

# Galaxy<sup>®</sup> 170 R CO<sub>2</sub> Incubator High-Temperature Disinfection (HTD) Evaluation Using Biologic Indicator Strips

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## Introduction

High-Temperature Disinfection (HTD) is an integrated function in the New Brunswick™ S41i CO<sub>2</sub> Incubator Shaker and a factory-installed option on Galaxy incubator models. The HTD process was designed for easy and reliable protection against contaminations, which are major concerns of incubator-based cell culture experiments. The HTD cycle reaches and maintains 120 °C for a period of four hours.

This protocol used the Galaxy 170 R incubator as an example, but data may be used to represent the S41i since the HTD processes are identical. The tests used biologic indicator strips to determine if the HTD cycle could kill bacteria strains including *Bacillus stearothermophilus* and *Bacillus atrophaeus*. The HTD test strips were placed in 18 different locations inside the incubator chamber.

## Materials and Methods

### Required equipment and accessories:

- > Galaxy 170 R CO<sub>2</sub> Incubator with High-Temperature Disinfection Option (Eppendorf)
- > Oxygen Sensor Removal Tool (Eppendorf, P0628-605)
- > Autoclave (Steris<sup>®</sup>, SV-16-H Prevac Steam Sterilizer)
- > *Geobacillus stearothermophilus* strip (Steris, Spordex<sup>®</sup> Bacterial Spore Strip, > 1.0 × 10<sup>6</sup> per strip)
- > *Bacillus atrophaeus* strip (Steris, Spordex Bacterial Spore Strip, > 1.0 × 10<sup>6</sup> per strip)
- > Tryptic Soy Broth (TSB, BD, 211822)
- > 50 mL conical tubes (Eppendorf, 0030122178)
- > Scotch<sup>®</sup> Glass Cloth Electrical Class “B” insulation tape (3M<sup>®</sup>, 27-34 in x 66 ft)

**Positive Control:** Incubate untreated strips according to manufacturer’s instructions: 55 °C ± 2 °C (*Bacillus stearothermophilus*) or 33 °C ± 2 °C (*Bacillus atrophaeus*) for 168 hours in 50 mL conical tubes containing 30 mL of sterile TSB. Check and record observations at 24 hour intervals.

**Negative Control:** Autoclave one of each strip at 121.5 °C/19.5 psig for 40 minutes; once cooled incubate autoclaved strips according to manufacturer’s instructions: 55 °C ± 2 °C (*Bacillus stearothermophilus*) or 33 °C ± 2 °C (*Bacillus atrophaeus*) for 168 hours in 50 mL conical tubes containing 30 mL of sterile TSB. Check and record observations at 24 hour intervals.

### TSB Sterilization and Media Control:

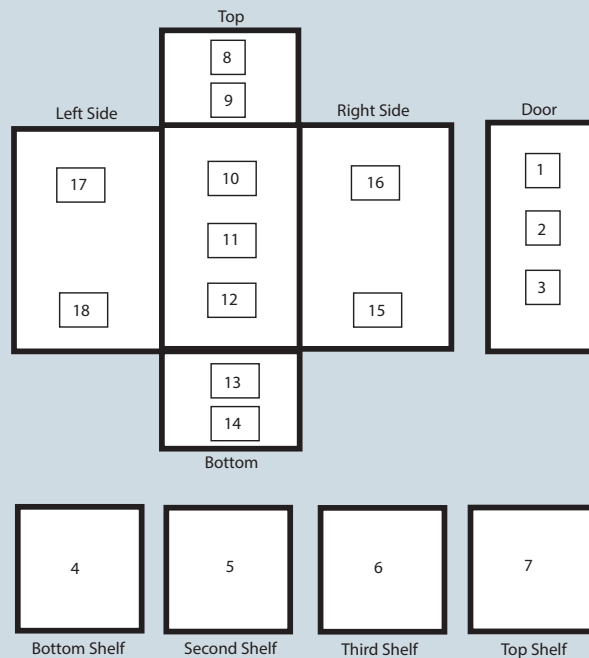
Prepare and sterilize one liter of TSB stock solution by manufacturer’s direction for aliquots.

**TSB Sterility Confirmation:** Incubate two tubes of TSB, 30 mL each; one at 55 °C ± 2 °C and one at 33 °C ± 2 °C for 168 hours in 50 mL conical tubes. Check and record observations at 24 hour intervals (if media is positive, the experiment may be compromised and should be run again to ensure that all results are accurate).

## Instructions

### Incubator Set-up:

- > Ensure that the incubator as well as the humidity tray are empty, clean and dry
- > In case an oxygen sensor is installed, remove it prior to running the HTD by following the steps below:
  - > Turn off the unit and unplug the power cord
  - > Pull the access cover off the outside wall of the incubator to gain access to the oxygen sensor
  - > Reach inside and disconnect the sensor by unplugging the connector (*Note: Be sure to grasp the white connector body, not the wire lead*)
  - > Using the sensor removal tool, unscrew the oxygen sensor by turning it counter-clockwise
- > During HTD cycle, the CO<sub>2</sub> valve is disabled; the temperature rises to 120 °C and is held steady for 4 hours; the incubator cools down to the previous temperature set point after 4 hours of heating
- > With the indicator strips secured by high-temperature tape in the designated locations, close the door
- > Press the USER menu button located on the front of the door and select DISINFECTION and press START
- > This will prompt the message: "IS CHAMBER CLEAN & DRY?"; press YES and the HTD cycle will start automatically



