

# Biological Indicators and Their Application in Clinical Sterilization 2010

**Spores Don't Lie®**



**SGM Biotech, Inc.**  
**10 Evergreen Drive, Suite E**  
**Bozeman, MT 59715**  
**Tel: 406-585-9535**  
**Fax: 406-585-9219**  
**email: [info@sgmbiotech.com](mailto:info@sgmbiotech.com)**  
**Web: [www.sgmbiotech.com](http://www.sgmbiotech.com)**  
**A Global Company**

**ST-Pack S.R.L.**  
**Llerena 3192 (C1427DEP)**  
**Buenos Aires, Argentina**

**Tel: +5411-4523-8555**

**Fax: +5411-4523-8555**

**Email: [stpack@stpack.com.ar](mailto:stpack@stpack.com.ar)**

**Web: <http://www.stpack.com.ar>**



**June 2010**



We make too many assumptions about sterilization processes.



Every time we make an  
**assumption** about a  
sterilization process we are  
setting ourselves up for failure.

It's **not if** – **but when.**



# Why is this?

- It takes too much time
- It costs too much money
- We don't really understand the risks we are taking

We are asked to make decisions. The more knowledge we have the better judgment decisions we make.



This seminar is designed to enhance your knowledge and understanding about sterilization and the use of biological indicators.



There are many scientists  
and engineers that  
**underestimate** the value  
of the use of bacterial  
spores in monitoring the  
sterilizing process.



We have three levels of  
evaluation of a  
sterilization process  
effectiveness.



- Physical measurements
- Chemical measurements
- Biological measurements



# Definitions

From ISO 11139:2006

- **Biological Indicator** – test system containing **viable microorganisms** providing a defined resistance to a specified sterilization process



Biological indicators are not immediate, they require time for incubation of the **viable microorganisms** so we can determine if they have survived the process.



**Physical measurements  
are the only way to  
calculate a sterility  
assurance level for any  
sterilization process.**



**A biological measurement is the only way to demonstrate that a sterilization process was effective.**



We use bacterial endospores (spores) to measure the effectiveness of the sterilization process.



# Why?

- Spores are the most **resistant** life forms to sterilization processes
- Spores are **stable**
- Spores are **predictable**
- Spores are **consistent**



# Spores

- The only tool available to integrate all critical process parameters whether known or unknown.



When the spores are  
killed the process is  
acceptable.



When the spores  
survive the process is  
not acceptable.

# What is a catastrophic sterilization failure?

- A positive BI when all physical measured parameters meet their specified values



The bacterial spore is the only device that is available to pick up a catastrophic failure in the process.



When a catastrophic failure occurs, there is a high probability that non-sterile goods are in the load.



- Physical measurements are necessary, but they can't detect catastrophic sterilization failures.
- BIs tell you when a cycle fails (positive BI). When a BI doesn't grow (negative) we assume it was killed.
- BI results are available between 3 and 10 hours.
- BIs are the most accurate determiners of acceptable sterilization.
- International standards have verbally eliminated “outliers” and “rogue” spores.
- BIs annual expense is much less than the cost of one hospital acquired infection.



When we remove the bacterial spore from the process monitoring data we lose the ability to detect catastrophic process failures.



# So why aren't BIs always used?

## Perception is:

- Physical measurements are more accurate and more dependable
- BIs are the messengers of bad news (positive BIs)
- BIs take too long to provide a result
- BIs are “not dependable”
- BIs have “rogue” spores
- BIs have “outliers”
- BIs cost a lot of money



More complex  
processes should  
require greater use of  
BIs.

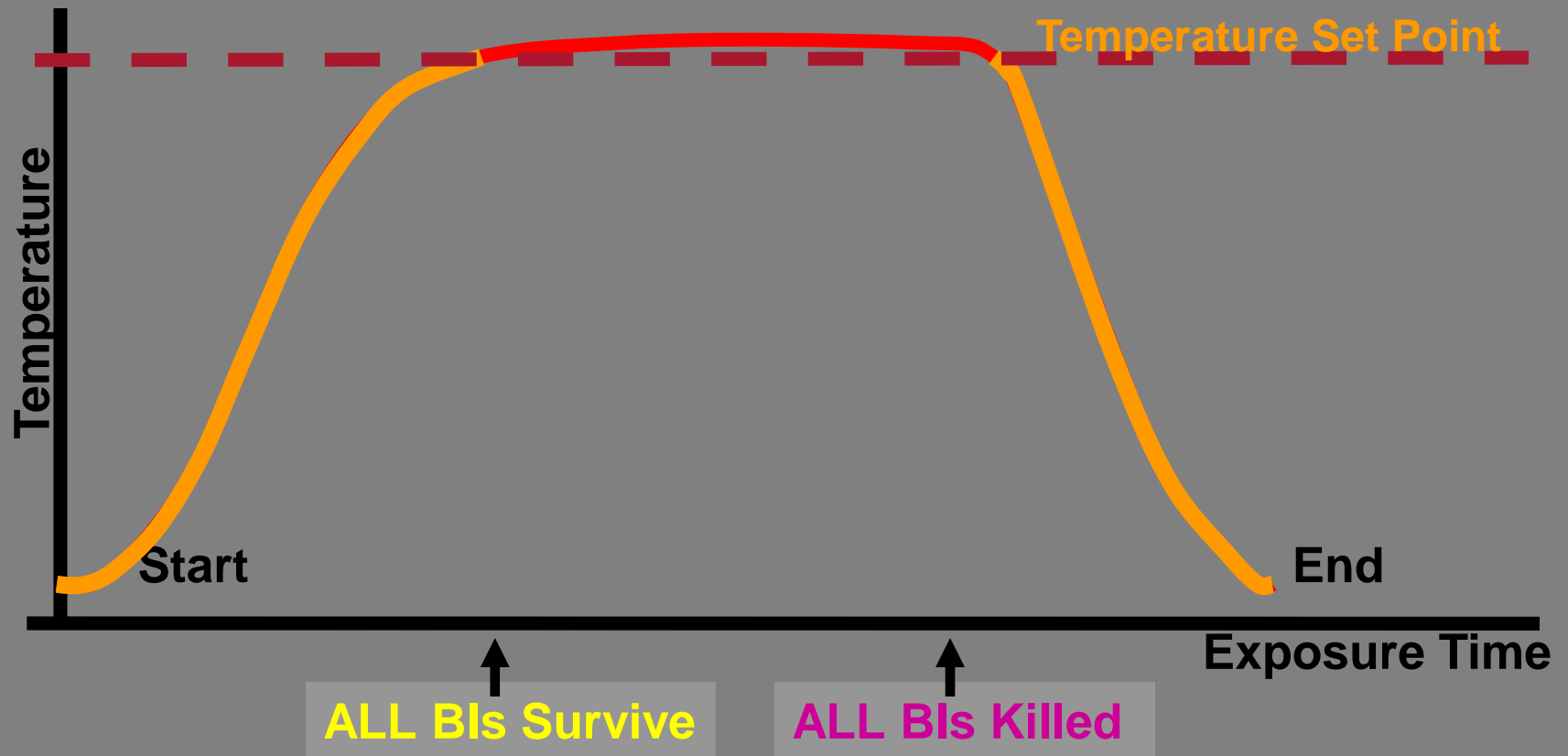


Processes that are **not**  
well understood should  
require greater use of  
BIs.



Why BIs work and  
instruments don't  
always work.

