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2 **Manuscript Title:** A Risk Based Approach to Variable Load Configuration Validation in Steam
3 Sterilization: Application of PDA TR1 load equivalence topic
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12 **Abstract:**

13 This article describes a method for achieving the load equivalence model described in PDA Technical
14 Report 1 (TR1), using a mass-based approach. The item and load bracketing approach allow for mixed
15 equipment load size variation for operational flexibility along with decreased time to introduce new items
16 to the operation. The article discusses the utilization of approximately 67 items/ components (Table IV)
17 identified for routine sterilization with varying quantities required weekly. The items were assessed for
18 worst case identification using four temperature related criteria. The criteria were used to provide a data-
19 based identification of worst case items, and/or item equivalence, to carry forward into cycle validation
20 using a variable load pattern. The mass approach to maximum load determination was used to bracket
21 routine production use and allows for variable loading patterns. The result of the item mapping and load
22 bracketing data is “a proven acceptable range” of sterilizing conditions including loading configuration
23 and location. The application of these approaches, while initially more time/test intensive than alternate
24 approaches, provides a method of cycle validation with long term benefit of ease of ongoing qualification,
25 minimizing time and qualification requirements for new equipment qualification for similar loads/use,
26 and for rapid and rigorous assessment of new items for sterilization.

27 **Keywords:** Steam Sterilization, Variable Load, Mixed Load, autoclave, validation, Risk based approach,
28 load equivalence, load bracketing, item bracketing

29 **Statement of Objectives or Hypothesis**

30 In an effort to minimize the frequent sterilization validation studies required for a multi-product facility,
31 where new product/component configurations requiring steam sterilization are routinely introduced, a
32 method was needed to objectively determine the worst case equipment/commodities for routine validation
33 testing. The validation method chosen had to fulfill several key objectives:

- 34 • Characterize and document all equipment and components requiring steam sterilization.
- 35 • Identify the subset of equipment and the subset of components that comprise a “worst case” load
36 relative to sterilization.
- 37 • Provide a mechanism to assess new components and equipment configurations and to determine
38 whether the item fits within the current qualification matrix, or if the item constitutes a new worst
39 case item.
- 40 • Provide a standardized test methodology such that all newly identified worst case equipment and
41 components could be tested, with the results being directly comparable to previous qualification
42 studies.

43 The general sterilization philosophy employed was the bracket approach, wherein both the maximum load
44 size (based upon mass) and the minimum load size, for each unique load type, were evaluated and
45 challenged to identify the worst case load for performance qualification testing. The item and load
46 bracketing approach allows for load size and load pattern variation for operations flexibility.
47

48 **Introduction**

49 A process improvement program was initiated to enable the introduction of new product processing
50 configurations to the production floor in a timely manner, while maintaining a high level of assurance that
51 the new component introduction did not compromise sterility of currently qualified components, or risk
52 contamination of the aseptic processing area.

53
54 Initial sterilization qualification studies had been performed using a fixed loading pattern approach as a
55 means to expedite the project schedule. While this approach expedited initial facility qualification and
56 licensing, operational issues resulting from the inflexibility of having fixed loading patterns emerged soon
57 after initiation of product manufacturing. Chief among the operational issues encountered were:

- 58 • As process changes were identified to optimize filtration and filling operations, seemingly
59 constant requests were being generated to change the previously qualified loads.
- 60 • Equipment/component preparation area operators, in an effort to accommodate production
61 demands, were frequently requesting re-evaluation of the loading pattern constraints, often on a
62 load-by-load basis. For example, could a filter cartridge that is typically sterilized on the top
63 shelf of a load located next to a coil of product tubing now be added to an existing load and
64 sterilized using the identical cycle parameters but located on the third shelf of an autoclave cart
65 next to a product filling manifold?
- 66 • The fixed load patterns lent themselves to “common sense” interpretations, resulting in load
67 patterns for which no qualification data had been acquired. For example, the filter cartridge that
68 was qualified loaded on the top shelf, in the front right corner, is found loaded in the middle of
69 the top shelf. Is this an issue, and if so, how much leeway does the operator have? Lacking
70 definitive data on how much variation is allowable without affecting sterilization efficacy, this is
71 a difficult, if not impossible question to adequately answer, and thus presents a significant
72 obstacle to effective training of equipment preparation operators. Although reasonable and
73 rational scientific thought supports the ability to relocate but not reorient objects, the fixed-load
74 approach initially utilized did not provide sufficient data to identify process boundary conditions.

75
76 To address the issues observed, a new qualification approach was developed. The approach chosen
77 applied the item bracketing and load bracketing concepts presented by PDA TR1, 2007 (1). The item
78 bracketing approach allows for the use of a worst case item(s) (i.e. greatest sterilization challenge for heat
79 penetration, mass, air removal, and/or condensate removal) to qualify items that are a lesser sterilization
80 challenge. The load bracketing is established by using predefined minimum and maximum loads. This
81 approach defined the minimum load as comprising the single item presenting the greatest sterilization
82 challenge, irrespective of mass, while the maximum load comprised an assembly of the ten items
83 identified as worst case (relative to sterilization difficulty), with additional items used create a maximum
84 load mass (i.e. a load mass greater than any anticipated for routine operations).

85
86 This approach requires that several key criteria are addressed during qualification, and during subsequent
87 process transfer to manufacturing:

- 88 • The sterilization cycle parameters used for validation utilize the overkill approach to assure any
89 variability in loading pattern or orientation is accounted for during routine production¹.
- 90 • Given the requirement for an overkill approach, multiple maximum and minimum loads *may* be
91 required, wherein heat labile (e.g. elastomeric materials, closures, etc.) and non-heat labile (e.g.
92 stainless steel parts, etc.) are segregated into separate load types. Where all equipment and

¹ Note: While the basic precepts of this validation methodology can be applied to other non-overkill validation approaches (e.g. F₀/Bioburden based sterilization, Sub-Lethal/Bioburden Reduction cycles, etc.), such approaches require additional bioburden monitoring, control, and evaluation components that were outside the scope of this case study.

