Effects of the Decontamination Solution DECON SHIELD on the Inactivation of Botulinum toxin A

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Abstract

Decontamination refers to the process of removing, reducing and/ or preventing the spread of contamination from persons, equipment and/ or other objects or structures involved in hazardous materials or health related activities. Contaminates which are harmful to us include chemical as well as biological substances such as bacteria, viruses and bacterial products like toxins. Removal of these contaminates is necessary in many industries including, Public health, Fire Brigade and the Police Service where exposure to hazardous substances is possible. Decontamination is an essential process which allows for the effective removal of these contaminates and safe site recovery. The current climate of Terrorism has drawn attention to the possibility of select biological agents such as Anthrax and Botulinum toxin being used against civilian populations. DECON SHIELD is a new solution which offers a non- corrosive, non-toxic yet effective option for decontamination of bacterial and chemical substances. This experiment tested the effectiveness of this solution in inactivating Botulinum toxin A by applying the solution to 10ul of active toxin. The inactivation of the toxin was tested at the end of select contact times. Results indicated that DECON SHIELD was successful in inactivating Botulinum toxin A after one minute contact time.

Introduction

Decontamination methods are a vital component of all microbiological and chemical processing, whether it is in a laboratory or in the field, it is important to have an appropriate solution which is capable of inactivating toxic or harmful substances, including biological agents, thereby rendering the surface or item free from contaminants and safe for continued work or transport. Many of the decontamination solutions available are effective yet harmful to humans, due to the toxic nature of the chemicals or are destructive by way of corrosion to particular surfaces and items.

To date the most difficult bacterial agent to effectively decontaminate is *Bacillus anthracis,* the causative agent of Anthrax. This is due to the fact that Bacillus species produce a spore which has a protective coat enabling it to form resistance to heat, ultra violet light and most chemicals. Due to this fact there are limited decontamination solutions which are effective against this agent.

DECON SHIELD is a new decontamination solution on the market, supplied by Martin International in Canberra. This non- corrosive, non-toxic solution is biodegradable and can be used on surfaces, large and small items and even outside environments. Incorporated within the solution is a wetting agent which acts to break the surface tension of the cell membranes allowing the delivery of the active biocide.

Recent studies have indicated that DECON SHIELD is effective at inactivating *Bacillus anthracis* spores (Ames strain). This study sought to determine the effectiveness of this solution in the inactivation of the biological toxin produced by the bacteria *Clostridium botulinum*.

Botulinum toxin is the most toxic substance known to man, with an ID50 (intoxication dose of 50% population exposed) of 0.001ug/kg body weight. In fact it is more toxic than the man-made substance Sarin nerve agent.

Strains of the anaerobic bacterium *Clostridium botulinum* can produce seven different botulinum toxins (A-G). The most common of which is Botulinum toxin A, this experiment sought to determine the effectiveness of DECON SHIELD in the inactivation of Botulinum toxin A.

Materials and Methods

All experimental work was conducted within a Personal Containment Laboratory Class III (PC3) located within the Queensland Health Services Microbiology Laboratory.

DECON SHIELD comprised of two separate solutions, was mixed together at the time of use. Once made up the solution has an active life span of 7 days. The solution was stored in a cool dry area away from direct sunlight and heat.

The solution was prepared by mixing 1Part A with 18 parts water first and then adding 1Part B. The solution was agitated lightly for one minute prior to use.

Botulinum toxin production

A stored frozen vial of Botulinum toxin A was thawed in a water bath and used to inoculate cooked meat broth. The cooked meat broth was then incubated for 24 hours until bacterial growth was evident. Macroscopic and microscopic identification of growth and bacteria were recorded. 100ul of the inoculated broth was transferred to a centrifuge tube and spun for 20 minutes at high speed. The supernatant which contains the toxin was used for the experiment.