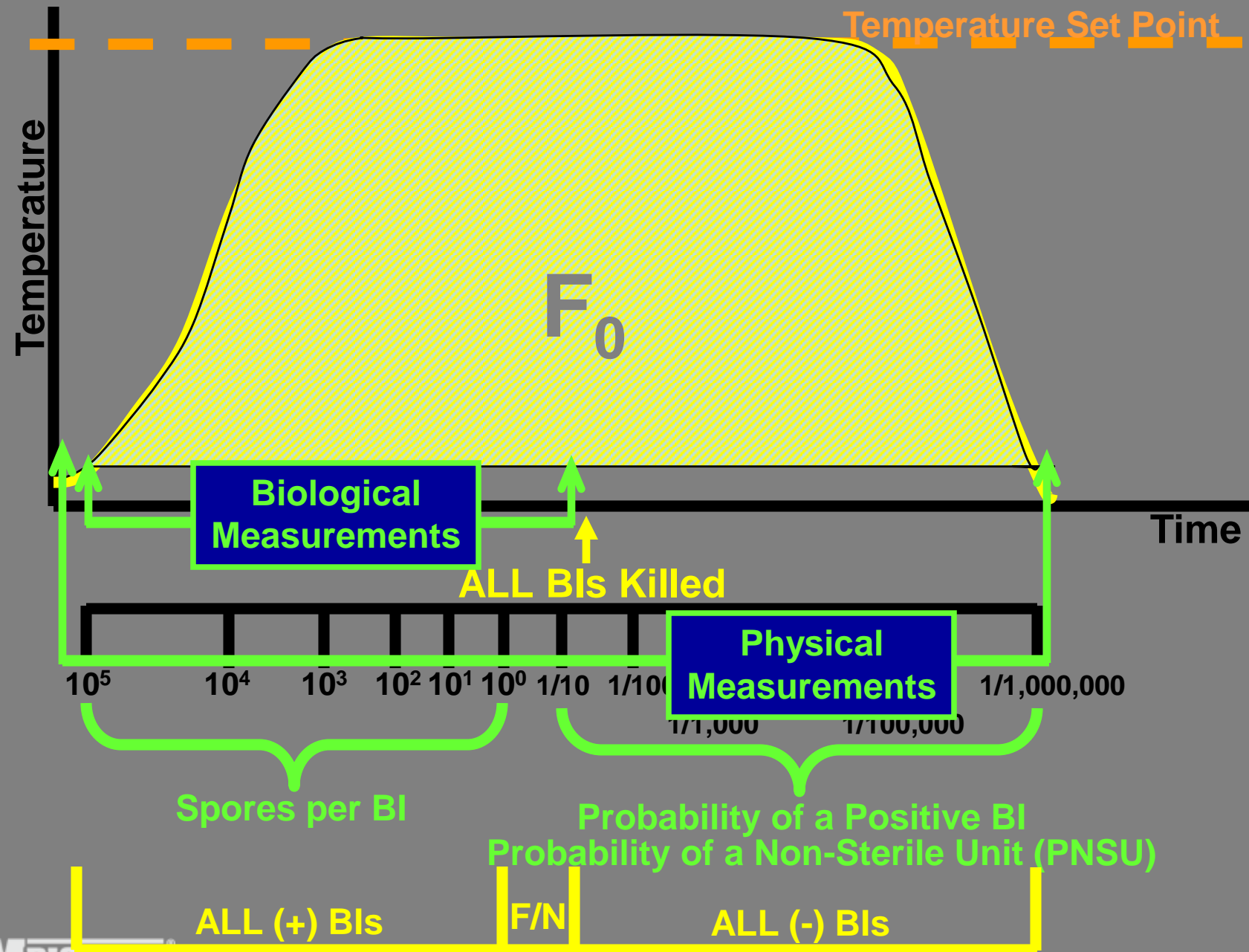
# Dissection of a Sterilization Cycle



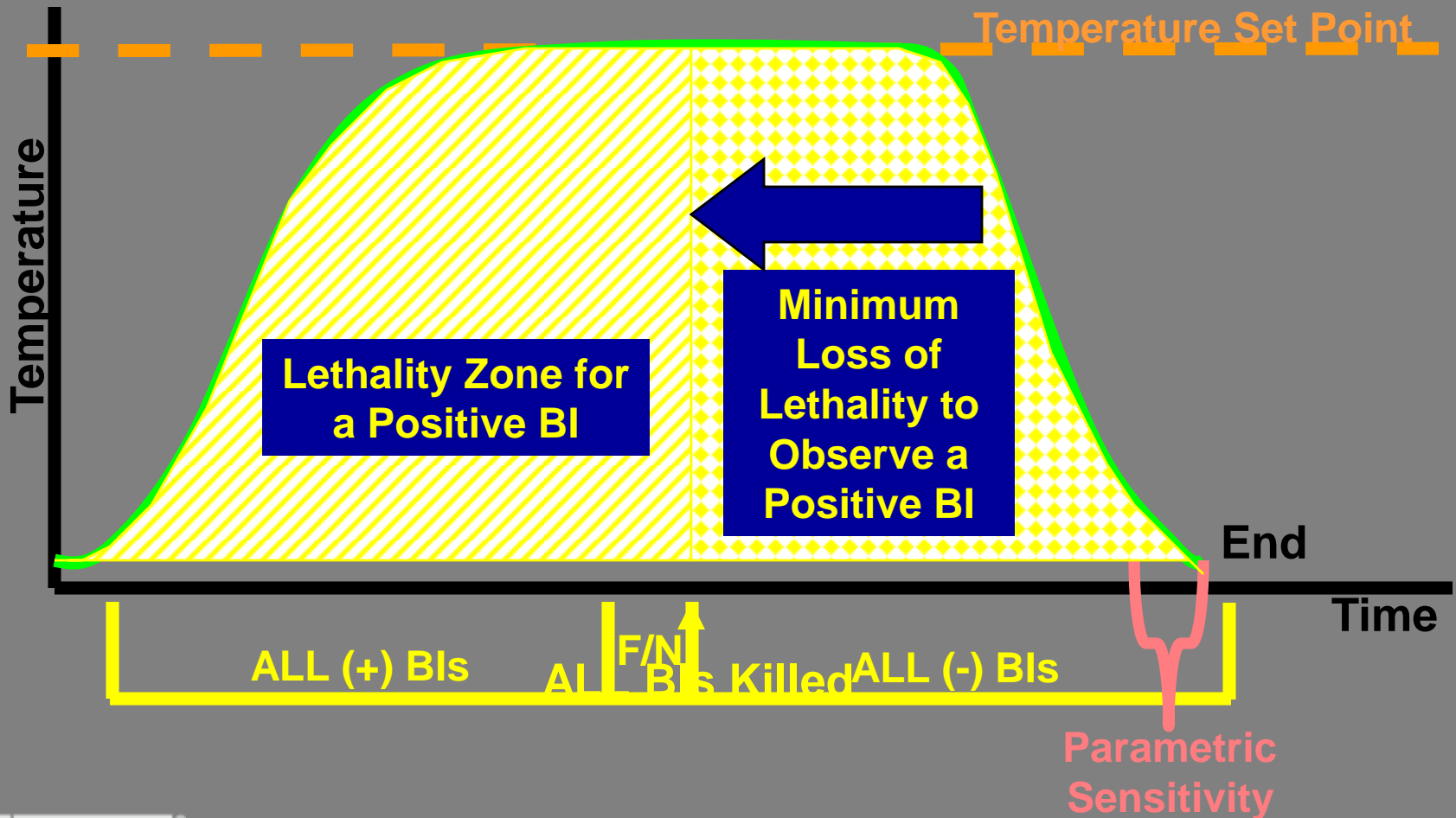
# Dissection of a Sterilization Cycle

half cycle approach

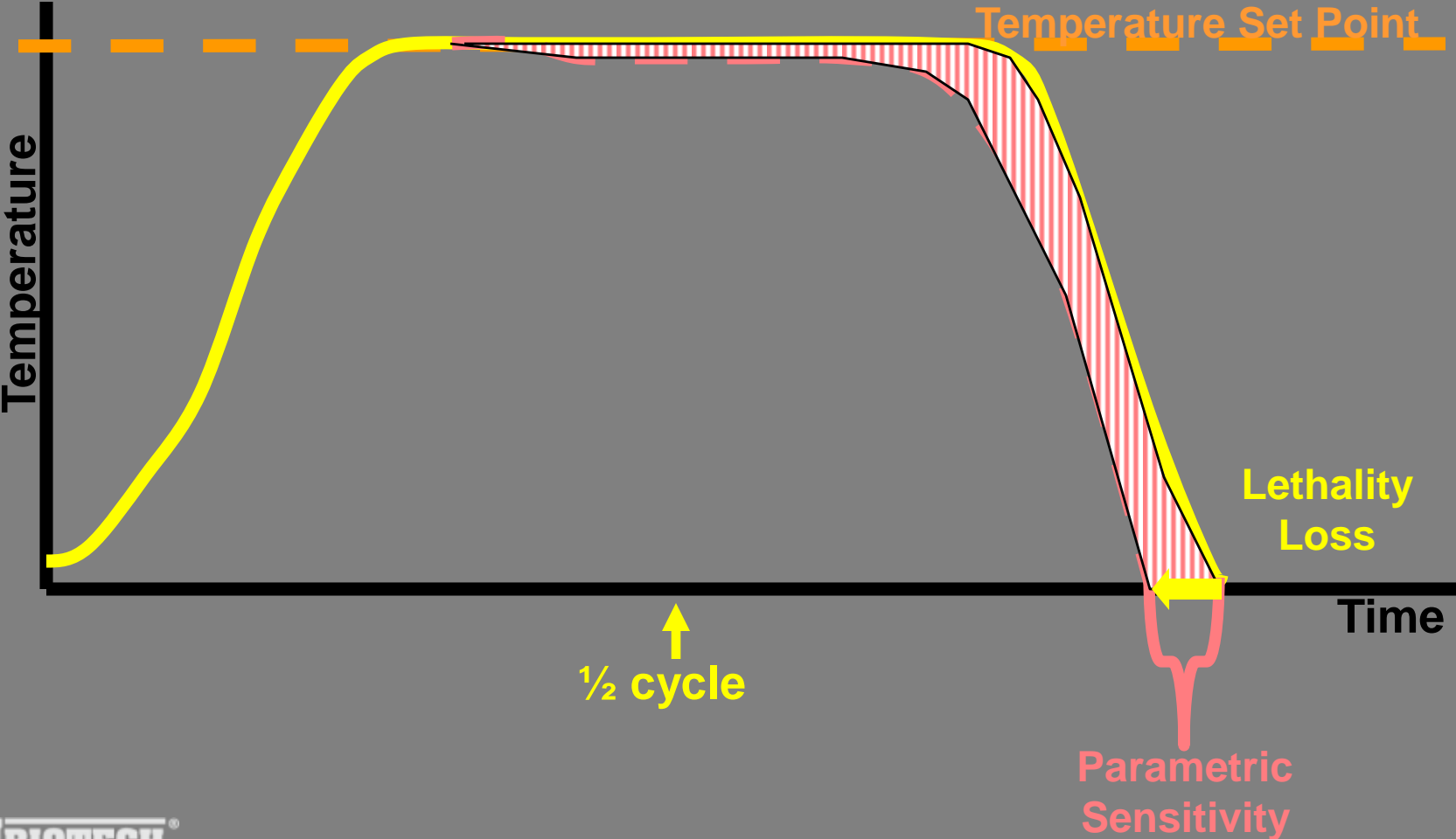




# If BI is Positive in a Validated Cycle



# Sensitivity of Parametric Measurements

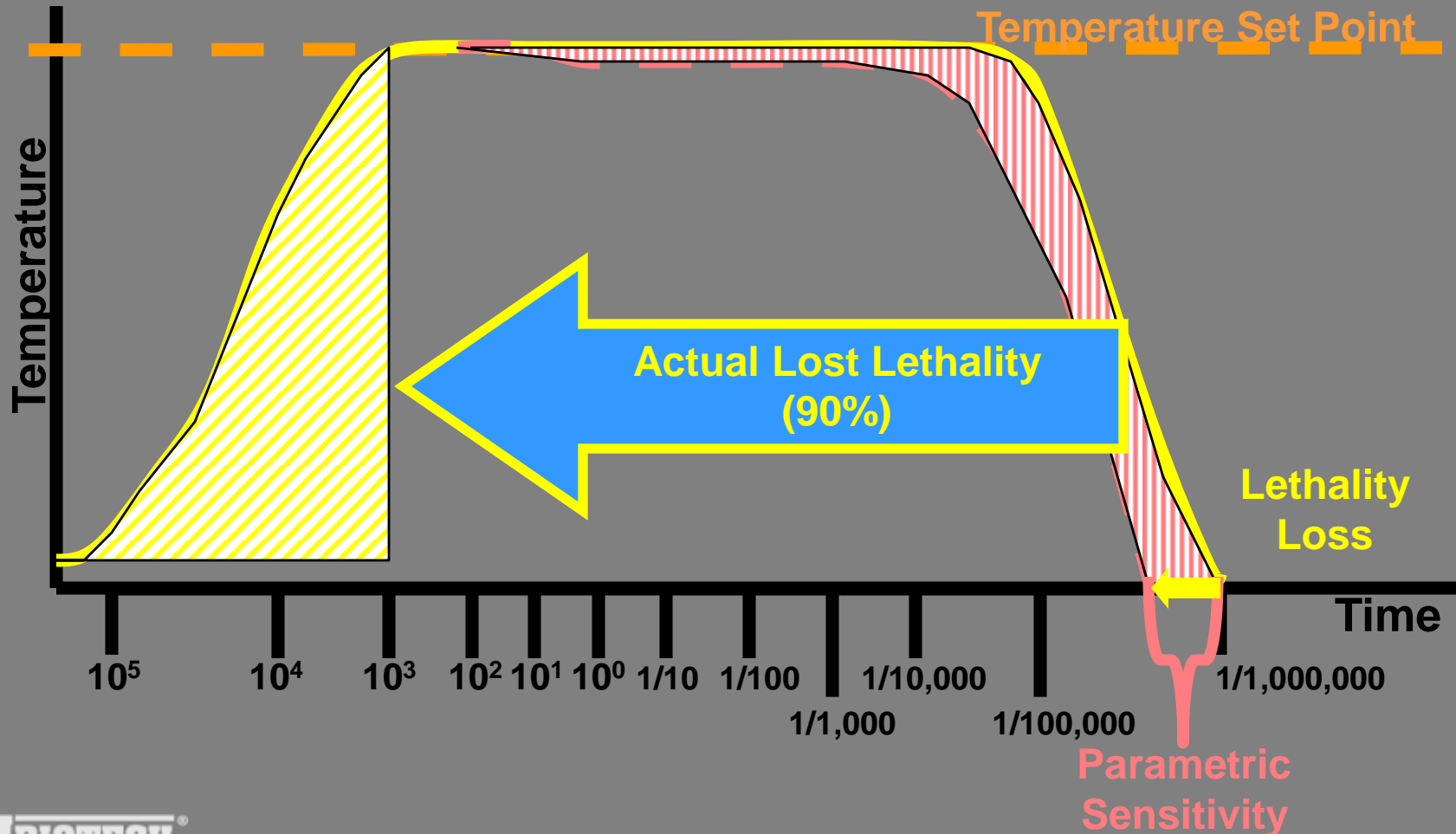


We all know that residual  
air creates problems in  
steam sterilizers.

\*Bowie & Dick test used daily.



# Positive BI in Air Pocket (steam process) or Positive BI in Low Moisture Area (EtO process)



# Summary

- Process failures are easily identified when parametric measurements are **not** within specifications.
- Parametric measurements are necessary to support Sterility Assurance Levels (SAL).
- Biological Indicators are only capable of identifying **catastrophic** sterilization failures.
- **If** BIs are not used you will not be able to identify these **catastrophic** failures.
- Any process that does not use both (parametric and biological) measurements is **inadequate**.



- **In an adequate sterilization cycle all spores are killed and BIs test negative**
- **Sterilization failure results in surviving spores**



If you don't use BIs for routine monitoring, you have no means of detecting a catastrophic failure.



When a catastrophic failure occurs with a BI, there is a high probability that you have non-sterile goods.