93 components can withstand sterilization cycle parameters sufficient to provide a sterility assurance
94 level (SAL) of $\geq 10^6$, such segregation may not be necessary.

- 95 • The variability in loading pattern and orientation must be addressed through specific and detailed
96 procedural controls since the process of loading porous load autoclaves for aseptic
97 filling/manufacturing facilities is manual.
- 98 • The documentation system must clearly define the items that were qualified with a maximum
99 quantity that can be loaded, and must provide the ability to fully document the specific load
100 contents to allow verification of process compliance during batch record review.
- 101 • The system must allow for the contents of individual loads to vary, while maintaining consistent
102 sterilization cycle parameters and performance, ensuring a consistent level of sterility assurance.

103 Heat penetration mapping studies should be conducted on all items to identify the more difficult to heat
104 items (e.g., large mass, potential for trapped air or condensate collection, hoses with longest lengths or
105 any combination of these issues). When conducting item temperature mapping, it is important to consider
106 the types of challenges the item may represent (e.g., air removal versus significant mass) and to position
107 the temperature probes in slowest-to-heat locations (2). For larger load items, items of complex
108 construction, or items where mass distribution is highly non-uniform, multiple probes may be required to
109 ensure that the coolest location on/within each item is clearly defined. In some instances (e.g. large filter
110 housing/valve/tubing assemblies, large bags of closures, intermediate tanks, etc.), separate item-mapping
111 studies may be indicated as a precursor to load heat penetration studies. Such item-mapping studies
112 minimize the monitoring requirements for large or complex items during subsequent load heat penetration
113 tests, freeing up probes for monitoring of other load items, and thus decreasing the total number of heat
114 penetration test cycles required.

115
116 The methods described in this article result in a sterilization and documentation control system that:

- 117 • Has a controlled version of a batch preparation list. This list is attached to preparation procedures
118 where items are not separated out by specific load, but are listed individually. Each autoclave
119 load configuration is documented on the batch preparation list form during routine operation.
- 120 • The validation will be run using a minimum load and a maximum load to validate combinations
121 of different items to show consistency between configurations. The test method will demonstrate
122 that while it is recognized that the *preparation* and *orientation* of each load item is critical, the
123 item's *location* within the load is not critical.
- 124 • The maximum loads studies will be conducted with a load containing a total mass in excess of
125 that needed for routine manufacturing. This maximum mass will establish quantities of items that
126 may be processed in a single load.
- 127 • Based on the validation, a master list of maximum items in the load will be established.

128 129 **Method and Materials**

130 The methods used for this qualification comprised the following primary activities:

- 131 • Listing of all equipment / commodities to be steam sterilized.
- 132 • Evaluation of equipment and commodities to determine the most efficient and effective means
133 (relative to air removal, steam penetration, and maintaining a sterile barrier) of pre-sterilization
134 preparation.
- 135 • Heat penetration evaluation tests to identify the worst case (i.e. most difficult to heat) load items.
- 136 • Maximum Load (i.e. greatest mass) heat penetration / biological challenge studies using the worst
137 case load items.

- 138 • Minimum Load (irrespective of mass) heat penetration / biological challenge studies using the
139 single worst case load item.
- 140 • Evaluation of data and subsequent generation of loading, operation, and documentation
141 procedures for manufacturing use.

142 A listing of all commodity items requiring steam sterilization was developed using existing procedures
143 and batch record documentation. The component preparation instruction for each of the listed
144 commodities was reviewed for clarity and consistency, and preparation methods were standardized
145 wherever possible.

146 The initial worst case item heat penetration evaluation studies were performed for each unique load type
147 (e.g. stoppers, seals, filling equipment, sanitization supplies, etc.), and/or for each unique cycle type (i.e.
148 vacuum pre-purge, gravity displacement, etc.). While multiple autoclaves were used for this study, only
149 one autoclave was used for any one of the unique load types evaluated in order to ensure comparability
150 between test cycles (e.g. stopper studies in a single unit, equipment studies in a single unit, etc.). Since
151 the purpose of this test was to determine the *relative* heat-up characteristics of the equipment and
152 component items within each load, testing of all load types in each autoclave was not required for
153 determination of the worst case commodities.

154 Commodities were prepared by rinsing with WFI, then smaller items (less than two square feet in area)
155 were bagged and sealed in steam permeable bags, while larger items had all open ends/ports covered
156 using steam permeable draw tight bonnets (General Econopak 1030 series mini-muff). Each commodity
157 was weighed after preparation and prior to loading to record the mass of each load. Using the mass
158 approach to individual items and the maximum load size is in recognition that commodity heating, and
159 the generation of condensate in the load, is primarily mass dependent. The efficiency with which the
160 condensate is removed by the sterilizer is critical for adequate steam penetration of the load items (3).

161 The orientation of each commodity was documented to provide loading procedures to the operators
162 during performance qualification (PQ) testing and future routine operations. Items were oriented, where
163 practicable, to minimize the possibility of condensate collection, and maximize air removal efficiency.
164 Calibrated thermocouples were placed within each commodity to measure the temperatures achieved
165 during the cycle and to calculate a lethality value (F_0 calculated using a D-value of 1.0, z-value of 10 °C,
166 and reference temperature of 121.1°C²) achieved during the entire cycle for each commodity. Care was
167 taken to seal the wrapping material around the thermocouple penetration to prevent enhancement or
168 restriction of steam penetration into the item.

169 Following the evaluation runs of each commodity, the data was analyzed to determine the two (2) worst
170 case commodities within that load based on the following parameters: Longest lag time from Exposure
171 start to attaining $\geq 121.1^\circ\text{C}$, Shortest dwell period at $\geq 121.1^\circ\text{C}$, Lowest maximum temperature attained
172 during the exposure (dwell) period, and Lowest accumulated lethality (F_0). These two worst case items
173 were then allowed to cool to ambient conditions and placed in the next load along with untested
174 commodities for further comparison. Inclusion of the two worst case items into each subsequent load
175 provided a facility for evaluating cycle-to-cycle variability that could impact the evaluation process (i.e. if
176 the thermal profile was comparable for these two items, cycle to cycle, then thermal variability within the
177 remaining load items can be assumed to be component related, not cycle related). This process was
178 repeated until all commodities identified for analysis were tested at least once. The data from all runs was
179 then compared to identify the ten worst case commodities overall that would be used for performance
180 qualification testing of each sterilizer, using identical bagging/wrapping methods.

