NANOCLAVE
ULTRAVIOLET LIGHT DECONTAMINATION

The Nanoclave Cabinet

Fast, effective UVC disinfection in 60 seconds

Technical Specification Document

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The Nanoclave Cabinet

This document covers the technical specifications and standards of the Nanoclave Cabinet as well as general technical information.

The Nanoclave Cabinet is designed to disinfect all non-invasive medical equipment and electronic devices present in a healthcare environment. In order to ensure an effective disinfection it is imperative that users follow the instructions for use.

Standards and EN Directives:

- Tested and accredited by the British Standards Institution (BSI)
- Manufactured in accordance with Quality Systems registered in the UK by BSI conforming to ISO 9001:2008 & ISO 13485:2003.
- CE certified class IIa medical device
- Complies with Medical Device Directive 93/42/EEC
- Complies with relevant sections of the Health Technical Memorandum 20/30 (HTM 20/30) for washer disinfectors

Technical Specifications:

- Physical dimensions (mm):
  - External: Height: 1,600; Height (without stand): 890; Width: 890; Depth: 1,170
  - Internal: Height: 740; Width: 740; Depth: 1,000
- UVC Lamps
  - Contains 32 x 30W and 16 x 25W Sylvania Germicidal UVC lamps
  - Quality and safety assurances provided by Sylvania
  - Each lamp has a useful life of 8000 hours
  - Lamps emit short wave ultraviolet ‘C’ (UVc) radiation with a radiation peak at 253.7nm – effective for germicidal action
  - Each UVC tube has an internal protective coating that limits the depreciation of useful UVC radiation and eliminates production of ozone gas
- UVC dosage per cycle – Dispenses a UV irradiation dose of 52W per m2 for 60 seconds
- Power rating
  - 220 – 240 Volts AC, 50Hz, 2000W (110v 60Hz is available)
  - Steady state power consumption: 1,500W

Daily Checks, Traceability and Validation

- The daily start-up routine involves cleaning of main touch points on the Cabinet and basic checks to ensure the device is operating correctly.

- UV intensity is measured by a set of current sensors. If the UV lamps are not operating correctly, the unit will not run the cycle and the on-board computer will return an error. If there is a lamp failure on any one bulb, the unit will return an error. The risk of a malfunction resulting in a failure to disinfect is extremely low as long as routine maintenance is carried out by an authorised person. The current sensors are checked and re-calibrated every 6 months during the maintenance cycle.

- For microorganism validation, Nanoclave can provide spore kits with broth to be placed in the cabinet for exposure. To confirm that a specific device has been disinfected to a certain standard we recommend using a swabbing technique. Nanoclave can assist and advise on this process.

Advanced model only - NHS ready unit with traceability

- In addition to the above monitoring system, the advanced model features an independent system, which measures both the current and actual UVC output of the lamps. Current meters measure the current drawn by each bank of lamps and can detect the failure of any one lamp in the system. UVC output is measured using 6 UVC sensors; each measuring one representative lamp. All data collected is recorded onto an SD card and printed using a thermal printer. The data-logging feature is completely independent from the PLC device controller (which controls the operation of the Nanoclave Cabinet) for additional safety.

Safety Features

- The risk of exposure to UVC light is minimal provided that the instructions for use are followed and the Cabinet is maintained according to NanoClave Technologies' guidelines.

- The front (main) access door is protected by two manual clasps and an Allen Bradley Guardmaster Titan safety interlock switch (or "interlock"). Light integrity is maintained using a UV resistant seal which is checked for wear during the six monthly maintenance cycles.

- The interlock operates in a "fail-safe" mode. Power to the UVC lamps is routed via the Titan safety interlock. Unless the door is closed and the lock is engaged, no power is provided to the UVC lamps. In the unlikely event the interlock fails to keep the door shut, power would be cut off as soon as the door is opened.

- The rear door is used only for maintained purposes. It is marked accordingly and requires a tool key to be opened. Light integrity is maintained using a UV resistant seal, which is checked for wear during the six monthly maintenance cycles.

- Further to Nanoclave's safety features, the British Standards Institution (BSI) has certified the safety of the Cabinet and confirmed during tests that it is not possible to open the door of the Cabinet while in use.

Advantages of UVC as a disinfection process over existing methods

- Many devices, especially electronic devices, are sensitive to immersion in water or exposure to moisture, high temperatures or pressures. This prevents them from being processed in an autoclave or washer/disinfector. By contrast, UVC exposure occurs at room temperature, pressure and does not involve moisture or immersion and is therefore an ideal candidate for processing these more difficult to clean items.

- The item to be disinfected must be physically cleaned with a detergent wipe first to remove any visible debris. Once this is done, the Cabinet can irradiate all surfaces providing a gentle but effective disinfection process.

- All devices placed in the Cabinet are exposed to a single 60 second cycle of UVC irradiation. When used correctly, the Nanoclave Cabinet will provide at least a log 5 reduction on surfaces for a selection of hospital relevant bacteria like MRSA etc... Testing was not conducted to achieve a higher than log 5 reduction, as research shows that normal ward contamination levels rarely exceed log 2 (1,000 times less than the level at which we tested)[1].

Material Compatibility – effect of intense UV radiation on polymer plastics

- Extended exposure of polymers to UV light (such as leaving plastics in direct sunlight for several years) will cause them to physically degrade. Degradation is related to UV dosage and to the chemical composition of the material. Most coloured plastics contain substrates, which block the penetration of UV light and so are much more resistant to degradation than clear plastics.

- The exposure of UVC that items receive in the Cabinet, although intense, is for short periods of time and so dosage is relatively low. As a result, it is possible to easily run tests to simulate the amount of UV that a device is likely to receive during its lifetime.

- Nanoclave has conducted extensive tests on a variety of polymers, exposing them to doses that are equivalent to routine daily disinfection in the cabinet over a period of 10 years. At this level of exposure, no material degradation has been detected. Full test results are available on request.

[1] Nanoclave Cabinet - University College London Hospitals (UCLH) bacterial report 2010

- If a Trust requires further confirmation of material compatibility, Nanoclave recommends that devices which are to be disinfected inside the Cabinet are first tested. Nanoclave can facilitate this, and depending on the proposed frequency of disinfection, a full product life-time of testing can be completed in 40-60 hours.

Endoscopes and UVC

- The Nanoclave Cabinet is not suitable for the disinfection of flexible endoscopes. However, some concern has been raised about material compatibility with UV following two medical device alerts published on 10th May 2007 by the MHRA in the UK[2].

- The alert advised that Olympus endoscopes had suffered from surface degradation and cracking as a direct result of storage in Ultraviolet C (UVc) drying cabinets manufactured by AFOS ltd.

- The MHRA have stated that the AFOS drying cabinets in question were programmed incorrectly and were exposing endoscopes to UVC light for 8 hours per day as opposed to the recommended 15 minutes per 6hrs. According to reports by Olympus, the problems with endoscope deterioration became evident and widespread about 4 yrs after the installation of such cabinets[3].

- These reported issues are specific to Olympus endoscopes, which have been placed in the incorrectly programmed AFOS cabinets.

- Although no accurate data on exposure is available from AFOS, Nanoclave calculations show that the damaged endoscopes were exposed to a dosage equivalent to over 20yrs of daily disinfection in the Nanoclave cabinet[4].

- Nanoclave has conducted extensive tests on a variety of polymers, exposing them to doses that are equivalent to routine daily disinfection in the cabinet over a period of 10 years. At this level of exposure, no material degradation has been detected. Full test results are available on request.

Testing on Organic Soils

- For viral tests conducted at Great Ormond Street Children's Hospital - Adenovirus (the most resistant virus to UVC) was grown in a protein medium (faeces). The Nanoclave Cabinet performed well in decontaminating the Adenovirus inoculations, achieving Log 6 reductions.

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[2] Medicines and Healthcare products Regulatory Agency (MHRA) publications; MDA/2007/34 and MDA/2007/35

[3] Olympus flexible endoscope compatibility statement KMF 4395 – 2010

[4] Estimates based on intensity levels of 2.5w/m2 in endoscope cabinet equivalent to 20 times less than that of the Nanoclave cabinet

Prions

- The Nanoclave cabinet has not been tested for its efficacy in deactivating prions

Additional data on Nanoclave UV Cabinet

- The Nanoclave Cabinet is not a steriliser – it is a surface disinfector, achieving Log 5 reductions on all problematic pathogens on a variety of surfaces. Because of its unique patent protected 360 degree UV light technology it reaches the vents and recesses, etc. of medical equipment and electronic devices. It does not disinfect cavities of tubes/lumens and is not suitable for endoscopes (or any invasive equipment), scopes, scalpels, etc. It disinfects non-invasive pieces of equipment/medical devices.

- **Risk of toxic shock from residual endotoxins:** Devices to be disinfected must be physically cleaned using soap and water, before running the UVC disinfection cycle, which removes any large scale residual proteins. After the Nanoclave Cabinet cycle is complete, the inactivated residue of residual dead microorganisms will remain on the surface of the disinfected device. In some scenarios, exposing patients to high levels of inactive microorganisms or endotoxins can cause them to go into toxic shock. However, in this context the associated risks are minimal. Inactive residues are a significant problem if those residues enter the blood stream, but in the context of non-invasive devices, which should not come into contact with the blood stream, the risk of causing a large scale inflammation is low.

NANOCLAVE CABINET - KEY ADVANTAGES

- Simple to operate
- Fast effective process creating major time savings and rapid turnaround
- Disinfection achieved in 60 seconds
- Reliable – 360 degree full beam decontamination – nothing gets away
- Disinfection process more effective than bleach
- Major cost savings compared to other disinfection/decontamination services
- No regular consumables – means a cost-effective solution
- Safe – process takes place at normal room temperature and pressure
- Dry cleaning technology means no moisture, high heat, high pressure or chemical residues
- Highly robust device with a lifecycle of 20 years+
- Mobile – for moving between cleaning rooms, laboratories or hospital wards

EFFECTIVELY DISINFECTS

- Dialysis machines
- ECG (Electrocardiography) machines
- Defibrillators
- Syringe Pumps
- Infusion Systems
- TENs machines
- Ultrasound Equipment
- Drip Stands
- Blood Pressure machines/Sphygmomanometers
- Computers
- TVs & remote controls and more...

Instructions for use

1. Check that item to be disinfected is free from dirt and physical soiling
2. Put on aseptic gloves
3. Press the “OPEN” touch sensitive button on the PLC on the front door panel
4. Disengage both the lower and upper clasp securing the front door
5. Open the front door of the Cabinet Disinfection Unit
6. Place the single item to be disinfected in the approximate centre of the Cabinet Disinfection Unit
7. Close the front door of the Cabinet Disinfection Unit
8. Engage both the lower and upper clasps securing the front door
9. Press the “START” touch sensitive button on the PLC on the front door panel
10. Wait until the PLC screen shows the message “CYCLE COMPLETED SUCCESSFULLY, PRESS STOP TO OPEN DOOR”
11. If the PLC screen does not show this message, turn off the Cabinet Disinfection Unit by following the “End of shift” instructions and inform your supervisor
12. Press the “STOP” touch sensitive button on the PLC on the front door panel
13. Disengage both the lower and upper clasp securing the front door
14. Open the front door of the Cabinet Disinfection Unit
15. Remove gloves and place in waste bin
16. Remove the item from inside the Cabinet Disinfection Unit

End

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Report contents:

Laboratory assessment of the Nanoclave Cabinet (Nanoclave Technologies LLP; London, UK)

Part I: To demonstrate effectiveness against a range of nosocomial pathogens

Executive Summary

The Nanoclave Cabinet (Nanoclave Technologies LLP) produces large amounts of UVc light. Its purpose, through a "360^{o} full beam decontamination process" is to rapidly disinfect a wide variety of medical equipment and electronic devices.