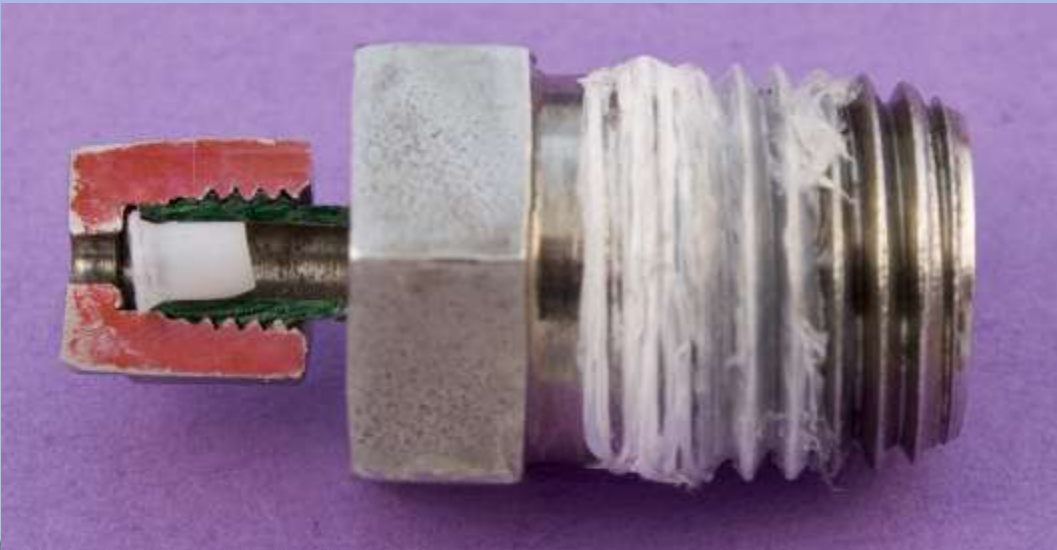
# Identifying Causes of Sterilization Failures

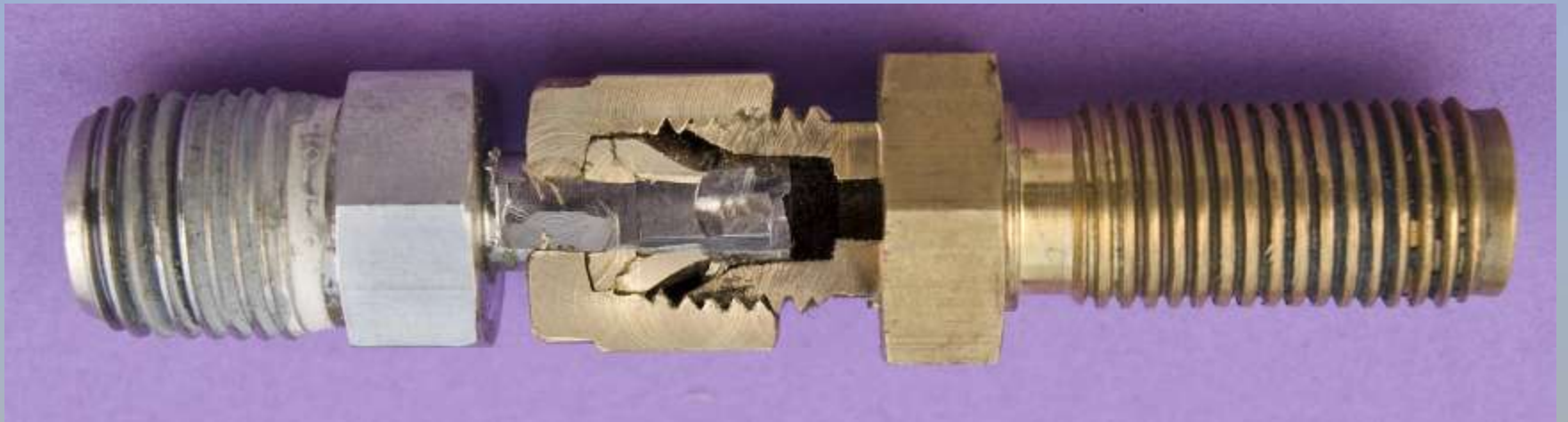


# Steam Sterilization Failures

- Load configuration
- Product/product container changed
- Poor air removal
  - Steam trap failure
  - Gasket failure
  - Check valve failure
  - Pipefitting leaking (all possibilities)
  - Vacuum pump wearing











# Steam

- Air is the most common cause for failure



# EO Sterilization Failures

- Load configuration changed
- Product changed
- Product package changed
- Poor humidification
  - Check ambient temperature and humidity
  - Check preconditioning
  - Check cycle phase change rates
  - Check chamber leak rate
  - Check chamber vacuum rate
  - Warmer than normal process temperature
- Pallet wrapping
- Product master cartons (general corrugate)



# EO

- Humidity (moisture) is the most common cause for failure





**Revolutionizing sterilization monitoring**

**Introduces**

**Smart-Read™ technology**



**Sterilization is a process that renders a product or material free from living microorganisms**



**Sterilization can not be  
expressed only as time and  
temperature**

**Sterilization is not as simple  
as 121°C for 15 minutes or  
132 C for 4 or 10 minutes**

**Sterilizing items in hospitals and clinics is becoming more complicated because of advances in medical instruments and supplies**



**A new biological indicator  
monitoring system that will  
simplify your sterilization  
monitoring program is now  
available**



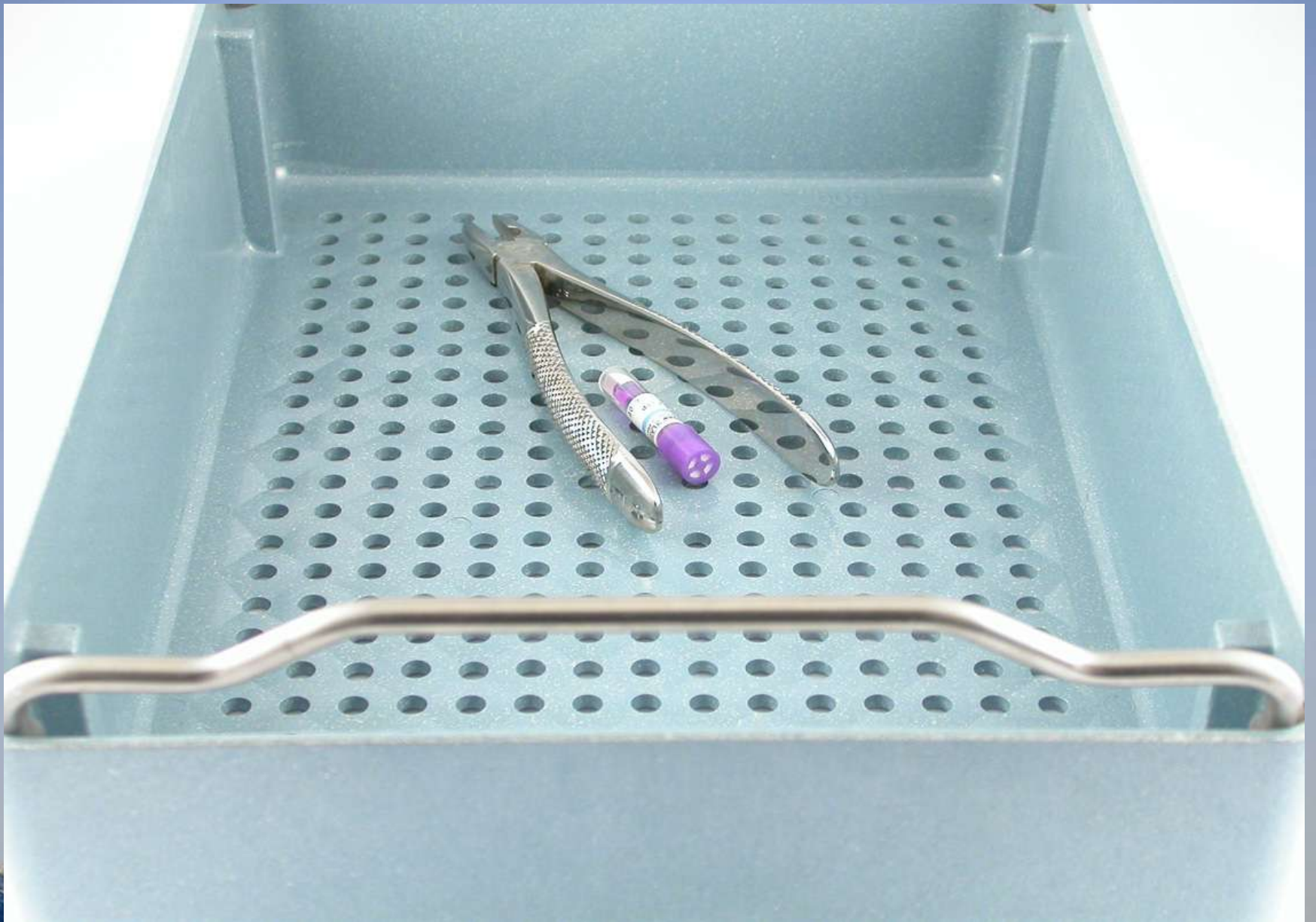
# **Smart-Read™ EZTest®** **Biological Indicator** **Monitoring System**

# ***Smart***-Read Technology

- **Smart-Read EZTest** biological indicators are positioned in the load according to facility procedure











22 10:15



22 10:07

**Retrieve  
exposed  
Smart-Read  
Bls from the  
load**



# ***Smart***-Read Technology

- The biological indicator is the only “tool” capable of integrating ***all*** critical process parameters.
  - Steam temperature
  - Pressure
  - Exposure time
  - Presence of trapped air
- It is recommended to use a minimum of one **S**mart-Read EZTest BI per cycle to guarantee greatest assurance of sterility

**This *TRUE* biological  
indicator monitoring  
system rapidly detects  
*ONLY* spore growth**



**Use the Smart-Read BI  
as you would use any  
other self-contained BI**

**Smart-Read does *NOT*  
rely on a correlated  
chemical or enzyme  
result to predict  
microbial activity**

# **Smart-Read EZTest<sup>®</sup> Biological Indicator Monitoring System**

- **Smart-Read EZTest biological indicator**
- **Smart-Well<sup>™</sup> incubator kit includes:**
  - **Smart-Well incubator**
  - **Printer**
  - **Thermometer**
  - **Record book**
  - **Stylus**
  - **Power packs and system manual**

# **Smart-Read EZTest<sup>®</sup> Biological Indicator Monitoring System**

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  - Stylus**
  - Power packs and system manual**



