# Waste management practices of infectious waste at the Reference Laboratory, Guyana.

Cecil Boston[1], Rajini Kurup[1], Tandika Allicock[1]

[1]Faculty of Health Sciences, University of Guyana, Georgetown, Guyana
Email: kurup_rajini@yahoo.com

**Abstract: Objective:** This research attempts to assess the degree and priority of action taken to minimize the risk posed by potential hazard. It is being undertaken to find out the sterility of treated waste that are sent out into the environment from (National Public health reference laboratory (NPHRL) and the means by which they are disposed. **Design and Methods:** A prospective study was carried out at NPHRL which targeted waste emanating from the Microbiology and Tuberculosis departments. A steam sterilizer was used to access the sterility of waste before disposal and the conditions that may affect the sterility of waste were examined. Waste loads of 15lbs, 10lbs and 5lbs were processed on different days. Thermal and Biological data were obtained using a chemical indicator (autoclave tape) and a biological indicator containing spores of *Geobacillus stearthermophilus* respectively. **Results:** Heat transfer was more efficient when waste was tested in stainless steel containers and single polypropylene autoclave bags rather than double. Growth of bacteria from residue was seen after exposure times of 10 and 15 minutes at 121°C. Growth of *Geobacillus stearthermophilus* was observed in waste processed in autoclave bags even after a cycle of 121°C for 45 minutes. **Conclusion:** Therefore, decontamination of infectious waste by autoclaving at 121°C for 10 minutes is insufficient due to the fact that conditions such as composition of waste, volume of waste, type of container used, and orientation in the autoclave contributes a great deal to the effective heat transfer during the autoclaving process. It is recommended therefore that waste be processes in smaller amounts in stainless steel containers and composition of waste load be standardized.

[Boston C, Kurup R, **Allicock T. Waste management practices of infectious waste at the Reference Laboratory, Guyana.** *Nat Sci* 2016;14(6):105-109]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 14. doi:10.7537/marsnsj14061614.

**Key words**: Waste management, infectious agents, hospitals, laboratories.

## 1. Introduction

Hospitals are one of the complex institutions which are frequently used by people from every walk of life in the society without distinction between age, sex, race and religion. This is over and above the normal inhabitants of hospital (patients and staff). All of them produce waste which is increasing in its amount and type due to advances in scientific knowledge and its creating impact. The hospital waste, in addition to the risk for patient and personnel who handle these wastes poses a threat to public health and environment (Rao, 2010).

Medical and Infectious waste is one of the current crisis in waste management and as such, major changes in medical waste disposal practices are expected to occur in the future due to regulatory requirement worldwide from local and international level. The increasing concern expressed by higher authorities and the general public about hazardous solid waste dictates that we identify methods for rendering our hazardous waste non-hazardous before disposal (MacKnight, 2010).

In developing an infectious waste management program for hospitals and laboratories one must realize that there is no one optimum plan. Several variables such as location, budget, available equipment and quality and nature of infectious waste must be considered. The EPA notes that potential hazards resulting from infectious waste are those that may occur when packaging integrity is compromised and infectious material is dispersed. It is said that medical waste may contain much higher concentration of organism in a more complex matrix and therefore the EPA recommends that infectious waste be treated before disposal in order to ensure protection from potential hazards. Steam sterilization techniques are recommended by the EPA for treatment of all infectious waste except pathological and animal bedding. The incineration technique was recommended for this type of infectious waste. Steam sterilization is one of the most effective methods for decontaminating biohazardous material. It combines heat and pressure to inactivate micro-organisms. This process has been used for sterilizing medical instruments in the hospitals and for the treatment of waste in laboratories for many years. However, this treatment does not normally include a destruction step in the treatment cycle. The solid waste remains recognizable after treatment although they may be adequately treated to inactivate all types of micro-organisms including bacterial spores.

Laboratory autoclave normally operates at a temperature of 121□C, a pressure of 15 pounds per square inch and a minimum cycle time of thirty

minutes. The effectiveness of an autoclave depends on time, temperature and direct steam contact with infectious agents. The most cost effectiveness means of treating the infectious waste of an institution might be a combination of treatment methods, both on and off site. Infectious waste that has been treated can be disposed of as a common solid waste in sanitary landfill.

