ULTRAVIOLET LIGHT AND THE IMPERFECT BIOLOGICAL INDICATOR

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ULTRAVIOLET LIGHT

A CLEAN TECHNOLOGY

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PURPOSE OF THE STUDY

To evaluate the effectiveness of Ultraviolet (UV) Light Pass Throughs and Ultraviolet Tunnels for surface sterilization using various indicators as quantifiable readout.

The study will first:

- Determine the D values using the DDK Scientific generated data utilizing the Spearman-Karber Method
- Evaluate the effectiveness of the DDK Scientific UV Tunnel

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MATERIALS and METHODS

The biological indicators (BIs) used were:

Bacillus atrophaeus:	ATCC 9372
Geobacillus stearothermophilus:	ATCC 12980
Bacillus pumilus:	ATCC 27142

The key requirements for any surface decontaminations study are:

- Biological indicators with precise concentrations
- Appropriate incubation media*
- Appropriate incubation time and temperature*

*As recommended by the BI vendor and the United States Pharmacopoeia (USP)

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BIOLOGICAL INDICATORS

INITIAL POPULATION

Each test organism had the following population

- $\geq$ 1.0×10^4 [cfu/carrier]
- $\geq$ 1.0×10^5 [cfu/carrier]
- $\geq$ 1.0×10^6 [cfu/carrier]

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BIOLOGICAL INDICATORS

CARRIER MATERIAL

- The carrier material for this investigation was 304 stainless steel inoculated with the specified organism.
- Prepared by Apex Laboratories

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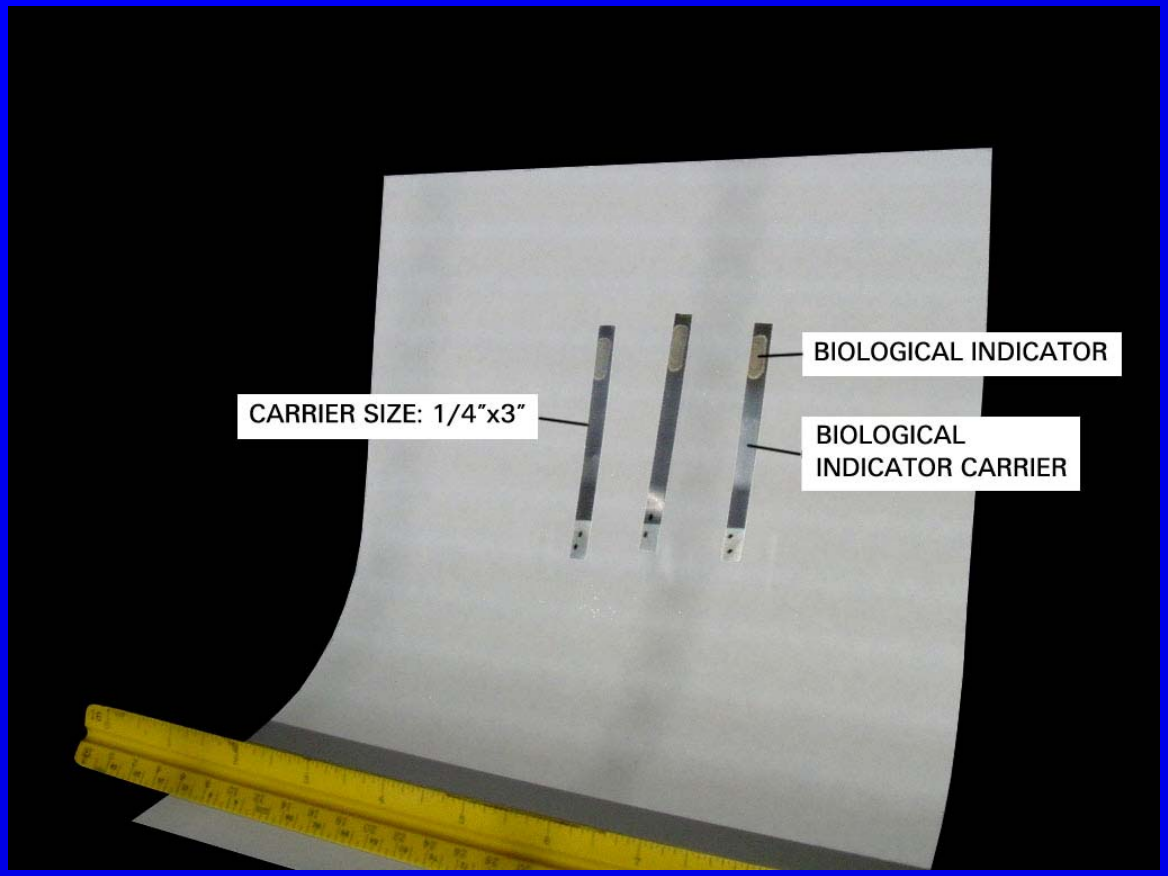
BIOLOGICAL INDICATORS