# Smart-Read EZTest<sup>®</sup> Biological Indicator Monitoring System

- **Smart-Read EZTest biological indicator**

- **Self-contained BI with *Geobacillus stearothermophilus* spores**

- **ONE indicator for all cycle types and temperatures**

- ✓ **121° to 135°C**

- ✓ **Pre-vacuum**

- ✓ **Gravity**

- ✓ **Flash**



# Smart-Read EZTest<sup>®</sup> Biological Indicator Monitoring System

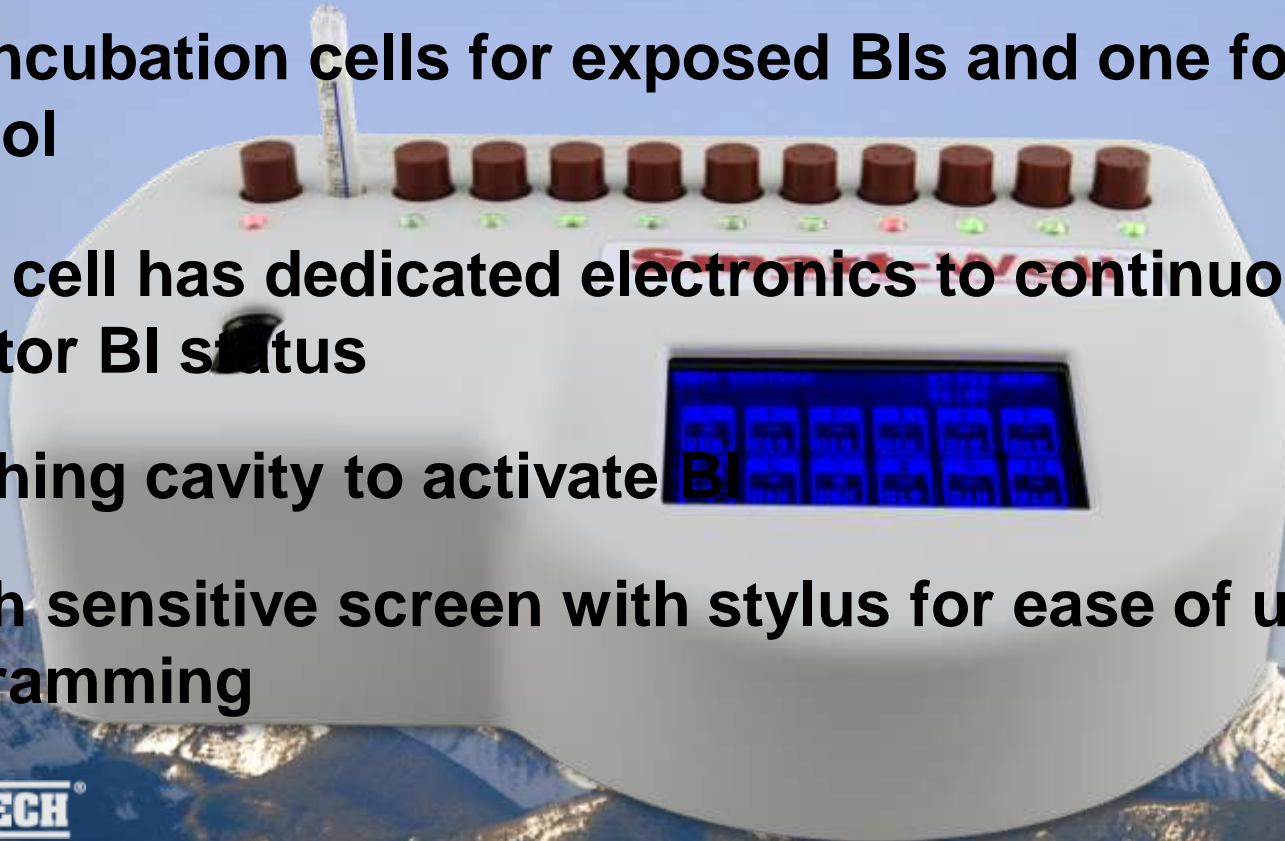
- **Smart-Well<sup>™</sup>** incubator kit includes:
  - **Smart-Well** incubator

Ten incubation cells for exposed BIs and one for positive control

Each cell has dedicated electronics to continuously monitor BI status

Crushing cavity to activate BI

Touch sensitive screen with stylus for ease of user programming



- **Printer** receives signal from **Smart-Well** and documents the exact time the positive result was obtained
- **Record includes:**

Always Printed

- Incubation start time/date
- Hours of incubation elapsed
- BI test result (positive/negative)
- Incubation cell number

User Programmable

- **Cycle type**
- **BI Lot number**
- **Sterilizer ID number**
- **User ID number**

SGM Smart-Well  
Time: 05:59  
ST: 05:59 11FEB2000  
TR: 00:02  
BI result: POSITIVE  
Cell#: 07  
Lot #: S????  
S...: #  
L...: #  
User: 107

# Printer



- Printer receives signal from **Smart-Well** and documents the exact time the positive result was obtained
- The rapid detection of biological growth is 100% visually verifiable by the operator

**Results are:**

**100% Accurate**

**100% Reliable**

**100% User Verifiable**

**The **S**nart-Well incubator  
detects *true* biological activity**

# Potential outcomes when chemical/enzyme methods are correlated to biological results:

**Chemical/enzyme reaction:**

- 1) Predicts sterility / spores grow (false-negative)**
- 2) Predicts sterility / spores do not grow**
- 3) Predicts sterilization failure / spores grow**
- 4) Predicts sterilization failure / spores do not grow (false-positive)**

# With **Smart-Read** Biological Indicator Monitoring System, the rapid reaction **IS** the biological activity

Results are either:

1. Positive BI indicates sterilization failure
2. Negative BI indicates adequate sterilization



# Definitions

From ANSI / AAMI / ISO 14161:2000

- **Sterile** – free from viable microorganisms
- **Sterilization** – validated process used to render a product free from viable microorganisms



# Definitions

From ISO 11139:2006

- **Chemical Indicator, non-biological indicator – system that reveals change in one or more predefined process variables based on a chemical or physical change resulting from exposure to a process**



# Definitions

From ISO 11139:2006

- **Biological Indicator** – test system containing viable microorganisms providing a defined resistance to a specified sterilization process



# Definitions

From U.S. FDA 510(k) for Biological Indicators, Draft Guidance

- **Biological Indicator – Device intended for use by a health care provider to accompany products being sterilized through a sterilization procedure and to monitor adequacy of sterilization. (continued)...**

# Definitions

From U.S. FDA 510(k) for Biological Indicators, Draft Guidance

**...The device consists of a known number of microorganisms, of a known resistance to the mode of sterilization, in or on a carrier and enclosed in a protective package. (continued)...**

# Definitions

From U.S. FDA 510(k) for Biological Indicators, Draft Guidance

**...Subsequent growth or failure of the microorganisms to grow under suitable conditions indicates the adequacy of sterilization.**



# Definitions

From U.S. FDA 510(k) for Biological Indicators, Draft Guidance

**The FDA Guidance Document specifies Exclusions:**

**“This document does not provide information on enzyme-type chemical indicators, also known as rapid readout indicators.” (continued)...**

# Definitions

From U.S. FDA 510(k) for Biological Indicators, Draft Guidance

**...Although some enzyme-type chemical indicators are incorporated into devices that also contain a biological indicator, the results of enzyme-type chemical indicators are read separately from (and typically before) the spore growth reading.”**

**Sterile, Sterilization, and  
Biological Indicator  
all contain the words  
“viable microorganisms”  
in their definition**

- **Smart-Read system:**

- ***IS*** a biological system that rapidly detects *spore* growth readings



# ***Smart***-Read Technology

- Use crushing cavity in **Smart-Well** incubator to activate **Smart-Read BIs**
- Insert **Smart-Read BI** into a **Smart-Well** incubation cell

