW <sup>(a) (b)</sup>	30 mL of rinsate in BPW	30	41.5	22-26	20
Turkey carcass sponge <sup>(a) (b)</sup>	1 sponge pre-moistened with up to 25 mL of BPW	25	41.5	24-26	20
Raw ground poultry (325 $\pm$ 32.5 g) rinsed in 1625 $\pm$ 32.5 mL BPW <sup>(b)</sup>	30 mL of product mixture in BPW	30	41.5	24-28	20
Chicken nuggets	25 g	225	41.5	24-28	20

- (a) If birds are treated with Cetylpyridinium chloride (CPC), it is necessary to add 5 mL per L of (Polysorbate 80; IUPAC: Polyoxyethylene (20) sorbitan monooleate CAS 9005-65-6) to the prepared 3M *Campylobacter* Enrichment Broth. Polysorbate 80 can be added to water before sterilizing or it can be added to sterile water before preparing 3M *Campylobacter* Enrichment Broth.
- (b) Alternatively, this matrix can be enriched with 30 mL of 2X blood-free Bolton Enrichment Broth (BF-BEB) for  $48 \pm 2$  h at  $42 \pm 1.0^\circ\text{C}$  in microaerobic conditions. Transfer 20  $\mu\text{L}$  of sample to 3M Lysis Solution.
- (c) 3M *Campylobacter* Enrichment Broth should be used within 24 h of preparation. Medium should be at ambient temperature ( $25\text{-}30^\circ\text{C}$ ) before use.
- (d) Before collecting enrichment sample for analysis, gently massage the bottom of bag. **After collecting the sample, roll down the bag to prevent exposure of the enrichment to air.** Additional sample may be required for re-testing or confirmatory steps.

**Preparation of the 3M<sup>TM</sup> Molecular Detection Speed Loader Tray**

1. Wet a cloth or disposable towel with a 1-5% (v:v in water) household bleach solution and wipe the 3M Molecular Detection Speed Loader Tray.
2. Rinse the 3M Molecular Detection Speed Loader Tray with water.
3. Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.
4. Ensure the 3M Molecular Detection Speed Loader Tray is dry before use.

**Preparation of the 3M<sup>TM</sup> Molecular Detection Chill Block Insert**

Place the 3M Molecular Detection Chill Block Insert directly on the laboratory bench: The 3M Molecular Detection Chill Block Tray is not used. Use the block at ambient laboratory temperature ( $20 - 25^\circ\text{C}$ ).

**Preparation of the 3M<sup>TM</sup> Molecular Detection Heat Block Insert**

Place the 3M Molecular Detection Heat Block Insert in a 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  $100 \pm 1^\circ\text{C}$ .

**NOTE:** Depending on the heater unit, allow approximately 30 minutes for the 3M Molecular Detection Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial immersion thermometer, digital thermocouple thermometer, not a total immersion thermometer) placed in the designated location, verify that the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^\circ\text{C}$ .

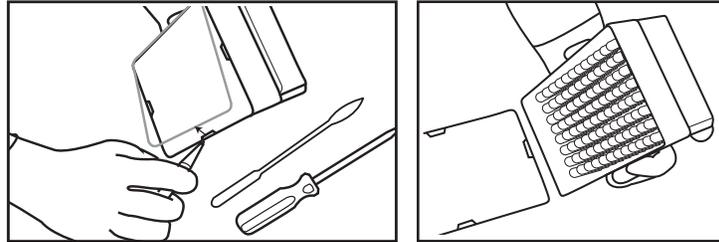
**Preparation of the 3M<sup>TM</sup> Molecular Detection Instrument**

1. Launch the 3M<sup>TM</sup> Molecular Detection Software and log in. Contact your 3M Food Safety representative to ensure you have the most updated version of the software.
2. Turn on the 3M Molecular Detection Instrument.
3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection System User Manual for details.

**NOTE:** The 3M Molecular Detection Instrument must reach Ready state before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the instrument is ready to start a run, the status bar will turn GREEN.

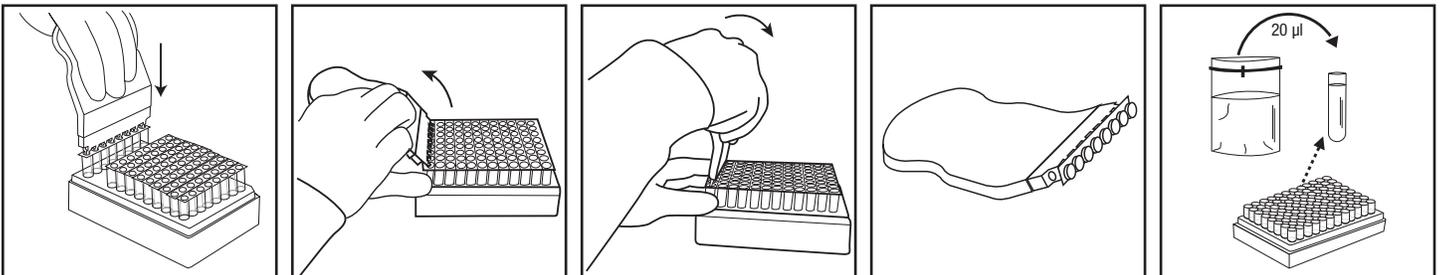
**Lysis**

Remove the bottom of 3M Lysis Solution Rack with a screwdriver before placing in the 3M Molecular Detection Heat Block Insert.



