Simplate®
Campylobacter Color Indicator

Introduction

Simplate® for *Campylobacter* (C-CI) method is used for the confirmed detection and quantification of *Campylobacter coli* and *Campylobacter jejuni* in poultry rinses and meat. The medium/sample mixture is dispensed into a Simplate® device and incubated for 48 h. The medium changes color in the presence of *Campylobacter.* The *Campylobacter* count is determined by counting the wells with changed color and referring to the Simplate® Conversion Table. Colored wells indicate presumptive positive results for *Campylobacter.* When viewed under a UV light, colored wells that do not fluoresce indicate confirmed positive results for *Campylobacter*. The Simplate® device is packaged separately.

Single Test Medium

Kit Components

100 individually-packaged dehydrated C-CI medium containers.

Multiple Test Medium

Kit Components

50 multi-test dehydrated C-CI medium containers. Each container is sufficient for 10 tests.

A. Sample Preparation

a. For Poultry Rinse

Invert the carcass to allow excess blood to drain from sample (very important). Place the carcass into an appropriate size plastic bag and add 400 mL* of Butterfield's Phosphate Buffer or Buffered Peptone Water. Rinse the interior and the exterior of the carcass thoroughly for 1 minute. After rinsing, transfer at least 40 mL of sample to a sterile specimen container. Sample may be refrigerated or held on ice for not more than 24 h. **Do not freeze.**

***Note:** For turkey rinse samples increase the volume of Butterfield's Phosphate Buffer or Buffered Peptone water to 600 mL.

b. For Poultry Meat

Allow excess blood to drain from sample. Homogenize 50 g of meat in 450 mL of Butterfield's Phosphate Buffer or Buffered Peptone Water. This is a 10-fold dilution. If an alternate sample size is specified in your testing procedure or standard, prepare a 10% weight to volume suspension.

c. If necessary, prepare 10-fold serial dilutions appropriate for the anticipated population of the sample.

B. Test Procedure (FOR SINGLE TEST)

a. Reconstitution and Sample Addition

1. **For poultry rinse 1.0 mL sample size**

   Resuspend powdered medium with 9.0 mL of sterile deionized water. Add 1.0 mL of sample and mix well. **DO NOT** count this reconstitution as a dilution.

2. **For poultry rinse 0.1 mL of sample size**

   Resuspend powdered medium with 9.9 mL of sterile deionized water. Add 0.1 mL of sample. This is an additional 10-fold dilution of sample from **A(a)**.

3. **For poultry meat 0.5 mL of sample size**

   DO NOT use 1.0 mL sample volume for analyzing poultry meat.

   Resuspend powdered medium with 9.5 mL of sterile deionized water. Add 0.5 mL of sample. This is an additional 2- fold dilution of sample from **A(b)** or **A(c)**.

4. **For poultry meat 0.1 mL of sample size**

   Resuspend powdered medium with 9.9 mL of sterile deionized water. Add 0.1 mL of sample. This is an additional 10-fold dilution of sample from **A(b)** or **A(c)**.

b. Add 0.025 mL of rifampicin additive and 0.040 mL of hemin additive to each container (see Appendix I). The final volume of liquid in the media/sample mixture container should be 10 ± 0.2 mL. Mix well.

c. Remove the lid from the Simplate® device and pour the sample/medium mixture onto the center of the plate. Immediately replace the lid.

B. Test Procedure (FOR MULTIPLE TESTS)

a. Reconstitution of C-CI medium

Empty contents of one container into 100 mL of sterile deionized water. Mix well to completely dissolve. Add 0.25 mL of rifampicin additive and 0.40 mL of hemin additive to each 100 mL of Simplate® resuspended medium (see Appendix I).

b. Sample addition

1. **For poultry rinse 1.0 mL sample size**

   Remove lid from Simplate® device. Pipette 1.0 mL of sample onto the center of the device. Overlay the sample with 9.0 mL of medium. **DO NOT** count this reconstitution as a dilution.

2. **For poultry rinse 0.1 mL of sample size**

   Remove lid from Simplate® device. Pipette 0.1 mL of sample onto the center of the device (Figure 2). Overlay the sample with 9.9 mL of medium. This is an additional 10 - fold dilution of sample from A (a).

