

**Evaluation of a decontamination strategy for a CO<sub>2</sub>  
incubator using Fertisafe and Sterile Water.  
Galaxy R CO<sub>2</sub> Incubator**

**TEST REPORT**

**Study Report Commissioned by Research Instruments Ltd**

Research Instruments Ltd.  
Falmouth, Cornwall  
,October 2007

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## I. DESCRIPTION OF THE STUDY

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- **Title:** **Evaluation of a decontamination strategy for a CO<sub>2</sub> incubator using Fertisafe and Sterile Water.**
- **Internal reference:** **Galaxy R CO<sub>2</sub> Incubator**
- **Sponsor:** **RI-MDR002**  
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- **Test periods:** *Contact:* Bill Brown  
July – October 07

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## II. AIM OF THE STUDY

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To determine whether the operating conditions of the Galaxy R CO<sub>2</sub> Incubator could maintain acceptable environmental conditions and thus be suitable to incubate mammalian cultures after a standard washing/disinfection procedure using Fertisafe and Sterile Water.

### **III. PRINCIPLE**

Performance qualification for the Galaxy R CO<sub>2</sub> Incubator consisted of carrying out tests to establish the ability to retain a good incubation environment and determine the effectiveness for up to 7 days between cleaning.

The challenge is to maintain a safe non-toxic environment for the mammalian cells while discouraging proliferation of opportunist microbes, which may gain entry to the incubator. The cabinet cannot realistically be deemed a sterile environment, with the abundance of good conditions for the microbes to proliferate 37 C, high humidity. However a reasonable sanitization protocol can lead to minimised microbial challenge without being toxic to the cultured cells.

Test challenge models were employed using culture microbes, environmentally introduced microbes and the mammalian culture medium.

Incubator design is of polished stainless steel interiors, these surfaces must remain in a good state and not become corroded or pitted. Salty deposits from Fertisafe must therefore be rinsed away with sterile water.

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#### IV. EQUIPMENT AND MATERIALS

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**a) Fertisafe: MD005/6**

**b) Fertisafe wipes:**

MPD 006 manufactured on 13/09/07.

**c) Microbial strains:**

Pseudomonas aeruginosa ATCC 15442, Don Whitley Scientific  
Bacillus subtilis var niger NCTC10073, Don Whitley Scientific  
Staphylococcus Aureus ATCC 6538, Don Whitley Scientific  
Aspergillus Niger ATCC 16404, Don Whitley Scientific  
Candida Albicans ATCC 10231, Don Whitley Scientific

The microbial strains correspond to the reference microbial strain of the CEN and AFNOR standards in force for the determination of the anti microbial activities of disinfectants.

**d) Sterile Water:**

For swabs:  
Deionised water Steam Sterilized 121°C for 15 minutes  
For Incubator:  
Baxter's water lot 06J28B29

**e) Sterile polyester wipes:**

Polyester sterile wipes.

**f) Recovering diluent:**

Maximum Recovery Diluent (Peptone Saline Diluent  
Steam Sterilized 121°C for 15 minutes

**g) Embryo handling media:**

Vitrolife G-MOPS™ plus supplemented with HSA sterile filtered SAL  $10^{-3}$  endotoxin level <0.25 EU/ml.

**h) Endoscope washer disinfectant test soil:**

HTM 2030 ref 17.34 manufactured 10/10/2007 batch 1-113

**i) Maintaining and counting medium:**

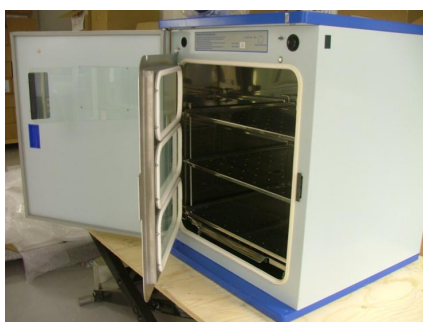
Tryptone Soy Agar  
Sabouraud Dextrose Agar

Steam Sterilized 121°C for 15 minutes

**j) Swabs for Microbiological Monitoring:**

Sterilin sterile swabs

**k) Galaxy R CO<sub>2</sub> Incubator:**



**Figure 1:** Galaxy R CO<sub>2</sub> Incubator  
(Doors open view)



**Figure 2:** Galaxy R CO<sub>2</sub> Incubator  
(Water reservoir)

➤ <b>Name:</b>	Galaxy R CO <sub>2</sub> Incubator
➤ <b>Description:</b>	Cell tissue culture incubator
➤ <b>Model:</b>	170-300
➤ <b>Serial N°:</b>	8998
➤ <b>Manufacturer:</b>	RS Biotec

The incubator cabinet consists of an inner chamber containing 3 shelves and a reservoir tray. An inner door, which is screwed shut during normal operation, has 3 smaller glass doors that give access to each shelf separately. There is also an outer door.