National Standard methods, which include Standard Operating Procedures (SOPs), algorithms and guidance notes, promote high quality practices. Performance of standard methods depends on the quality or reagents and equipment. Laboratory and hospitals should ensure that these have been validated and shown to be fit for purpose. Therefore, internal and external quality assurance procedures should be in place. NPHRL as well as other hospitals and laboratory in Guyana at present do not use biological indicators for quality control testing of the steam sterilization method in the decontamination of infectious waste and thus the steam sterilizer cannot be properly validated. Therefore, other parameters that can affect the sterilization process resulting in infectious waste not being sterilized properly cannot be asses properly. The autoclave indicator tape (chemical indicator) that is used currently and mechanical indicators (digital displays, gauges) does not prove decontamination effectiveness. The autoclave tape only indicates that the outside of the container came to temperature, it does not reflect time conditions inside the load, and therefore, it only shows that autoclaving has been performed.

Biological indicators such as the spores of the thermophilic bacterium, *B. stearothermophilius* are highly resistant to thermal inactivation and thus may provide answers to whether wastes are properly decontaminated before disposal. Studies have shown that various parameters such as the type of containers, bags and the volume of waste can affect the heat transfer in the decontamination and sterilization process thus affecting the aim of the entire process. Therefore, there is a need for the biological indicators to assess the level of sterility of waste before it is sent into the environment.

## 2. Methodology

### Description of study

This research was carried out at the National Public Health Reference Laboratory located at Georgetown. This institution is a part of the public Sector of Guyana. The study focused on the Microbiology and Tuberculosis departments since these two department deal mainly with pathogenic microorganism.

### Study Design

A prospective study was carried out and the variables were collected simultaneously.

### Variables

Independent: Autoclaving Cycle and Processing techniques.

Dependent: Sterility of waste

The way infectious waste are process before treatment (bagging method, volume of waste, content of waste load, type of containers used and orientation in autoclave) and the time temperature profile chosen (Autoclaving Cycle) for decontamination affects the level of micro-organisms being eliminated or denatured.

### Method of measuring variables

Interviews, on site observation and testing were mainly used to obtain the relevant information.

### Data Analysis

The data obtained was analyzed using Microsoft Excel.

### Material and Method

The study was accomplished using commercially available plastic autoclave bags measuring 24″x 36″ (Fisher brand). These bags are constructed of polypropylene material and are designed to withstand a maximum temperature of 140□C. Standardized waste loads of 5lbs, 10lbs and 15lbs from the Microbiology and TB departments were used; however no attempts were made to standardize the contents of waste in each load for TB department. Waste consisted of contaminated disposable Petri-plates, blood culture bottles, Biochemical tubes (microbiology department), sputum cups, tubes, slide, gauze and lab coats (TB department). An average of 90 % of these plates and containers were contaminated with viable and vegetative organisms (*Staphylococcus* sp, *Streptococcus* sp, *Proteus s*p, *E.coli, M. tuberculosis, etc).* Bags were placed into a vacuum assisted steam sterilizer (model 400/500 HC series). The interior dimensions of this sterilizer was 17.5″ x 17.5″ x 27.6″ and it reached a temperature of 121°C in an average of 15 minutes after the initiation of the cycle.

The waste from the Microbiology department was processed in two different ways. Stainless steel containers were used strictly for biochemical tubes whereby, polypropylene autoclave bags were used for petri-plates and blood culture bottles. Waste loads were placed directly into steam sterilizer without the addition of water to containers since the machine utilizes a fixed amount of water to generate steam.

The sterilization efficacy was monitored by Biological Indicators, while waste load was monitored for accurate heat transfer by a chemical indicator (autoclave tape). The Biological system used was 3M Attest (1292 Rapid Biological Indicator). Each Attest ampoule contained a suspension of *Geobacillus*

*stearothermophilus* spores in culture which were denatured at a temperature of 121□C for 30 minutes and above. After the test cycles, biological indicators were removed and incubated in a low temperature incubator at a temperature of 60 □C for 48 hours. The internal temperature of the incubator was monitored by an external traceable digital thermometer. Positive cultures were determined by a colour change of the pH indicator in the ampoule from purple to yellow. An unprocessed biological indicator was used as a positive control.