3. **For poultry meat 0.5 mL of sample size**

   DO NOT use 1.0 mL sample volume for analyzing poultry meat.

   Remove lid from Simplate® device. Pipette 0.5 mL of sample onto the center of the device (Figure 2). Overlay the sample with 9.5 mL of medium. This is an additional 10-fold dilution of sample from A (b) or A(c).

4. **For poultry meat 0.1 mL of sample size**

   Remove lid from Simplate® device. Pipette 0.1 mL of sample onto the center of the device (Figure 2). Overlay the sample with 9.9 mL of medium. This is an additional 10-fold dilution of sample from A (b) or A(c).

c. Immediately replace the lid.

d. Gently swirl to distribute the sample/medium mixture into all the wells (Figure 3). The plate may be held with both hands and tilted slightly to help distribute the liquid into the wells.

e. If necessary, tap the Simplate® device GENTLY on a hard surface to remove any air bubbles which may have become trapped in the wells (Figure 4). DO NOT be concerned if partially filled wells are present. Wells containing partial volume of liquid will turn positive in the presence of viable *Campylobacter.*

f. Pour off excess medium by holding the lid against the plate on either side of the sponge cavity. Tip the plate toward you to allow liquid to drain into the sponge (Figure 5). Observe the background color of the wells. Background is defined as the color of the sample/medium mixture inside the wells.

g. **DO NOT** invert the Simplate® device. Incubate in the dark for 48–52 h at 42 °C in a microaerophilic (5% O2, 10% CO2, and 85% N2) environment.

Figure 1

For single test, pour sample/ medium mixture onto the center of the plate.

Figure 2

For multiple tests, pipette sample onto center of plate. Add rehydrated medium to make a final volume of 10 ±0.2 mL.

Figure 3

Cover plate, gently swirl to distribute the sample into all of the wells.

Figure 4

Tap plate GENTLY on a hard surface to remove air bubbles.

Figure 5

Holding the cover, tip the plate toward you to allow liquid to drain.

C. Reading and Interpretation of Results

a. After incubation, observe red-colored liquid in the wells. Count all the red wells (the intensity of the color in the wells may vary). Some wells may be encircled by a red ring or contain red particles at the bottom of the well. This is acceptable. All red wells are presumptive positive for *Campylobacter*.

b. Count the number of red wells that fluoresce blue by holding UV light (366 nm wavelength) approximately 5 cm (2 inches) above the Simplate® device. Occasionally, there may be wells that are not red but fluoresce. DO NOT count these since they are not presumptive for *Campylobacter*.

NOTE: Some meat samples can cause the medium to fluoresce slightly. If this occurs, repeat test with a lower sample volume or count only the fluorescent wells where the intensity of the fluorescent reaction significantly exceeds the background reaction.

c. To determine the population, perform the following calculations:

1. Count the number of red wells on the plate.
2. Subtract the number of fluorescent red wells from step C (b) (fluorescent wells are confirmed negative results) to determine confirmed positive results for Campylobacter.
3. Use the Simplate® Conversion Table to determine the total number of microorganisms per plate.

d. To calculate the number of **microorganisms per g (mL),** multiply the count in **C(c)** 3 by the appropriate dilution factor: (see sections **A** and **B**).

D. Product and Storage Information

a. Store dehydrated medium away from direct light between 2–30 °C.

b. DO NOT use expired medium.

c. Store containers of reconstituted medium without rifampicin and hemin in the dark between 15 and 25 °C and use within 12 h.

d. Handle and dispose of incubated medium in a decontamination container and sterilize according to Good Laboratory Practices.

Appendix I: Preparation of Simplate® Campylobacter - CI Additives

Rifampicin Additive

Add 0.25 g of rifampicin slowly into 60–80 mL of ethanol, swirling repeatedly. Powder may not dissolve until the addition of water. Add sterile deionized water to a final volume of 100 mL and continue swirling until powder is completely dissolved. Store up to 1 year at -20 °C.

Hemin Additive

Add 10 mL of a 1N NaOH solution to 90 mL of deionized water. Add 0.5 g hemin to liquid and mix until powder is completely dissolved. Filter sterilize. Alternatively, the solution can be autoclaved for 15 min at 121 °C. Store up to 3 months at 4 °C.

Manufacturing Entity

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