Efficiency of various incubator decontamination methods
A comparative Analysis between high heat & H₂O₂ decontamination methods
Common Contamination in the Lab
Bacteria, Viruses, Mold and Yeast, Mycoplasma

Mycoplasma Contamination:
A serious threat to cell culture samples

Most common form of cell culture contaminant; in the US accounts for > 15% of cultures.
Smallest free living organism (0.2-0.3μm). Approximately 180 different species exist.
No cell wall cannot be seen under phase contrast microscopy.
Affects virtually every aspect of cell behavior and growth, even micro-arrays.

Mycoplasma Prevalence:
Independent test document high incidence

<table>
<thead>
<tr>
<th># CULTURES TESTED</th>
<th># POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDA</td>
<td>20,000</td>
</tr>
<tr>
<td>BIONIQUE TESTING LABS</td>
<td>11,000</td>
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<tr>
<td>MICROBIOLOGICAL ASSOCIATES</td>
<td>2,863</td>
</tr>
<tr>
<td>ATCC</td>
<td>5,362</td>
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</tbody>
</table>

Methods of Decontamination in Incubators

High Heat
- Generally can run for several hours up to a day
- Different manufacturers have different approaches: Moist heat at 90°C to dry heat at 120°C

H₂O₂ Vapor diluted to 6% concentration
- All interior components remain inside the incubator to be decontaminated concurrently with the interior surfaces
- H₂O₂ decontamination is effective against every known contaminant

UV Light
- Well-known procedure for disinfection of bio-safety cabinets used in cell culture laboratories
- Can be included in several incubator models for further active background contamination control

Sources:
How efficient are the available methods for decontaminating cell culture incubators?

Sample micro-organisms for common contaminant types were tested:

<table>
<thead>
<tr>
<th>MICROBE</th>
<th>TYPE</th>
<th>OXYGEN REQUIREMENT</th>
<th>GRAM REACTION/CELL MORPHOLOGY</th>
<th>PHYSIO-CHEMICAL RESISTANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma orale</td>
<td>Bacteria</td>
<td>Facultative anaerobe</td>
<td>Gram Negative/Micrococcal</td>
<td>High</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Bacteria</td>
<td>Facultative anaerobe</td>
<td>Gram Positive/Cocci</td>
<td>Low</td>
</tr>
<tr>
<td>Acholeplasma Laidlawi</td>
<td>Bacteria</td>
<td>Facultative anaerobe</td>
<td>Gram Negative/Micrococcal</td>
<td>Medium</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Fungi</td>
<td>Facultative anaerobe</td>
<td>Yeast/Filamentous</td>
<td>Low</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>Bacteria</td>
<td>Obligate aerobe</td>
<td>Gram Positive/Rod</td>
<td>High</td>
</tr>
</tbody>
</table>

The following incubators and decontamination methods were tested:

**Panasonic**

$H_2O_2$ decontamination:
Cycle time of 2 hours with additional prep time depending on individual requirements.

**Brand A**

Dry heat decontamination at 120°C:
10-12 hours downtime with 4 hour heat cycle.

**Brand B**

Moist heat decontamination at 90°C:
25 hour downtime with 9 hour heat cycle.
Measuring the Efficiency of Each Decontamination Method

**Study Protocol**

1. Dilute each microorganism to a specific CFU
2. Inoculate sample on steel coupons
3. Place coupons on upper and lower shelves of each test incubator
4. Decontaminate each incubator according to manufacturer’s protocols
5. Rinse each decontaminated coupon to recover viable organisms
6. Plate on appropriate media to determine final viable count
7. Compare input vs. final viable counts against a non-decontaminated control
8. Calculate log reduction in viability for each microorganism and method tested

**Measuring the Effectiveness of Recovery Techniques**

- **Time control coupon**
  - Control set incubated at room temperature
  - Incubated for same duration of sterilization cycle
  - Determine recovery effectiveness/cell death over elapsed time

- **Spike recovery control**
  - Known concentration of each organism inoculated on a steel coupon
  - Grow the coupon with appropriate medium
  - Serially dilute and plate
  - Determine final viability

Measuring the Effectiveness of Recovery Techniques*

* Each spike control showed efficient recovery indicating that the techniques performed to recover organisms were effective.
Decontamination Efficiency Results:

All three incubator decontamination methods reduced bioburden, but the efficiency of each when compared to the non-decontaminated controls showed that H$_2$O$_2$ was the fastest and most effective decontamination method.

**H$_2$O$_2$ Vapor**

1. Increased Efficiency
2. 100% Kill Rate with at least a 6 Log Reduction

All of the decontamination protocols eliminated bioburden for each microorganism tested. However, H$_2$O$_2$ vapor was able to effectively reduce the same amount of bioburden with in 2 hours as compared to 10-12 hours for Brand A and 25 hours for Brand B.