A controlled independent laboratory study was conducted to assess the ability of the Nanoclave Cabinet to eradicate methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE), *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Clostridium difficile* from a range of difficult-to-clean surfaces and/or items of clinical equipment.

Each test surface was placed in the Nanoclave Cabinet and exposed to two 30-second irradiation cycles. The Nanoclave Cabinet was capable of reducing the level of MRSA, VRE, *A. baumannii* and *Kleb. pneumoniae* by at least 99.999% but was less effective against *C. difficile* spores. Bacterial numbers on 41 of the 51 target sites (80%) were consistently reduced to below detectable levels but the decontamination of Velcro on a blood pressure cuff and deep recesses associated with a tympanic thermometer was less effective. However, those sites that proved difficult to decontaminate using the Nanoclave Cabinet, particularly those associated with the blood pressure cuff, were also difficult to disinfect using antimicrobial wipes.

Decontamination of surfaces and equipment in the near patient environment is often poor because domestic staff do not clean items on or near the patient, yet these are often the most heavily contaminated areas in the ward. The results of this study suggest that the Nanoclave Cabinet could provide rapid and reliable decontamination of patient-related equipment and could play an important role in preventing the spread of hospital-acquired infection.

Experimental Protocol

Test organisms and preparation of bacterial suspensions

Testing involved five potential nosocomial pathogens:

Methicillin-resistant *Staphylococcus aureus* (MRSA; EMRSA-15 variant B1)

Vancomycin-resistant *Enterococcus faecalis* (VRE)

Multi-resistant *Acinetobacter baumannii* (MRAB; OXA-23 clone 1)

Extended beta-lactamase (ESBL) producing *Klebsiella pneumoniae*

Clostridium difficile 027 (spores)

Prior to each experiment, a single colony of MRSA, VRE, MRAB or *Kleb pneumoniae* was aseptically transferred into 10 ml sterile nutrient broth. A stationary-phase culture (~10^8 cfu/ml) was obtained by incubating the bacteria at 37°C for 18 h. After incubation, the culture was transferred to a sterile universal container and centrifuged at 3000 rpm for 10 min. The supernatant was discarded and the remaining pellet re-suspended in 10 ml sterile ¼-strength Ringer's solution (an isotonic salt solution).

Similarly, a previously prepared *C. difficile* spore suspension (ribotype 027) was centrifuged at 3000 rpm for 10 min and re-suspended in 10 ml sterile ¼-strength Ringer's solution.

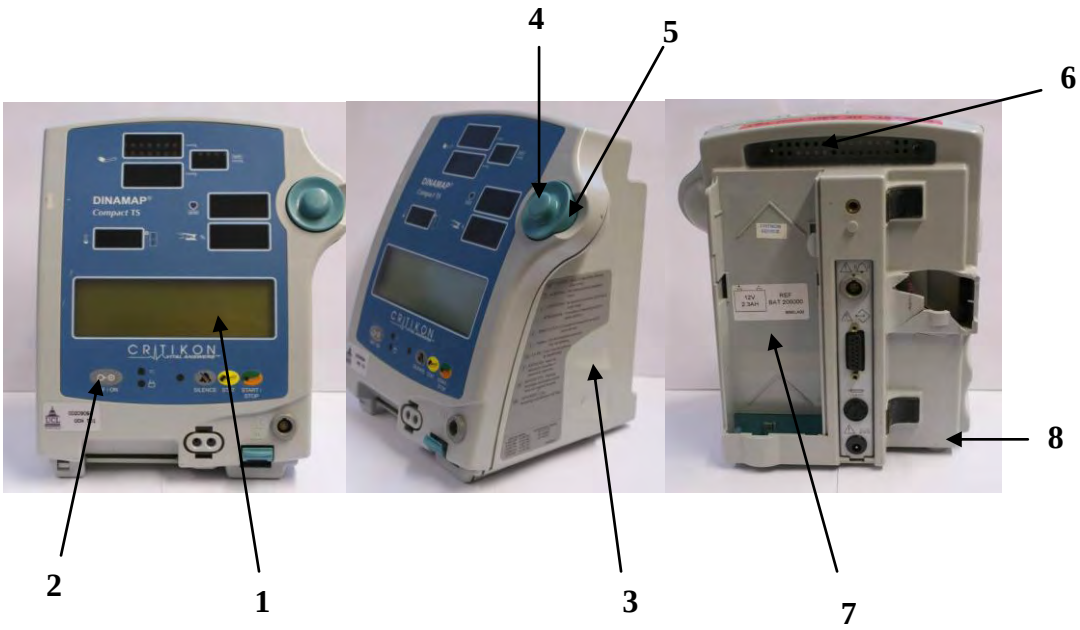
Test surfaces and sample points

Testing involved a number of 'difficult-to-clean' surfaces and/or items of clinical equipment of the type and in the condition of those likely to be found in the ward environment. Each test surface was marked with individual sample points (Figures 1a-1h).

Preparation of test surfaces

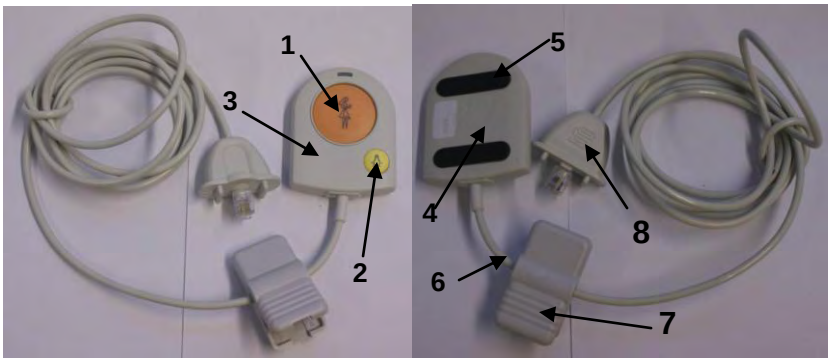
Prior to each experiment, each test surface was cleaned using a microfibre cloth (soaked with hot water), left to air-dry under ambient conditions and disinfected using 70% alcohol spray. This in-house validated cleaning protocol consistently reduced residual microbial numbers to 0 cfu/1.5 cm^2 and gave ATP bioluminescence readings of < 50 RLU (i.e. after cleaning no residual microbial contamination remained on the surfaces and all microscopic organic matter had been removed).

Figure 1a: Electronic blood pressure gauge (aka Dinamap) and associated sample points



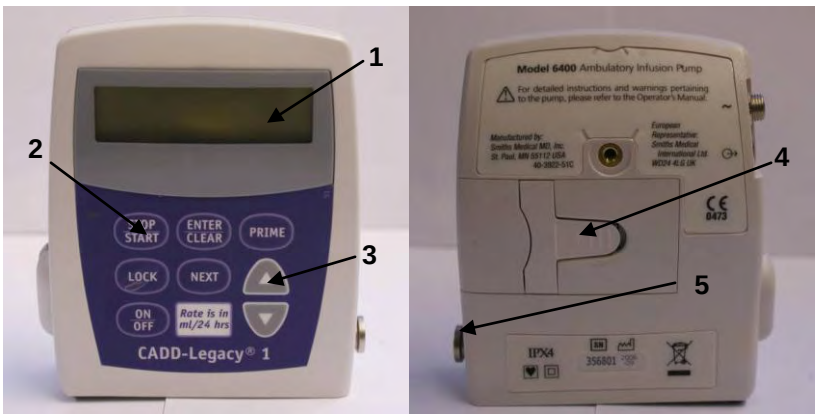
1. Main display panel
2. On/off button
3. Side grip
4. Centre grip of control dial
5. Outer edge of control dial
6. Underside surface of handle
7. Underside surface of printer cover
8. battery compartment wall

Figure 1b: Patient call button and associated sample points



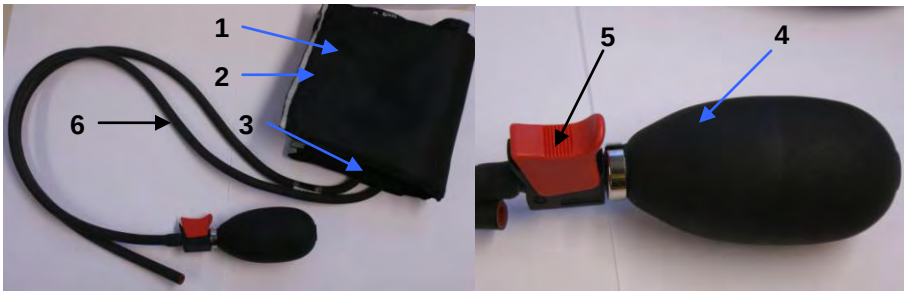
1. Orange call button
2. Yellow “lights” button
3. Front panel
4. Back panel
5. Rubber grip
6. Cable
7. Cable clip finger-grip
8. Data cable finger-grip

Figure 1c: Infusion pump and associated sample points



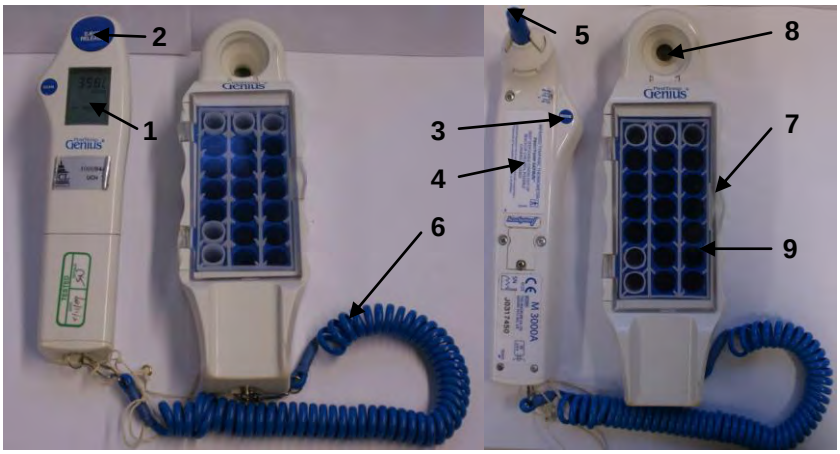
1. Digital display screen
2. Start/stop button
3. Up/down arrows button
4. Battery cover panel
5. Locking screw