BIOLOGICAL INDICATOR

- A biological indicator is a living organism
- A biological indicator is:

  "an indicator not an absolute"
- Since biological indicators are living organisms we some times have clumps of microorganisms (rogues)

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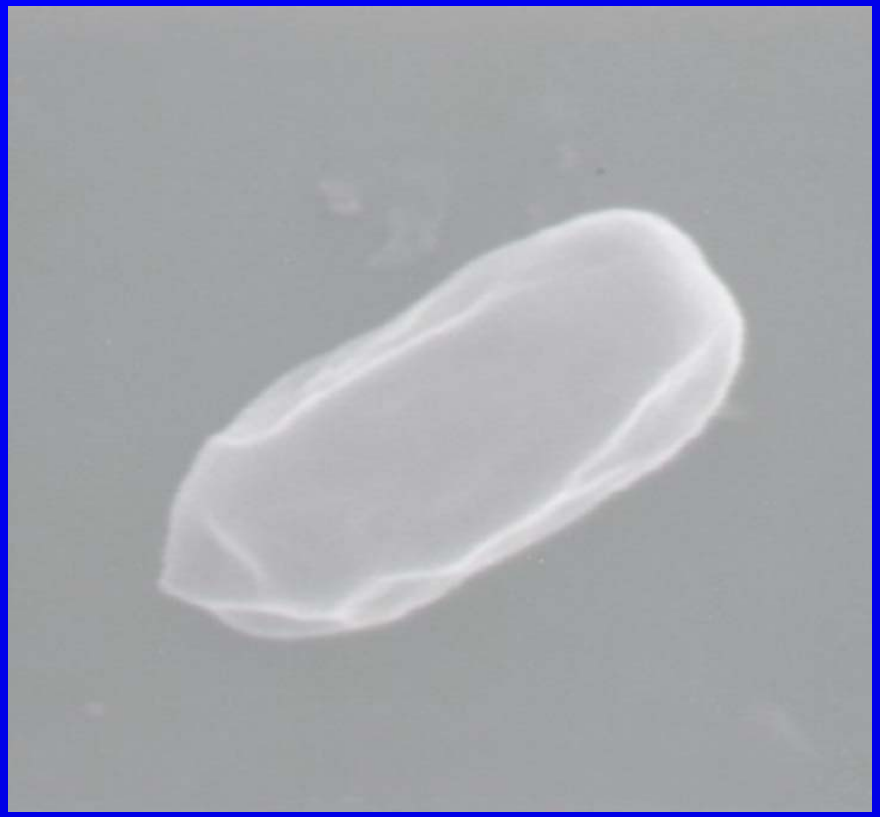
BIOLOGICAL INDICATOR

A biological indicator unit (BI) is a measuring device with the following characteristics:

- Totally self contained
- Preset calibration, not adjustable
- Non-indicating during measurement
- Result is: on-off, growth – no growth