² For this case study, 121.1°C was chosen as the baseline sterilization temperature, although it should be noted that other baseline temperatures may be employed to address specific component requirements and/or validation strategies.

181 Following the identification of the ten worst case commodities, the minimum and maximum worst case
182 loads (of each unique load type) were assembled and processed for three consecutive successful PQ runs
183 to verify that the load preparation, load configuration and sterilizer parameters were satisfactory for
184 routine operation. For each of the three PQ runs the commodities were moved within the load to verify
185 that the commodity was successfully sterilized regardless of its' position in the load and its' nearest
186 neighbor commodity. Each load item was tested, as near as practicable, in the front, middle, and back of
187 the chamber, and in the bottom, middle, and top of the chamber (e.g. bottom-front, middle-middle, and
188 top-back).

189 **Results**

191 The initial assessment of components requiring sterilization was completed through operator interviews
192 and batch record reviews. It was critical to identify the commodities required to be steam sterilized, and to
193 document the preparation methods used for sterilization of those items. For the method of variable load
194 validation to achieve consistent results it is important to control the commodity preparation, including
195 pre-rinsing and bagging/wrapping, along with the commodity orientation during sterilization.

196 Various commodity orientations were tested during the initial development studies to verify that either the
197 orientation was not critical to the sterilization of an individual item, or that the item orientation is critical
198 for complete draining and efficient steam penetration/air removal. Areas of steam penetration concern
199 were selected for thermocouple monitoring. These areas included areas such as domed sections with non-
200 symmetrical openings that may trap air, in the center of tubing lengths and in areas of greatest mass
201 concentration of the individual commodity. All commodities used in the challenge tests were prepared
202 (wrapped, bagged, wet, dry, etc.) in the same manner as for manufacturing use. Each commodity was
203 loaded in the same physical orientation (i.e. horizontal, vertical, inverted, etc.) during each of the
204 qualification runs, while the location of the item was varied from front to back and top to bottom.

205 Thermal equivalence between loads of varying load configuration and mass was evaluated by comparing
206 the penetration thermocouple data obtained from the various loads, when processed using identical
207 sterilization parameters, in a single autoclave (Refer to Table I Cycle Sterilization Parameters). The heat
208 penetration data demonstrated that the thermal profile for each of the items tested was equivalent at each
209 tested location (i.e. front, middle, back – top, middle, bottom of chamber), verifying that the load “cool
210 spot” was load item dependent, not load position dependent. The thermal profile data were also evaluated
211 to identify the 10 worst case items, relative to all other items planned for steam sterilization (Table II
212 Cool Point Determination for Commodities). This result was verified by repositioning the load items
213 during the replicate runs and verifying that the worst case item remained the worst case relative to the
214 other load items. The data presented in Table II details the number of times each individual item was
215 identified as the worst case item, relative to the other load items, for each of the specific assessment
216 criteria. It is clear from the data in the table that two filters, each with a long section of tubing with a
217 valve on one end, were the overall worst case items based on the total number of times the items they
218 were observed within the top ten coolest items in a load. The other items identified in the top ten varied in
219 their ranking depending on the criteria used for comparison. For example the cleaning hose did not have a
220 lag time at the beginning of the exposure period indicating quick heat-up characteristics but did not
221 achieve a temperature as close to the load setpoint, relative to other items, resulting in a lower
222 accumulated lethality. The hose was also quick to cool down resulting in a shorter total time above
223 121.1°C relative to other items and therefore also contributed to the lower accumulated lethality for this
224 item. Comparing the hose results with those of the stoppering outer bowl demonstrates that the more
225 massive outer bowl took longer than the hose to achieve the sterilization temperature of 121.1°C but was
226 able to achieve a higher maximum temperature and higher accumulated lethality. The observation that the
227 worst case items maintained a constant thermal profile and that profile was consistent relative to the
228 thermal profiles of the other items in the load, irrespective of load configuration or item location, provides
229 the basis for elimination of fixed loading patterns in routine sterilization. The items in shaded rows of

230 Table II were within the top ranking for worst case items within a single test run but did not consistently
231 rank in the top 10 relative to the other items tested. Although the 25 foot section of silicone tubing did not
232 rank in the top 10 worst case items it was selected for PQ testing since the results were very similar to the
233 worst case items ranked 8 through 10, two of which were the same item prepared in different ways. Based
234 on the cool point determination study data, a total of four PQ runs (3 maximum loads and 1 minimum
235 load) was executed to validate the process. Since the minimum and maximum loads are determined to be
236 equivalent relative to delivery of a $\geq 10^6$ SAL, loads of intermediate size are deemed equivalent as well.

237 The thermal data from the Cool Point Determination study identified the 10 worst case items to be
238 challenged during PQ execution. The worst case items along with additional items (dummy items for
239 mass) were assembled to achieve a total load mass that exceeded that anticipated for routine production
240 (Refer to Table III Proposed PQ Challenge Maximum Load). Cool Point Determination studies for the
241 minimum load were not required due to the small volume of materials loaded, the relatively small area
242 occupied by the load contents, and the high percentage of the load being monitored (i.e. $\geq 50\%$).
243 Therefore, a total of four PQ runs (3 maximum loads at 175 Kg and 1 minimum load at 0.5 Kg) were
244 executed to validate the process. The item identified in the Maximum Load studies as the hardest to heat
245 item was used for the Minimum Load Qualification.