Figure 1d: Blood pressure cuff and associated sample points



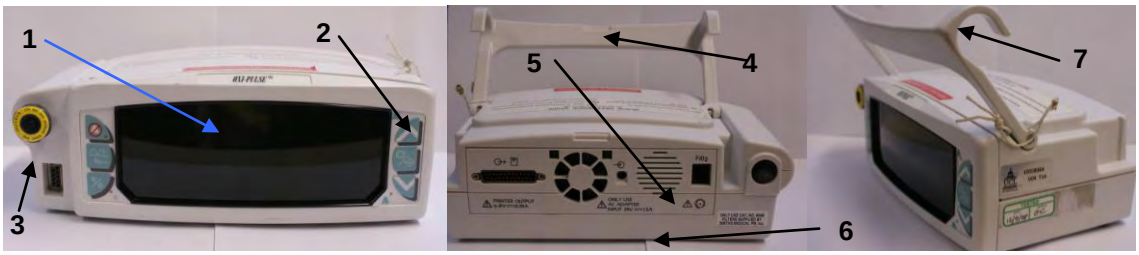
1. Velcro (hook-side)
2. Velcro (loop-side)
3. Inner Cuff Surface
4. Pump
5. Pressure-release switch
6. Pump Tubing

Figure 1e: Tympanic thermometer and associated sample points



1. Digital Screen
2. Eject Button
3. Mode Button
4. Underside Surface of Handset
5. Infra-red Sensor Window
6. Extension Coil
7. Earpiece Container Lid
8. Probe Receptor
9. Earpiece Holder

Figure 1f: Pulse oximeter (oxygen SATS probe base unit) and associated sample points



1. Digital screen
2. Up/down arrow button
3. Side panel of unit
4. Underside of carrying handle
5. Rear panel
6. Underside of unit
7. Inner surface of handle hook

Figure 1g: Computer keyboard and associated sample points

1. Mouse scroll button
2. Mouse ‘click’ button
3. Keyboard “enter” key
4. outer surface of casing

Figure 1h: TV remote control and associated sample points

1. Power button
2. “Enter” Button
3. Front Surface
4. Back Panel

Basic test procedure

Non-exposed control samples:

1. For each test surface, 10 µl of bacterial (or spore) suspension (~10^6 cfu) was inoculated onto each sample point and, rather than being left as a droplet, spread over a 1cm^2 test area
2. Each test area was sampled using a pre-moistened cotton-tipped swab
3. Each swab was placed in 9ml of ¼-strength Ringer solution and vortexed to release the bacteria
4. The resulting bacterial suspension was diluted 100-fold and 100µl of the diluted sample plated onto a pre-poured blood or, for *C. difficile*, Braziers agar plate

Test samples:

1. For each test surface, 10 µl of bacterial (or spore) suspension (~10^6 cfu) was inoculated onto each sample point and, rather than being left as a droplet, spread over a 1cm^2 test area
2. The test surface was placed in the Nanoclave and exposed for 30 sec to the UV light source
3. The test surface was rotated (to allow all target sites to be exposed to the UV) and the irradiation cycle repeated
4. After exposure, a pre-moistened cotton-tipped swab was used to sample each test area
5. Each swab was placed in 1ml of ¼-strength Ringer solution and vortexed to release the bacteria
6. 100µl of the resulting bacterial suspension was plated onto a pre-poured blood (or Braziers) agar plate

All agar plates were incubated at 37^{o}C under appropriate atmospheric conditions for 24-48 hours. Resulting colonies were enumerated and the efficacy of the Nanoclave Cabinet calculated:

Effectiveness of the Nanoclave Cabinet (Log reduction)	=	Mean number of bacteria/spores recovered from 'control' surfaces	-	Mean number of bacteria/spores recovered from test surfaces

Modifications to the above protocol will be discussed where appropriate. Each experiment was repeated to validate the results obtained.

Results

Is the Nanoclave Cabinet equally effective against a range of nosocomial pathogens?

A flat stainless steel surface was contaminated with high numbers (~10^6 cfu) of test organism and placed in the Nanoclave Cabinet. In all cases, exposing the surface to two 30-second UV cycles reduced bacterial numbers to below detectable levels (Figure 2). The minimum detection limit (i.e. the sensitivity) of the sampling technique was such that it cannot be assumed that bacterial numbers were reduced to 0. However, it can be concluded that when used to decontaminate a flat surface the Nanoclave Cabinet was capable of reducing the level of MRSA, VRE, MRAB and *Klebsiella pneumoniae* by at least 4.65 log values (99.99%; Table 1).

Table 1: Ability of the Nanoclave Cabinet to decontaminate a flat, stainless steel surface

	Mean (n = 5) number of organisms recovered (log cfu)		
	Exposure time: 0 sec	**Exposure time: 2 x 30 sec**	**Log reduction**
MRSA	5.65	< 1	> 4.65
VRE	5.89	< 1	> 4.89
MRAB	5.81	< 1	> 4.81
ESBL *Kleb pneumoniae*	6.16	< 1	> 5.16
C. difficile	5.46	2.83	2.63

UVc irradiation was less effective against *C. difficile* spores. Two 30-second cycles resulted in a 2.63 log reduction in spore numbers (Table 1; Figure 2). Increasing the cycle time had little effect (Figure 3 (pg 8)).

Nonetheless, in order to demonstrate a 5-log reduction, it was necessary to inoculate each sample point with unrealistically high levels of bacteria (at least 10^6 (1 million) cfu/cm^2). Regular cleaning and good hand hygiene compliance reduces the risk of cross-contamination. This research team has conducted extensive sampling within the ward environment; results suggest that except in outbreak settings bacterial levels on high contact sites rarely exceed 10^2 cfu/cm^2. When the number of *C. difficile* spores present on a stainless steel surface equated to 10^4 cfu/cm^2 or less (100 times greater than realistic levels), two 60-second UV cycles reduced spore numbers to below detectable levels (Figure 4 (pg 9)).

Figure 2: Mean number of organisms recovered from a contaminated stainless steel surface after it was placed in the Nanoclave Cabinet and exposed to UV for 2 x 30 second cycles. Recovery from non-exposed surfaces is also illustrated. (n = 5; error bars indicate the standard deviation)

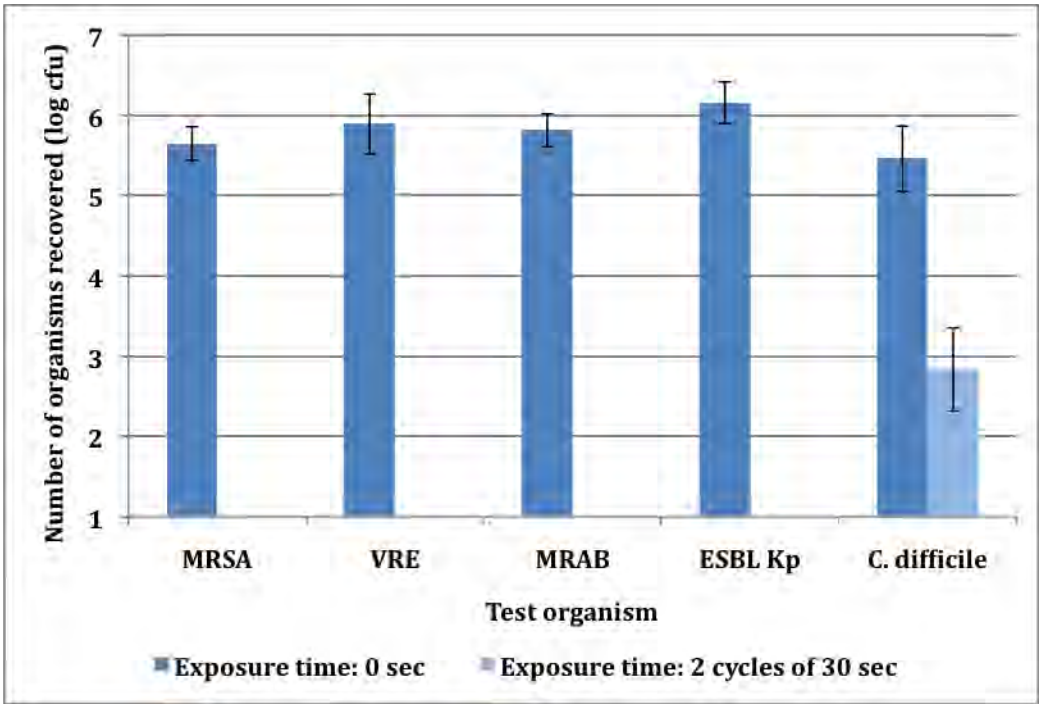


Figure 3: Efficacy of the Nanoclave Cabinet against *C. difficile* spores: the effect of cycle duration. (n = 5; error bars indicate the standard deviation)

