Chapter 6

Disposal of Testers

6.1. Disposal of used testers

Bart testers, when charged with a sample and incubated, are likely to now contain active bacterial populations whether the tester has gone positive by a recognized reaction for the specified bacteria or not. There is a global recognition that such cultured bacteria now present at least a theoretical risk to society and needs to be disposed of safely to ensure any hygiene risk is significantly reduced or eliminated. Because of these potential risks testers need to be disposed of in a responsible manner to ensure that any cultured microbes are eliminated or reduced to an insignificant risk. There are two approaches to achieve this reduction: (1) sterilization of the testers to ensure all of the cultured microbes are dead; or (2) sanitization that kills all of the microbes that are likely to generate some form of hygiene risk. The term "microbe" is used here to include not just the bacteria but also molds (fungi), yeast, protozoa and viruses.

The manner in which Bart testers are routinely used involves the testing of natural or industrial samples that do normally contain microbes that are either active or passive in the individual samples. These microbes are in large part normal members of the environment that is all around us from the soils, to streams, rivers, lakes and oceans and even in places like the deep oceans, deep sediments, muds, brines, sedimentary rocks and even up in the clouds and further out in the blackness of space within frozen ice meteorites. Indeed the edge of the microbial universe is not known and very poorly understood. Essentially we operate as humans within a cocoon of microbial activities which allows the planet to function the way it does!

Carrying out a Bart test (which is simple to do) creates conditions within that sample encourages the activities and growth of selected microbes from within that sample. These would be potentially interest and concern not necessarily with the direct hygiene risk but with the potential for nuisance activities. In the Bart testers there is not a direct focus on hygiene risk in part because this is already done when detecting coliform bacteria and E.coli. Those

technologies are well established while the newer Bart technologies are searching for the microbes that do create economic challenges within all natural and engineered systems. For safe disposal the hygienically significant microbes cultured within the testers needs to be eliminate (sterilised) while the other microbes do not create such a direct risk and need to be sanitised.

In the practise of using Bart testers to check for bacterial activity the number of bacteria isolated in the sample, grown in the Bart tester and needing now safe disposal is infinitely smaller than the bulk mass of those same bacterial types that are active in that environment that was sampled. However it is environmentally responsible to either sterilise or sanitise the completed Bart testers in order to eliminate the risk of back contamination of the environment or the indirect risk of causing nuisance societal risks. Methods of disposal follow either the sterilization or sanitization pathways of environmental and social responsibility.

For the field tester essentially all of the microbial risk is contained within the inner vial where the microbes become active and can grow. In the gap between the inner and outer vial in the field tester the principal risk relates to odors that may have been generated in the inner vial now becoming trapped within the outer vial. Such odors might be released during sterilisation or sanitization and so the testers for treatment and disposal should be bagged and minimally sealed with some form of acceptable tie. For the inner tester there is not the protection of the outer vial and cap to restrict the releases of odors. That is one important reason why laboratory testers should be used in that type of setting i.e. so that any odors released are dissipated quickly into the atmosphere of the laboratory. Again the lab testers may to be sealed with a suitable bag to prevent leakages of culture fluids and gases during sterilization or sanitization.

6.2. Sterilisation of used BART testers.

Sterilization means that methods are applied to totally eliminate all known living microbes from the finished testers that are going to be disposed of safely within the normal garbage collection methods available to the user of the Barts. Steam sterilization has been around since the early nineteenth century when Napoleon employed that technology to preserve meats during the invasion of Russia. Unfortunately at that time there was not realisation of the implication of

putting lead in the solder for sealing the cans!

Since that time steam sterilization has become a standard practise within the food and health industry achieving excellent standards of performance. In microbiology pressurized steam sterilization has become an accepted standard practise for rendering materials sterile. Here the standard is commonly set at 121^{o}C with 15p.s.i. for twenty minutes. Greater bulk materials required to be sterilised commonly result in longer exposure times. For Bart users wishing to dispose of the used testers than steam sterilization (also known as autoclaving) is a very good technique. In the home a equivalent exists in the domestic steam sterilisers used for canning (that is essentially rendering the food sterile and free from microbiologically influenced spoilage. For the Bart user then steam sterilization offers a very acceptable of removing risks that could be associated with positive testers within which microbes have grown as a normal part of the detection process. Steam sterilization (autoclaving) used testers would mean that the polystyrene inner and outer vials would collapse releasing their (now sterile) contents It is important to bag the testers and seal them in a manner that would not cause the bag to rupture. Microbiological facilities routinely will bag, seal and sterilize waste materials to allow safe landfill-type disposal. Here the bag can be replaced with glass or heat resistant plastic (e.g. polycarbonate) that is then sealed using a heat resistant screw cap that is sealable (by compression of a washer). If the Bart user wished to use such a sterilization method then local medical, veterinary, food laboratories would be able to advise on the best method for packing the testers and getting them to the facility.