246 During the maximum load tests, all penetration thermocouples accumulated ≥ 15 minutes F_0 . The
247 minimum F_0 was 33 minutes for Run number 2B as shown by the minimum temperature for that specific
248 run (Refer to Figure 1). There were no criteria during PQ testing to maintain a minimum temperature or
249 lag time after the initiation of the exposure period. The cycle exposure period was started based on
250 distribution temperatures within the chamber and chamber drain remaining above 121.1°C and within a
251 1.5°C spread. It is important to note that efforts (e.g. equipment disassembly or cycle development)
252 should be made to minimize any lag time in accordance with the EU expectations (4). However, the
253 methods employed to meet this lag time requirement should be carefully evaluated to ensure that the
254 overall sterility assurance of the process is not adversely affected (e.g. by requiring an inappropriate
255 number of aseptic connections (5)).

256 In all runs the distribution temperature spread was less than 1°C within the first minute after starting the
257 exposure period (refer to Figure 3). This assured an even temperature distribution within the chamber to
258 provide equal exposure of the loaded items to steam during the cycle. The equal exposure of items within
259 the chamber provides support for the ability to load any item at any shelf position within the chamber and
260 not influence the sterilization capability.

261

262 Discussion

263 For the method of variable load validation to achieve consistent results it is important to control the
264 commodity preparation, including pre-rinsing and bagging/wrapping, along with the commodity
265 orientation during sterilization. Control of these items is necessary to minimize equilibration times of the
266 commodities. Extended equilibration times, in addition to resulting simply from heterogeneous load
267 contents, may also be indicative of inadequate air removal or steam penetration, and as such, should be
268 evaluated and addressed (through cycle parameter modifications, preparation procedure modifications,
269 etc.) where indicated, to ensure that variability is minimized. This is of special concern during
270 developmental and cool point mapping/penetration studies relying solely on thermometric data, where dry
271 heat cannot be distinguished from moist heat. While there is an understandable desire to create assemblies
272 of items for sterilization, to reduce the number of aseptic connections required in the aseptic processing
273 area, care must be taken to ensure that the pre-assembled sections do not create excessive difficulties in
274 air removal and steam penetration. In order to reduce or eliminate equilibration times, especially when
275 complex wrapped/bagged items are being processed, care should be taken to; orient items for efficient air
276 removal (e.g., hoses not pinched, maximizing surface area for steam penetration, inversion of items with
277 large internal volumes, etc.), increase the number of vacuum or positive steam pulses, add hold steps
278 during vacuum and/or steam pulses, increase depth of vacuum pulses, and/or optimize steam exposure to

279 load items by creating reasonable assemblies. In this qualification, non-wetting bags/covers were used for
280 equipment preparation. However, in the case where wet-able sterilization wraps are utilized, it is
281 important to recognize that the first vacuum air removal pulse will be the most efficient (as the wrap
282 becomes hydrated and thus less steam permeable) following the first steam backfill. In this case, both the
283 depth and duration of the first vacuum pulse should be maximized.

284 Initial development studies verified that while the physical orientation of an individual load item may be
285 critical (for air removal and steam penetration), its location within the chamber did not affect its thermal
286 profile. The data presented in Table II – Cool Point Determination for Commodities demonstrated the
287 need for multiple assessment criteria to determine item and load equivalence, especially to provide
288 sufficient definition of item performance, on a cycle by cycle basis, to evaluate whether observed
289 variability was cycle specific or load item / location specific. Since this was an inter-item comparative
290 study using identical sterilization cycle parameters and similar preparation procedures, it was observed
291 that for some items the lag time (to $\geq 121.1^{\circ}\text{C}$) did not correlate with delivered lethality. For example,
292 item A has a shorter lag time than item B, but may achieve a lower overall mean kinetic temperature, and
293 therefore a lower lethality (assuming comparable steam penetration levels) than item B. This disparity to
294 equilibrate quickly during exposure can be due to a number of factors, such as the specific heat of the
295 various items due to their materials of construction, density and thermal conductivity, air removal
296 efficiency, and localized superheat conditions (e.g. large hydrophilic absorbent pack in a breather bag).
297 Thus two items of equal mass loaded into the sterilizer may have dissimilar thermal profiles where, for
298 example, one is made of dense high heat capacity and highly heat conductive material, such as stainless
299 steel, while the other is made of a lower density and low specific heat, and lower heat conductive material
300 such as polycarbonate. Thus, while it takes more energy to heat 1 kg of an item with a higher specific
301 heat than the item of comparable mass with lower specific heat, the lower specific heat / lower heat
302 conduction item may take longer to heat. Additionally, while the maximum steam input to the autoclave
303 chamber (e.g. during initial heatup) is constant, an item that has a very high specific heat and conductance
304 will be condensing steam rapidly compared to other low specific heat and low heat conductance load
305 items. As the steam collapses around the high mass/specific heat item, the localized low pressure acts as
306 a “pump” to backfill the area with steam. Thus while the autoclave controls based (typically) on one
307 temperature monitoring point, the heat input may vary dramatically on a localized basis. A similar
308 situation may be encountered when highly absorbent materials are sterilized, resulting in localized
309 superheat conditions until the materials quickly reach an equilibrium hydration point. In this case of
310 highly absorbent materials (e.g. wipes, batch record materials, etc.), evaluation of just a single comparison
311 criterion (e.g. minimum temperature), may fail to detect item issues such as localized superheat that
312 evaluation of heatup times and maximum temperatures achieved, in conjunction with minimum
313 temperature and F_0 , might identify. These issues highlight the criticality of using multiple evaluation
314 criteria when determining worst case components within highly mixed loads. . This process verifies that
315 the identified “cold spot(s)” move in the chamber with the worst case item (6). The final assessment of an
316 item will be performed using a biological indicator to verify the desired steam penetration has been
317 achieved since “the bugs don’t lie” (7). It should also be noted that while this study used only the
318 Lethality accumulated during the stabilized dwell period for evaluation purposes, approaches
319 incorporating lethality accumulated during heat up and cool down can be equally valid, especially for heat
320 labile components where exposure should be minimized. In the context of an overkill approach for non-
321 heat labile components, such as this case study, ignoring heat up and cool down lethality contributions
322 simply increases overkill. Furthermore, the requirement to maintain a certain minimum temperature
323 during the exposure period (i.e. not less than 121°C) for the cycle to be acceptable is not critical to the
324 process as it relates lethality or sterility assurance. The maintenance of moist heat conditions appropriate
325 for sterilization, along with total cycle lethality (as calculated thermometrically), should be considered the
326 critical minimum criteria for cycle acceptance, not simply minimum temperatures achieved.

