Thermal Disinfection Validation: The Relationship Between A_0 and Microbial Reduction

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Abstract

Validating a thermal disinfection process for the processing of medical devices using moist heat via direct temperature monitoring is a conservative approach and has been established as the A_0 method. Traditional use of disinfection challenge microorganisms and testing techniques, although widely used and applicable for chemical disinfection studies, do not provide as robust a challenge for testing the efficacy of a thermal disinfection process. Considerable research has been established in the literature to demonstrate the relationship between the thermal resistance of microorganisms to inactivation and the A_0 method formula. The A_0 method, therefore, should be used as the preferred method for validating a thermal disinfection process using moist heat.

Disinfection, which is defined as reducing the number of viable microorganisms on a product to a level previously specified as appropriate for its intended further handling or use, can be achieved thermally by the action of moist heat.[1] Thermal disinfection during the processing of medical devices, typically performed in a washer-disinfector, is widely used for two purposes. The first is for reducing product bioburden (disinfection) either as a terminal step (e.g., for noncritical or semicritical devices) or prior to packaging and sterilization (e.g., for critical devices) in preparation for patient use. The second is to render the devices safe for handling for central service professionals during inspection and packaging.[2,3] Thermal disinfection requirements therefore should consider the potential levels of microbial contamination on reusable devices after use, the desired level of reduction to render those devices safe for handling and for their intended purpose, and the reliability of the disinfection process to consistently achieve that endpoint.

The microbial load on device types after patient use has been established in the literature and can vary depending on the typical clinical use of the device. For example, critical (surgical) devices, on average, have demonstrated relatively low levels of viable microorganisms (bioburden level $<10^2$ colony-forming units [CFU]/cm^2).[4] However, these same studies have shown the concentration of other testing analytes (e.g., protein, total organic carbon, hemoglobin) to be more noteworthy. Although the data indicate that residual clinical soil (e.g., human secretions, blood, tissue) can harbor microorganisms, the incoming product bioburden levels are far below the microbial populations challenged during an overkill sterilization process (e.g., moist heat or gaseous processes).

Conservative sterilization processes have been demonstrated to achieve at least a 12-$\log_{10}$ reduction of microorganisms with a known higher resistance versus typical bioburden.[3,5] Cleaning, which is defined as the removal of contamination from an item to the extent necessary for its further processing and its intended subsequent use, is an important step to render the device ready for sterilization and will further reduce the levels of microorganisms prior to sterilization. Therefore, with critical devices, adequate cleaning followed by sterilization is the minimum requirement to ensure the device is safe for patient use.

It is not likely that, for the intended use of the device, a disinfection process is strictly necessary as an intermediate step prior to sterilization. A benefit may exist to having an interim disinfection step to render the device safe for handling during inspection and packaging for sterilization. For example, the expectation in the Occupational Safety and Health Administration's Bloodborne Pathogens standard 29 CFR 1910.1030 is that an

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employer will minimize the occupational exposure to bloodborne pathogens.

Thermal disinfection has been used by sterile processing departments as a universal precaution to reduce the risk of exposure to processing personnel postcleaning. Although routine thermal disinfection at less than 100°C (212°F) may not be effective in deactivating all types of microorganisms (e.g., certain types of bacteria spores), it is a reliable and consistent disinfection process. As the temperature increases above a certain point (typically ≥70°C or 158°F), so does the activity against microorganisms, with variable intrinsic and acquired resistance mechanisms to heat.[3] Thermal disinfection therefore will provide processing personnel with a minimized risk of bloodborne pathogens exposure.

In other situations, the microbiological load can be much higher (e.g., with flexible endoscopes used in the gastrointestinal system[6]) or more variable (e.g., with noncritical devices or surfaces depending on their use[7]). Where practical, thermal disinfection is still viewed as the preferred and more reliable method to render these devices safe for use due to its known efficacy against microbial pathogens.[5] Chemical disinfection generally is only considered if thermal disinfection cannot be applied (e.g., due to thermo-sensitivity of device or surface materials).