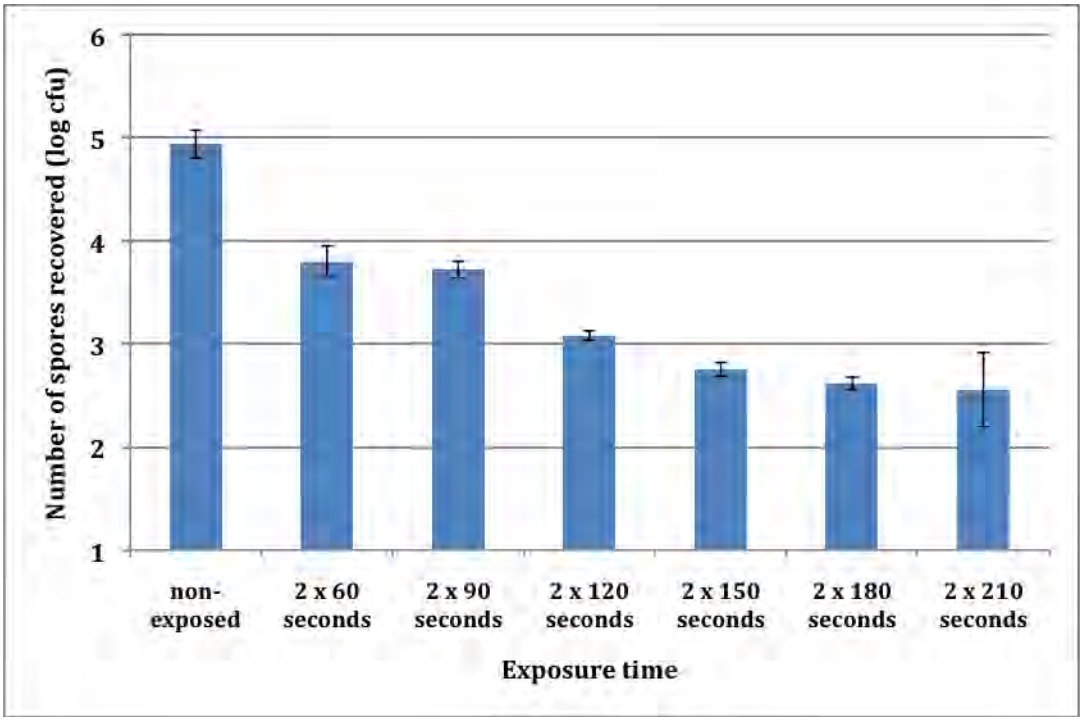
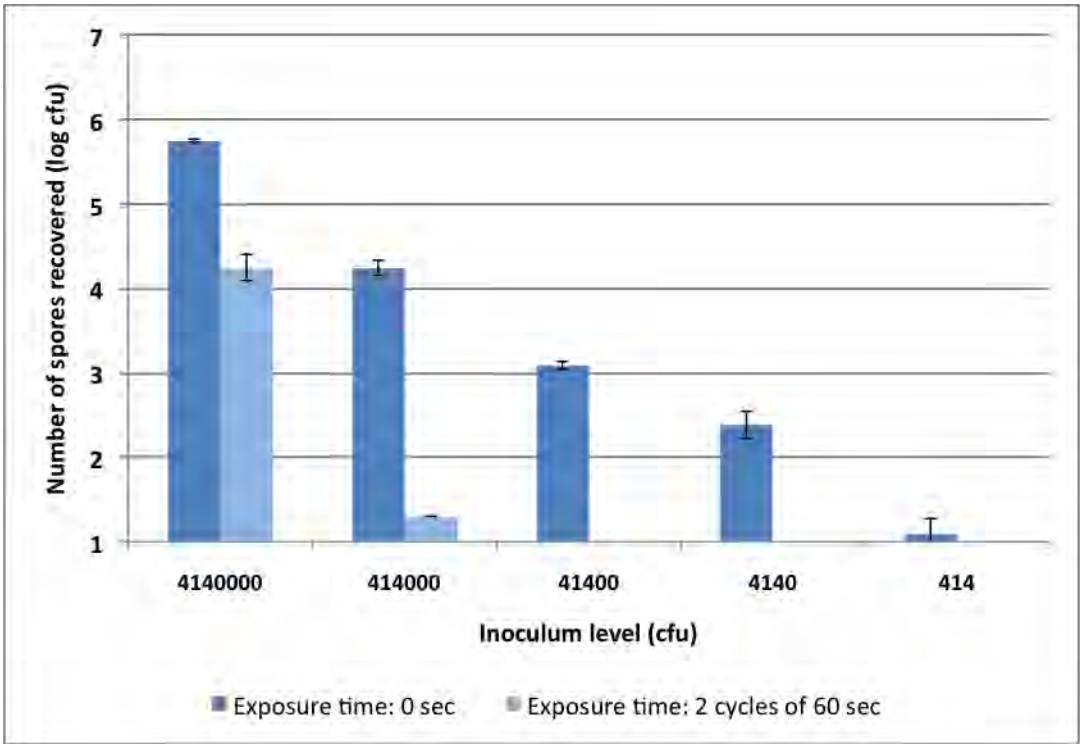


Figure 4: Efficacy of the Nanoclave Cabinet against *C. difficile* spores (n = 3; error bars indicate the standard deviation)



Does surface type affect the efficacy of the Nanoclave Cabinet?

The eight items of 'difficult-to-clean' clinical equipment (Figures 1a-1h) were contaminated with high levels (~10^6 cfu) of MRSA, VRE, MRAB or *Klebsiella pneumoniae*. Each test surface was placed in the Nanoclave Cabinet and exposed to UV irradiation for 30-seconds. Each piece of equipment was rotated to ensure all sample points were exposed to the UV and the irradiation cycle repeated. Log reduction was calculated as previously described. Results are presented in Tables 2a-2h and are based on two (MRSA, VRE, *Kleb. pneumoniae*) or three (MRAB) replicate experiments.

The results demonstrate that the Nanoclave Cabinet is capable of reducing bacterial numbers on a variety of surface types by at least 5 log values. Overall, the level of bacterial contamination on 41 of the 51 target sites (80%) was consistently reduced to below detectable levels and/or by at least 4.7 log values.

Table 2a: Ability of the Nanoclave Cabinet to decontaminate an electronic blood pressure gauge (Dinamap)

	Log reduction achieved after two 30-second cycles							
	sample point							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
MRSA	> 4.42	5.26	> 5.08	> 5.00	> 5.13	4.45	> 5.05	> 5.14
VRE	> 5.22	> 5.17	> 5.23	> 5.11	> 5.17	> 5.14	> 5.23	> 5.15
MRAB	> 5.51	5.93	> 5.47	> 5.54	6.00	3.45	> 5.61	> 5.50
ESBL *Kleb pneu*	> 5.19	> 5.01	> 5.93	> 5.01	> 5.03	2.74	> 5.04	> 5.07

Table 2b: Ability of the Nanoclave Cabinet to decontaminate a patient call button

	Log reduction achieved after two 30-second cycles							
	sample point							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
MRSA	> 5.04	> 5.03	> 5.12	> 4.85	> 4.80	> 4.86	> 4.87	> 5.12
VRE	> 5.08	> 5.17	> 5.18	> 5.21	> 5.14	> 5.21	> 5.17	> 5.13
MRAB	> 5.53	> 5.48	> 5.44	> 5.42	5.33	> 5.50	> 5.45	> 5.60
ESBL *Kleb pneu*	> 5.07	> 4.96	> 5.05	> 5.06	> 4.92	> 5.21	> 5.13	> 5.25

Table 2c: Ability of the Nanoclave Cabinet to decontaminate an infusion pump

	Log reduction achieved after two 30-second cycles				
	sample point				
	1	**2**	**3**	**4**	**5**
MRSA	> 4.86	> 4.94	> 4.72	> 4.77	> 4.87
VRE	> 5.35	> 5.08	> 5.01	> 5.25	> 4.95
MRAB	> 5.58	> 5.52	> 5.51	> 5.48	> 5.44
ESBL *Kleb pneu*	> 5.06	5.00	5.37	2.95	> 4.98

Table 2d: Ability of the Nanoclave Cabinet to decontaminate a blood pressure cuff

	Log reduction achieved after two 30-second cycles					
	sample point					
	1	**2**	**3**	**4**	**5**	**6**
MRSA	2.53	2.48	1.93	> 4.86	> 4.91	> 4.97
VRE	2.66	3.45	2.13	> 5.08	> 4.83	> 5.01
MRAB	3.18	3.83	2.58	> 5.40	> 5.10	> 5.22
ESBL *Kleb pneu*	3.22	3.35	3.22	> 5.09	> 4.84	> 5.35

Table 2e: Ability of the Nanoclave Cabinet to decontaminate a tympanic thermometer

	Log reduction achieved after two 30-second cycles								
	sample point								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
MRSA	> 4.99	5.35	1.72	> 5.18	1.74	> 5.29	> 5.31	4.41	4.59
VRE	> 5.25	> 5.21	> 5.48	> 5.23	1.53	> 4.99	> 5.15	2.56	2.42
MRAB	> 5.51	5.72	2.81	> 5.53	2.12	> 5.47	> 5.67	3.91	3.47
ESBL *Kleb pneu*	> 4.98	> 5.11	> 4.99	> 5.09	1.04	> 5.03	> 4.97	2.45	2.51

Table 2f: Ability of the Nanoclave Cabinet to decontaminate a pulse oximeter (oxygen SATS probe base unit)

	Log reduction achieved after two 30-second cycles						
	sample point						
	1	**2**	**3**	**4**	**5**	**6**	**7**
MRSA	> 5.42	> 5.23	> 5.29	> 5.23	> 5.21	> 5.22	> 5.07
VRE	> 4.98	> 4.98	5.57	> 5.06	5.15	> 5.24	> 4.91
MRAB	> 5.43	> 5.40	> 5.40	> 5.25	> 5.49	> 5.40	> 5.53
ESBL *Kleb pneu*	> 4.92	> 4.91	> 4.85	> 4.95	> 4.77	> 5.09	> 4.90

Table 2g: Ability of the Nanoclave Cabinet to decontaminate a computer keyboard

	Log reduction after two 30-second cycles			
	sample point			
	1	**2**	**3**	**4**
MRSA	> 4.79	> 4.94	> 4.81	> 4.92
VRE	3.79	> 5.03	> 4.93	> 5.02
MRAB	4.68	> 5.72	5.59	> 5.77
ESBL *Kleb pneu*	> 5.21	> 5.21	> 5.11	> 5.21

Table 2h: Ability of the Nanoclave Cabinet to decontaminate a TV remote control

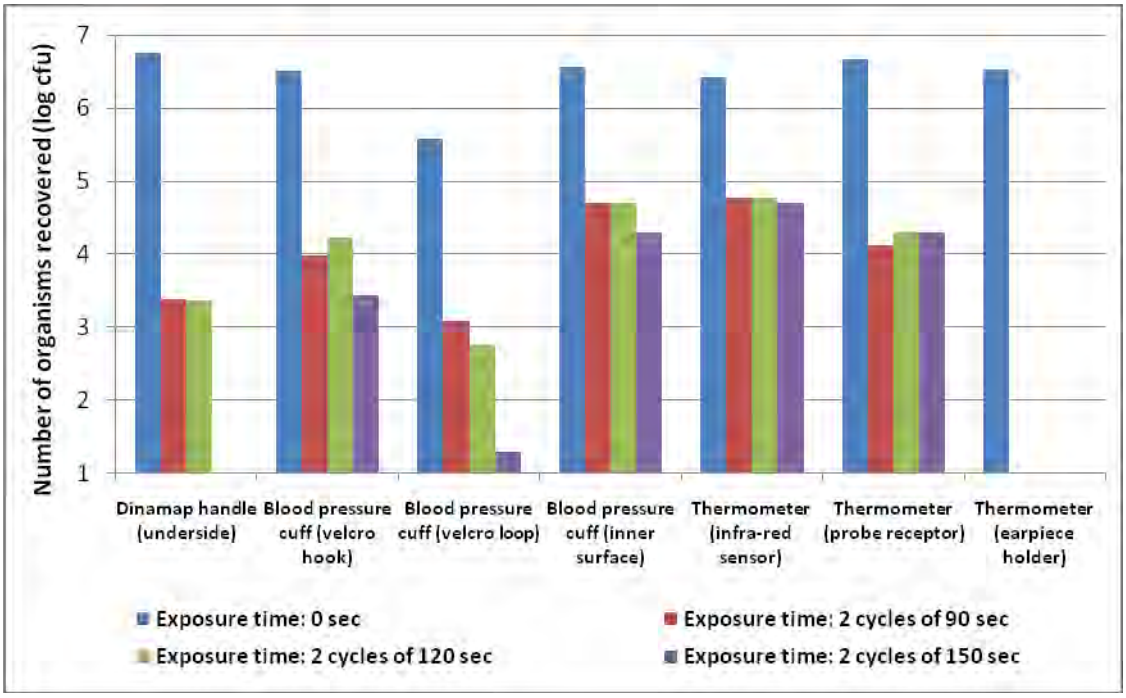
	Log reduction after two 30-sec cycles			
	sample point			
	1	**2**	**3**	**4**
MRSA	> 5.20	> 4.97	> 4.98	> 4.98
VRE	> 5.00	> 5.19	5.08	> 5.02
MRAB	> 5.36	> 5.59	> 5.78	> 5.49
ESBL *Kleb pneu*	5.39	> 5.12	> 5.12	> 5.07

The Nanoclave Cabinet was most effective in decontaminating the patient call button, the oximeter and the TV remote control. Regardless of contaminating organism, exposing these pieces of equipment to two 30-second UV cycles reduced bacterial numbers on all target sites to below detectable levels (Table 2b, 2f, 2h).