The Biological indicators were placed at the top and bottom of waste load in bags and at the top of stainless steel containers. A syringe was used to draw molten agar that remained after completion of cycle from Microbiology department. This was placed onto blood agar plates and incubated aerobically at 35□C for 48 hours and anaerobically at 35□C for 96 hours. Gram stain was done to identify organism from plates that had growth along with other tests to identify species of organism. A positive $2^{+}$ sputum sample was placed onto Nutrient agar and Lowenstein and Jensen media after completion of waste from TB department. Plates were incubated at 35□C for 24 hours and Six weeks respectively. A smear was also made from the sample and stained using Ziehl – Neelsen to detect growth of the Bacilli and other micro-organism.

## 3. Results and Discussion

The NPHRL has a policy for the decontamination of all biohazardous waste generated from the laboratory (NPHRL-100-P10 Waste Management). However, at present only biohazardous waste from the Microbiology, Tuberculosis and Haematology Departments are being decontaminated before disposal in the sanitary landfill. The wastes are processed as they build up and after processing it is taken outside the building for storage until removal by Mayor and City Council.

Waste from each department was processed on separate days and all used a standard time temperature profile of 121□C for ten minutes. The content of each waste load was not standardized.

In the first series of experiment, attempts were made to identify growth of viable and vegetative micro-organisms. After 15 minutes growth of micro-organisms was observed. Figure 1 shows the steps that were taken to identify the organism (*Corynebacterium* species) from the plates that were incubated at 35□C for 24 hrs. However the particular species were not identified.

Attempts were also made to identify the location most difficult for steam and heat to circulate within the autoclave and the waste load. After a cycle of 121□C for 10 minutes results obtained were similar as seen by Rutala and colleagues on the Decontamination of Laboratory Microbiological Waste where, the greatest delay in heating was observed when thermocouple was placed 5.5 cm from bottom of the bags and where the bags nearest to the door heated more slowly than the middle and back. This may be as a result of the direct contact of steam with bags located at the back and the composition and volume of the waste load along with the orientation of waste inside steam sterilizer.

Large amount of waste and bags packed close together requires extra time for heat to penetrate them and thus the processing time will need to lengthen to as much as 60 minutes to ensure sterility. Sterilization is most effective when the organisms present in waste are either contacted directly by steam or are in small volumes of aqueous liquid (water). It is more efficient and safer to run two uncrowned loads than one crowded load.

Figure 1: Flow Chart for Identification of Micro-organism from Infectious waste residue

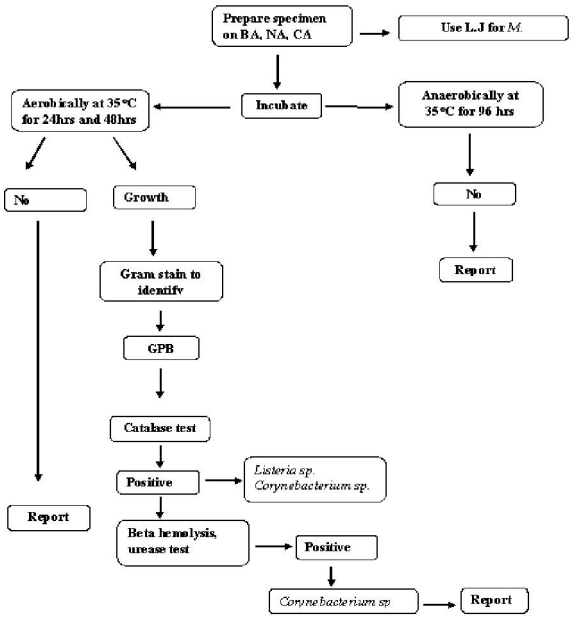


Figure 1

Figure 2a shows that at an exposure time of 10 minutes 50% of the autoclave tape (chemical indicator) changed completely and that was located outside of the waste load, whilst 25% of the ones located inside the bag had no change and 25% changed moderately. After the exposure time was adjusted, 50% of the tape located inside the bags changed moderately and the other 50% located outside the load changed completely.

Figure 3b shows that 50% of the indicator strip on the Biological indicators had faint color change but dropped by 30% after the exposure was increased. Moderate color change was seen by only 25% at 10 minutes but majority (60%) after 15 minutes. Biological ampoules were not getting into direct

contact with steam. This may be as a result of the bagging method. Placing waste in two bags rather than one hindered heat transfer under identical test conditions.