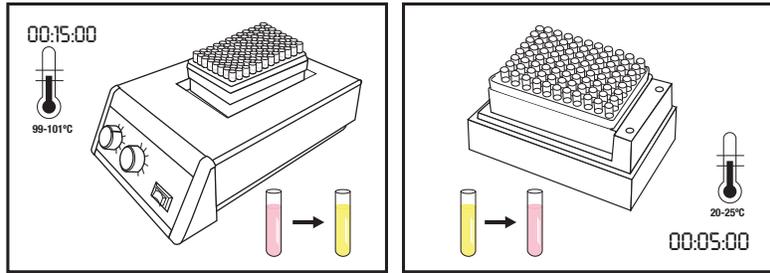
1. Allow the 3M Lysis Solution tubes to warm up by setting the rack at ambient temperature (20-25°C) overnight (16-18 hours). Alternatives to equilibrate the 3M Lysis Solution tubes to ambient temperature are to set the 3M Lysis Solution tubes on the laboratory bench for at least 2 hours, incubate the 3M Lysis Solution tubes in a 37 ± 1°C incubator for 1 hour or place them in a dry double block heater for 30 seconds at 100°C.
2. Invert the capped tubes to mix. Proceed to next step within 4 hours after inverting.
3. **Remove the enriched sample from the incubator.**
  - 3.1.1 Gently massage the bottom of the enrichment bag before transferring the sample to the 3M Lysis Solution tube.
  - 3.1.2 Additional sample may be required for re-testing or confirmatory steps. After collecting the sample, roll down the bag to minimize headspace and prevent exposure of enrichment to air. If confirmation of presumptive results is required, proceed with confirmatory steps as soon as presumptive result is obtained.
4. One 3M Lysis Solution tube is required for each sample and the NC sample (sterile enrichment medium).
  - 4.1 3M Lysis Solution tube strips can be cut to desired tube number. Select the number of tubes or 8-tube strips needed. Place the 3M Lysis Solution tubes in an empty rack.
  - 4.2 To avoid cross-contamination, decap one 3M Lysis Solution tube strip at a time and use a new pipette tip for each transfer step.
  - 4.3 Transfer enriched sample to 3M Lysis Solution tubes as described below:
 

Transfer each enriched sample into an individual 3M Lysis Solution tube **first**. Transfer the NC **last**.
  - 4.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one 3M Lysis Solution tube strip - one strip at a time.
  - 4.5 Discard the 3M Lysis Solution tube cap - If lysate will be retained for retest, place the caps into a clean container for re-application after lysis.
    - 4.5.1 For processing of retained lysate, see Appendix A.
  - 4.6 Transfer 20 µL of sample into a 3M Lysis Solution tube.
5. Repeat steps 4.4 to 4.6 as needed, for the number of samples to be tested.



6. When all samples have been transferred, transfer 20 µL of NC (sterile enrichment medium e.g. BPW) into 3M Lysis Solution tube. Do not use water as a NC.
7. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100 ± 1°C.
8. Place the uncovered rack of 3M Lysis Solution tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ± 1 minutes. During heating, the 3M Lysis Solution will change from pink (cool) to yellow (hot).
  - 8.1 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 Molecular Detection Instrument.

9. Remove the uncovered rack of 3M Lysis Solution tubes from the 3M Molecular Detection Heat Block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The 3M Molecular Detection Chill Block Insert, used at ambient temperature without the 3M™ Molecular Detection Chill Block Tray, should sit directly on the laboratory bench. When cool, the 3M Lysis Solution will revert to a pink color.
10. Remove the rack of 3M Lysis Solution tubes from the 3M Molecular Detection Chill Block Insert.

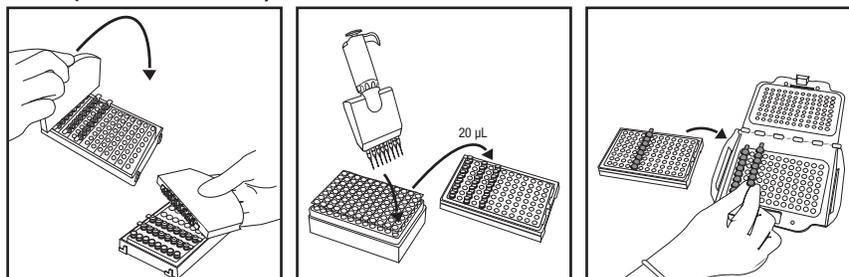


### Amplification

1. One 3M Molecular Detection Assay 2 - *Campylobacter* Reagent Tube is required for each sample and the NC.
  - 1.1 Tube strips can be cut to desired tube number. Select the number of individual 3M Molecular Detection Assay 2 - *Campylobacter* Reagent Tube or 8-tube strips needed.
  - 1.2 Place tubes in an empty rack.
  - 1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.
2. Select one 3M Reagent Control Tube and place in rack.
3. To avoid cross-contamination, decap one 3M Molecular Detection Assay 2 - *Campylobacter* Reagent Tube strip at a time and use a new pipette tip for each transfer step.
4. Transfer each of the lysate to a 3M Molecular Detection Assay 2 - *Campylobacter* Reagent Tube and 3M Reagent Control Tube as described below:

Transfer each sample lysate into individual 3M Molecular Detection Assay 2 - *Campylobacter* Reagent Tube **first** followed by the NC. Hydrate the 3M Reagent Control Tube **last**.

5. Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the 3M Molecular Detection Assay 2 - *Campylobacter* Reagent Tubes - one strip at a time. Discard cap.
  - 5.1 **Transfer 20 µL of sample lysate from the upper ½ of the liquid (avoid precipitate) in the 3M Lysis Solution Tube into corresponding 3M Molecular Detection Assay 2 - *Campylobacter* Reagent Tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.**
  - 5.2 Repeat step 5.1 until individual sample lysate has been added to a corresponding 3M Molecular Detection Assay 2 - *Campylobacter* Reagent Tube in the strip.
  - 5.3 Cover the 3M Molecular Detection Assay 2 - *Campylobacter* Reagent Tubes with the provided extra caps 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.
  - 5.4 Repeat steps 5.1 to 5.3 as needed, for the number of samples to be tested.
  - 5.5 When all sample lysates have been transferred, repeat 5.1 to 5.3 to transfer 20 µL of NC lysate into a 3M Molecular Detection Assay 2 - *Campylobacter* Reagent Tube.
  - 5.6 **Transfer 20 µL of NC lysate into a 3M Reagent Control Tube.** Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
6. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray. Close and latch the 3M Molecular Detection Speed Loader Tray lid.



7. Review and confirm the configured run in the 3M Molecular Detection Software.
8. Click the Start button in the software and select instrument for use. The selected instrument's lid automatically opens.

9. Place the 3M Molecular Detection Speed Loader Tray into the 3M Molecular Detection Instrument and close the lid to start the assay. Results are provided within 60 minutes, although positives may be detected sooner.
10. 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 solution for 1 hour and away from the assay preparation area.

**NOTICE:** To minimize the risk of false positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes 3M Molecular Detection Assay 2 - *Campylobacter* Reagent, 3M Reagent Control, and 3M Matrix Control Tubes. Always dispose of sealed reagent tubes by soaking in a 1 - 5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

## Results and Interpretation

An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analysed automatically by the software and are color-coded based on the result. A Positive or Negative result is determined by analysis of a number of unique curve parameters. Presumptive Positive results are reported in real-time while Negative and Inspect results will be displayed after the run is completed.

Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the appropriate reference method confirmation<sup>(1,2)</sup>, beginning with transfer from the primary 3M *Campylobacter* Enrichment Broth enrichment to selective *Campylobacter* plates with microaerophilic incubation, confirmation of isolates using appropriate biochemical, microscopic and serological methods. For the best maintenance of the enrichment, roll down the enrichment bag after collecting a sample.

**NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular Detection Assay 2 - *Campylobacter* amplification reagents have a “background” relative light unit (RLU) reading.

In the rare event of any unusual light output, the algorithm labels this as Inspect. 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using your preferred method or as specified by local regulations<sup>(1,2)</sup>.

## Appendix A. Protocol Interruption: Storage and re-testing of heat-treated lysates

1. To store a heat-treated lysate, re-cap the 3M Lysis Solution Tube with a clean cap (see Lysis section, 4.5)
2. Store at 2 to 8°C for up to 72 hours.
3. Prepare a stored sample for amplification by inverting 2-3 times to mix.
4. Decap the tubes.
5. Place the mixed lysate tubes on 3M Molecular Detection Heat Block Insert and heat at 100 ± 1°C for 5 ± 1 minutes.
6. Remove the rack of 3M Lysis Solution tubes from the 3M Molecular Detection Heat Block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes.
7. Continue the protocol at the **Amplification** section detailed above.

## References:

1. Microbiology Laboratory Guidebook. U. S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) Microbiology Laboratory guidebook 41.04. Isolation and identification of *Campylobacter jejuni/coli/lari* from poultry rinse, sponge and raw product samples. August 1, 2016.
2. ISO 10272-1. Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1. Detection method.
3. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.
4. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
5. ISO 7218. Microbiology of food and animal feeding stuffs - General rules for microbiological examination.
6. 3M Installation Qualification (IQ) / Operational Qualification (OQ) Protocols and Instructions for 3M Molecular Detection System. Contact your 3M Food Safety representative to obtain a copy of this document.
7. ISO 11133. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.
8. U. S. Department of Agriculture (USDA). Food Safety and Inspection Service (FSIS) Directive 10, 250.1. *Salmonella* and *Campylobacter* verification program for raw meat and poultry products. September 20, 2013.

## Explanation of Symbols

[www.3M.com/foodsafety/symbols](http://www.3M.com/foodsafety/symbols)

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34-8723-8358-2