Two operating programs are available:

- Cycle N°1 – *continuous incubation mode*: this is set at standard conditions of temperature (e.g. 37 C) humidity and CO<sub>2</sub> with alarms and controls to maintain pre-set parameters.
- Cycle N°2 – *heat decontamination cycle*: this is set to heat up to 120 C internal temperature of the chamber and hold for 4 hours before returning to 37 C

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## V. METHOD

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### a) Internal contamination of incubator with surrogates

In carrying out tests to verify the efficacy of the Fertisafe disinfecting process it is necessary to evaluate the removal of micro-organisms, which may occur and demonstrate that this removal efficacy is reproducible within acceptable limits.

The method employed used of stainless steel surrogate plates to simulate the load items, and inoculated solutions were placed directly onto surrogate surfaces to monitor the efficacy of the process. Contaminated with maximum recovery diluents (MRD) containing about  $10^4$  to  $10^9$  microbes per ml in order to reproduce the situation where trays are contaminated. The surfaces were submitted to a standard cleaning-disinfection's with Fertisafe ANK

Bacteria were recovered by:

Following Fertisafe decontamination using liquid wipes and spray, representative microbiological swab tests were carried out to determine the sterility of the surrogates. Hands were washed and gloves worn at all times to ensure sterility.

Swabs were suspended in 10 ml maximum recovery diluent (MRD) and sonicated at 45 MHz for 10 minutes at  $35^\circ\text{C}$  before dispensing 0.1 ml MRD onto counting medium. After 48 hours of incubation at  $37^\circ\text{C} \pm 1^\circ\text{C}$  the number of colony forming units (cfu) on each plate was counted and results expressed as the number of viable bacteria per swab.

Variations were carried out using hand sprays and dry sterile polyester wipes and then Fertisafe and water wipes made up in sterile tubs. G-MOPS™ plus supplemented with HAS was used to simulate the possible contamination of spills in the incubator. Microbe solutions containing 0.1% test soil were made to mimic soil contamination.

### B Operational tests for the Incubator

Temperature control. Continuous data logging of temperature and relative humidity gave satisfactory limits (not shown).

## VI. RESULTS

Micro organisms	Log
Aspergillus Niger	6.44
Pseudomonas aeruginosa	8.53
Bacillus Subtilis	6.32
Staphylococcus Aureus	9.13
Candida Albicans	7.17

**Table I: Micro organisms with log cfu/ml used in the tests.**

These are the stock solutions used in the following tests more dilute concentrations were made up with MRD when required.

Spray / wipe	Viable Bacteria per Swab		
	Number	Log	% Removed
Fertisafe	0	0	100
Water	8	2.90	96.8
Control	248	4.39	0

**Table II: Swab tests of steel surfaces used to evaluate the effect spray and wipe with Fertisafe and water on bacillus subtilis.**

Surfaces were sprayed so as to give a uniformly wet surface over the test areas, which were then wiped “dry” with one dry wipe per area. The lack of sporicidal activity of water accounts for not all the spores being removed but the 97% removal demonstrates the importance of good mechanical cleaning.

Spray /Wipe	Contact time / minutes	Viable Bacteria per Swab		
		Number	Log	% Removed
Fertisafe	1	0	0	100
Fertisafe	2	0	0	100
Fertisafe	3	0	0	100
Fertisafe	4	0	0	100
Water	1	11	3.04	92.4
Water	2	7	2.85	95.2
Water	3	9	2.95	93.8
Water	4	10	3.00	93.1
Control	0	145	4.16	0

**Table III: Swab tests of steel surfaces used to evaluate the effect wash and wipe with Fertisafe and water on bacillus subtilis with contact time of liquid as a variable.**

Contact time of water with spores was not expected to any change in spores remaining the 92.4-95.2% variation is the variability of the swab method. The Fertisafe should show and increase in spore removal with time but even after one-minute contact time no spores were detected.