Disinfection Efficacy

When thermal disinfection is specified in the device manufacturer's instructions for use (IFU), the disinfection process must be validated to demonstrate the effectiveness of the cycle to inactivate microorganisms for that specific device.[8] Chemical disinfection claims and validations traditionally are performed by inoculating the most difficult-to-disinfect locations with a known titer of challenge vegetative microorganisms (e.g., *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Mycobacterium species*) in the presence of soil and calculating the log reduction postdisinfection. The levels and types of microorganism inactivation are the basis for disinfection claims (e.g., in many countries defined as low-, intermediate-, and high-level disinfection) to describe the effectiveness of the process).[9,10] Unfortunately, using a similar strategy for thermal disinfection validation may not appropriately or reliably challenge the device or load.

The disinfection levels established for chemical disinfection leverage the chemical resistance profile of microorganisms such as *Mycobacterium* species (e.g., *M. terrae*), which are known to represent some of the more challenging vegetative microorganism to inactivate.[11] It may not be appropriate to apply the same microorganism resistance profile to a thermal disinfection method using moist heat, even when a thermoresistant strain is used. This concept was confirmed in studies evaluating the resistance of microorganisms using thermal disinfection, suggesting that from the 19 microorganisms tested, only *Micrococcus luteus* showed any resistance (equivalent to a low-level disinfection claim).[12] This study showed that a bioburden reduction of a heat resistant *Mycobacterium* species may not be the most resistant challenge for a device during thermal disinfection validation studies. Other reports suggested the use of other bacteria due to their notable resistance profiles to thermal inactivation (e.g., *Enterococcus* species[13]).

The procedure for performing a bioburden reduction study in a liquid environment (e.g., washer-disinfector) also presents challenges. The most obvious is that the process of exposure within the washer-disinfector is designed for the physical removal of microorganisms (and other soil components) during the full washer-disinfector process. The method of direct inoculation of the challenge microorganism to a device surface used in chemical disinfection validations is impractical because a high population of the inoculum would wash off the device during the exposure cycle (even if just the disinfection phase). To circumvent this problem, glass ampoules are prepared with the inoculation titer and placed at worst-case locations within the load (e.g., load, load carrier) in the washer-disinfector. These ampoules are secured on the outside or inside feature of a device. Due to their size (Figure 1), they may only represent portions outside the device surfaces or loads. They also depend on heat transfer into the vials, which does not directly simulate exposure

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Figure 1. An example test ampoule used for microbial reduction challenges.

directly on a device surface. Such ampoules therefore are not a representative challenge.

The challenges of working with microorganisms during thermal disinfection are addressed by using thermometric testing, as described in ISO 15883-1:2006.[1] The reliability of thermometric testing is based on the known microbial resistance profiles of different types of microorganisms to thermal inactivation, which has been established in various industrial microbiology applications since the early 1900s.[5,11] Essentially, most bacteria, fungi, viruses, and protozoa are inactivated at temperatures in excess of 70°C or 158°F. The much higher temperature conditions defined for steam sterilization applications are to accommodate the specific inactivation of thermoresistant bacterial spores, which are outside the scope of this discussion on disinfection.[5,14]

As the temperature increases above levels of 70°C, the time to inactivate microorganisms decreases considerably—a concept similar to the well-established microbial inactivation profile used for steam sterilization and the basis for lethality modelling in heat inactivation studies (the F-value or F_0 for moist heat processes).[14,15] Similar to steam sterilization processes, the thermometric test for thermal disinfection is performed by challenging the disinfection phase of the washer-disinfector cycle with each load carrier, including a representative load to determine the thermal distribution of the chamber, load, and carrier load. The relationship of the recorded temperature to microorganism inactivation can be expressed using the A_0 calculation.[1] Unlike the size challenges with the ampoules, thermocouples may be placed at multiple locations to identify the greatest challenge for thermal disinfection within the chamber, chamber loads, and individual devices (Figure 2).