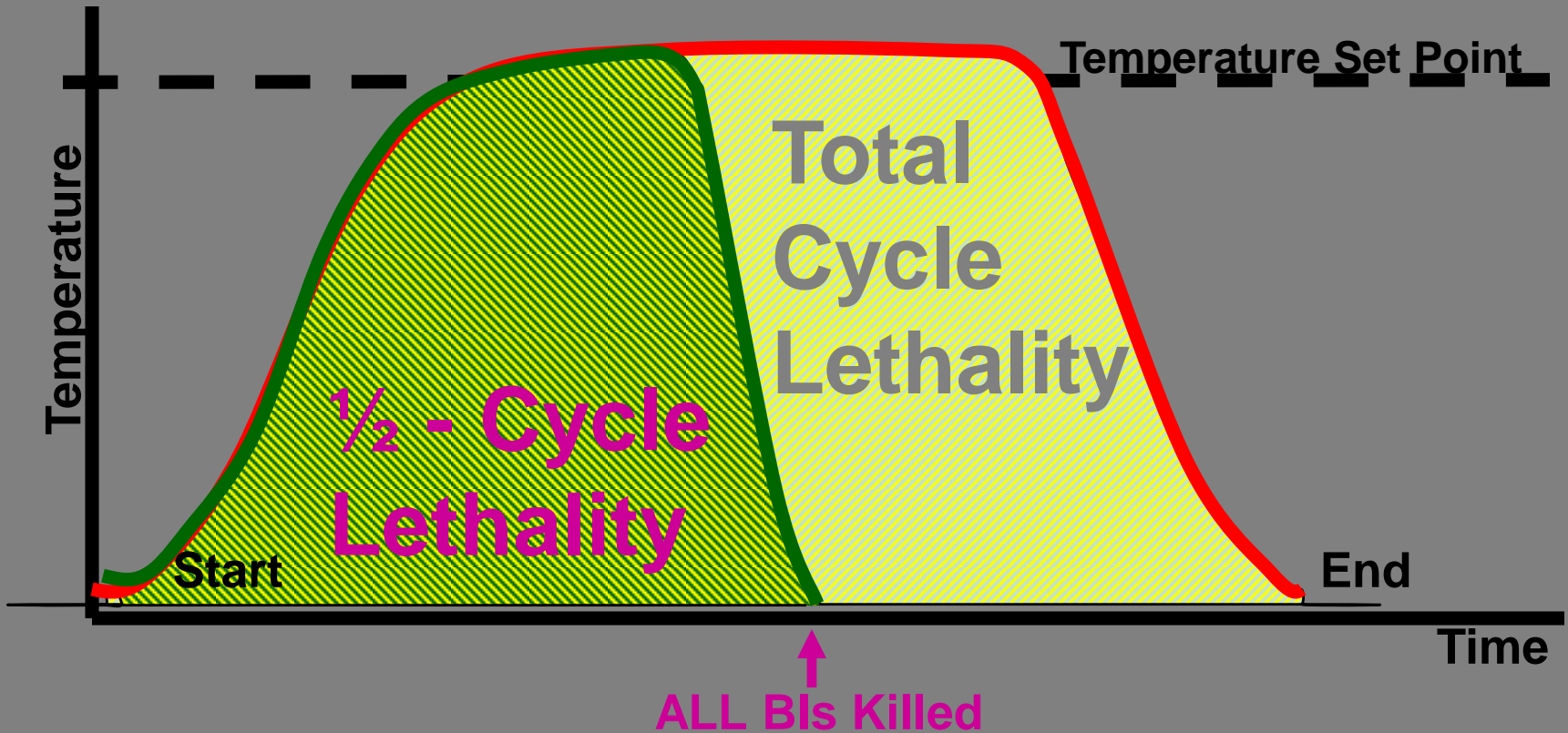
# ***Smart***-Read Technology



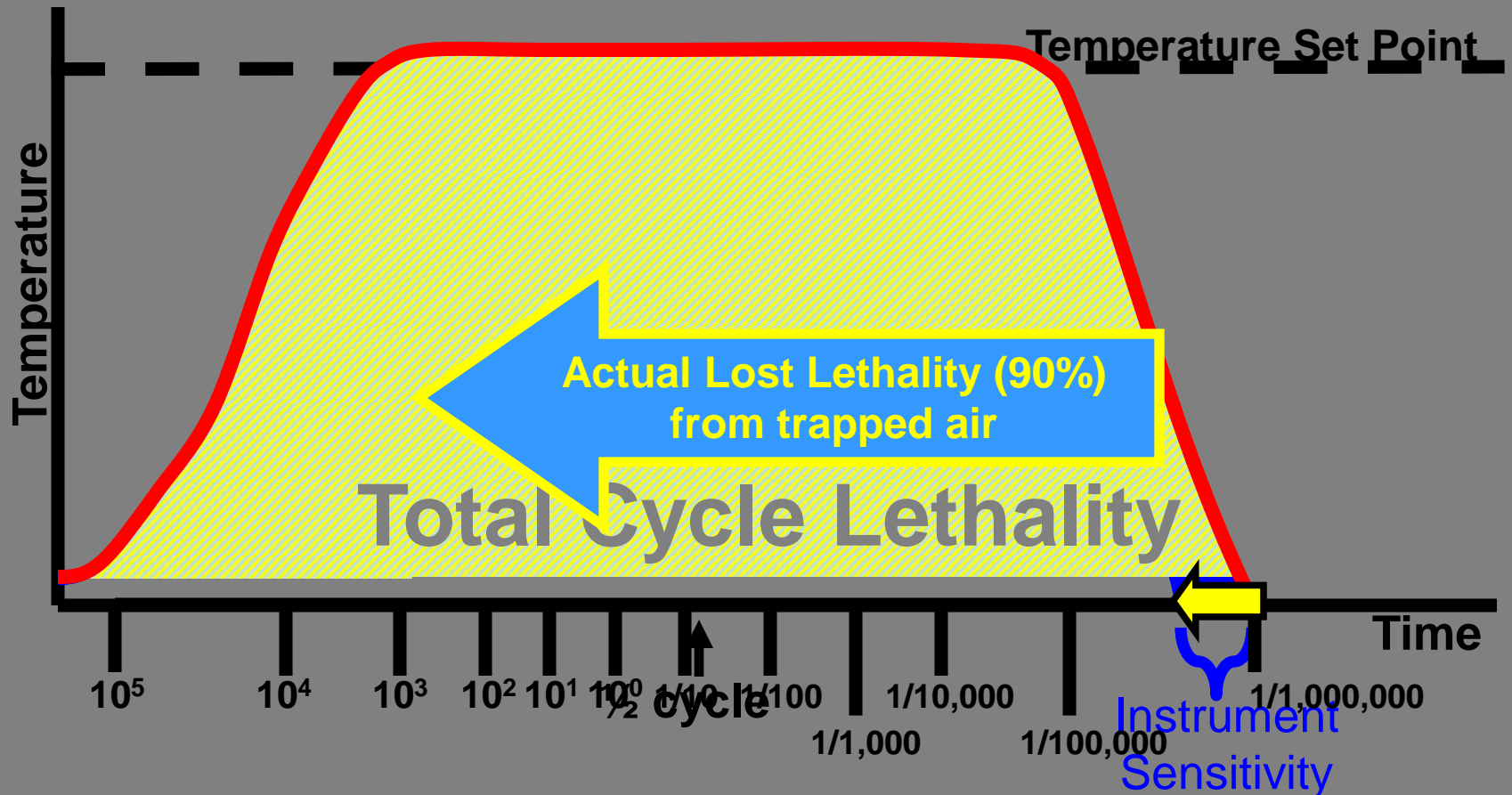
# Dissection of a Sterilization Cycle

Most common way to develop a cycle is the

**half cycle** approach



# Positive BI due to Trapped Air in Steam Process



# Biological & Physical Release

- **Biological Indicators identify catastrophic sterilization failure**
- **Physical measurements are necessary to support Sterility Assurance Levels (SAL)**
- **Both physical and biological measurements are necessary**
- **Any process that does not use both measurements is inadequate**

**A sterilization failure occurs  
when the *first* positive BI is  
detected**

**A positive BI is a catastrophic  
cycle failure**



# Why?

- **Cycles are designed to kill all BIs and include an additional safety factor**
- **The failed sterilization cycle must lose a significant portion of the cycle lethality where the BI is intended to survive**



# Summary of **Smart-Read EZTest** Biological Indicator Monitoring System

**The Smart-Read Biological Indicator Monitoring System provides rapid biological detection of failed sterilization cycles**

# Summary of **Smart-Read EZTest** **Biological Indicator** **Monitoring System**

**User convenience:**

**Once incubated, no additional  
manipulation at specific timed  
intervals**

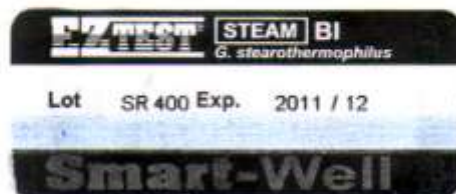
# Summary of **Smart-Read EZTest** **Biological Indicator** **Monitoring System**

```
SGM Smart-Well  
Temp: 60 +/- 2C  
Type of Test:  
  121C Prevac 465MIN  
ST: 10:31 08JUN2010  
TT: 03:35  
BI Result: POSITIVE  
Cell#: 03  
Lot #: 5400  
Sterilizer #: 561  
Load: _____  
_____  
_____  
_____  
User ID: SGM
```

**Automatic  
printed  
documentation**

# Summary of Smart-Read EZTest Biological Indicator Monitoring System

SGM Smart-Well  
Temp: 60 +/- 2C  
Type of Test:  
121C Prevac 465MIN  
ST: 10:31 08JUN2010  
TT: 04:11  
BI Result: POSITIVE  
Cell#: 04  
Lot #: 5400  
Sterilizer #: 561  
Load: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
User ID: SGM



Easy to peel unit  
label can be  
attached to printed  
document

# Summary of **Smart-Read EZTest** **Biological Indicator** **Monitoring System**

**ONE indicator for all steam cycle  
types and temperatures**

# Summary of **Smart-Read EZTest** Biological Indicator Monitoring System

The **Smart-Read BI** rapidly  
detects failures using true  
biological results to allow re-  
sterilization of the load

**These results allow  
timely re-sterilization  
of the load**



# Rapid Biological Detection by **Smart-Well Incubator**

10 hours

Meets U.S. FDA Reduced  
Incubation Time Protocol  
for sterile product release

# Summary of **Smart-Read EZTest** Biological Indicator Monitoring System

**NO False Negatives**

**NO False Positives**



# Summary of **Smart-Read EZTest** Biological Indicator Monitoring System

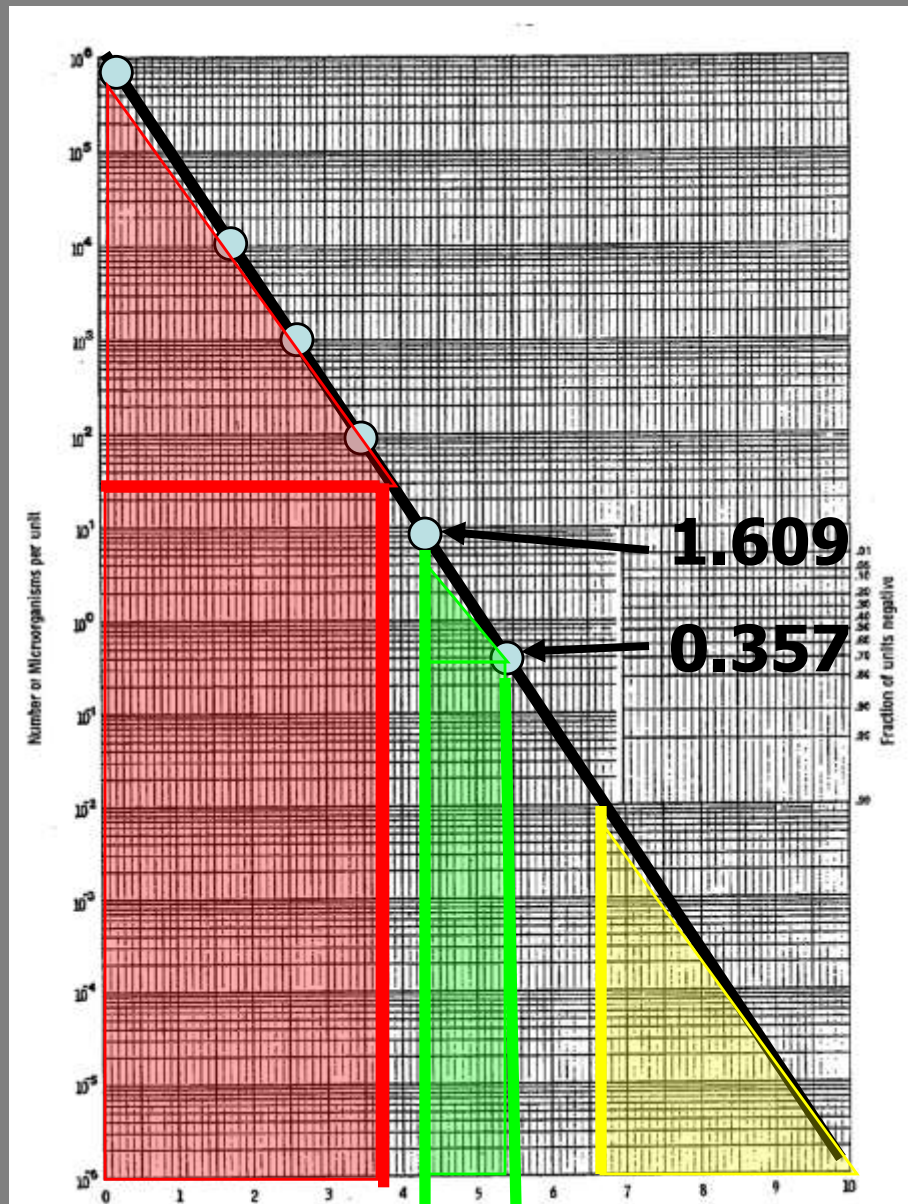
**Results are  
100% User Verifiable**

# Summary of **Smart-Read EZTest** **Biological Indicator** **Monitoring System**

**Releasing materials based upon  
biological results provides the  
greatest assurance of  
sterilization**

# Reduced Incubation Time USFDA (RIT) Protocol

- 100 units per test
- Perform test 3 times (3 different lots)
- Achieve fractional exposure
  - At least 30/100 survivors
  - No more than 80/100 survivors
- Read results daily up to 7 days
- When 97% of BI's read same as 7 days you have achieved the reduced incubation time



Survivor Curve

RIT  
Zone

Probabilistic Zone  
(No biological measurements)

