

A new Draft version of this document is available for comment in Appendix H of the document entitled Premarket Notifications [510(k)] for Biological Indicators Intended to Monitor Sterilizers Used in Health Care Facilities; Draft Guidance for Industry and FDA Reviewers at: <http://www.fda.gov/cdrh/ode/guidance/1320.pdf>

This guidance was written prior to the February 27, 1997 implementation of FDA's Good Guidance Practices, GGP's. It does not create or confer rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. This guidance will be updated in the next revision to include the standard elements of GGP's.

THE CENTER FOR DEVICES AND RADIOLOGICAL HEALTH, FDA
GUIDE FOR VALIDATION OF BIOLOGICAL INDICATOR INCUBATION TIME

Applicability of the Recommendation:

1. Types of BIs

This recommendation applies to all biological indicators (BIs), either prepared commercially or in-house, on any carrier, self-contained or on a strip.

2. Types of Sterilization Processes

This recommendation applies to BIs used in all types of sterilization processes.

3. Labeling of BIs by Manufacturer

This recommendation allows the BI manufacturer to label BIs for incubation times of less than 7 days provided that they validate the shorter incubation and specify the sterilization validation parameters in the labeling of the BI.

4. Users of BIs

This recommendation applies to both commercial users and health-care providers. If the BI user wants to reduce the number of incubation days to a period shorter than the labeled incubation time, or use sterilization parameters other than those labeled, it is incumbent upon the BI user to validate, in their system, that the number of days incubation meets the criteria described in the methodology section.

Methodology:

The Center for Devices and Radiological Health recommends that a firm may reduce the incubation time for biological indicators used in medical device sterilization processes, from the standard 7 or more days, PROVIDED THAT, at a minimum, the firm performs validation studies demonstrating the revised number of days of incubation are sufficient for product release according to the following testing methodology described below:

1. Obtain a minimum of 300 biological indicators. One hundred (100) BIs should be from each of 3 separate lots.
2. Using the parameters of the firm's sterilization cycle, expose the BIs in 3 partial sterilization cycles (100 BIs per partial cycle). Each of the test cycles will have 30% to 80% of the indicators surviving (i.e., test positive).
 - A. A partial cycle is one in which all sterilization parameters, except the time parameter, are met. The exposure time is much shorter than the standard sterilization cycle.

- B. Only the BIs from one lot are to be used in each partial cycle. Do not mix BIs from different lots.
- C. It would be preferable if the BIs were run in a device load. However, the inherent difficulties of achieving a partial cycle kill under such circumstances are well understood. Thus, the partial cycle can be run without the BIs being in the presence of devices.

NOTE: During all sterilization validation studies, a firm must consider the effects of the sterilant in combination with the device material on the indicator organism. If the materials are judged to have a significant effect on organism destruction, the BIs should be exposed to the sterilant in conjunction with the devices during the partial cycle studies.

- D. If there are fewer than 30% survivors or more than 80% survivors in any one run, this particular cycle is invalid and must be rerun to achieve the desired number of survivors.
 - E. Three partial cycles are the minimum number of testing cycles to be run. If the results of any one cycle are invalid (see D above), another partial cycle must be substituted for it.
3. After exposure incubate the BIs for a minimum of 7 days. Follow the BI manufacturer's instructions for incubating the BIs. Place the BIs in the growth media no more than 8 hours after removal from the sterilization chamber or removal from the sterilized load of devices. Record the number of positive BIs on either a daily basis or for the particular time interval of interest.
 4. Using the number of BIs that test positive on day 7 as the base of 100% grow out (denominator data), determine from the growth chart if the required number of BIs have grown out (numerator data) in the time interval in question. More than 97% of the base number of BIs must test positive in each partial cycle for the proposed incubation time to be acceptable.
 5. The greatest number of days of incubation required to obtain more than 97% positive BIs (based on the 7 day incubation time) in any one of the partial cycles is the minimum incubation time that will be allowed. Averaging the three (or more) partial cycle incubation times is not allowable (see example in Appendix).
 6. If the BI user has not or cannot validate the BI incubation period using the described methodology, then the user must remain with at least a seven day incubation period.

CHART: BIOLOGICAL INDICATORS

Number of Positive Biological Indicators Required to Achieve greater than 97% Level of Growth

Numerator											
Data (1)	30	31	32	33	33	34	35	36	37	38	
Denominator											
Data (2)	30	31	32	33	34	35	36	37	38	39	
Numerator											
Data	39	40	41	42	43	44	45	46	47	48	
Denominator											
Data	40	41	42	43	44	45	46	47	48	49	
Numerator											
Data	49	50	51	52	53	54	55	56	57	58	
Denominator											
Data	50	51	52	53	54	55	56	57	58	59	
Numerator											
Data	59	60	61	62	63	64	65	65	66	67	
Denominator											
Data	60	61	62	63	64	65	66	67	68	69	
Numerator											
Data	68	69	70	71	72	73	74	75	76	77	78
Denominator											
Data	70	71	72	73	74	75	76	77	78	79	80

(1) The numerator is the number of positive biological indicators that is greater than 97% of the denominator. If the numerator is equal to or greater than the one listed for the corresponding denominator (based on the total number of positive biological indicators on day 7), the length of the incubation time when this occurs is acceptable.

(2) The denominator is the total number of positive biological indicators on day 7 of incubation.

Appendix

EXAMPLE: A firm would like to reduce their BI incubation time to 3 days. Their testing data shows the following:

Partial cycle #1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Numerator	0	56	57	57	58	59	59
Denominator	59	59	59	59	59	59	59
Percent Growth	-	94.9	96.6	96.6	98.3	100	100

Partial cycle #2	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Numerator	1	34	35	35	35	35	35
Denominator	35	35	35	35	35	35	35
Percent Growth	2.9	97.1	100	100	100	100	100

Partial cycle #3	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Numerator	0	79	81	Test invalid because of number of positive BIs which are outside the allowable window			
Denominator							

Partial cycle #4	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Numerator	0	47	48	49	49	49	49
Denominator	49	49	49	49	49	49	49
Percent Growth	0	95.9	98.0	100	100	100	100

The firm would not be allowed to reduce their BI incubation time to 3 days because, of the three valid test cycles, not all tests achieved 97% grow out in 3 or fewer days. However, based on the criteria listed in point 5 of the testing methodology, the firm would be allowed to reduce their incubation time to 5 days. The 5 day incubation time in this example is the greatest number of days, of all the valid partial cycles, needed to grow out more than 97% of the denominator BIs.