A_0 Calculation and Microbial Reduction

Moist heat kills microorganisms by disrupting the structure and functions of the different macromolecules that make up their structure, such as nucleic acids, proteins, carbohydrates, and lipids.[11] The efficiency of this process can be measured thermometrically using the equation:

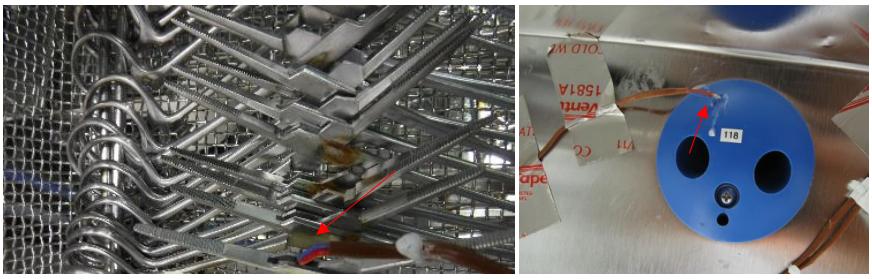

Figure 2. Examples of the positioning of thermocouples at various positions within the load and washer-disinfector for temperature distribution validation testing. Thermocouples are colored orange or brown.

$$A_0 = \sum 10^{\frac{T-80}{z}} = \Delta t$$

where A is the time equivalent in seconds at 80°C to inactivate microorganisms using thermal disinfection with a defined z value,[16] T is the temperature of the load, z is the change in temperature required to achieve a tenfold change in the rate of microbial inactivation by a moist heat disinfection process (i.e., 10°C), and Δt is the selected time period. This formula is the mathematical expression to support the relationship of microorganism reduction at a specific temperature. In general, the higher the temperature, the more kill will be achieved.[5,16]

Similar to other methods used to evaluate microbiological inactivation rates (e.g., F_0 method for steam sterilization), the A_0 method incorporates safety factors to conservatively measure the actual disinfection value. The z value of microorganisms increases as the resistance of the microorganism increases to the disinfection method. The z value of 10°C selected for the A_0 equation is representative of bacterial spores, which are the most resistant of all microorganisms and not the goal of the thermal disinfection cycle for reduction.[16] Although the A_0 value can consist of the sum of many subvalues (the combination of any time/temperature during the disinfection phase), additional safety is added to the calculation by only including the time associated with a 70°C or greater temperature range in the calculation, even when it has been established that microorganism inactivation occurs at temperatures below this value.[16] The A_0 method therefore can be considered a conservative process based on the effectiveness of microbial inactivation.[12]

To describe the conservative capability of the A_0 method, the following example can be

considered. The A_0 is calculated using thermocouple data from a thermal disinfection cycle, where the minimum temperature allowed for the cycle is 90°C for 60 seconds. The calculated A_0 value results in 600 using the equation:

$$A_0 = \Sigma 10^{(90-80)/10} \times 60 = 600$$

where the dry time or exposure times with temperatures less than 90°C are not included. However, if the A_0 calculation is applied to the entire cycle (to include all cycle times where temperatures exceed 70°C, as depicted in Figure 3) a substantially higher value is achieved when accounting for all phases of the washer-disinfector cycle in which microbial inactivation may occur.