327 Equivalence between loads of varying size for the same size chamber can be established by comparing the
328 penetration thermocouple F_0 range obtained from minimum and maximum loads using the same

329 sterilization parameters in an autoclave. Data from the distribution thermocouple graph (Figures 2 and 3)
330 demonstrates that all areas within the chamber are equally exposed to steam. Penetration thermocouple
331 data graph (Figure 1) demonstrates equivalent steam penetration into all loaded commodities for all runs.
332 This demonstrates equivalence in all runs where the commodity locations were changed. If the data
333 demonstrates equivalence, a total of four runs (3 maximum loads and 1 minimum load) can be used to
334 validate the process (9). If the minimum and maximum loads are determined to be equivalent, then loads
335 of intermediate size can be deemed equivalent as well.

337 The similarity of the distribution data for runs within the same autoclave (Figure 2) coupled with the
338 pattern of penetration thermocouple data demonstrates the consistency of the assessment method within
339 an autoclave. The confirmation of this consistency was established through the repositioning of load items
340 in each of the replicate runs. The item repositioning data obtained during the performance qualification
341 supports the ability to eliminate fixed loading patterns in routine sterilization. This consistency of
342 response of an item to the sterilization conditions provides the ability to assess an item in one autoclave
343 and use that information to support that its' response in the other autoclave will be similar.
344 Experimentation has shown that the item that is most difficult for steam to penetrate in the component
345 mapping studies will most likely be the cold spot within the autoclave regardless of its location (8, 10).
346 Therefore, the heating characteristics of any given item are dependent on that items' composition,
347 preparation, and orientation, and is independent of the autoclave used³.

348 Once the validation has been completed and the listing of worst case items has been developed, a risk
349 based assessment method can be developed to assess new items to be sterilized. The assessment method
350 should include a risk review (11) of the intended preparation process (rinsing and wrapping) relative to
351 the established item process, configuration within the load and similarity to other items previously
352 validated. The risk assessment may be able to justify no validation testing is required for new items to be
353 sterilized based on the equivalence to previously validated items. For example, validation testing of a bag
354 of 3000 stoppers may not be required if previous validation testing has been completed for equivalent
355 sized stopper bags of 2000 and 5000 stoppers that were filled with stoppers made of rubber compounds
356 with equal or higher substrate-specific D-values.

357 The risk evaluation is accomplished by grouping items to be sterilized based on their shape, materials of
358 construction, preparation procedures and pre-assembly methods (12). The preparation and pre-assembly
359 methods for new equipment or components may be modified to conform with those of previously
360 qualified, similar items, to mitigate or reduce risks relative to both sterilization efficacy, and maintenance
361 of sterility during subsequent aseptic assembly and/or use procedures. If the risk assessment results in an
362 item being classified as requiring additional evaluation (temperature mapping in a cycle including
363 existing worst case items) since it appears dissimilar to previously qualified items, then the original
364 assessment criteria and worst case items data from Table II will be used to perform a sterilization study to
365 directly compare the new item to existing item sterilization data to determine if the new item is indeed a
366 new worst case. Identification of a new worst case item will require requalification of the sterilization
367 cycle with the new items.

368
369 Review of the data presented here indicates a strong correlation between the thermometric properties of
370 an item and the achieved lethality (as verified by Bioindicator challenges) after identifying the appropriate
371 commodity preparation methods and loading orientation. Based on the observed correlation, only using
372 the thermometrically calculated item lethality in a comparison study of a new item to an existing item (in
373 lieu of the four assessment criteria used in this study) may be justified. This approach appears feasible for
374 items with similar configuration, preparation methods, and mass as previously qualified components.
375 New items that are unique in configuration, and may present heat up and steam penetration challenges
376 outside the those previously observed, should be analyzed using the multiple assessment criteria until

³ Assuming previously demonstrated equivalence between the autoclaves.

377 sufficient data exists to identify similarities/dissimilarities relative to previously qualified commodities.
378 Once this characterization is completed, future studies may then rely on thermometric lethality data.
379

380 The methods described in the article result in a sterilization and documentation control system where:

- 381 1. A controlled version of a batch preparation list is developed and used during manufacturing. This list
382 is attached to preparation procedures where items are not to be separated out by specific load but
383 listed individually. The load they are autoclaved in is documented on the batch preparation list form
384 during routine operation and included with batch documentation.
- 385 2. The approved component list can be maintained through the site change control program and new
386 items added. The list would also serve as a part of the annual sterilization validation summary for the
387 site.
- 388 3. For non-batch specific loads, a separate form was created where items could be written in to
389 document what went through the autoclave. The non-batch specific form is stored in an equipment
390 file tracking everything processed through that autoclave.
- 391 4. For loads or items requiring assembly, the assembly instructions and configurations were captured in
392 SOP or preparation instructions for assembly prior to steam sterilization. For example a pump
393 assembly that includes the product supply manifold, pumps, filling nozzles and associated tubing.
- 394 5. The validation would be run using a minimum load (cart and a piece of tubing for example) and a
395 maximum load and then combinations of different items to show consistency between configurations.
- 396 6. A maximum load based on mass was validated to establish an item and load bracket of parts. This
397 established the maximum quantity of each item validated provided the personnel adhere to the
398 preparation and loading orientation/configuration criteria to facilitate air removal and steam
399 penetration. This would be the identified load for annual re-qualifications.
- 400 7. Based on the validation, a master list of maximum items in the load can be maintained and used to
401 assess new product or component configurations being introduced to the facility.
402

403 The result of the item mapping and load bracketing data is “a proven acceptable range” of sterilizing
404 conditions. The critical control points for maintenance of these conditions are the component preparation
405 procedures, commodity orientation in the load, and commodity proximity to other items allowing for
406 adequate steam penetration, and the maximum allowable load size based upon mass. These sterilization
407 conditions result in items that are sterile, functional, allow for operational flexibility and do not
408 compromise product sterility.
409

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412 technical review and input to the article. This information was previously presented at the 2006 PDA
413 Annual meeting in Anaheim, California.
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 12. PDA Technical Report 44 – Risk Management, 2008, Section 3.3.2 pg. 10.