**Panasonic H$_2$O$_2$ Decontamination:**

*Except B.subtilis and S.aureus*

- At least 6 log reduction for all contaminants
- 100% kill rate
- 3 hour cycle time compared to 12-24 hours for the other incubators
- Complete decontamination in the shortest period of time
**H₂O₂ vapor eliminates Mycoplasma**

**Faster than high heat**

When tested against *Mycoplasma orale*, H₂O₂ vapor decontamination produced a greater reduction in bioburden than the alternative protocols and in a significantly shorter time.

*Mycoplasma orale*

(Compared to Time Control)
**Results: Acholeplasma laidlawii**
(Compared to Time Control)

**Results: Candida albicans**
(Compared to Time Control)

**Results: Staphylococcus aureus**
(Compared to Time Control)

**Results: Bacillus subtilis**
(Compared to Time Control)

$\text{H}_2\text{O}_2\text{ vapor}$ effectively and efficiently eliminated all the bioburden for each of the test organisms in the shortest period of time.

The titers of controls for Candida albicans and Acholeplasma laidlawii decreased slightly during the tests.

For Staphylococcus aureus, it decreased for 2 hours and 10-12 hours but increased overtime for 25 hours.

For Bacillus subtilis, an increase in titer was observed between 2 hours and 10-12 hours and a decrease was observed between 10-12 hours and 25 hours.
H₂O₂ decontamination results in a drastic reduction across all contaminants.

<table>
<thead>
<tr>
<th></th>
<th>TIME CYCLE</th>
<th>LOG REDUCTION BETWEEN TIME CONTROL AND UPPER SHELF COUPON</th>
<th>LOG REDUCTION BETWEEN TIME CONTROL AND LOWER SHELF COUPON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANASONIC</td>
<td>2 hr</td>
<td>6.4</td>
<td>6.4</td>
</tr>
<tr>
<td>BRAND A</td>
<td>10-12 hr</td>
<td>6.1</td>
<td>6.1</td>
</tr>
<tr>
<td>BRAND B</td>
<td>25 hr</td>
<td>5.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Acholeplasma laidlawii</td>
<td>2 hr</td>
<td>5.3</td>
<td>5.3</td>
</tr>
<tr>
<td>BRAND A</td>
<td>10-12 hr</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>BRAND B</td>
<td>25 hr</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Mycoplasma orale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANASONIC</td>
<td>2 hr</td>
<td>6.2</td>
<td>6.2</td>
</tr>
<tr>
<td>BRAND A</td>
<td>10-12 hr</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td>BRAND B</td>
<td>25 hr</td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANASONIC</td>
<td>2 hr</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>BRAND A</td>
<td>10-12 hr</td>
<td>6.1</td>
<td>7.5</td>
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<tr>
<td>BRAND B</td>
<td>25 hr</td>
<td>6.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2 hr</td>
<td>6.6</td>
<td>6.6</td>
</tr>
<tr>
<td>BRAND A</td>
<td>10-12 hr</td>
<td>6.4</td>
<td>6.4</td>
</tr>
<tr>
<td>BRAND B</td>
<td>25 hr</td>
<td>6.6</td>
<td>6.6</td>
</tr>
</tbody>
</table>

The H₂O₂ vapor method produced a greater log reduction than alternative methods for Candida albicans, Acholeplasma laidlawii and Mycoplasma orale, based upon the resultant viable cells recovered for each corresponding time control coupon.

Although an increase in visibility was observed over time for Bacillus subtilis and Staphylococcus aureus, the H₂O₂ vapor decontamination method effectively and efficiently eliminated all bioburden for these microorganisms.

**Conclusion:**

To effectively ensure all possible contaminants are significantly reduced or eliminated, H₂O₂ vapor is the preferred method of decontaminating a cell culture incubator. Dry heat and moist heat each reduce the contamination level, but do not have the same effectiveness across the full range of common contaminants.
Conclusion:

Each decontamination method demonstrated an effective reduction in contamination levels. However, the H₂O₂ vapor method was observed to be more efficient, as it reduced the same amount of bioburden in 2 hours that dry heat did in 10-12 hours and moist heat did in 25 hours. It was also more versatile at removing the full range of contaminants tested.

4 take-home points for H₂O₂ decontamination:

Efficiency
H₂O₂ decontamination is faster and more efficient than high heat decontamination.

Increased Productivity
Cell culture productivity can be increased by using H₂O₂ vapor due to the rapid decontamination cycles.

Versatile
H₂O₂ vapor is effective on a wide variety of contaminants including Mycoplasma, ensuring that whatever your incubator may come in contact with can be eliminated.

Consistent
H₂O₂ vapor system is consistent in decontaminating the entire incubation chamber (upper and lower shelf) as well as all interior parts at once.