An example of a washer-disinfector cycle with the A_0 value calculated during the performance of the cycle, as depicted in Figure 3, is as follows

- Intermediate Rinse: 70°C/0.5 min(conservative estimate using minimum phase temperature)
  $A_0 = 10^{(70-80)/10} \times 30 = 3$
- Rinse: 90°C/5min
  $A_0 = {}^{10(90-80)/10} \times 300 = 3{,}000$
- Disinfection: 90°C/1 min
  $A_0 = {}^{10(90-80)/10} \times 60 = 600$
- Dry: 90°C/9min
- $A_0 = {}^{10(90-80)/10} \times 540 = 5{,}400$
- Sum of A_0 for entire washer-disinfector cycle:
- $A0 = \Sigma({}^{10(70-80)/10} \times 30) + ({}^{10(90-80)/10} \times 300) + (10^{(90\times 80)/10} \text{ x } 60) + (10^{(90\times80)/10} \times 540) = 9{,}003$

Although the ISO 15883-1 standard only applies the A_0 concept to the disinfection phase of the washer-disinfector cycle, any temperature above 70°C in the cycle will achieve disinfection. Therefore, when looking at the total disinfection efficacy in the rinse, disinfection, and drying phases of the cycle, the conservative estimate for the total cycle A_0 value is 9,003.

Of note, during the drying phase, moist heat (steam) is created during the process; therefore, this phase of the process can also be considered cumulative to the A_0. Figure 3 shows the temperature and duration of exposure for devices during the entirety of the washer-disinfector cycle. If the accumulative kill is calculated using all subparts of the cycle, then the A_0 method demonstrates a very conservative approach for thermal disinfection.

Data from peer-reviewed literature over many years initially were used to establish the relationship between moist heat and microbial reduction using thermal disinfection, especially from the use of heat for the pasteurization of foods. During the past 20 years, further research has confirmed the thermoresistant profiles of microorganism in device disinfection studies and applied these to the A_0 method (Table 1).

Testing for statistical significance has been performed to demonstrate the correlation and highly predictable behavior between microbial reduction and the A_0

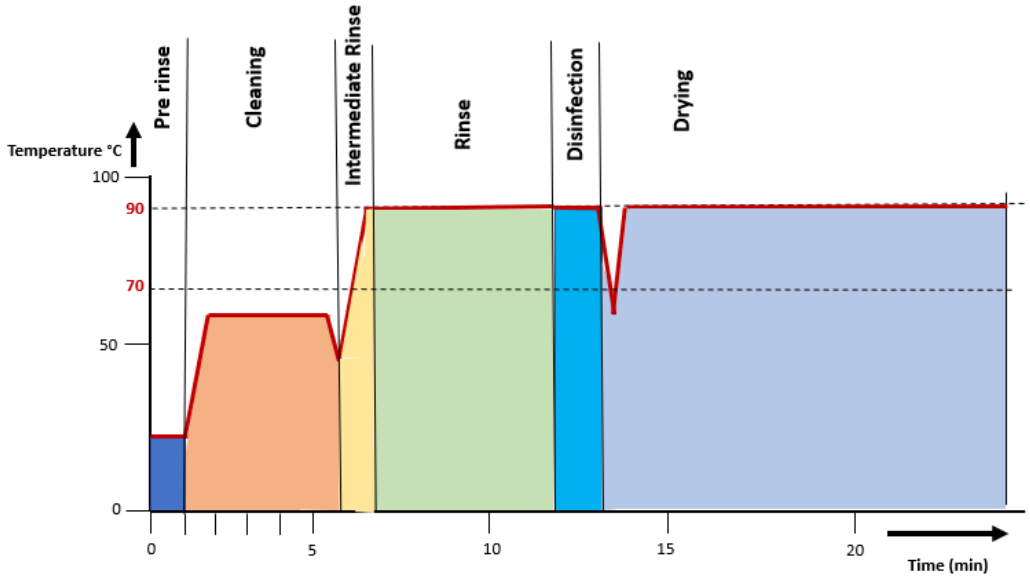

Figure 3. Example of a washer-disinfector cycle with calculated A_0 by phase.

value. In fact, the minimum A_0 value (i.e., A_0 = 60) was achievable even when microorganisms were tested in the presence of blood or biofilms.[12,17] The data within these literature references clearly show that using the A_0 method to demonstrate microbial inactivation of microorganisms is far superior to the traditional microbiological reduction method adopted from chemical disinfection efficacy studies.