The Nanoclave Cabinet was less effective in decontaminating the blood pressure cuff and the tympanic thermometer (Table 2d and 2e). Although, two 30-second UV cycles reduced bacterial numbers on some sites to below detectable levels, on others, bacterial numbers were reduced by less than 2 log values. Three such 'hot spots' were associated with the blood pressure cuff (1 (Velcro hook-side); 2 (velcro loop-side); 3 (inner cuff surface); Figure 1d) and the tympanic thermometer (5 (infra-red sensor screen); 8 (probe receptor); 9 (earpiece holder); Figure 1e). In comparison to other target sites, the Nanoclave Cabinet was also less effective in decontaminating the handle (underside surface) of the electronic blood pressure gauge (Table 2a; Figure 1a).

Each ‘hot spot’ was contaminated with a representative organism (*Acinetobacter baumannii*) and exposed to UV irradiation for increasing periods of time. After two 90-second cycles, bacterial levels on the thermometer earpiece holder were reduced to below detectable levels (Figure 5). However, increasing the exposure time had little effect upon the number of bacteria contaminating the other thermometer ‘hot spots’. After two 150-second cycles (i.e. after a total exposure time of 5 minutes), the Nanoclave had reduced bacterial levels on the infra-red sensor and the probe receptor by just 1.7 and 2.4 log values respectively (Figure 5). Exposing the blood pressure cuff to two 150-second cycles reduced the number of bacteria contaminating the loop-side of the velcro fastener by 4.3 log values. In contrast, bacterial levels on the hook-side were reduced by 3 log values and those on the inner surface of the cuff by just 2.2 log values (Figure 5). After a total exposure time of 5 minutes, the number of bacteria contaminating the underside of the Dinamap (electronic blood pressure gauge) handle was reduced to below detectable levels.

Figure 5: Efficacy of the Nanoclave Cabinet against *Acinetobacter baumannii*: cycle duration and the decontamination of identified ‘hot spots’.

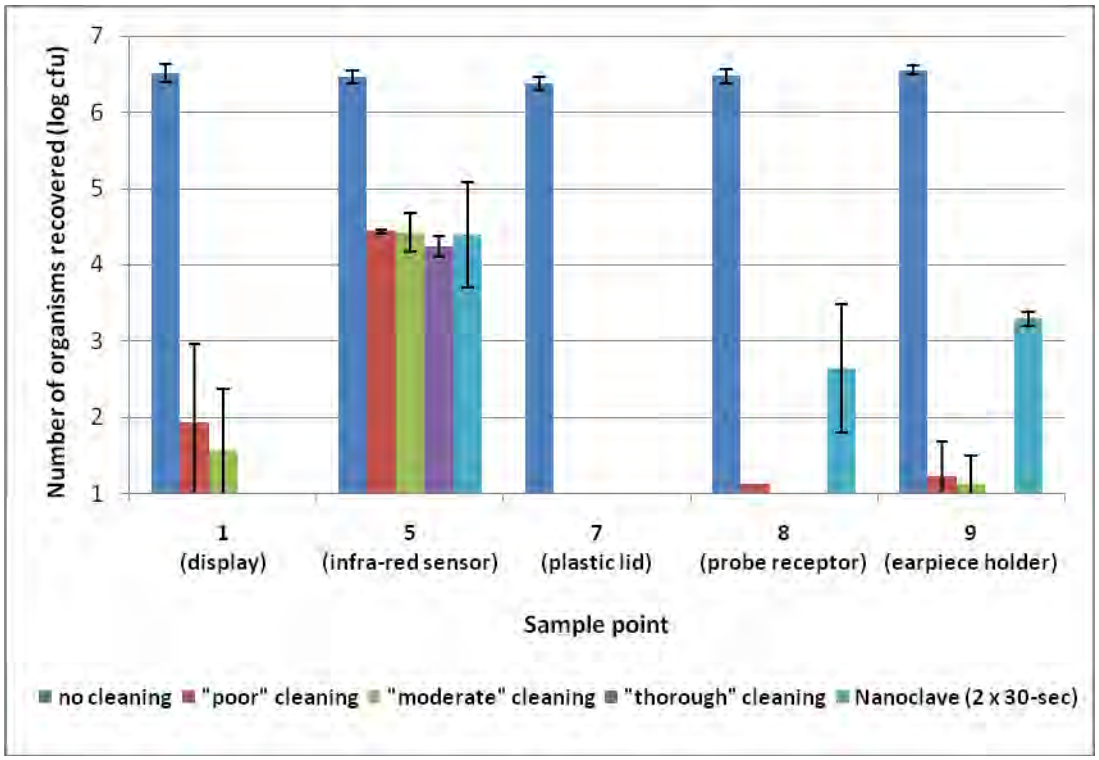


How does the efficacy of the Nanoclave Cabinet compare with that of antimicrobial wipes?

Three items of ‘difficult-to-clean’ clinical equipment (blood pressure cuff, tympanic thermometer, patient call button) were inoculated with a representative organism (*Acinetobacter baumannii*). Selected sample sites were cleaned ‘poorly’ (one wiping stroke), ‘moderately well’ (two wipes) or ‘thoroughly’ (four wipes) using an antimicrobial wipe (active ingredients: stabilized peroxides, synergized benzalkonium chloride). During the

sampling procedure, to neutralise the effects of the active ingredients, swabs were placed in 1ml of neutralising solution (phosphate buffered saline incorporating 3% Tween 80 (w/v), 0.3% lecithin (w/v), 0.1% sodium thiosulphate (w/v)). Reduction in bacterial numbers was calculated as previously described and was compared to that achieved using the Nanoclave Cabinet.

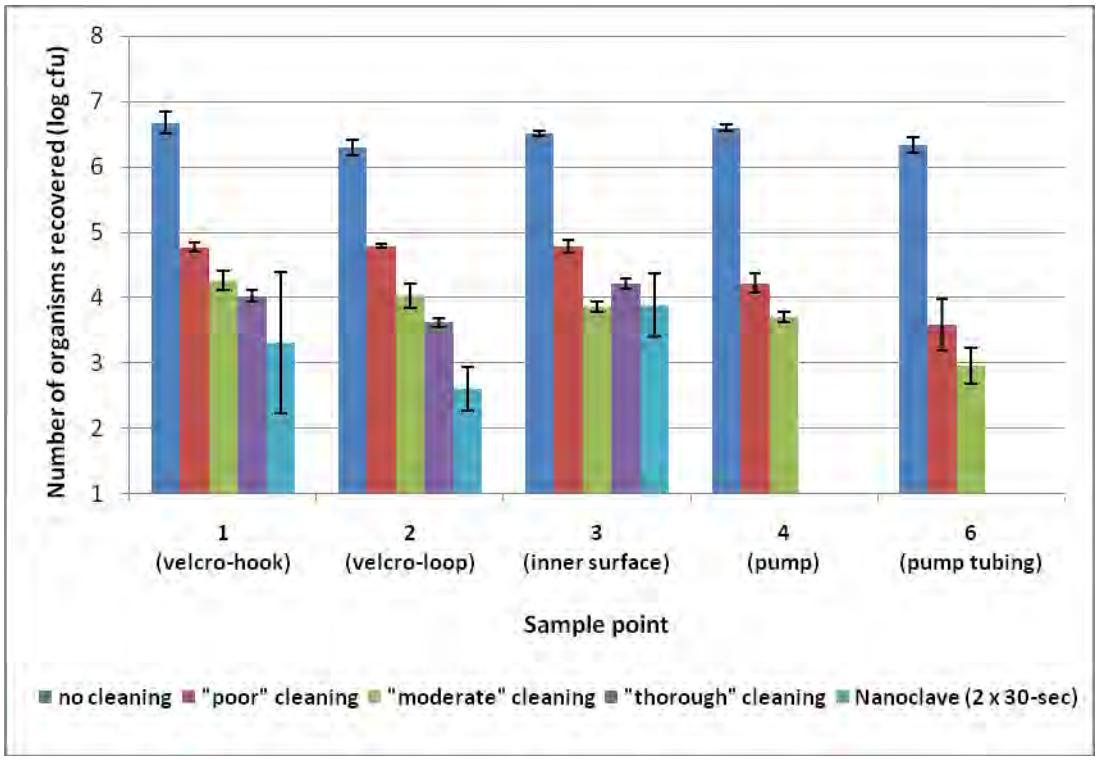
Figure 6a: Mean number of organisms recovered from a contaminated tympanic thermometer after it was cleaned using an antimicrobial wipe. Recovery after exposure to two 30-second UV cycles is also illustrated (n = 3; error bars indicate the standard deviation).



'Thoroughly' cleaning the tympanic thermometer with an antimicrobial wipe reduced the number of bacteria on most sample points to below detectable levels (Figure 6a). In comparison to when the surfaces were exposed to two-30 second UV cycles, a single wiping motion (defined as a 'poor' clean) was less effective in reducing contamination levels on the display panel (sample point 1; Figure 1e) but more effective when used to disinfect the probe receptor and earpiece holder (two of the previously identified 'hot spots'). When used to decontaminate the third identified 'hot spot', (the infra-red sensor; sample point 5; Figure 1e) neither antimicrobial wipes nor the Nanoclave Cabinet were particularly effective in reducing bacterial numbers. Whilst two 30-second UV cycles achieved a 2.12 log reduction, even 'thorough' cleaning with an antimicrobial wipe only reduced bacterial numbers by 2.14 log values (Figure 6a).