The key factor here is getting the used testers safely to the sterilization facility while final disposal should then be the laboratory undertaking the sterilization. One common type of bag used is plastic made of 3mil polypropylene. This is called a “Biohazard Autoclavable Bag” and commonly is heavy duty, puncture resistant and double thick and is stable up to 135^{o}C. They come in various sizes and include seals of various types. During the autoclaving the bags have to be loosened to allow steam to penetrate into the testers and sterilize the contents. These biohazard bags come in a range of sizes and for most Bart user the small sized bags should be adequate (e.g. 8” x 12”, 20 x 30cm; 12” x 24”, 30 x 60cm; 19” x 24”, 48 x 61cm; and 24” x 36”; 61 x 92cm). It is important to note that these bags bear heat sensitive labels that change color to confirm that the contents have been sterilised.

If a user chooses to sterilise the used testers using a smaller steam sterilizer then the biohazard

autoclave bags should still be used. The advantage of doing that is that the label will indicate when the contents have been sterilized and routine disposal would now be acceptable.

6.3. Sanitization of used BART testers.

Many Bart users undertake tests in support of routine monitoring and treatment management practices and so do not generate such a bulk of used testers that would warrant sterilization by steam. Sanitization is not so all encompassing of autoclaving because now the objective is to kill only that fraction of the microbes that could cause a hygiene or nuisance risk. Although easier to achieve the question can always be asked as to whether the hygiene risk and nuisance microbes (HRNM) have been killed by the process or at least rendered relatively harmless. Heat remains the preferred delivery system but now the aim is to differentially kill the HRNM effectively. This has long been a long standing challenge in microbiology and was resolved by Pasteur in the nineteenth century by the application of lower levels of heat that would not achieve sterilization but would have a significant impact on the HRNM. Pasteur's first targets were the HRNM that made French beer inferior to German, and then milk since it was spoiling so quickly particularly in the rapidly urbanizing regions accompanying the industrial revolution. It was found that using lower heat temperatures (pasteurization) could impact negatively on the activities of the HRNM. For beer these meant that French beer would now be treated and stored and produce similar qualities to German. For the French people in the growing urban areas it meant that no longer were cows being marched along the streets to be milked at the doorstep but could now be bottled and then shipped to the customers.

Microwave generating ovens are relatively cheap and can be a convenient way to sanitize used Bart testers. There are certain limitations to the use of the microwave oven that should be exclusively used for the sanitization of used testers. It is recommended that the dedicate microwave carries the following sign prominently displayed: "THIS MICROWAVE OVEN IS DEDICATED TO THE USE OF USED BART TESTERS AND SHOULD NOT BE USED FOR ANY OTHER PURPOSE SUCH AS THE HEATING OF FOOD AND BEVERAGES". Choice of microwave is up to the user and it should have a heating capacity of 800 to 1,000 watts and placed away from any kitchen or food area.

Sanitization means to improve the hygiene risk to selectively eliminate of those potentially pathogenic microorganisms from the material of concern (e.g. used Bart testers). Here

sanitization involves the application of sufficient heat (e.g. pasteurization) to significantly destroy cells that may harbour hygienic risks. The objective is to heat the subjected (e.g. used testers) up to a sufficiently high temperature to destroy at least two orders of magnitude (i.e. 99%) of the microbial cells that are capable of growth without involving germination. Commonly this involves taking the temperature up by >40^{o}C. In practise Pasteur introduced temperatures of greater than 60^{o}C to pasteurise milk and beer. For used Bart testers it has been found that effective pasteurization quickly when the temperature in the cultured tester rises rapidly into the 65 to 80^{o}C range. This achieved by microwaving the used tester for between ninety seconds and two minutes. Standard sanitization of the used testers involves a number of steps defined below. The objective is to wrap the testers tightly using duct tape, place them on top of four paper towel sheets in a microwaveable safe defrost-vent bag using a multiple zipper, place the open bag (zipper open) into a 5.8L plastic tray without lid, place in microwave and apply heat (follow the guide in Table 6.3.1). At the end of the microwave generated sanitization the testers will each have internal temperatures ranging from 65 to 80^{o}C. Seal the bag by closing the two zippers and place a self-adhesive sticker on the outside that reads "CONTENTS HAVE BEEN EFFECTIVELY MICROWAVED BY PROTOCOL DBI 631 AND IS NOW SAFE FOR DISPOSAL WITH GARBAGE IN THE REGULAR MANNER". This label should; be signed, dated and include the most suitable phone number and e mail address for the person undertaking the sanitization.

6.3.1 Specifications for sanitization protocol

This list includes all of the component parts that would be used in the sanitization of used Bart testers for safe disposal with the garbage.

- Defrost-vent multipurpose double zipper plastic storage bag with dimensions 26.8 x 27.3 cms designated as "Large". Further information on these types of bags can be found at www.ziploc.ca
- Good quality 2" (5.4 cms) duct tape.
- Clear polypropylene 5.83L 11.6 x 20.8 x 34.2cms tray (example. Rubbermaid model 701H 2) – lid not required for sanitization.
- Microwave 800 to 1,000 watts timing in minutes and seconds.
- Disposable latex gloves and safety glasses.

- Bart testers should all contain the standard 15mL of sample that would have been subjected to testing by culture.