Wipe	Viable Bacteria per Swab			
	Contact time / days	Number	Log	% Removed
Fertisafe	1	0	0	100
Fertisafe	2	1	3.00	99.8
Fertisafe	3	0	0	100
Fertisafe	4	4	3.62	99.4
Water	1	52	3.71	91.9
Water	2	96	3.98	84.9
Water	3	126	4.10	80.0
Water	4	133	4.12	79.1
Control	0	644	4.80	0

**Table IV: Swab tests of steel surfaces used to evaluate the effect wipe with Fertisafe or water on bacillus subtilis with contact time of bacteria solution as a variable.**

Since a contact time of a minute appeared satisfactory for Fertisafe wipes impregnated with Fertisafe and water were used to simplify the method. The control in table IV is not really accurate as the number of spores was expected to increase with time. If the cleaning efficiency of water is assumed to be constant there can be seen in table IV an increase in spores with time. It is safe to assume the same is happening on the Fertisafe test areas that show even after six days of possible growth Fertisafe wipes still remove 99.4% of the spores. Note surface allowed to dry in air before swabbing.

Micro organisms	Wipe	Viable Bacteria per Swab		
		Number	Log	% Removed
Aspergillus Niger	Fertisafe	0	0	100
Aspergillus Niger	Water	2	2.30	95.5
Aspergillus Niger	Control	44	3.60	0
Staphylococcus Aureus	Fertisafe	0	0	100
Staphylococcus Aureus	Water	3	2.50	95.9
Staphylococcus Aureus	Control	73	3.90	0
Candida Albicans	Fertisafe	4	2.60	98.0
Candida Albicans	Water	9	3.00	95.5
Candida Albicans	Control	200	4.30	0

**Table V: Swab tests of steel surfaces used to evaluate the effect wash and wipe with Fertisafe and water on three micro organisms with Fertisafe and water as variables as a variable.**

Aspergillus Niger and Candida Albicans were chosen as fungi and yeasts respectively that are common in the atmosphere and could cause contamination of the incubator. Staphylococcus aureus is typical of bacteria carried by people on their skin so again could cause contamination. Only Candida was not removed completely with a Fertisafe wipe, whether this is a resistance of Candida or poor wiping cannot be distinguished at this point.

Micro organisms	Wipe	Viable Bacteria per Swab		
		Number	Log	% Removed
Bacillus Subtilis	Fertisafe	1	2.00	99.9
Bacillus Subtilis	Control	728	4.86	0
Aspergillus Niger	Fertisafe	0	0	100
Aspergillus Niger	Control	17	3.23	0
Staphylococcus Aureus	Fertisafe	0	0	100
Staphylococcus Aureus	Control	8	2.90	0
Candida Albicans	Fertisafe	0	0	100
Candida Albicans	Control	708	4.85	0

**Table VI: Swab tests of steel surfaces used to evaluate the effect of Fertisafe wipe with micro organisms in mammalian growth media G-MOPS™ plus.**

Table VI shows the efficacy of Fertisafe as a disinfectant for micro organisms in a growth media possible being used in the incubator. For contamination to spread the microbes' need a food source an obvious source would be accidentally spilt media in the incubator. The one colony forming unit found for bacillus subtilis could be due to contamination in processing the samples. Both control and sample were left in the incubator for 24 hours prior to disinfection and analysis.

Micro organism	Log	Remaining log	% Removed
Pseudomonas Aeruginosa	8.53	4.12	99.996
Staphylococcus Aureus	9.13	2.95	99.99993
Pseudomonas Aeruginosa	4.85	0	100
Staphylococcus Aureus	4.60	0	100

**Table VII: Effect of micro organism concentration on Fertisafe wipe efficiency.**

When tested at  $10^8$  per ml, Fertisafe wipes are more effective at reducing *S. aureus* than *P. aeruginosa* with an average of 133 *P. aeruginosa* colonies remaining compared to 9 *S. aureus* colonies. At the lower levels of  $10^4$ , Fertisafe wipes completely remove all organisms from the surface for both species.

Micro Organism	Log	Remaining log	% removed
Pseudomonas aeruginosa.	8.53	4.65	99.9868
Staphylococcus aureus	9.13	3.20	99.9998
Candida albicans	7.17	2.34	99.9985
Bacillus subtilis	6.32	1.62	99.9980

**Table VIII: Effect of 0.1% test soil on Fertisafe wipe efficiency.**

Contaminating the microbe solution with soil decreases the efficiency of the microbe removal. Plates were allowed to dry for 30 minutes before wiping longer drying time or a higher % of soil will lead to less efficient removal. In the case of heavily soiled areas two or more wipes are recommended one to remove the soil the other to sterilise the surface.