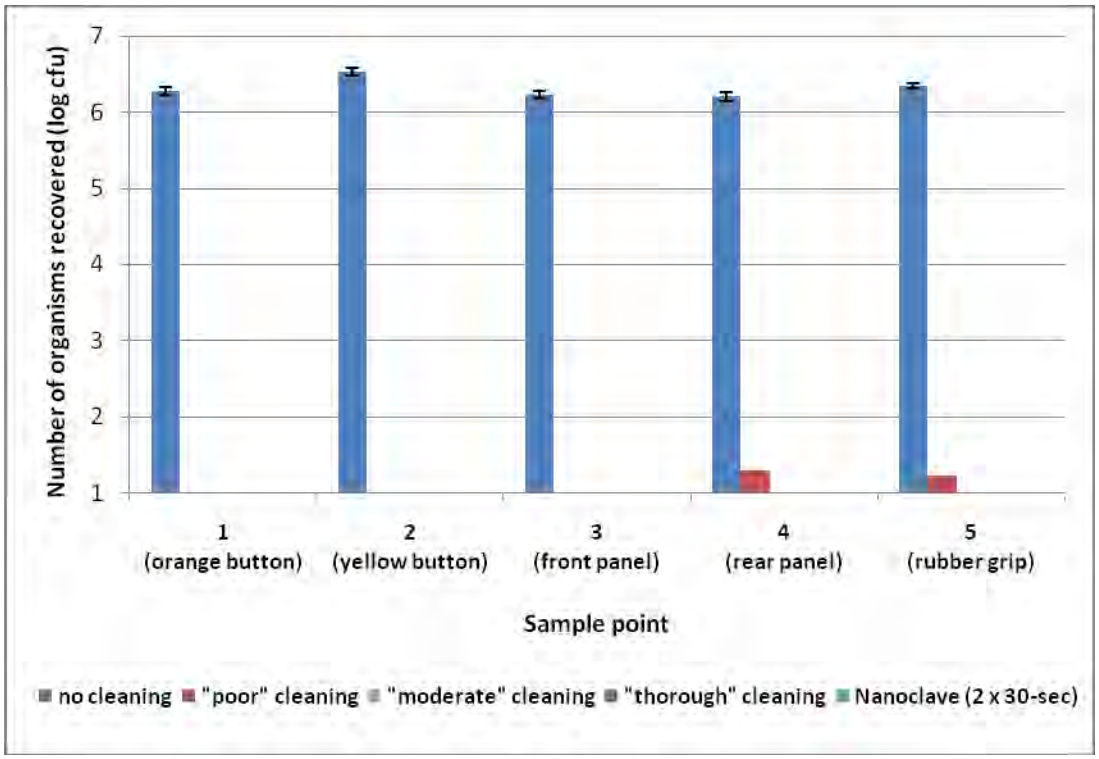
When used to decontaminate a blood pressure cuff, the Nanoclave Cabinet reduced the number of bacteria on the pump and pump tubing by more than 5 log values. 'Thorough' cleaning using an antimicrobial wipe achieved a similar log reduction but less effective wiping (i.e. 'poor' or 'moderate' cleaning) only reduced bacterial numbers by between 2.4 and 3.4 log values (Figure 6b). In comparison to these sites, the antimicrobial wipes were less effective in disinfecting the inner cuff surface and both sides of the Velcro fastening (Figure 6b). When used to decontaminate these surfaces the Nanoclave Cabinet was also ineffective (Figure 5). Although surface contamination decreased as the thoroughness of wiping increased, the results imply that the antimicrobial wipes were less effective than the Nanoclave Cabinet in decontaminating these sample sites.

Figure 6b: Mean number of organisms recovered from a contaminated blood pressure cuff after it was cleaned using an antimicrobial wipe. Recovery after exposure to two 30-second UV cycles is also illustrated (n = 3; error bars indicate the standard deviation).



When used to decontaminate a patient call button, the Nanoclave Cabinet reduced the number of bacteria on all target sites to below detectable levels and/or by at least 5 log values. Cleaning using an antimicrobial wipe was equally effective although a 'poor' wiping technique allowed residual organisms to persist on the rear panel and rubber grip (Figure 6c).

Figure 6c: Mean number of organisms recovered from a contaminated patient call button after it was cleaned using an antimicrobial wipe. Recovery after exposure to two 30-second UV cycles is also illustrated (n = 3; error bars indicate the standard deviation).



Conclusions

Under the experimental conditions described here the Nanoclave Cabinet effectively decontaminated a range of artificially contaminated 'difficult-to-clean' items of clinical equipment. Two 30-second UV irradiation cycles were sufficient to reduce MRSA, VRE, *Acinetobacter baumannii* and *Klebsiella pneumoniae* numbers by at least 5 log values (99.999%). Although the Nanoclave Cabinet was less effective against *C. difficile* spores, when the organism was present in realistic numbers (i.e. at levels more likely to be recovered from the ward environment ($< 10^2$ cfu/cm^2)), two 60-second irradiation cycles reduced the number of spores to below detectable levels. In general, exposing the test surfaces to two 30-second irradiation cycles was as effective in reducing bacterial numbers as cleaning the surfaces with an antimicrobial wipe.

Supplementary experiments

Ability of the Nanoclave Cabinet to decontaminate dialysis equipment

Parts of a dialysis machine were obtained from Fresenius Medical Care. Each 'module' was marked with individual sample points (Figures 7a-7c), inoculated with a representative test organism (*Acinetobacter baumannii*) and irradiated as previously described. Experiments were repeated in triplicate. Exposing each module to two 30-second UV cycles reduced bacterial numbers on most sample points to below detectable levels (i.e. the Nanoclave reduced bacterial numbers by at least 5.3 log values; Table 3). Post-exposure, residual microorganisms were recovered from a 'release button' associated with module 1 (sample point 2). Nonetheless, a 4.7 log reduction was achieved.

Figure 7: Dialysis equipment and associated sample points

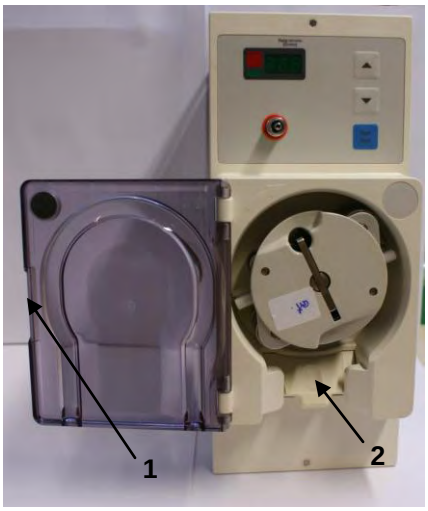


Figure 7a: Module 1

1. Cover to motor chamber
2. Release button in chamber

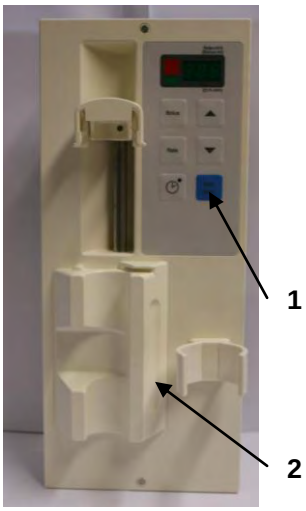
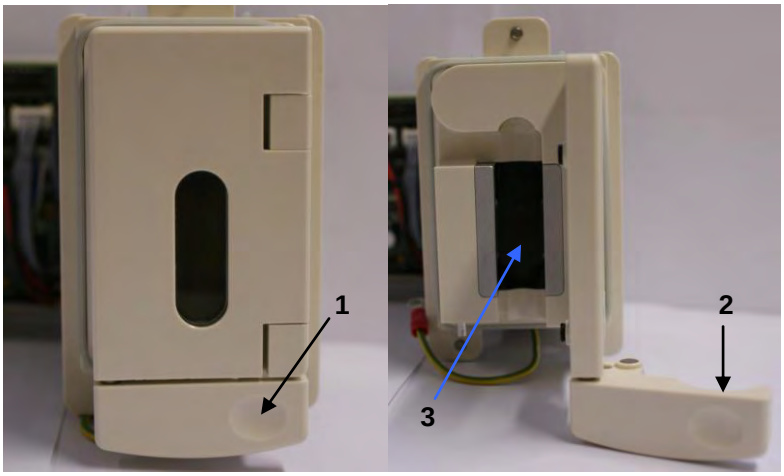


Figure 7b: Module 2

1. Start/stop button
2. Syringe holder finger grip

Figure 7c: Module 3 and associated sample points



1. Outer Grip of Door Release 2. Inner Grip of Door Release

3. Rubber Housing in Chamber

Table 3: Ability of the Nanoclave Cabinet to decontaminate dialysis equipment

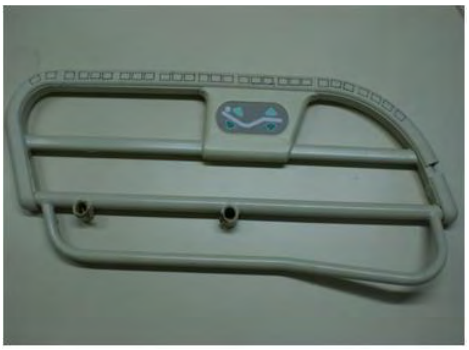
	Log reduction after two 30-second cycles		
	1	**2**	**3**
module 1	> 5.51	4.73	-
module 2	> 5.51	> 5.34	-
module 3	> 5.34	> 5.53	> 5.55

Ability of the Nanoclave Cabinet to decontaminate bed rails

Two different bed rails (Figures 8a and 8b) were contaminated with a representative organism (*Acinetobacter baumannii*) and placed in the Nanoclave Cabinet. In both cases, exposing the rails to two 30-second UV cycles reduced bacterial numbers to below detectable levels (i.e. the Nanoclave reduced bacterial numbers by at least 5.5 log values).

Figure 8a: Stainless steel bed rail

Figure 8b: Moulded plastic bed rail



This is an independent report commissioned and funded by Nanoclave Technologies LLP

Investigation conducted by Dr Shanom Ali PhD

Report prepared by Dr Ginny Moore PhD

Report issued by Dr Peter Wilson MD FRCP FRCPath

31st August 2010

Part II: To demonstrate effectiveness of the Nanoclave Cabinet against Adenovirus

Background

Adenovirus is a double stranded DNA virus that is associated with respiratory, ocular and gastrointestinal disease, especially in children. It is a recognised significant pathogen within immunocompromised patients receiving hematopoietic stem cell transplantation (HSCT), where acquisition or reactivation can lead to high morbidity and mortality. Adenovirus once excreted can survive and remain infectious within the environment for up to 35 days. As a double stranded DNA virus it has also been demonstrated to be the most resistant of the viruses tested when exposed to UV in water [1]

Experimental Protocol

Viral testing involved the use of Adenovirus species A (serotype 31) in cell culture medium inoculated onto surfaces from a stock suspension with a viral genome concentration of approximately 2.9^{10}/ml (as determined by Real Time Polymerase Chain Reaction (PCR) using standards provided by NIBSC). During this analysis viral detection was undertaken by PCR which detects both viable and none viable virus dependent on the integrity of the DNA present on the surface. Levels of retrievable virus are given in Cycle Threshold (CT) values, with a 3.3 CT increase equating to a 1 $\log_{10}$ reduction in detectable viral genome (Table 1). A PCR value of 45 equates to undetectable as this is the end point of the assay.

Table 1: Theoretical table demonstrating the correlation between CT value and retrievable viral genomes present, as calculated from the stock suspension CT

Reduction	Viral genomes/ml	CT value
Neat cell culture	29,000,000,000	12
1 log reduction	2,900,000,000	15.3
2 log reduction	290,000,000	18.6
3 log reduction	29,000,000	21.9
4 log reduction	2,900,000	25.2
5 log reduction	290,000	28.5
6 log reduction	29,000	31.8

During this analysis two types of flat surfaces were analysed, stainless steel and ceramic.