Table 6.3.1 Protocol DBI 631 recommended sanitization times

BART #*	Diameter (cms)**	Sanitization time (seconds)***	Active volume in testers (mL)
9	<11 - <16	90	135
10	<12 - <16	93	150
11	<12 - <17	96	165
12	<13 - <19	99	180
13	<14 - <19	102	195
14	<15 - <20	105	210
15	<16 - <20	108	225
16	<17 - <21	111	240
17	<17 - <22	114	255
18	<18 - <23	117	270

Note: number of Bart testers (*) may include lab and field testers but the volume per tester remains at 15mL; diameter of the duct taped testers will vary (**) within the diameter range shown depending the number of labs to fields being sanitized; sanitization time is in seconds (***) and should not be extended beyond those times since the objective is to get the contents in the testers up to the range from 65 to 80^{o}C. It is recommended to observed through the glass door of the microwave and shut down the sanitization if any of the testers begin to boil (this means that sanitization has now been achieved)

6.3.2 Protocol DBI 631 for sanitization of used Bart testers

Follow the order listed in Protocol DBI 631 to complete a sanitization treatment of nine to eighteen field or lab testers in any combination:

a. Take the tester for sanitization and keep them upright and form into a tight “circle” on a clean dry surface. Make sure that all of the caps (outers in the field and inners on the lab) are reasonably tightly screwed down.
b. Use duct tape to bind the testers all together. Use the adhesive side of the tape against the testers and ensure that all testers are taped using between one and a half and two circles of tape. The testers should now be held secure by the tape.
c. Lift the taped roll of testers and place in the zipper lockable microwave safe defrost and vent bag. Do not seal the multipurpose double zipper.
d. Place the bagged rolls of testers into the 5.83L polypropylene tray and place in the microwave on the tray in a central position so that the tray will turn during microwaving.
e. Close microwave door. Use Table 6.3.1 to determine the sanitization time (90 to 117 seconds). Enter the number appropriate to the number of testers to be sanitized (e.g. 14 testers would require 105 seconds (1 minute, 45 seconds).
f. Double check to make sure that all of the testers are tightly bound into the duct taped roll. Testers that are not in tight proximity to the other testers could overheat and melt.
g. Press the START button. The tester roll will run on the turntable and the internal temperatures will rise to between 65 and 80^{o}C. The timing specified is for 800 to 1,000 watt microwaves. At the very most there may be limited boiling in the outer testers but not in the middle of the roll. Under the rare circumstance that boiling is heard in the last few seconds then this would be acceptable.
h. Once the timing has been completed do not open the microwave door for five minutes to allow the testers to begin to cool and stabilise.
i. Remove the tray from the microwave and cautiously close the double zippers on the bag. It is recommended that gloves be worn for this stage in the rare case there is any sudden release of steam.
j. Allow the bag and contents to cool down for sixty minutes to room temperature.
k. Take a sanitization label and fill in the date, contact information and sign and then apply the label to the side of the bag. Use a permanent black marker (fine tipped) to write on the label.
l. Dispose with regular garbage as sanitized laboratory waste.

6.4 Disinfection of Bart testers prior to disposal

Disinfection of testers uses Trichloro-S-Triazontone (15g) as a stabilized chlorine tablet that liberates 90% of its weight as chlorine. One tablet is adequate to disinfect nine to 15 Bart testers provided that the bagged testers are disposed in regular garbage. Chlorination disinfection only starts when the garbage is compacted either in the collection truck or at the landfill site.

6.4.1 Specifications for sanitization protocol

This list includes all of the component parts that would be used in the sanitization of used Bart testers for safe disposal with the garbage.

- Defrost-vent multipurpose double zipper plastic storage bag with dimensions 26.8 x 27.3 cms designated as "Large". Further information on these types of bags can be found at www.ziploc.ca
- Good quality 2" (5.4 cms) duct tape.
- DIS- BART disinfector containing one tablet of stabilized chlorine in an white outer bottle (vial) with a dark green cap.

6.4.2 Protocol DBI 641 for disinfection of used Bart testers

Follow the order listed in Protocol DBI 641 to complete a sanitization treatment of up to a total of eighteen field or lab testers in any combination:

a. Take the testers for disinfection and keep them upright and form into a tight "circle" on a clean dry surface. Make sure that all of the caps (outers in the field and inners on the lab) are reasonably tightly screwed down. Place one DIS- Bart disinfector in to the middle of the clustered testers making sure that the green cap is up and that all of the other testers also have the cap facing up.
b. Use duct tape to bind the testers all together. Use the adhesive side of the tape against the testers and ensure that all testers are taped using two circles of the testers with the duct tape. The testers should now be securely held by the tape.
c. Lift the taped roll of testers and place in the zipper lockable microwave safe defrost and vent bag. Seal the multipurpose double zipper.
d. Place a disinfection label on the outside of the bag on the side just below the closed zippers. Using a permanent black marker (fine tipped) pen then write on the label giving the date, contact information and signature.

e. Dispose with regular garbage. Chlorine will not be released until the package is compacted in some manner. At that time of compaction then the chlorine will be released and disinfect the used testers. Note that there is sufficient chlorine to neutralise all of the sodium thiosulfate in the testers and still disinfect the contents of the used testers.