Conclusion

The application of microbial reduction for the thermal disinfection process does not accurately account for the efficiency of the cycle. The A_0 method is a far more conservative method and allows for greater scientific understanding of the true distribution of temperature in a typical washer-disinfector (and associated load). The method also applies a greater safety factor to ensure the safe handling or use (when applicable) of devices after cleaning using a washer-disinfector validated under the requirements of the ISO 15883 standard series. The relationship between A_0 and microbial reduction has been well documented in the literature. Using the A_0 method with thermometric profiling is a practical way to establish the relationship between heat and microbial inactivation during validation testing and should be considered the preferred method of validating thermal disinfection processes.

Reference No.	Summary
17	The data presented in the study showed highly predictable inactivation kinetics (log reduction) to correlate with the A_0 value (different times/temperatures). An A_0 value as low as <60 demonstrated a ≥5-log reduction with the microorganisms tested. Higher temperatures performed better due to decreased heat resistance of tested microorganisms.
12	The A_0 concept has been well established and documented in the literature within the food, pharmaceutical, and healthcare industries. This article reviews several studies that are directly applicable to healthcare. The studies showed substantial inactivation (log reduction ≥6) of tested microorganisms (including *Mycobacterium thermoresistibile*) at an A_0 of 60, and most vegetative bacteria and fungi were readily inactivated at temperatures in excess of 55°C. Fungi and certain types of protozoa (including most bloodborne pathogens, such as enveloped viruses) generally were inactive above temperatures of 65°C. The formation of biofilm has little to no impact on heat resistance of microorganisms to moist heat disinfection.
18	The experimental data permitted the conclusion that an A_0 value of 600 is sufficient for disinfection of surgical instruments. This value has inherent safety factors embedded in the calculation, bacterial inactivation capability, and sum of cycle subvalues (e.g., heat-up phase for thermal disinfection in washer-disinfectors). As the principle of overkill should be applied to disinfection as it is to sterilization, the A_0 value of 600 provides appropriate overkill for thermal disinfection.
19	In this study, which compared thermal disinfection of enveloped and nonenveloped viruses in the presence of interfering factors (packed erythrocytes in bovine serum albumin), it was confirmed that enveloped viruses (e.g., human immunodeficiency virus, hepatitis B virus, influenza) were rapidly inactivated by at ≥70°C in line with the A_0 concept, but some parvoviruses showed greater resistance. However, parvoviruses were inactivated at thermal disinfection conditions of 80°C for 10 minutes and 90°C for one minute even under soiled conditions. A recommendation to increase the minimum temperature for thermal disinfection (from 65°C to 70°C) was given.
20	This study used the A_0 method to investigate the reduction of heat resistant *Enterococcus* spp. and *Bacillus subtilis* (a spore former surrogate for *Clostridium difficile*) using moist heat for human waste containers. Microorganisms were treated at different temperatures and time periods to evaluate inactivity under various soiling conditions. The results of the current study showed that unprotected enterococci (in phosphate-buffered saline) were inactivated at 70°C for 200 seconds (one-third of recommended A_0 value of 60), thus allowing adequate margin of safety; however, additional time/temperatures may be required if soil is present causing thermal insulation. As expected, the spores showed increased heat resistance, but the inactivation of spores is neither feasible nor generally required in a disinfection process; therefore, other strategies (e.g., cleaning and abundant rinsing) are crucial to avoid cross-contamination.
21	This study explored the relationship between temperature and time for microbial inactivation within a hemodialysis unit. The concept of A_0 allowed for calculations of all possible combinations of time and temperature to achieve a certain degree of disinfection in a more efficient and economical way, adapted to the needs of each hemodialysis unit through its programming.

Table 1. Literature review of microorganism reduction using moist heat disinfection.

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