Basic test procedure

Non-exposed control samples:

1. For each control (non-Nanoclave exposed) test surface, 10 µl of neat viral cell culture (~10^9 viral genomes) was inoculated onto each sample point. The test area consisted of 25 sampling points inoculated within a 5cm^2 test area (250 µl in total)
2. Each 5cm^2 test area was sampled using a cotton-tipped swab pre moistened with molecular grade water
3. Each swab was placed in 500 µl of molecular grade water and vortexed to release the virus particles
4. 200 µl of the resulting suspension was removed and extracted for PCR using the Qiagen mini prep extraction kit and eluted into 100 µl
5. 10 µl of extract was processed using a semi quantitative Adenovirus real time PCR [2]

Test samples:

1. For each test surface, 10 µl of neat viral cell culture (~10^9 viral genomes) was inoculated onto each sample point. The test area consisted of 25 sampling points inoculated within a 5cm^2 test area (250 µl in total)
2. The test surface was placed in the Nanoclave and exposed for sequential time periods to the UV light source
3. After indicated exposure time, each 5cm^2 test area was sampled using a cotton-tipped swab pre moistened with molecular grade water
4. Each swab was placed in 500 µl of molecular grade water and vortexed to release the virus particles
5. 200 µl of the resulting suspension was removed and extracted for PCR using the Qiagen mini prep extraction kit and eluted into 100 µl
6. 10 µl of extract was processed using a semi quantitative Adenovirus real time PCR [2]

All PCR's were run with a negative extraction, as well as negative and positive controls. Positive controls were utilised to monitor assay performance across runs.

Results

Stainless steel surface

A flat stainless steel surface was contaminated with high numbers of Adenovirus (as described), placed in the Nanoclave Cabinet and exposed to UV in 30 second bursts for up to 6 minutes. Sampling was undertaken after each exposure period. Actual CT values at each test point are shown in Figure 1. Average results are presented in Table 2.

The ability of Nanoclave exposure to degrade Adenovirus DNA is shown by an increase in the CT value with accumulated exposure time.

Figure 1: Individual PCR CT values from all sampling points during analysis of Adenovirus inoculated stainless steel following accumulated exposure in Nanoclave Cabinet. (Replicate numbers vary due to size of the metal surface and number of initial inoculations possible)

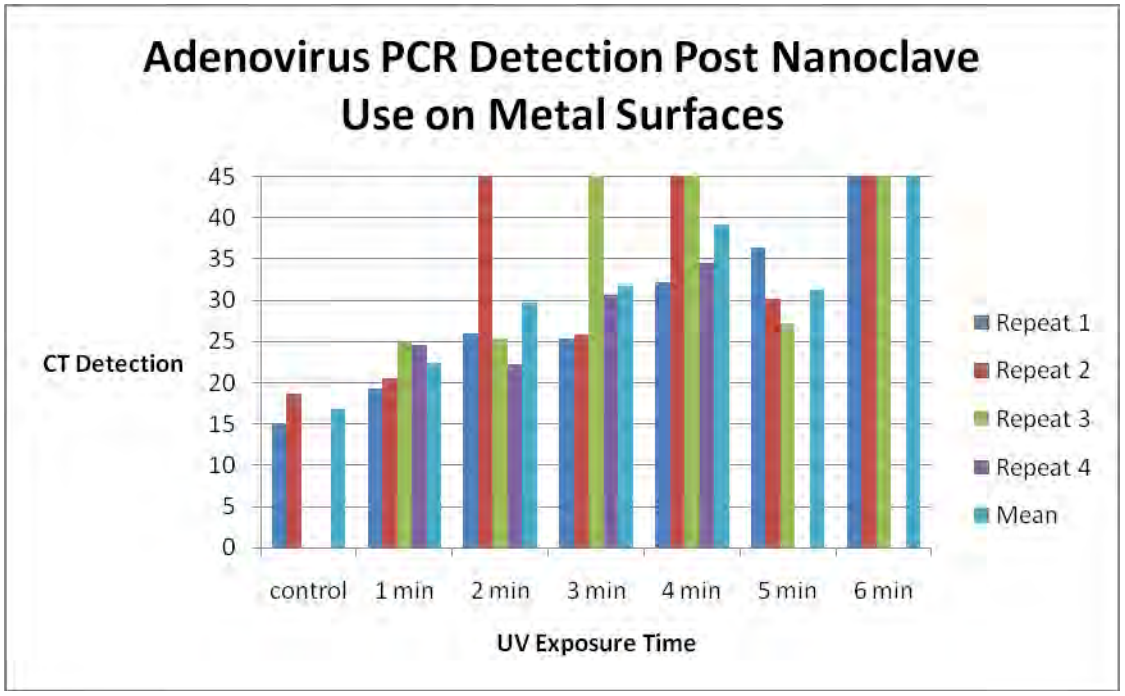


Table 2: CT values of Adenovirus PCR performed on swabs from inoculated test areas of stainless steel after accumulative exposure to UV light in the Nanoclave Cabinet.

		Exposure time					
	Control	1 min	2 min	3 min	4 min	5 min	6 min
Mean CT	17	22	25	27	33	31	undetectable

After a 3 minute exposure a > 3 log reduction in detected viral DNA occurred. After 6 minutes of exposure DNA was undetectable (> 6 $\log_{10}$ reduction).

Ceramic surface

Flat ceramic tile surfaces were contaminated with high numbers of Adenovirus, placed in the Nanoclave Cabinet and exposed to UV in 30 second bursts for up to 6 minutes. Sampling was undertaken after each exposure period. Actual CT values at each test point are shown in Figure 2. Average results are presented in Table 3.

Figure 2: Individual PCR CT values from all sampling points during analysis of Adenovirus inoculated ceramic surface following accumulated exposure in Nanoclave Cabinet.

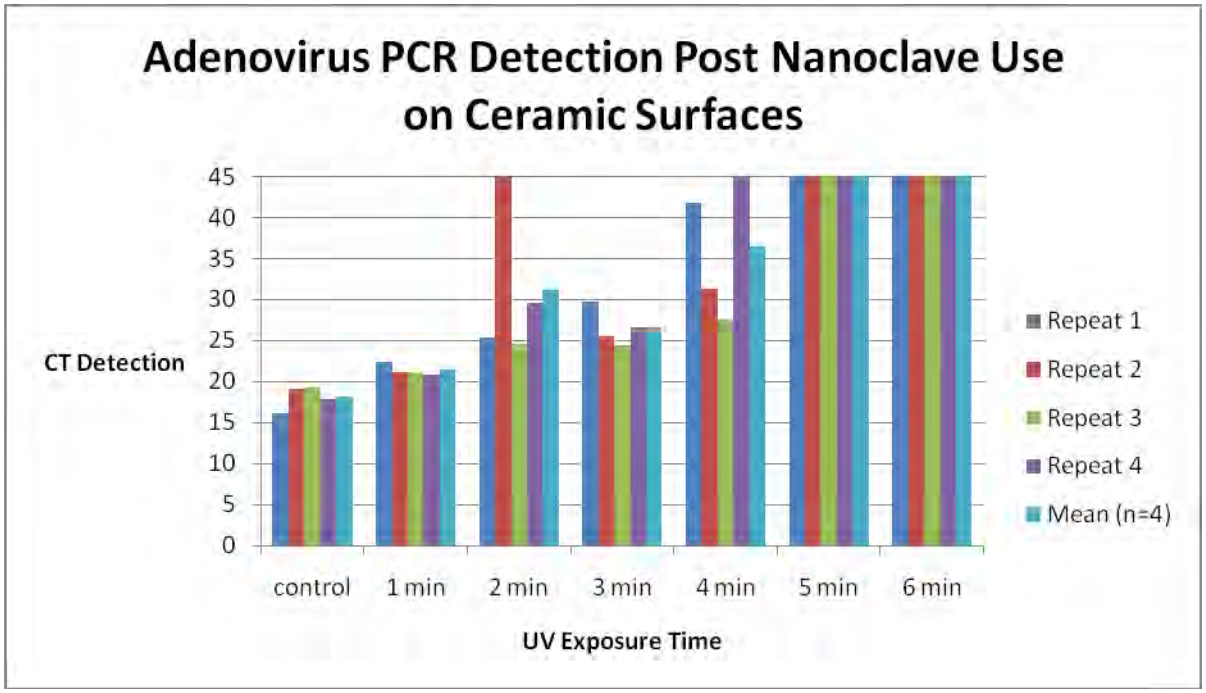


Table 3: CT values of Adenovirus PCR performed on swabs from inoculated test areas of ceramic material after accumulative exposure to UV light in the Nanoclave Cabinet.

		Exposure time					
	Control	1 min	2 min	3 min	4 min	5 min	6 min
Mean CT (n=4)	18	22	27	27	34	undetectable	undetectable

After a 3 minute exposure a > 3 log reduction in detected viral DNA occurred. After 5 minutes of exposure DNA was undetectable (> 6 $\log_{10}$ reduction).

Conclusion

Under the experimental conditions described, using flat stainless steel and ceramic test surfaces, the Nanoclave Cabinet led to the degradation of Adenovirus DNA, inoculated from viable culture material, such that it became undetectable by a sensitive PCR. A high level of DNA was consistently rendered undetectable on both of these surface types after a 6 minute UV exposure.

Precise comment cannot be given on the minimum required exposure to achieve a > 6 log reduction in viable virus. As Adenovirus is likely to become non-viable before DNA becomes non-detectable by PCR, the exposure time required for reduction in viable organisms may be less than is demonstrated in the current experiments.

References

1. Hijnen WAM, Beerendonk EF and Medema GJ. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo) cysts in water. *Water research* 2006; 40: 3-22
2. Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *Journal of Medical Virology* 2003; 70:228-39

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Investigation conducted by Elaine Cloutman-Green BSc, MRes, MSc

Report prepared by Elaine Cloutman-Green BSc, MRes, MSc

Report issued by Dr John Hartley MRCP, FRCPath

14th November 2010
Great Ormond Street Hospital for Children NHS Trust

University Hospitals Birmingham NHS
NHS Foundation Trust

ASSESSMENT OF THE MICROBIOLOGICAL EFFICACY OF THE NANOCLAVE CABINET

FOR

NANOCLAVE TECHNOLOGIES

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NOVEMBER 2011

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OBJECTIVE

To assess the performance of the Nanoclave Cabinet against four species of bacteria and *Clostridium difficile* spores in the presence of low organic soiling.

The Nanoclave cabinet is designed to disinfect non-invasive medical and electronic equipment eg infusion pumps, blood pressure monitors and cuffs.. The equipment is subjected to UVC light for 60 secs.

Previous tests have not included any organic matter with the test organisms. Therefore, these tests were designed to mimic the scenario of low levels of soil remaining on the items due to inadequate cleaning prior to processing in the cabinet.

The principle of these tests was to expose stainless steel discs inoculated with the test organisms to UVC light for 60 secs in the Nanoclave cabinet. The manufacturer's instructions for use were followed.

European standards for assessing the efficacy of chemical disinfectants describe testing under clean (0.03% bovine serum albumin) and dirty (0.3% bovine serum albumin and 0.3% sheep red blood cells) conditions. The tests on the Nanaoclave cabinet were carried out under the conditions described as clean i.e. in the presence of 0.03% bovine serum albumin.

