sterilization or depyrogenation method should be automatically recorded during each phase of the sterilization/depyrogenation cycle (e.g., continuous chart recorder or print out at least once a minute).

3. Sterilizer Monitoring and Control I/Es should be calibrated before the operational qualification (OQ) study and routinely according to a defined calibration schedule

4. External Monitoring and Recording I/Es (e.g., data logger and thermocouples) should be calibrated before and after the OQ study, and before and after the performance qualification (PQ) study.

5. One Thermocouple should be placed adjacent to the temperature controlling I/E in static, batch type sterilizers. Load patterns and/or equipment diagrams with indication of thermocouple placement should be documented as a part of qualification studies.

   For continuous belt depyrogenation tunnels, thermocouples should be fed into the tunnel (i.e., trailing thermocouples) in containers distributed across the belt, at least in the first and last rows of each load.

6. Load Pattern and/or Equipment Diagrams with indication of biological indicators (BI) and/or endotoxin indicators (EI) placement should be documented as a part of the qualification studies.

7. Certificate of Analysis (COA) for Commercially Prepared BIs should contain the following information:
   - Organism identification;
   - Source (supplier);
   - *D*-value and *z*-value;
   - Survival time and kill time;
   - Spore population per carrier (or mL);
   - Expiration Date; and
   - Storage conditions.

8. Custom Prepared BIs for moist heat sterilization or dry heat sterilization should be tested prior to use for survival time and kill time under the conditions in which they will be used. *D*-values and kill time required for complete inactivation of prepared BIs should be determined experimentally. BIs having high *D*-values and/or large spore populations can result in some survivors when using the overkill sterilization cycle.

   The following equation illustrates the effect *D*-values and population size may have on kill time for a given steam sterilization cycle. Kill Time $_{(\text{min at } 121)} = D_{121}$ x$(\text{Log}_{10} N_0 + 4)$ $D_{(121C)}$ = time in minutes required to reduce the microbial population by ninety (90) percent or one log cycle at a reference temperature of 121$_o$C.

   $N_0$ =Initial BI sporepopulation.
   *D*-value studies should be performed using the Biological Indicators-Resistance Performance Test (e.g., as indicated in the USP).

 

15. DH Sterilization Cycle Development should include the following:
    - Runs conducted using BIs (i.e., *Bacillus atropheus* formerly referred to as *Bacillus subtilis var. niger*) which have a predetermined spore population (e.g., $10^5$ to $10^6$), *D*-value, and kill time;
    - Calculation of $F_H$ in the coolest location in the load to determine the degree of lethality as a function of process parameters; and
    - Incubation of BIs at 30ºC-35ºC withno growthafter seven(7) days or at the temperature and time interval recommended by the BI supplier.

16. DH Sterilization (e.g., 140ºC -180ºC) OQ/PQ Studies should be performed and include, and not be limited to, the following:
    - A minimum of three (3) temperature distribution runs on an empty chamber to confirm heating uniformity and identify the slowest-to-heat zone;
    - Heat penetration runs on each different load configuration, including minimum and maximum loads, to identify cold spots and the worst case load configuration; and
    - A minimum of three (3) consecutive, successful runs using the worst case load configuration using minimum cycle parameters and BIs and meeting all validation acceptance criteria.

17. DH Depyrogenation (e.g., > 180ºC) OQ/PQ Studies for DH ovens should be performed and include, and not be limited to, the following:
    - A minimum of three (3) temperature distribution runs on an empty chamber to confirm heating uniformity and identify the slowest-to-heat zone;
        - Heat penetration runs on different load configuration using a matrixing approach (e.g., smallest and largest glass vials) including minimum and maximum loads, to identify cold spots and the worst case load configuration; and
        – A minimum of three (3) consecutive, successful runs using the worst case load configuration using minimum cycle parameters and EIs and meeting all validation acceptance criteria.

18. DH Depyrogenation OQ/PQ Studies for continuous belt tunnels should consist of a minimum of three (3) consecutive, successful runs based on a matrix approach for varying vial sizes, using minimum cycle parameters, and EIs and meeting all validation acceptance criteria, to confirm heating uniformity in each tunnel zone and across the belt, and to identify tunnel cold spots.

19. DH Tunnel Temperature Distribution Runs should evaluate and determine the following:
    – Effects that tunnel load (e.g., empty, partially full, and full) has on temperature distribution;
    – Positioning of baffle plates or gates in each tunnel zone for each container size; and
    - Belt speed for each container size.

 