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Table I – Cycle Sterilization Parameters	
Exposure Time:	Production: 35 minutes Validation: 26.5 minutes
Exposure Set-point:	123.0 °C
Pre-vacuum Pulses:	6
Pre-vacuum Depth:	1.0 PSIA

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Table II – Cool Point Determination for Commodities					
Equipment Description	Number of Times in the Coolest 10 Items - By Parameter				
	Total F₀	Maximum Temperature Attained	Lag Time from Expose to ≥ 121.1°C	Time at ≥ 121.1°C	Total Times in Top Ten Coolest
Filters KA4NFP1	5	5	1	5	16
Filters SLK7002PFRP	4	4	0	4	12
1.5 inch Cleaning Hose with valve on one end (Teflon with Stainless Steel over-braided hose)	5	3	0	2	10
Stoppering Outer Bowl, stainless steel	2	2	1	4	9
50cc pump (spare, no tubing attached)	1	4	0	3	8
Vent Filters KA2PFRP1	3	2	0	3	8
25cc pump assembly (includes manifold, pump, nozzle with associated tubing)	2	1	0	4	7
50cc pump assembly (includes manifold, pump, nozzle with associated tubing)	4	0	0	3	7
25' Braided Silicone Tubing	3	2	0	1	6
MPGL20CL3 Vent Filter (bag closed)	1	1	0	2	4
MPGL20CL3 Vent Filter (bag open)	1	1	0	2	4
Tool box (set up tools)	1	0	0	3	4
25' Silicone Tubing	0	2	0	1	3
Stainless Steel "U" Tube	1	1	0	0	2
6" Silicone Tubing		2	0	0	2
Stoppering Inner Disk, stainless steel	1	1	0	0	2
Pressure Indicator w/swage lock valve	1	1	0	0	2
Spill Tray (small) w/ tubing	1	1	0	0	2

Tool box (fill tools)	1	1	0	0	2
Vacuum Manifold w/ tubing (large)	0	1	0	1	2
25cc Spare Pumps 1	1	0	0	0	1
6cc pump assembly (includes manifold, pump, nozzle with associated tubing)	1	0	0	0	1
Stopper Feeder Chute, stainless steel	0	1	0	0	1
Stopper Hopper, stainless steel	1	0	0	0	1
Intermediate Tank with inlet and outlet tubing attached	0	1	0	0	1
Stoppering Transfer Disk, stainless steel	0	0	0	1	1
Vacuum Manifold w/ tubing (small)	0	1	0	0	1
Intermediate Tank Vent Filter Capsule	0	1	0	0	1
The items in the NON-shaded areas are the recommended items for TC/BI challenge during PQ based on the frequency with which they are observed as being the hardest to heat.					

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Table III – Proposed PQ Challenge for Filling Equipment Maximum Load

Load Configuration			
Item Description	Number in Load	Avg. Mass (gm)	Total Mass (gm)
Filters KA4NFP1*, **	4	520.1	2080.4
Filters SLK7002PFRP*	5	1250.5	6252.5
1.5 inch Cleaning Hose with valve on one end (Teflon with Stainless Steel over-braided hose)*	1	4356.0	4356.0
Stoppering Outer Bowl, stainless steel*	1	20990.2	20990.2
50cc pump (spare, no tubing attached)*	2	1055.4	2110.8
Vent Filters KA2PFRP*	2	149.7	299.4
25cc pump assembly (includes manifold, pump, nozzle with associated tubing)*	1	11720.3	11720.3
50cc pump assembly (includes manifold, pump, nozzle with associated tubing)*	1	13354.2	13354.2
25' Braided Silicone Tubing*	1	1427.9	1427.9
MPGL20CL3 Vent Filter (bag closed)*	1	249.1	249.1
MPGL20CL3 Vent Filter (bag open)*	1	241.3	241.3
Tool box (set up tools)*	1	7515.0	7515.0
100cc Sample Tray	1	635.2	635.2
20cc Sample Tray	1	209.8	209.8
25cc Spare Pumps	2	824.1	1648.2
3" Nozzle Bracket	1	854.8	854.8
Batch Record	4	478.0	1912.0
Box of Screws	1	2228.0	2228.0
Bulk Container Bracket	1	2086.4	2086.4
Fill Needles (spare)	4	36.7	146.8
Inner Disk	1	7914.2	7914.2
Intermediate Tank	1	16260.5	16260.5
Lyo Rake (large)	2	1114.7	2229.4
Lyo Rake (small)	5	582.9	2914.5
Pressure Indicator w/swage lock valve	1	636.2	636.2
Ring Gate Boxes w/ 19 gates	8	4603.9	36831.2
Spare Bracket, 1.5"	1	418.6	418.6
Spill Tray (large) w/ tubing	1	1849.1	1849.1
Spill Tray (small) w/ tubing	1	1449.2	1449.2
Spray Wand	1	331.2	331.2
Stopper Track	1	5211.4	5211.4

Tool box (fill tools)	1	4032.7	4032.7
Vacuum Manifold w/ tubing (large)	1	3357.0	3357.0
Vacuum Manifold w/ tubing (small)	1	1163.0	1163.0
Vacuum Plugs	1	183.1	183.1
Vacuum Wheel	1	8293.2	8293.2
Z spacers (set of 4)	1	1395.0	1395.0
		Total (g)	174,788
* One (1) of each of these items to be monitored with TC's and BI's.			
** One of this item used as the minimum load			