TEST METHOD

Test organisms

Staphylococcus aureus	NCTC 10788
Pseudomonas aeruginosa	NCTC 6749
Escherichia coli	NCTC 10418
Enterococcus hirae	NCTC 12367
Clostridium difficile	NCTC 11209 (spore suspension)

The test suspensions were prepared as described in EN 1276 and EN 13704 for bacteria and spores respectively. A suspension containing at least 10^8 cfu/ml was obtained for each test organism.

Organic load

0.03% bovine serum albumin (BSA) sterilized by membrane filtration

Test method

The test organism (20 µl) in the presence of the organic load was dried onto sterile stainless steel discs (1 cm diameter) at 30^{o}C for 1 hour 20 minutes. Two test pieces were attached to each surface of a plastic cube i.e. 12 test pieces per cycle and placed in the cabinet for exposure to UVC light. The discs were therefore, exposed in horizontal and vertical positions. After processing the test pieces were cultured by aseptically transferring them into 10 ml of tryptone soya broth containing sterile glass beads. After 1 min of vortexing, the broth was ten fold diluted and the broth and the dilutions plated on to tryptone soya (bacteria) or blood agar (Clostridium difficile spores). After the required incubation time the number of surviving test organism enumerated. Untreated test discs were cultured to establish the pre counts. The test was repeated 3 times for each test organism.

Position of test pieces

Top	1A and 1B
Side 1	2A and 2B
Side 2	3A and 3B
Side 3	4A and 4B
Side 4	5A and 5B
Bottom	6A and 6B

RESULTS

There are no standard test methods or acceptance requirements for this type of equipment. Therefore, as this system is an alternative to the use of chemical disinfectants, the test requirements for these were used as the basis for the acceptance criteria. The tests used for bactericidal activity e.g. EN 13727 require a 5 $\log_{10}$ reduction and for sporicidal activity e.g. EN 13704 require a 3 $\log_{10}$ reduction.

The $\log_{10}$ reductions obtained for each test organism are shown in table 1. Individual reductions for each test organism are shown in tables 2 – 6.

The results obtained demonstrate that the Nanoclave Cabinet with 60 seconds exposure to UVC light gave a >5 $\log_{10}$ reduction with bacteria and a >3 $\log_{10}$ reduction with spores of C. difficile.

Table 1

Summary table for all test organisms

	Mean log_{10} reduction factor obtained				
Sample	***S. aureus***	***P. aeruginosa***	***E. coli***	***E. hirae***	***C. difficile***
Pre count	7.44	6.23	6.13	7.37	6.31
1	>7.11	>6.06	6.13	5.82	3.63
2	>7.06	>6.06	>5.41	6.24	3.73
3	>7.27	>6.06	>5.80	>6.52	3.55
4	7.44	>6.06	5.96	>5.83	3.29
5	6.93	6.23	>5.96	>6.7	3.65
6	>7.27	6.23	>5.75	>5.8	3.47
Mean	**>7.18**	**>6.12**	**>5.84**	**>6.15**	**3.55**
SD	**0.624**	**0.485**	**0.486**	**0.659**	**0.470**

Table 2

***Staphylococcus aureus* NCTC 10788**

	Log_{10} reduction factor obtained			
Sample	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean**
1A	7.44	7.44	7.44	
1B	6.44	>6.44	7.44	>7.11
2A	7.44	7.44	6.14	
2B	7.44	>6.44	7.44	>7.06
3A	7.44	>6.44	7.44	
3B	7.44	7.44	7.44	>7.27
4A	7.44	7.44	7.44	
4B	7.44	7.44	7.44	7.44
5A	4.38	7.44	7.44	
5B	7.44	7.44	7.44	6.93
6A	7.44	7.44	7.44	
6B	>6.44	7.44	7.44	>7.27
Mean				**>7.18**

Table 3

***Pseudomonas aeruginosa* NCTC 6749**

	Log_{10} reduction factor obtained			
Sample	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean**
1A	>5.23	6.23	6.23	
1B	6.23	6.23	6.23	>6.06
2A	>5.23	6.23	6.23	
2B	6.23	6.23	6.23	>6.06
3A	6.23	6.23	>5.23	
3B	6.23	6.23	6.23	>6.06
4A	>5.23	6.23	6.23	
4B	6.23	6.23	6.23	>6.06
5A	6.23	6.23	6.23	
5B	6.23	6.23	6.23	6.23
6A	6.23	6.23	6.23	
6B	6.23	6.23	6.23	6.23
Mean				**>6.12**

Table 4

***Escherichia coli* NCTC 10418**

	Log_{10} reduction factor obtained			
Sample	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean**
1A	6.13	6.13	6.13	
1B	6.13	6.13	6.13	6.13
2A	6.13	4.83	6.13	
2B	>5.13	>5.13	>5.13	>5.41
3A	6.13	6.13	>5.13	
3B	>5.13	6.13	6.13	>5.80
4A	6.13	6.13	5.13	
4B	6.13	6.13	6.13	5.96
5A	6.13	6.13	6.13	
5B	>5.13	6.13	6.13	>5.96
6A	>5.13	6.13	6.13	
6B	4.83	6.13	6.13	>5.75
Mean				**>5.84**

Table 5

***Enterococcus hirae* NCTC 12367**

	Log_{10} reduction factor obtained			
Sample	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean**
1A	6.07	5.05	5.89	
1B	6.37	6.07	5.47	5.82
2A	7.37	6.37	5.67	
2B	6.07	5.89	6.07	6.24
3A	7.37	6.07	6.07	
3B	7.37	>6.37	5.89	>6.52
4A	>6.37	5.67	5.77	
4B	4.72	>6.37	6.07	>5.83
5A	7.37	6.37	6.37	
5B	7.37	>6.37	>6.37	>6.70
6A	4.70	6.37	6.07	
6B	5.67	>6.37	5.59	>5.80
Mean				**>6.15**

Table 6

***Clostridium difficile* NCTC 11209**

	Log_{10} reduction factor obtained			
Sample	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean**
1A	3.77	2.90	3.59	
1B	4.71	3.19	3.61	3.63
2A	4.53	3.85	3.40	
2B	3.68	3.42	3.48	3.73
3A	4.20	3.75	2.76	
3B	4.36	3.36	2.88	3.55
4A	3.61	3.27	3.16	
4B	3.56	3.06	3.06	3.29
5A	3.20	4.03	3.08	
5B	4.11	3.97	3.48	3.65
6A	3.24	4.03	3.40	
6B	3.67	3.46	3.00	3.47
Mean				**3.55**

CONCLUSION

The Nanoclave Cabinet achieved a >5 $\log_{10}$ reduction in the presence of low level soiling with *S. aureus*, *P. aeruginosa*, *E. coli* and *E. hirae* dried onto stainless steel discs. A >3 $\log_{10}$ reduction was achieved in similar test conditions with *C. difficile*.

The position of the tests pieces did not appear to influence the reductions obtained.

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CR Bradley **MAC Wilkinson**
Laboratory Manager **Biomedical Statistician**

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Dr AP Fraise
Director

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ASSESSMENT OF THE MICROBIOLOGICAL EFFICACY OF THE NANOCLAVE CABINET

OBJECTIVE

A preliminary screen to assess the performance of the Nanoclave Cabinet against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the presence of high organic soiling.

The Nanoclave cabinet is designed to disinfect non-invasive medical and electronic equipment e.g. infusion pumps, blood pressure monitors and cuffs. The equipment is subjected to UVC light for 60 secs.

The manufacturer recommends that all devices are cleaned prior to processing in the cabinet. These tests were designed to mimic the worse-case scenario of high levels of soil remaining on the items due to the absence of cleaning.

The principle of these tests was to expose stainless steel discs inoculated with the test organisms to UVC light for 60 secs in the Nanoclave cabinet. The manufacturer's instructions for use were followed.

European standards for assessing the efficacy of chemical disinfectants describe testing under clean (0.03% bovine serum albumin) and dirty (0.3% bovine serum albumin and 0.3% sheep red blood cells) conditions. The tests on the Nanaoclave cabinet were carried out under the conditions described as dirty i.e. in the presence of 0.03% bovine serum albumin.

TEST METHOD

Test organisms

Staphylococcus aureus	NCTC 10788
Pseudomonas aeruginosa	NCTC 6749

The test suspensions were prepared as described in EN 1276. A suspension containing at least 10^8 cfu/ml was obtained for each test organism.

Organic load

0.3% bovine serum albumin and 0.3% sheep red blood cells (sterile).

Test method

The test organism (20 µl) in the presence of the organic load was dried onto sterile stainless steel discs (1 cm diameter) at 30°C for 1 hour 20 minutes. Two test pieces were attached to both the upper and lower surface of a plastic cube, i.e. 4 test pieces per cycle, and placed in the cabinet for exposure to UVC light. After processing the test pieces were cultured by aseptically transferring them into 10 ml of tryptone soya broth containing sterile glass beads. After 1 min of vortexing, the broth was ten fold diluted and the broth and the dilutions plated on to tryptone soya agar. After the required incubation time the number of surviving test organisms were enumerated. Untreated test discs were cultured to establish the pre counts. The test was repeated 3 times for each test organism.

Position of test pieces

Top	1A and 1B
Bottom	6A and 6B

RESULTS

There are no standard test methods or acceptance requirements for this type of equipment. Therefore, as this system is an alternative to the use of chemical disinfectants, the test requirements for these were used as the basis for the acceptance criteria. The tests used for bactericidal activity e.g. EN 13727 require a 5 $\log_{10}$ reduction.

The $\log_{10}$ reductions obtained for each test organism are shown in table 1. Individual reductions for each test organism are shown in tables 2 and 3.

The results obtained demonstrate that the Nanoclave Cabinet with 60 seconds exposure to UVC light gave a >5 $\log_{10}$ reduction with bacteria.

Table 1

Summary table for all test organisms

	Mean log_{10} reduction factor obtained	
Sample	***S. aureus***	***P. aeruginosa***
Pre count	6.94	6.76
1	6.44	>5.55
6	5.94	>6.43
Mean	**6.19**	**>5.99**
SD	**0.762**	**0.771**

Table 2

***Staphylococcus aureus* NCTC 10788**

	Log_{10} reduction factor obtained			
Sample	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean**
1A	5.94	5.94	6.94	
1B	6.94	6.94	5.94	6.44
6A	6.94	5.94	5.94	
6B	6.94	5.09	4.76	5.94
Mean				**6.19**

Table 3

***Pseudomonas aeruginosa* NCTC 6749**

	Log_{10} reduction factor obtained			
Sample	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean**
1A	5.76	4.50	5.46	
1B	>5.76	5.06	6.76	>5.55
6A	6.76	6.76	6.76	
6B	5.76	>5.76	6.76	>6.43
Mean				**>5.99**

CONCLUSION

The Nanoclave Cabinet achieved a >5 $\log_{10}$ reduction with *S. aureus* and *P. aeruginosa* dried onto stainless steel discs in the presence of high level soiling.

The position of the tests pieces did not appear to influence the reductions obtained.

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