Time / days	Count	Count / ml
1	0	0
2	0	0
3	1	10
4	0	0
5	0	0
6	0	0

**Table IX: micro organisms found in incubator reservoir. Numbers of days are since the end of inoculation tests.**

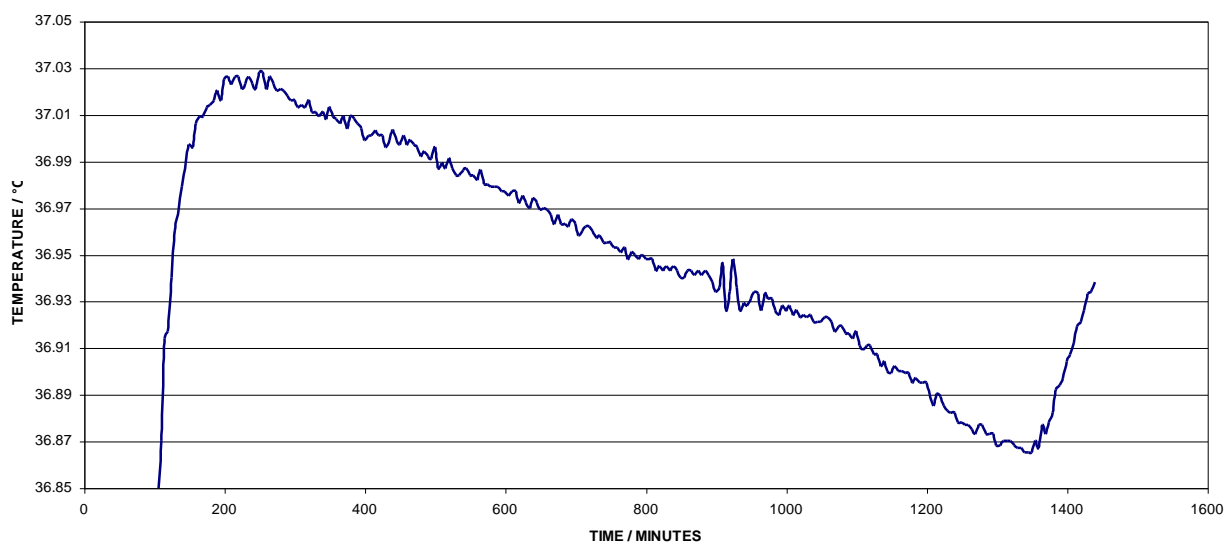
The one colony forming unit found for day 3 was probably an air contaminate whilst processing the sample as no further growth was seen with time up to six days.

Micro organisms	Start Log	cfu	End Log	% Removed
Bacillus Subtilis	6.32	0	0	100
Pseudomonas Aeruginosa	8.53	0	0	100
Aspergillus Niger	6.44	0	0	100
Staphylococcus Aureus	9.13	0	0	100
Candida Albicans	7.17	0	0	100

**X: Micro organisms found in incubator after Cycle N<sup>o</sup>2 – heat decontamination cycle.**

Heat decontamination was carried out twice in the testing once before any microbes were introduced and the secondly after microbe analysis had been completed. As can be seen in table IX the thermal decontamination is highly effective even against bacillus subtilis which is the standard organism specified by the US Pharmacopoeia to validate sterilisation.

**TEMPERATURE TIME VARIATION FOR INCUBATOR**



**Fig 3. Temperature profile of incubator normal mode.**

The graph for the temperature profile looks as if there is a major change but the temperature axis is only 0.2°C. So the temperature control oscillates between 37.03 and 36.87°C a 0.16°C spread. Relative humidity started at 65% for lab air to 100% within 40 minutes after which no variation was seen.

Days	CFU
1	0
2	0
3	3
4	6
5	11
6	8

**XI: Micro organisms found in incubator on settle plates over six days one plate removed every day for six days.**

Settle plates don't actually show much except the quality of the lab air. The results will be proportional to the amount of time a door is open and the number of times its open. By definition an incubator cannot be a sterile environment (no growth) but can be kept clean to prevent contamination of samples.

## VII. DISCUSSION - CONCLUSION

Fertisafe wipes and spray plus wipes proves highly effective at disinfecting the surfaces in the incubator. Running with water the slight salt deposit does not show up on the shelves. If running the incubator without water, sterile water can be used to clean off the white residue left by Fertisafe though this extra step could introduce contamination. Salt deposits from Fertisafe do not present a major problem, as the high grade stainless steel will not rust.