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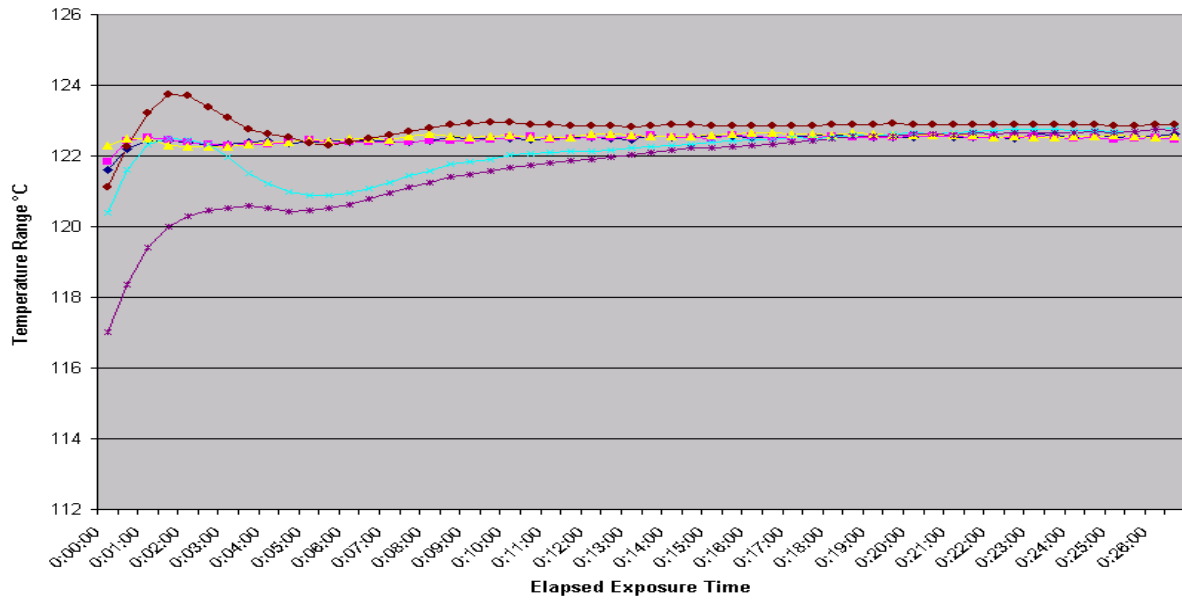
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Table IV – Overall Equipment Listing			
Item Name	Mass (gms)	Item Name	Mass (gms)
"U" Tube	427.6	MPGL20CL3 Vent Filter (bag open)	242.6
1.5" Teflon Gaskets	9.0	Nozzle Bracket (1 - 3" and 1- 1.5")	1273.4
1.5" Triclamps 6/bag	1787.5	Stopper Outer Bowl	20961.4
1.5" Triclover Clamps 2/bag	616.7	Overseals 10/pack 20mm	14.5
1.5" Triclover Gaskets	7.2	Overseals 10/pack 28mm	23.8
100cc Sample Tray	635.2	Filters KA4NFP1	549.8
20cc Sample Tray	209.8	Filters SLK7002PFRP	1216.3
20L Nalgene Carboy w/ lid	1550.0	Pressure Indicator w/swage lock valve	636.2
25' Braided Silicone Tubing	1394.9	Product Tubing	1394.9
25' Silicone Tubing	1333.0	Retainer Clips 6/ pack	294
25cc pump assembly	11720.3	Ring Gate Boxes w/ 19 gates	4595.4
25cc Spare Pumps 2	819.0	Sample Thief	272.4
3' Nozzle Bracket	854.8	Segment Tags	256.5
4' 0.75 braided hose w/ 1.5 Triclamp fittings	1643.2	Small Stir Bar	13.2
5 Gallon Glass Carboy	5990.5	Spare Bracket	418.6
5 Liter Glass Carboy	1536.0	Spill Tray (large) w/ tubing	1849.1
50cc pump (spare)	1063.5	Spill Tray (small) w/ tubing	1449.2
50cc pump assembly	13354.2	Spray wand	325.0
5L Nalgene Carboy w/ lid	156.9	Stopper # 8 (small)	27.6
6cc pump (spare)	317.1	Stopper Feeder Chute	1634.4
6cc pump assembly	9269.8	Stopper Hopper	7010.2
Assorted Screws in s/s box	2228.0	Stopper Track	5211.4
Batch Record	478.0	Stoppering Track	5211.4
Box of Screws	2228.0	Stopper # 12	82.0
Bulk Container Bracket	2086.4	Tool box (fill tools)	4051.6
Fill Needles (spare)	36.7	Tool box (set up tools)	7615
Inner Disk	7914.2	Transfer Disk	2690
Intermediate Bulk Tank (w/ frame)	18346.9	Vacuum Manifold w/ tubing (large)	3357
Intermediate Tank	16260.5	Vacuum Manifold w/ tubing (small)	1163.0
Large stir bar magnetic 1	105.0	Vacuum Plugs	211.3
1.5 "Cleaning Hose with valve on one end	4356.0	Vacuum Wheel	8293.2
Lyo Rakes (large)	1125.0	Vent Filters KA2PFRP1	126
Lyo Rakes (small)	582.9	Z spacers (set of 4)	1395.1
MPGL20CL3 Vent Filter (bag closed)	249.1		

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455 **Figures**
456
457 **Figure 1**