Sterilizing Value, Minutes

1.609

0.357

# RIT Protocol

- No more than 80/100 positives

OR

at least 20 sterile BIs

- No less than 30/100 positives

OR

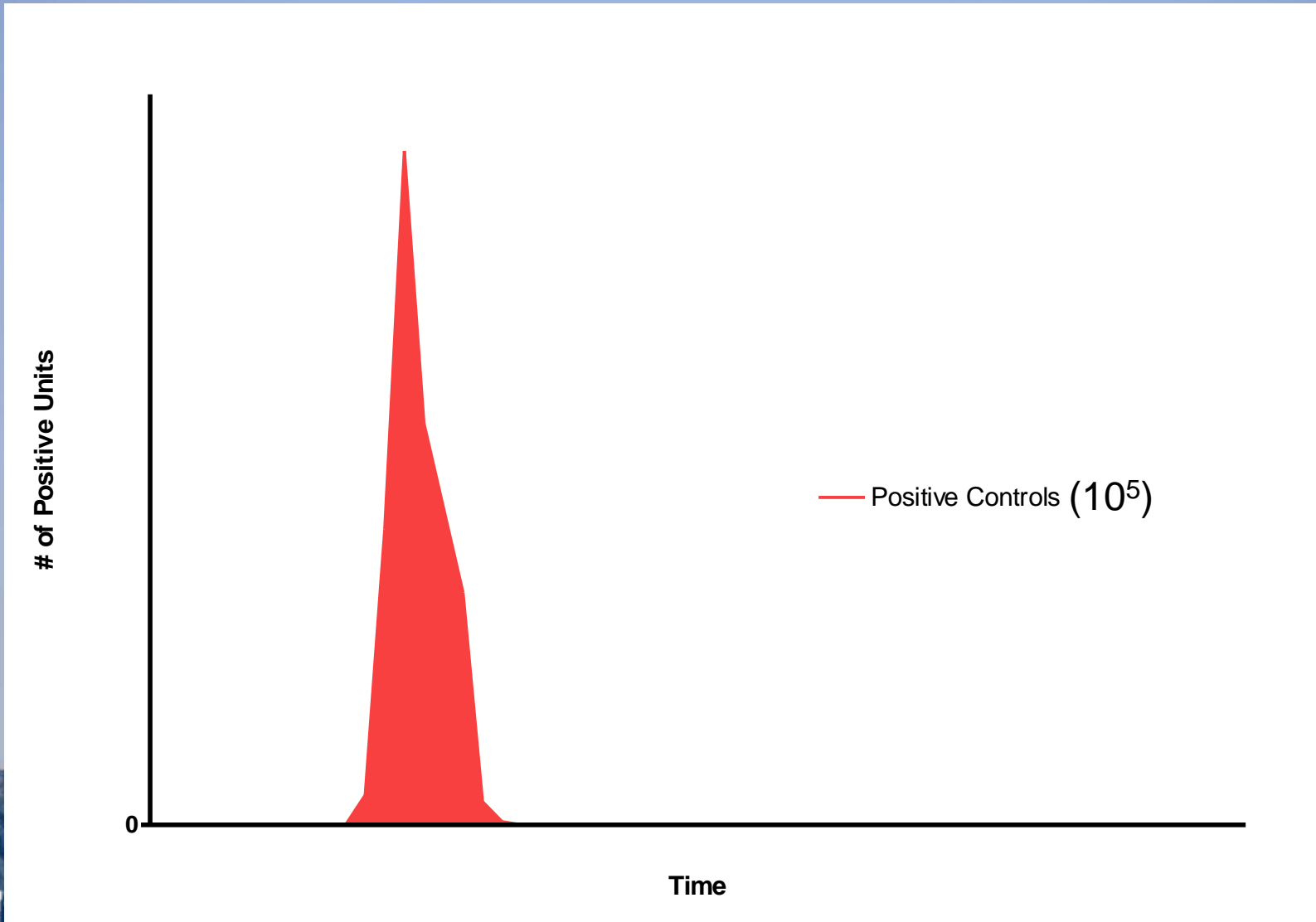
no more than 70 sterile BIs



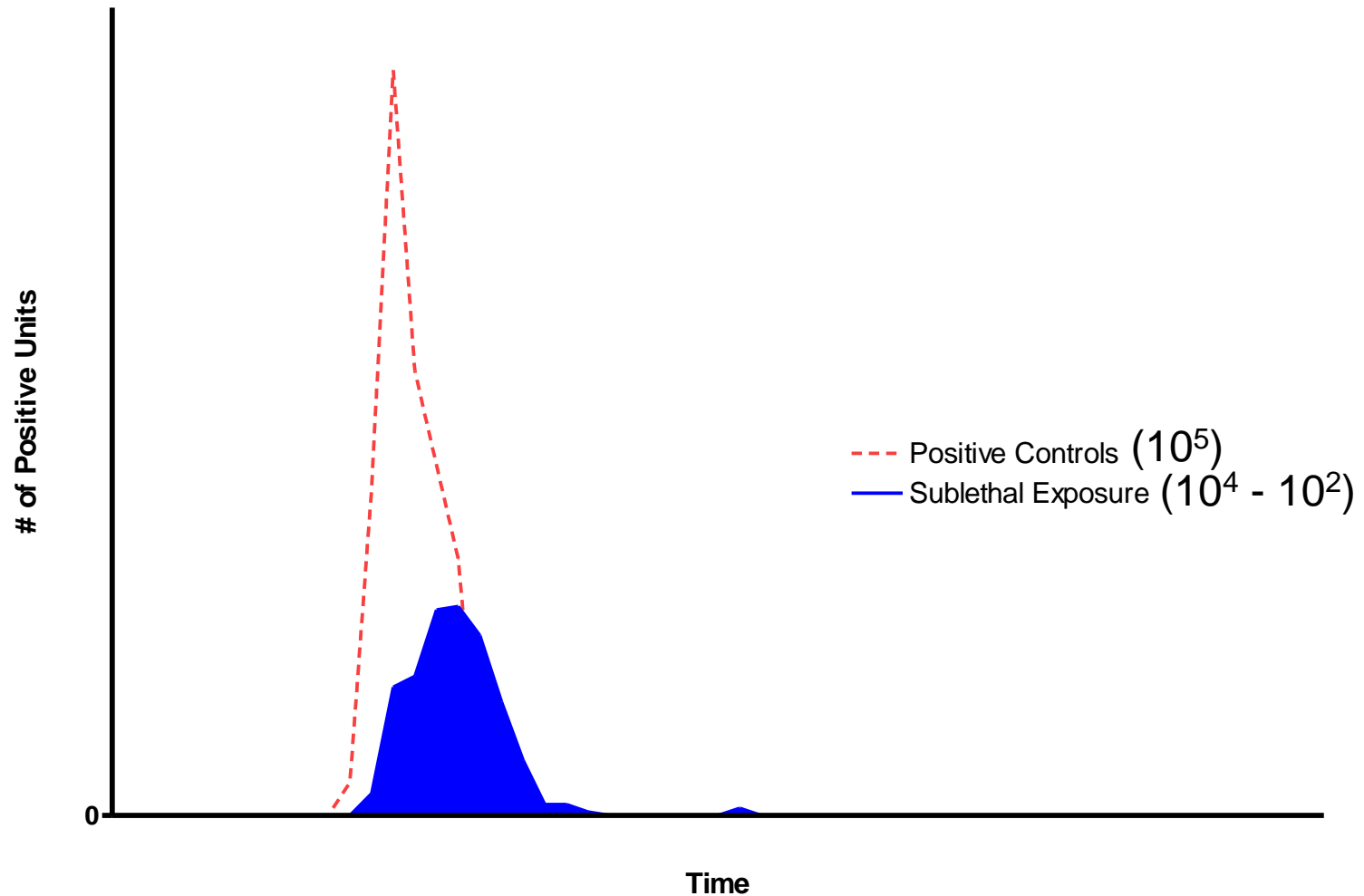
**The more spores  
present on the BI the  
faster a positive  
sample will be  
detected**



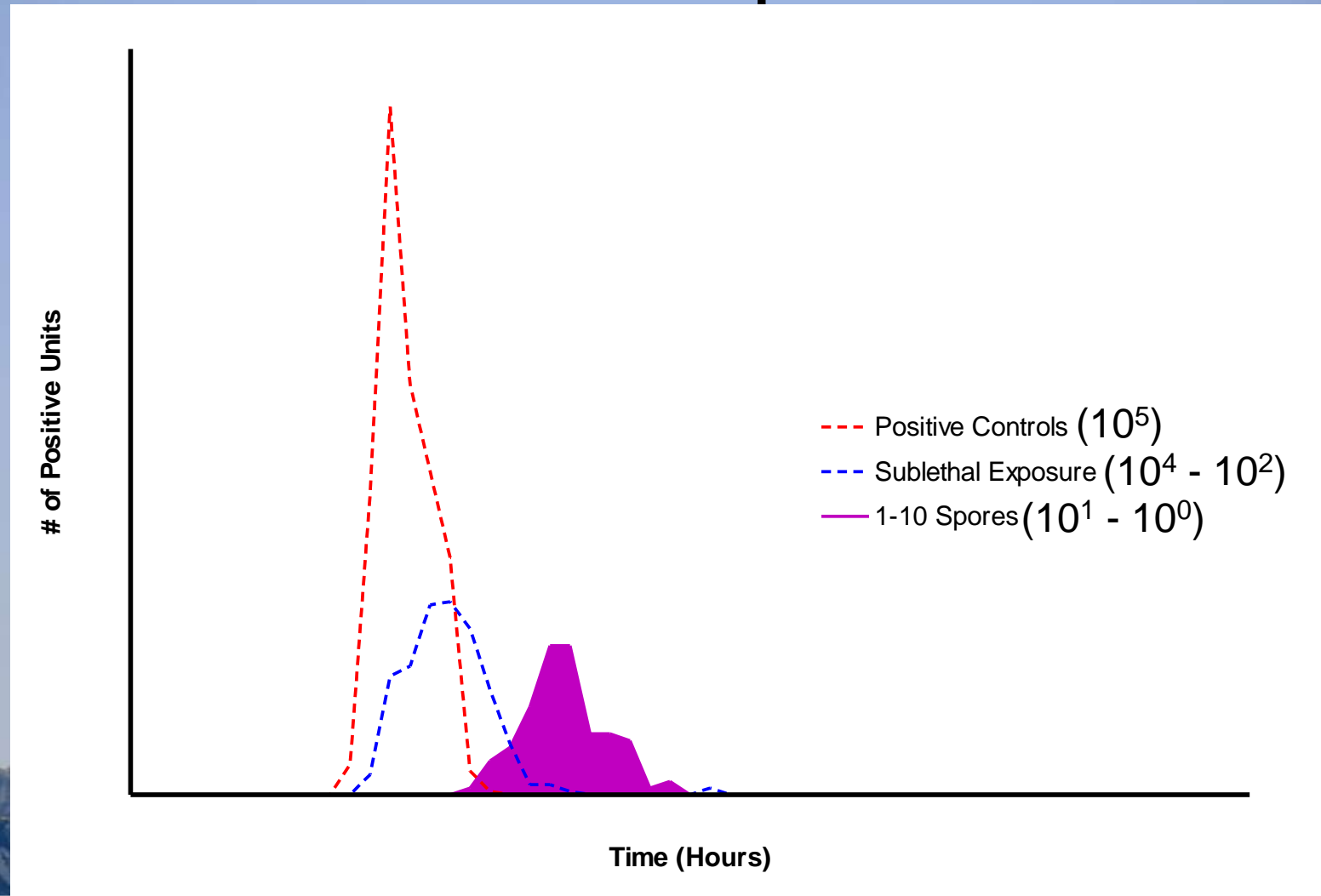
# Positive Controls ( $10^5$ spores/BI) Incubation Time



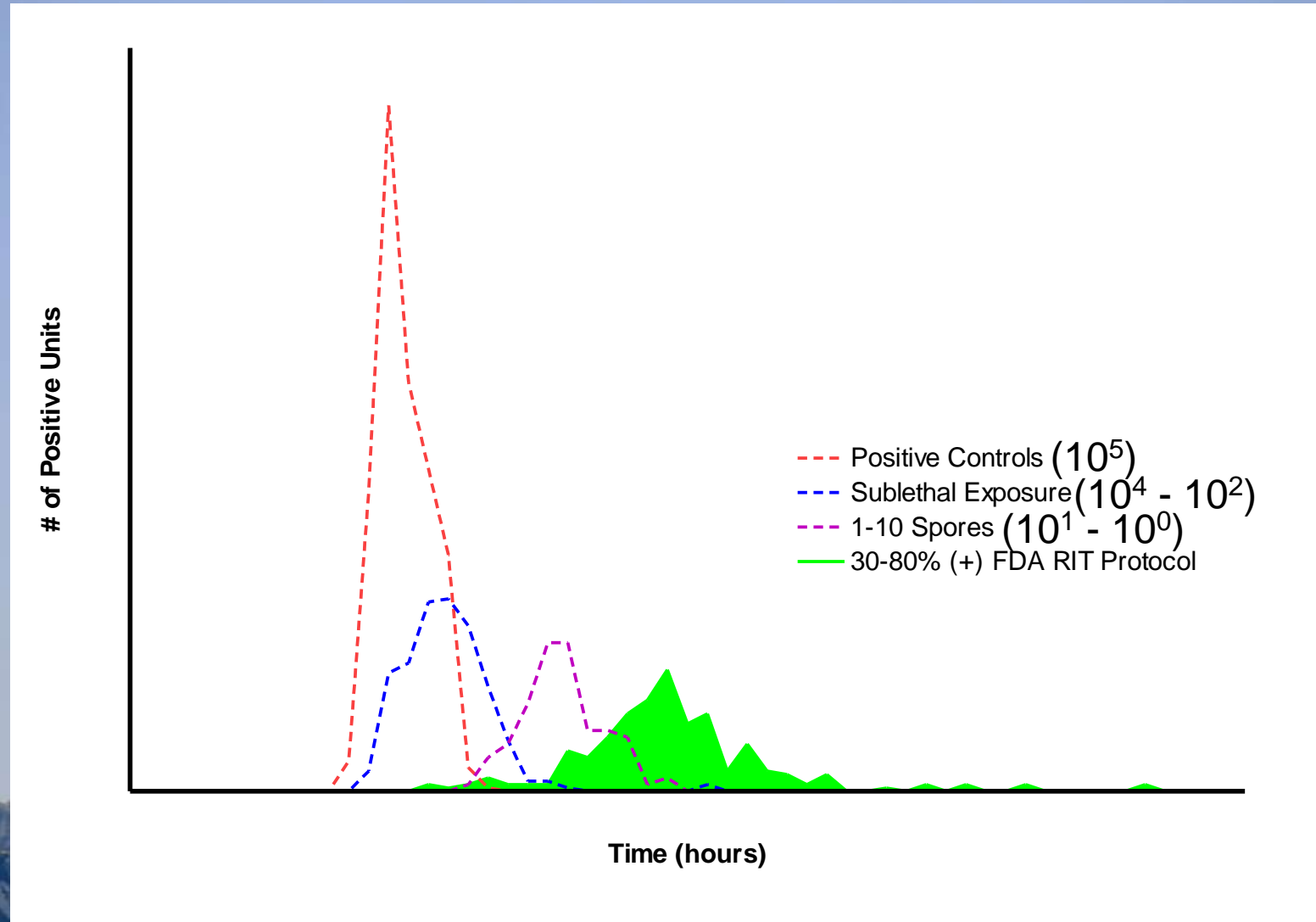
# Sub-lethal exposed units between $10^4$ and $10^2$ spores/BI