**Figure 1:** Location of Biologic Indicator Strips

- > When HTD is completed, turn the incubator power OFF and carefully retrieve all strips from the incubator while wearing lab gloves
- > Place and seal each strip in a labeled 50 mL conical tube and the follow-up testing should be conducted by a microbiology lab
- > In the microbiology lab, prepare and incubate the strips according to manufacturer's instructions: 55 °C ± 2 °C (*Bacillus stearothermophilus*) or 33 °C ± 2 °C (*Bacillus atrophaeus*) for 168 hours in 50 mL conical tubes containing 30 mL sterile TSB; check and record observations at 24 hour intervals

## Results and Discussion

Indicator Strip Number	Location	Hours (Approximate) P = Growth N = No Growth						
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
1	Top front glass	N	N	N	N	N	N	N
2	Middle front glass	N	N	N	N	N	N	N
3	Bottom front glass	N	N	N	N	N	N	N
4	Bottom shelf middle	N	N	N	N	N	N	N
5	Second bottom shelf middle	N	N	N	N	N	N	N
6	Third shelf middle	N	N	N	N	N	N	N
7	Top shelf middle	N	N	N	N	N	N	N
8	Front top of chamber	N	N	N	N	N	N	N
9	Back top of chamber	N	N	N	N	N	N	N
10	Top back of chamber	N	N	N	N	N	N	N
11	Middle back of chamber	N	N	N	N	N	N	N
12	Bottom back of chamber	N	N	N	N	N	N	N
13	Back bottom of chamber	N	N	N	N	N	N	N
14	Front bottom of chamber	N	N	N	N	N	N	N
15	Bottom right side of chamber	N	N	N	N	N	N	N
16	Top right side of chamber	N	N	N	N	N	N	N
17	Top left side of chamber	N	N	N	N	N	N	N
18	Bottom left side of chamber	N	N	N	N	N	N	N
<b>Negative Control</b>	Autoclave killed	N	N	N	N	N	N	N
<b>Media</b>	Incubated without strip	N	N	N	N	N	N	N
<b>Positive Control</b>	Media plus untreated strip	P	P	P	P	P	P	P

**Table 1:** TSB culture results of *Bacillus stearothermophilus* strips upon completion of HTD; P = Growth Observed; N = No Growth

Indicator Strip Number	Location	Hours (Approximate) P = Growth N = No Growth						
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
1	Top front glass	N	N	N	N	N	N	N
2	Middle front glass	N	N	N	N	N	N	N
3	Bottom front glass	N	N	N	N	N	N	N
4	Bottom shelf middle	N	N	N	N	N	N	N
5	Second bottom shelf middle	N	N	N	N	N	N	N
6	Third shelf middle	N	N	N	N	N	N	N
7	Top shelf middle	N	N	N	N	N	N	N
8	Front top of chamber	N	N	N	N	N	N	N
9	Back top of chamber	N	N	N	N	N	N	N
10	Top back of chamber	N	N	N	N	N	N	N
11	Middle back of chamber	N	N	N	N	N	N	N
12	Bottom back of chamber	N	N	N	N	N	N	N
13	Back bottom of chamber	N	N	N	N	N	N	N
14	Front bottom of chamber	N	N	N	N	N	N	N
15	Bottom right side of chamber	N	N	N	N	N	N	N
16	Top right side of chamber	N	N	N	N	N	N	N
17	Top left side of chamber	N	N	N	N	N	N	N
18	Bottom left side of chamber	N	N	N	N	N	N	N
<b>Negative Control</b>	Autoclave killed	N	N	N	N	N	N	N
<b>Media</b>	Incubated without strip	N	N	N	N	N	N	N
<b>Positive Control</b>	Media plus untreated strip	P	P	P	P	P	P	P

**Table 2:** TSB culture results of *Bacillus atrophaeus* strips upon completion of HTD; P = Growth Observed; N = No Growth

The test results indicated that the HTD cycle of the Galaxy 170 R CO<sub>2</sub> incubator was able to effectively decontaminate the incubator’s chamber.

The *Geobacillus stearothermophilus* test strips chosen for this procedure were widely used for evaluation of the effectiveness of a steam sterilization cycle of an autoclave. Due to the spore strips having a documented population and corresponding kill times, they are used for validation of laboratory autoclaves. The starting mean population of bacteria per strip was 2.3 x 10<sup>6</sup> which was killed entirely through the Galaxy 170 R CO<sub>2</sub> incubator’s HTD cycle.

The *Bacillus atrophaeus* test strips chosen for the second part of this test were typically used for evaluation of the effectiveness of a “dry heat” cycle of an autoclave. The starting mean population of bacteria per strip was 3.1 x 10<sup>6</sup> which was also killed entirely through the incubator’s HTD cycle. The results presented within this protocol clearly demonstrated the effectiveness of utilizing HTD cycle to achieve bacteria decontamination for the Galaxy 170 R CO<sub>2</sub> Incubator.

**Ordering information**

Description	Order no. International	Order no. North America
New Brunswick™ S41i CO <sub>2</sub> Incubator Shaker	S41I230011	S41I120010
Galaxy® 170 R CO <sub>2</sub> Incubator with HTD	CO17311001	CO17211005
Galaxy® 170 S CO <sub>2</sub> Incubator with HTD	CO17111001	CO17011005

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