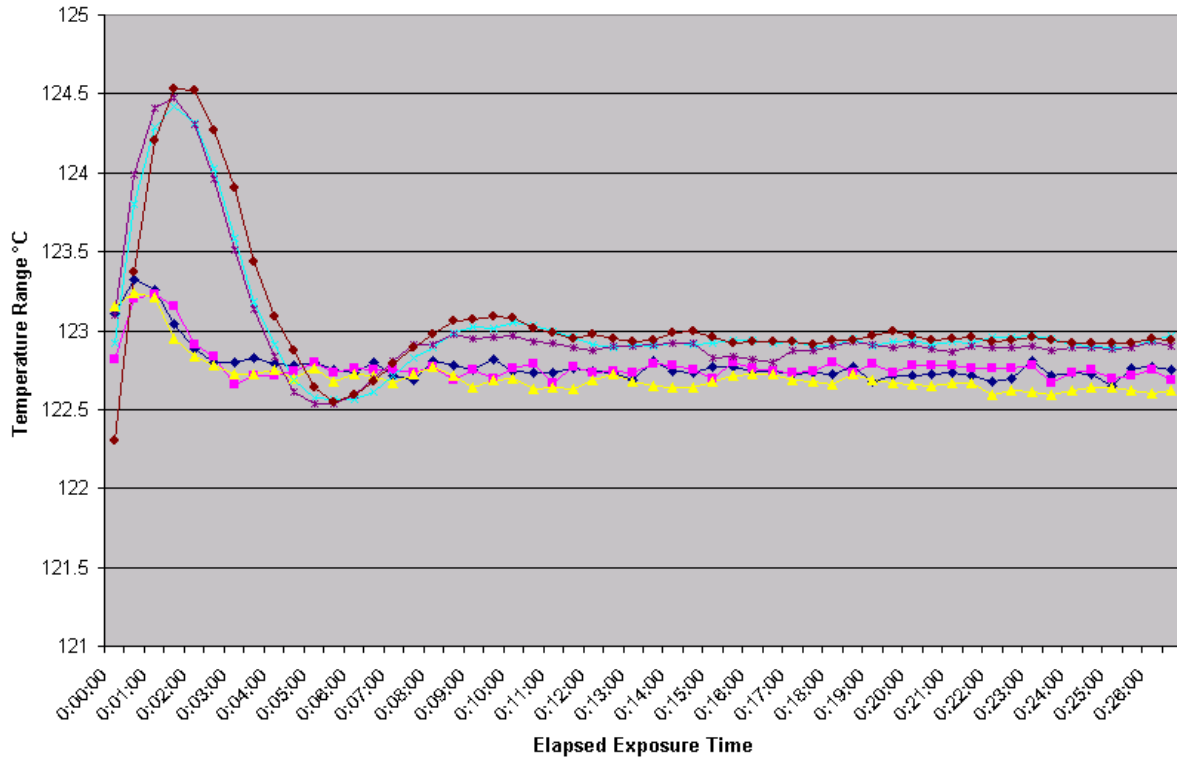
Fill Equipment - Max Load Minimum Temperatures - From all Penetration TC's > 121.1°C until Exposure Cycle End.



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462 **Figure 2**

Fill Equipment - Max Load Minimum Temperatures - From all Distribution TC's > 121.1°C until Exposure Cycle End.

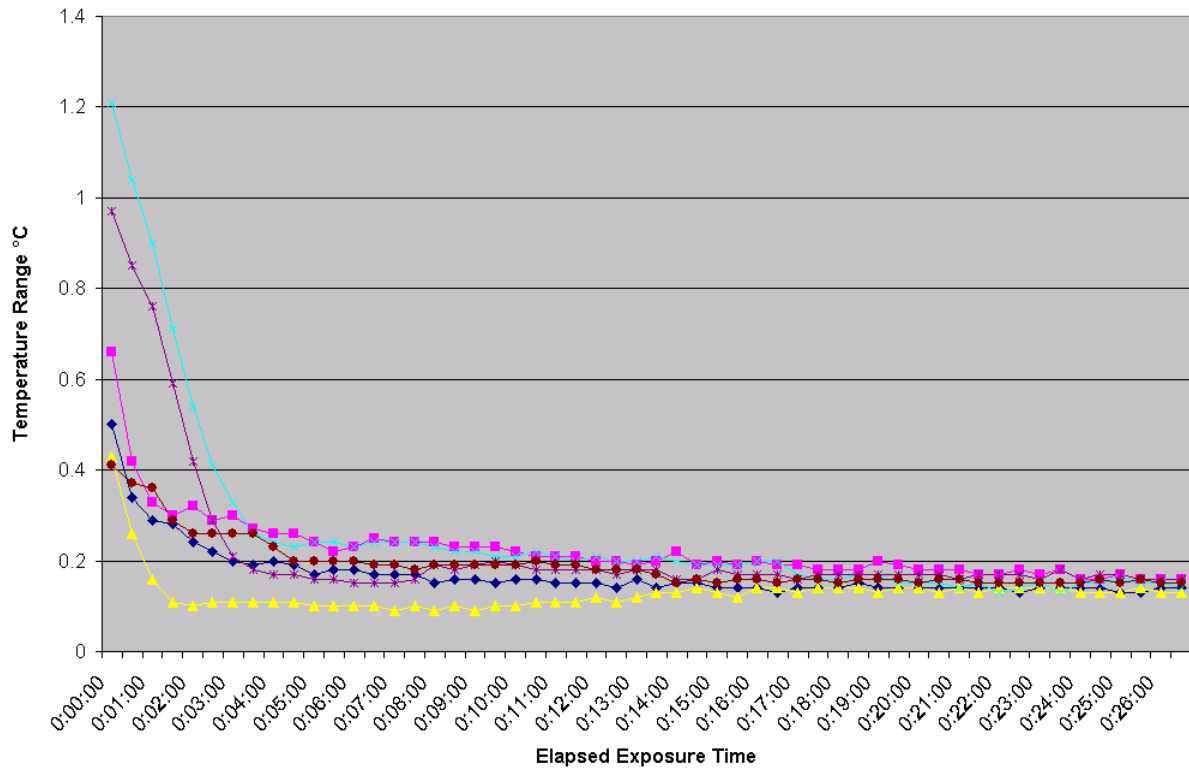


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Figure 3

Fill Equipment - Maximum Load - Temperature Range of all Distribution TC's > 121.1°C to Exposure Cycle End



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Figure 1, 2, 3 Legend



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