Method of use for Fertisafe wipes is: Wipe surface carefully and leave to dry in air. If area heavily soiled use first wipe to remove all visible soil and second to further wet the risk areas. One wipe was used for each surrogate, which represents a worst case scenario when a second wipe has not being carried out.

## VIII. CLEANING PROTOCOL

From the results of the microbiological surrogates a cleaning protocol was formulated to give the optimum sterilisation. The steps are as follow:

- a) Wearing gloves and good handling techniques are important to successful cleaning.
- b) Check incubator temperature to check it is not on a thermal decontamination, turn incubator off.
- c) Remove the two screws retaining the inner stainless steel door so it can be swung fully open as in fig 4.
- d) Remove inner stainless steel door by releasing the two plastic U clips on the hinges.

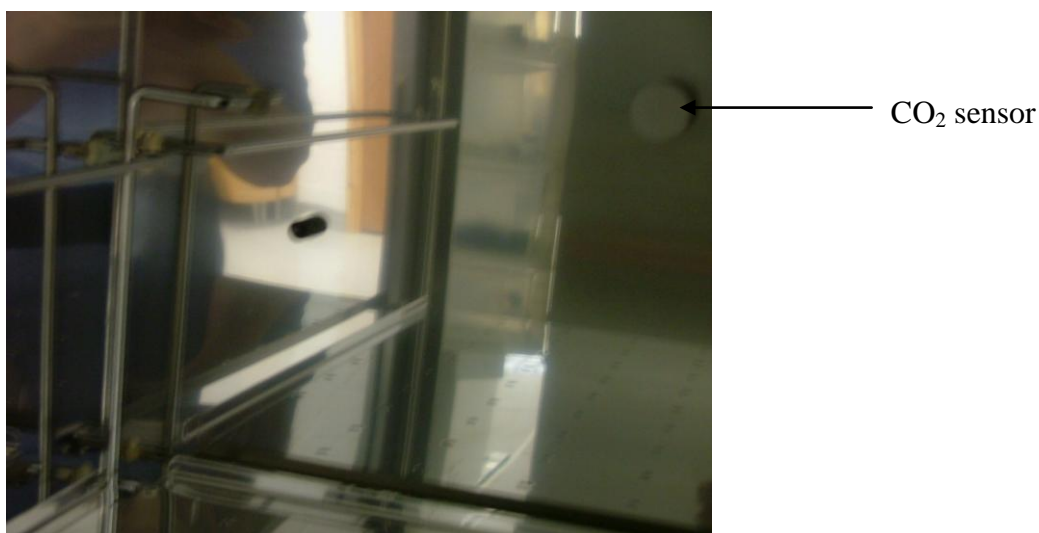


**Figure 4. opening of the inner door.**

- e) Remove water tray and drain water (if present), remove all three trays then remove both rack side pieces.



**Figure 5. Water tray (bottom) partly removed.**



**Figure 6. Left hand rack prior to removal.**

- f) Remove black cover from top right hand corner of the incubator chassis see fig 5. Place cover over CO<sub>2</sub> sensor see figure 6.
- g) **Using Fertisafe spray:** Spray internal surfaces making sure to get behind the door seal at the front and the water tray back stop at the back.
- h) **Follow up with Fertisafe wipes:** wipe all internal surfaces with wipes once with one wipe and then repeat, if wipe still has soil (discoloured) use a third wipe repeat until last wipe appears clean. Make sure the door seal recess and the underside of the water tray backstop is cleaned.
- i) After five minutes contact time spray with sterile water and wipe with a sterile polyester wipe. Alternatively wipe with polyester wipe dampened with sterile water.
- j) Repeat procedure with rack pieces when dry reassemble in incubator clean trays and insert back into the incubator

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- 
- k) Repeat procedure with the inner stainless steel door open each glass door to clean around the door seal. Open each glass door to clean around the door recess and seal.
  - l) Replace door ensuring hinge clips are secure. Remove CO<sub>2</sub> sensor cover and replace in holder. Close doors and fasted with securing screws.
  - m) Check and recalibrate CO<sub>2</sub> levels if needed.

If the inner surfaces have substantial soiling parts can be washed with a sponge and a detergent rinsed with tap water prior to Fertisafe cleaning.

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## VIII. ACKNOWLEDGEMENTS

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The staff at Research Instruments Limited, for providing equipment and advice, particularly David Lansdowne. Ronny Janssens for providing an incubator decontamination protocol with the wise council “Incubators cannot be kept sterile and are not considered to be a sterile environment.”

I acknowledge that this is a true and accurate record of a study carried out by an independent test house

David Lansdowne