When microbiology waste was processed in stainless steel containers, there was a 100% colour change of both chemical and biological indicators (figure 3c) and only 20% growth of *B. stearthermophilus* after incubation of 48 hours. This may be due to the excellent conduction of heat through the walls of stainless steel.

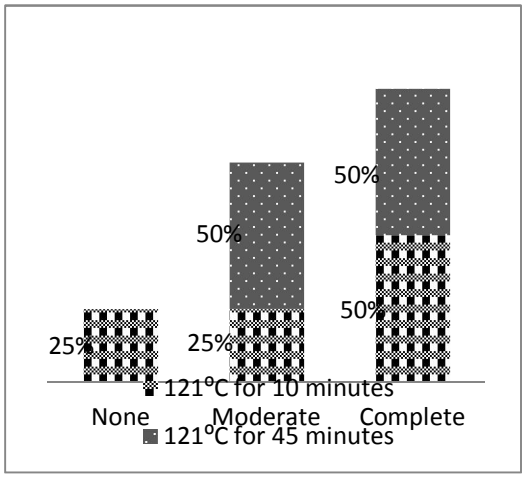


Figure 2a: Comparison of TB waste processed at two different cycles

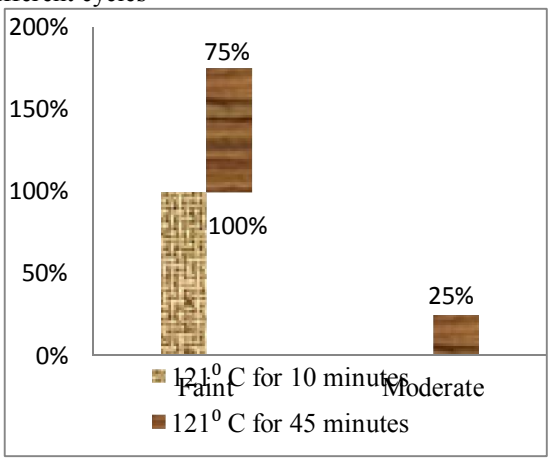


Figure 2b: Comparison of TB waste processed at two different cycles

It was evident that even after 15 minutes and 45 minutes exposure time, 100% growth of *B. stearthermophilus* were still observed from microbiology and TB waste processed in polypropylene bags. Results showed that 75% of the three day incubation period for each cycle had 100% positive growth while the other 25% had 71% positive growth.

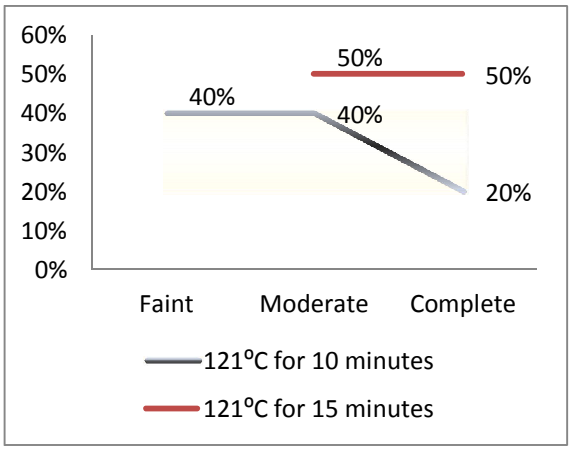


Figure 3a: Comparison of Microbiology waste processed in Autoclave bags at two different cycles

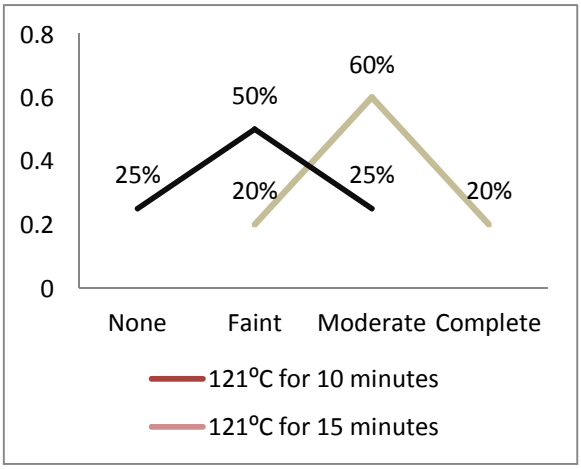


Figure 3b: Comparison of Microbiology waste processed in Autoclave bags at two different cycles