# Sub-lethal exposed units between $10^1$ and $10^0$ spores/BI

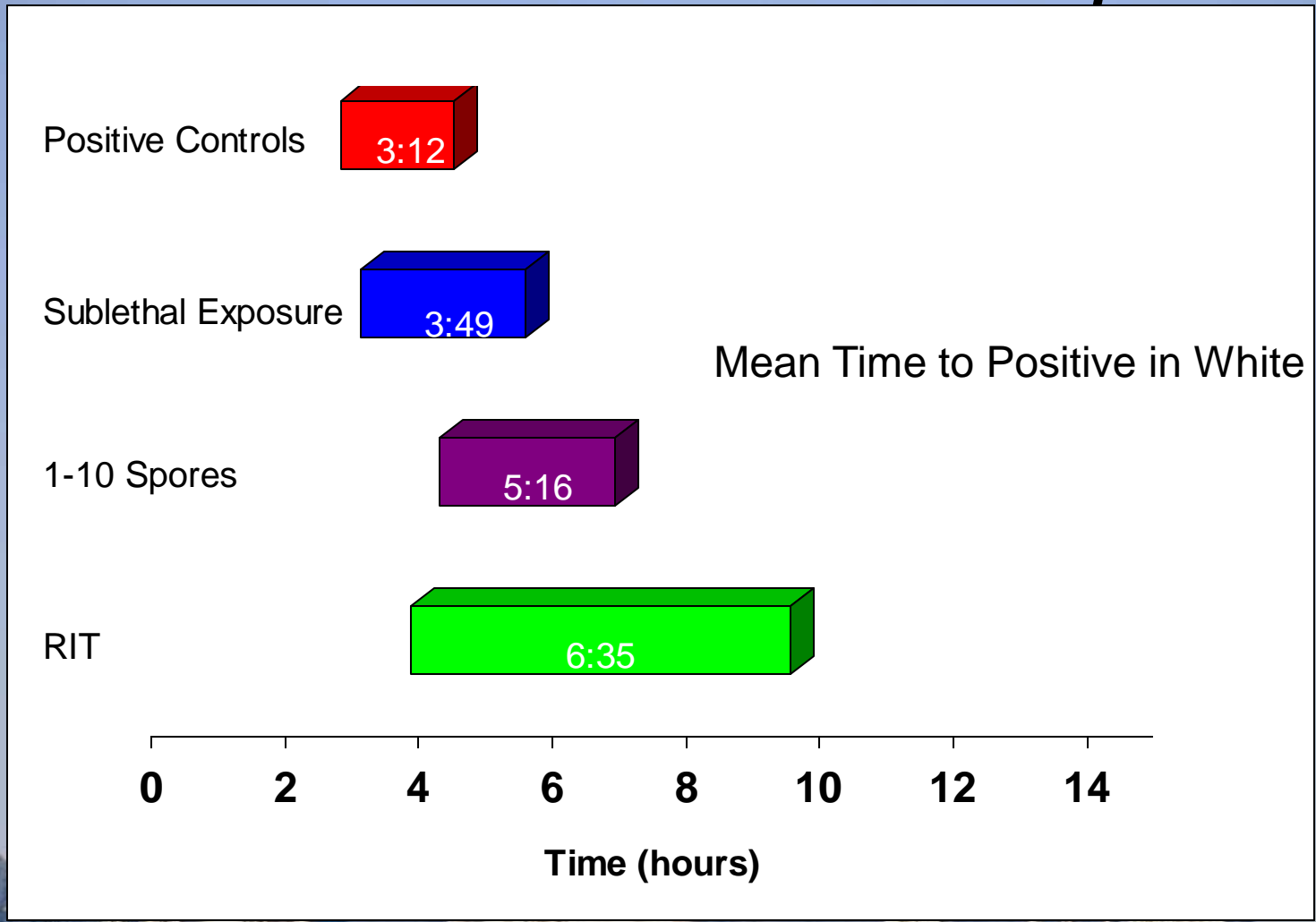


# Quantal zone (FDA RIT Protocol) between 30 to 80 positive BIs per 100 tested

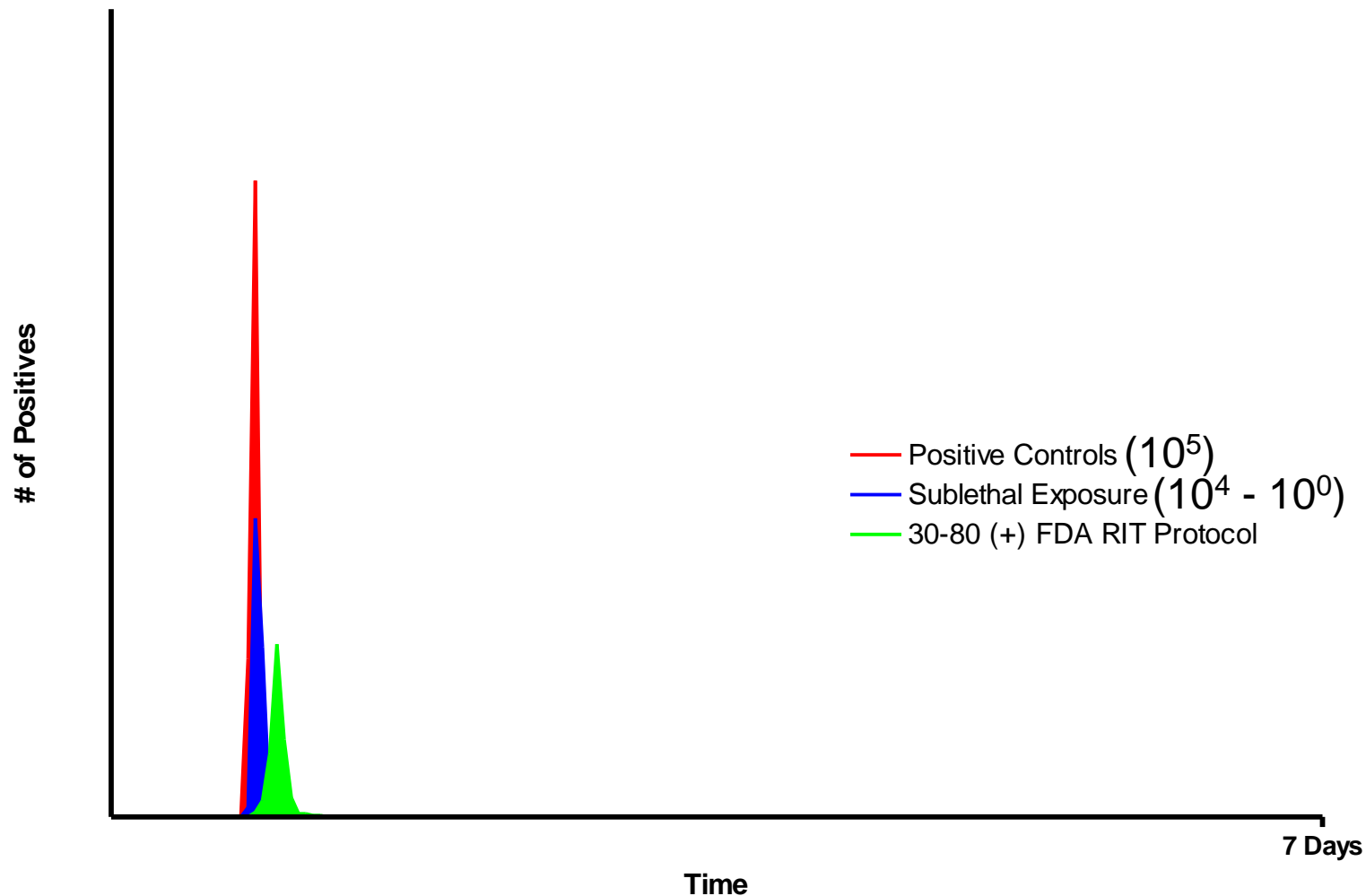


# Time to Positive Incubation Test

## *Geobacillus stearothermophilus*



# All data to date fits this picture when media is controlled



# Lethality Maps





# Kaye Validator 2000

## Thermocouple Data Acquisition System

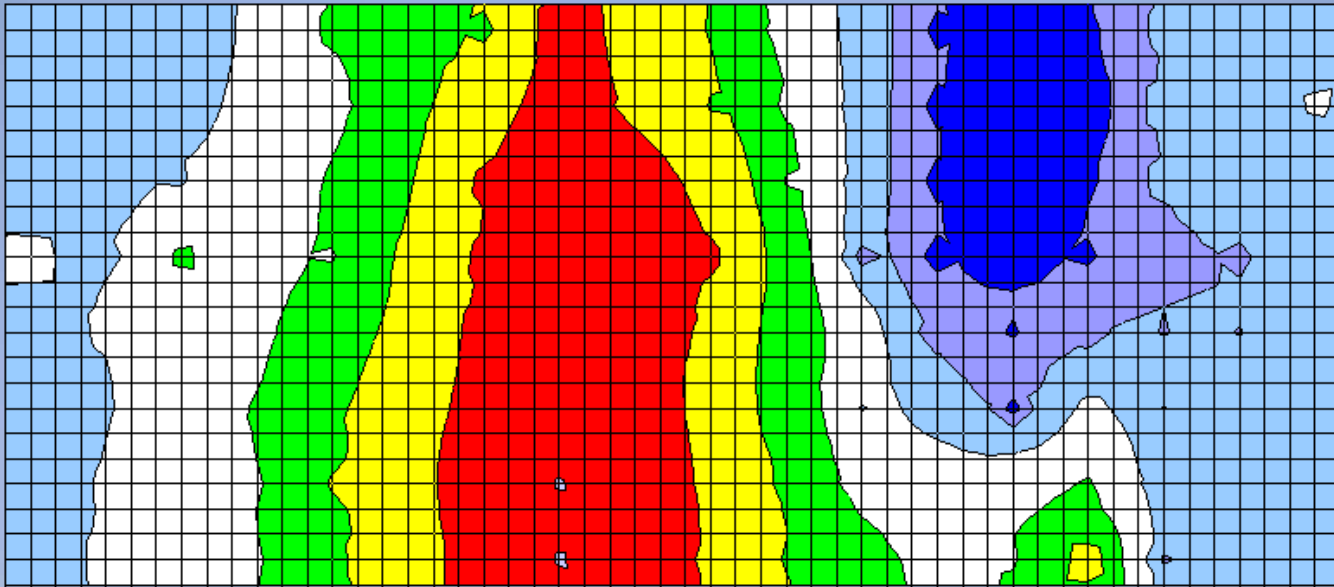


Maps Use  $F_o$   
Calculated From  
Each Thermocouple  
Location



# Lethality Map – Flat Surface Display

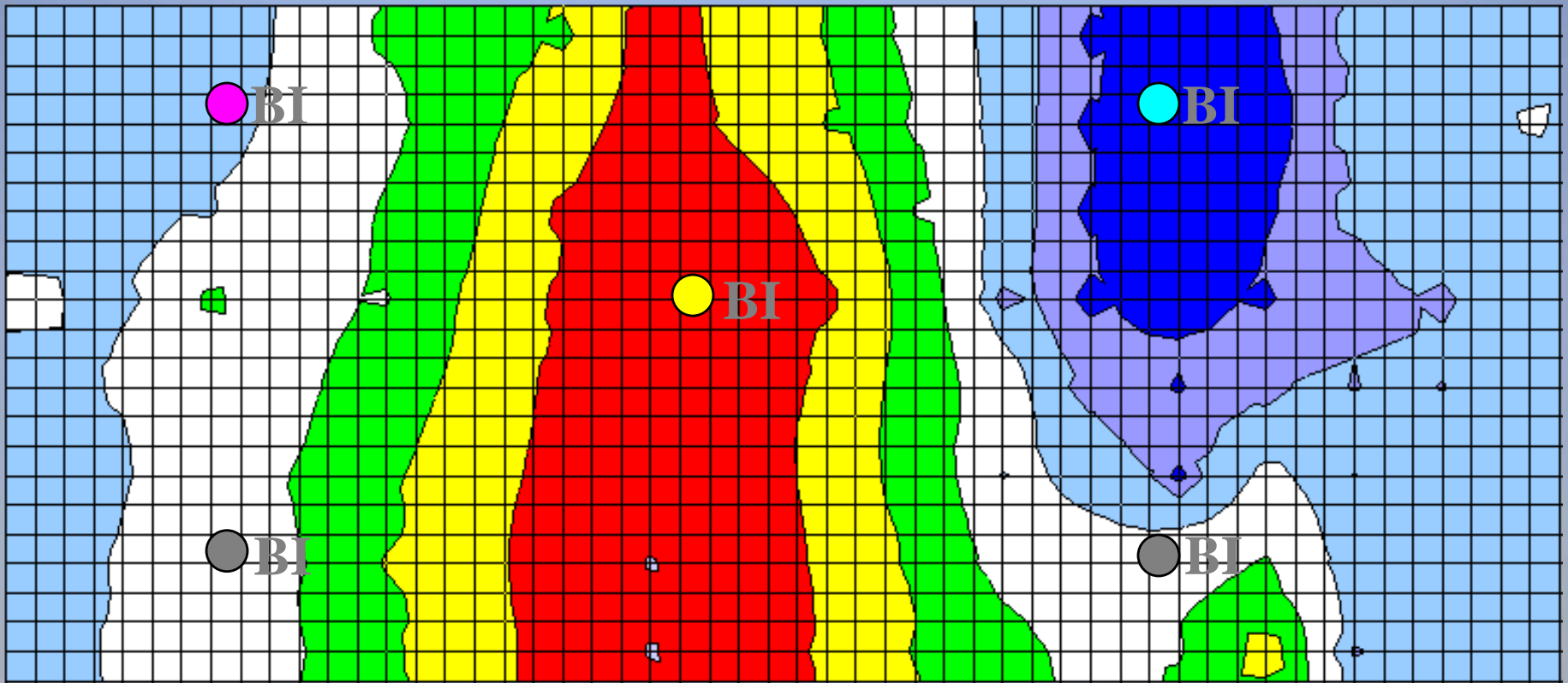
## 9% Thermocouple Density



When BI's are individually dispersed throughout the load, there is a good chance each BI is exposed to different conditions and therefore a replicate of "one"



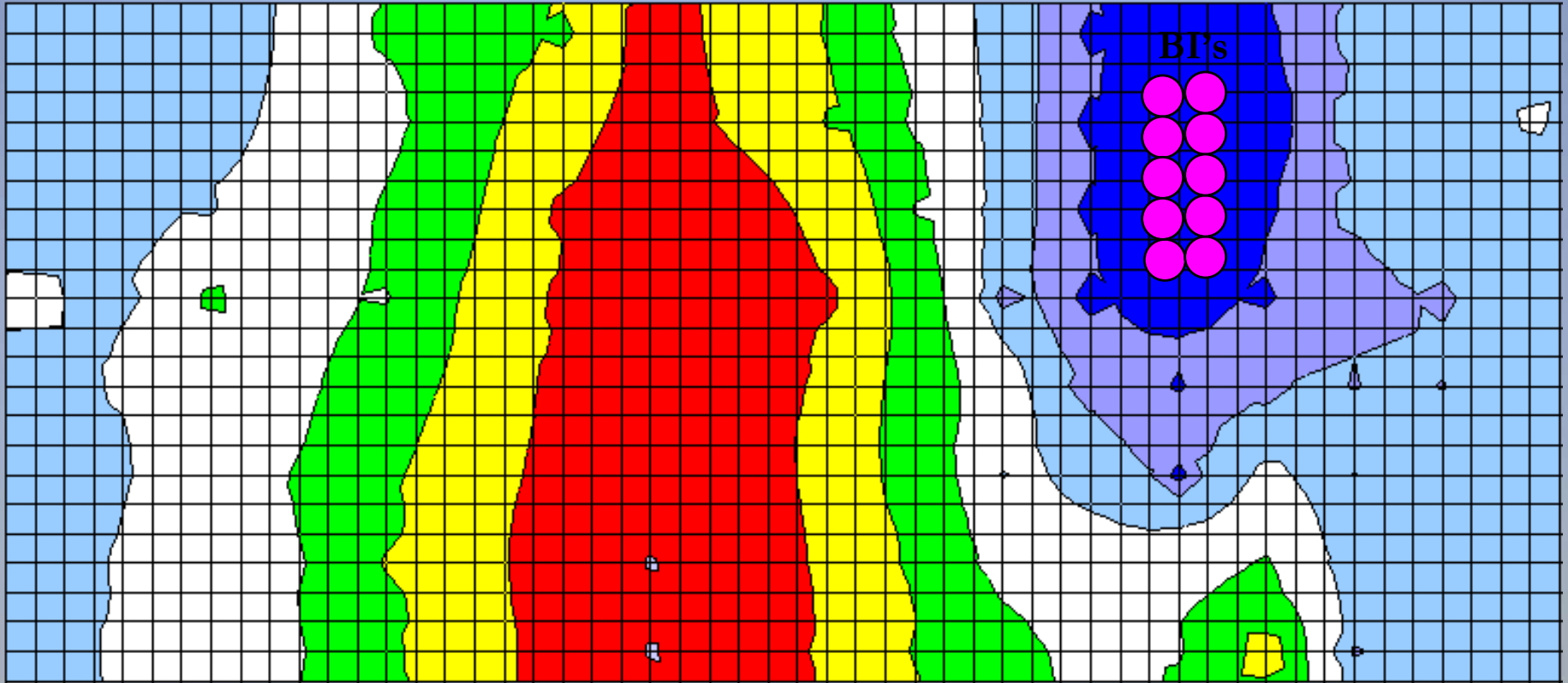
# Random BI Distribution



Cluster BI's in the  
areas documented  
to be “worst case”



# Cluster BI Distribution



Hospitals typically lack the equipment and skilled personnel to perform this classical validation.



Biological validation is  
the next best approach.

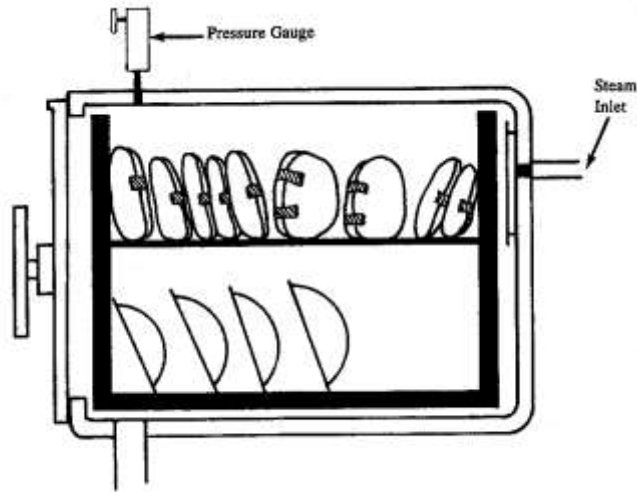


Use biological indicators to identify “worst case location” in the sterilizer.