The supernatant was tested for the presence of B. toxin A using the Rapid Analyte Measurement Platform (RAMP), a hand held instrument and test kits for the detection of Botulinum toxin A.
This result was positive and recorded as Control Sample 1.

Experimental design

The effectiveness of the decontamination solution as well as the time required for effective inactivation was conducted by labelling Petri dishes with the date and allocated contact times; 1 minute, 2 minutes, 5 minutes, 10 minutes, and 15 minutes respectively. 10ul of the concentrated B. toxin solution was transferred via pipette to the dry Petri dishes. The Petri dished were then sprayed with an even distribution of the DECON SHIELD and allowed to stand for the set contact time. A timer was set which indicated when this time was complete.

At the completion of each contact time the Petri dish was swabbed thoroughly and the swab was transferred to the buffer solution of the RAMP test. If the Botulinum toxin was still present in levels above the limit of detection the RAMP test would indicate Positive. If the Botulinum toxin levels were present in levels lower than the limit of detection which would indicate no activity, the RAMP test was negative. Refer to Table One for test results. In addition a test tube was set up with 0.5ml of supernatant containing the B. toxin and 0.5 ml of DECON SHIELD solution. This was agitated lightly and allowed to stand for one minute. This sample was tested for the presence of active B. toxin using the RAMP at the end of this allocated contact time.

Two Petri dishes were set up with 10ul of supernatant containing the toxin and were labelled as Control Sample 2. These dishes would not undergo decontamination and were tested at the completion of the experiment.

Results

Table 1. **Post decontamination results**

Method	Contact time	Test repeated	RAMP Botox Result
B. toxin A 10ul sample: spray decon solution	1 min	3	Negative (-)
	2 mins	2	Negative (-)
	3 mins	2	Negative (-)
	5 mins	1	Negative (-)
	10 mins	1	Negative (-)
B.toxin : spray	**Contact time**	**Repeated**	**Result**
	15 mins	1	Negative (-)
B. toxin A 0.5ml: 0.5 ml	1 min	2	Negative (-)
Control Sample 1	Botox sample tested prior decon		Positive (+)
Control Sample 2	Positive sample tested post experiment		Positive (+)

Discussion

The Rapid Analyte Measurement Platform (RAMP) is a hand held instrument used for the detection of B. toxin A from environmental samples. Based on immunoassay technology the RAMP test kit indicates the presence of B. toxin when an antibody-antigen complex is indicated via florescence detection. The detectable limit for
B. toxin is 5 ng per sample (equivalent to 2.5 ng delivered to the test cartridge). This limit of detection allows for a rapid and effective means of toxin detection.

The supernatant was initially tested for the presence of B. toxin and was positive using the RAMP. This indicated the successful extraction of the toxin. The effectiveness of a decontamination solution needs to take into account not only the chemical makeup and active mechanisms of the solution but the amount of time the solution needs to be in contact with the contaminate for the effective inactivation of these agents. At present the effective contact time for bleach (common decontamination solution) ranges from 10-20 minutes dependant on the type of contaminant.

Contact times selected ranged from 1-15 minutes to determine the minimum effective contact time required. Results indicate that this solution is effective after just 1 minute. Control sample 2 underwent the same conditions as the experimental samples yet did not undergo decontamination. This control sample indicated the presence of active toxin in the test solution, after both minute and 15 minutes, reflecting the fact that active toxin was present prior to decontamination. The results indicate that no active toxin was present after applying DECON SHIELD solution with a contact time of 1 minute or greater. This may be reflective of the solutions ability to disrupt the cell membrane and allow the active ingredients to penetrate. The reduction in contact time is a valuable quality of this new solution.

This experiment has demonstrated an effective contact time of one minute for the inactivation of Botulinum toxin A. This research suggests that DECON SHIELD solution is effective at inactivating botulinum toxin A at concentrations above 5ng. Further research needs to be conducted to determine the effectiveness of this solution on other potential chemical and biological agents.

References

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