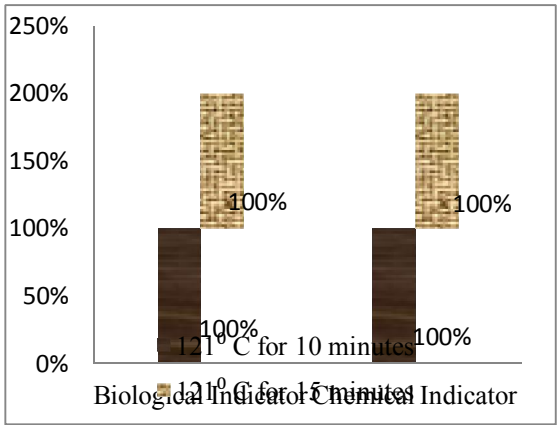


Figure 3c: Comparison of Microbiology waste processed in Stainless steel containers at two different cycles

The autoclave contains a steam jacket where steam is maintained. As steam flows from the steam

jacket into the sterilizing chamber, cool air is forced out and the load is preheated. A special valve then increases the pressure to 15 psi and the temperature rises to 121.5□C and at this point the chamber and the load is heated to the exposure temperature. The superheated water molecules rapidly conduct heat into micro-organism. Saturated steam is maintained at this temperature for the required exposure time thus providing the conditions necessary to achieve sterilization. As the cycle winds down, the chamber temperature is still above the boiling point of water (100□ C) for an average of 15 minutes and thus any micro-organism remaining after an exposure time of 15 minutes can still be denatured. The exposure time for destruction of bacterial spores and other micro-organism is reduced unless like TB waste, the content or objects are dense, an exposure time for at least 30 minutes may be required.

## 4. Conclusion

In conclusion, decontamination of infectious waste by autoclaving at 121□C for 10 minutes is insufficient even though waste may not contain micro-organisms that are highly resistant to heat due to the fact that conditions such as composition of waste, volume of waste, type of container used, and orientation in the autoclave contributes a great deal to the effective heat transfer during the autoclaving process.

Sterilization by autoclaving is invariably successful if properly done and may be reliable if specific minimal conditions are met, otherwise micro-organism will remain viable.

**Corresponding Author:**
Dr. Rajini Kurup
Faculty of Health Sciences
Turkeyen Campus
University of Guyana
E-mail: kurup_rajini@yahoo.com

4/27/2016

## References

1. Lauer JL, Battles DR and Vesley D, 1982. Decontaminating infectious laboratory waste by autoclaving. Appl Environ Microbiol 44 (3):690-694. Accessed on 28th April 2010.
2. Lee CC and Huffman LG, 1996. Medical waste management/incineration. Journal of Hazardous Materials. 48 (1-3):1-30. Accessed on 28th April 2010.
3. Rao SKM, Ranyal RK, Bhatia SS, Sharma VR, 2004. Biomedical waste management: An infrastructural survey of hospitals disposal of laboratory waste; MJAFI. 60(4)379-383. Accessed on 28th April 2010.
4. MacKnight, T.Kamrin.1993.Problems of medical and infectious waste.Envtl.L.23.785. Accessed on 28th April 2010.
5. Ozanne, Gerard Huot, Roger, and Montpetit, Claude. 1993. Influence of packaging and processing conditions on the decontamination of laboratory biomedical waste by steam sterilization. Appl. Environ. Microbiol. 59 (12):4335-4337. Accessed on 28th April 2010.
6. Rutula WA, Stiegel MM, and Sarubbi FA Jr. 1982. Decontamination of laboratory microbiological waste by steam sterilization. Appl. Environ. Microbiol.43 (6):1311-1316. Accessed on 28th April 2010.
7. Rutala, A. Williams, PhD, MPH. Mayhall, C. Glen, MD. 1992. Medical Waste. Infection Control and Hospital Epidemology.38 (1):37-39. Accessed on 28th April 2010.
8. WHO.1983.Management of waste from hospitals and other health care establishment. Euro reports and studies 97. Accessed on 28th April 2010.
9. Standard Unit, Evaluations and Standard Laboratory, Centre for infection. Risk Assessment: safe disposal of laboratory waste; QSOP34. 2.2. Accessed on 28th April 2010.