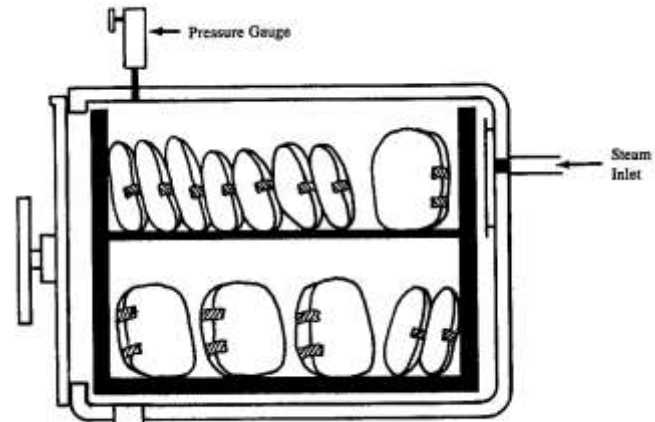


Prepare typical loads to  
be used in these tests.

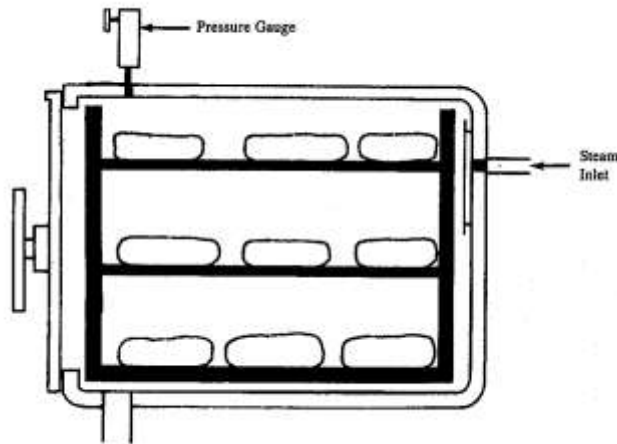




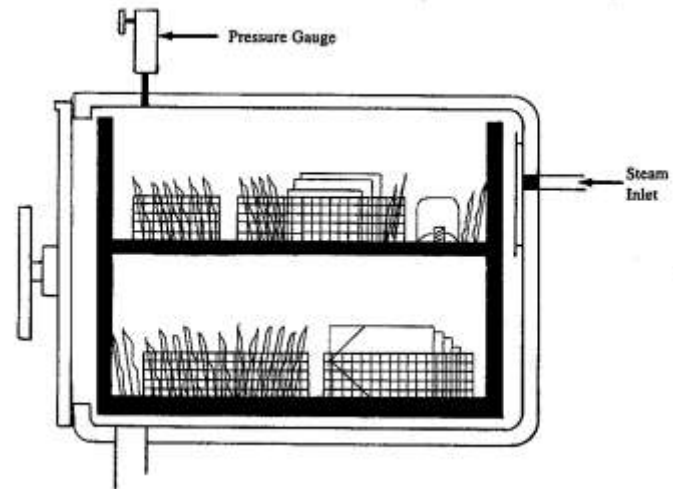
Mixed Load



All-textile Load



Wrapped Instrument Load:  
Perforated or wire-mesh bottom trays



Loading paper/plastic peel-down  
packaging, using wire-type baskets to  
keep the packages in position

Place two BI  
(replicates) into your  
best estimate location  
as the “worst-case” in  
each pack.



Document the loading pattern. Document contents of each pack and where the two BIs are located.



# Perform Abbreviated Cycles

- Shortest cycle expected results = all BIs (+)
- Longer cycle expected results = some packs all BIs (+); some packs all BIs (-)
- Longer cycle than above expected results = all BIs (-)
- Double the above cycle time, use only packs and locations that showed the last to die BIs; place 2 BIs in this pack
  - Expected results = worst case pack all BIs (-)
  - Repeat this test three times



# Dissection of a Sterilization Cycle

## half cycle approach



Repeat this series of tests with each different load configuration you have identified.



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