BIOLOGICAL INDICATORS
BACILLUS ATROPHAEUS



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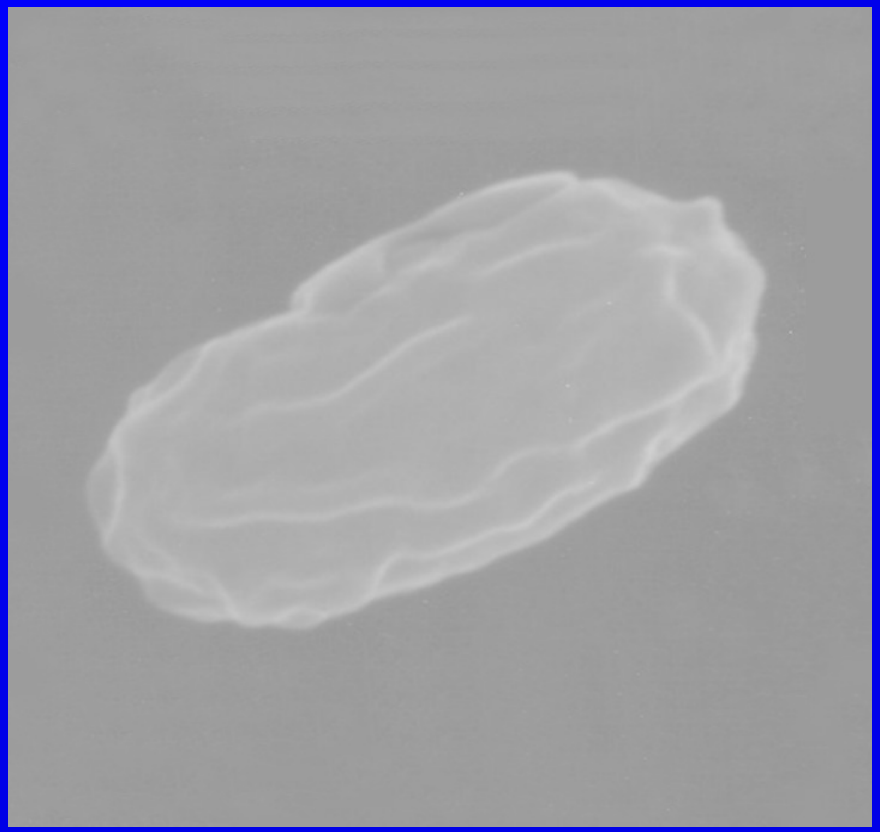
BIOLOGICAL INDICATORS
GEOBACILLUS STEAROTHERMOPHILUS



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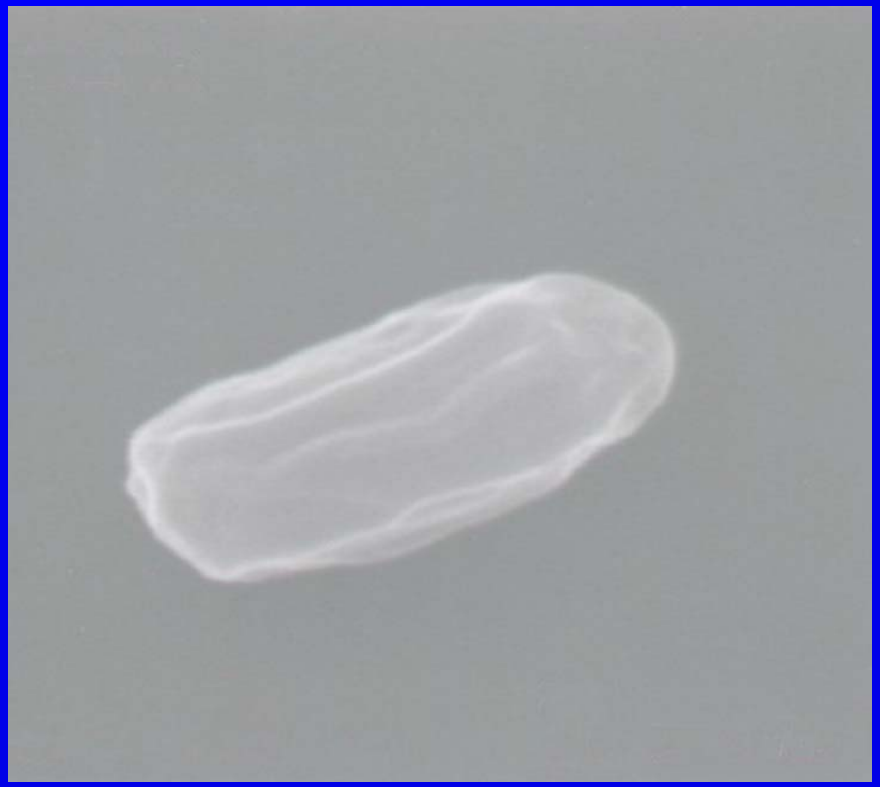
BIOLOGICAL INDICATORS
BACILLUS PUMILUS



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UV TEST TUNNEL

- The UV Test Tunnel used for this investigation has the ability to adjust the distance of the UV Light from the exposed BI, also the Test Tunnel has a variable speed conveyor
- For these test the distance was fixed and the conveyor was turned off
- All the BIs were placed manually in the UV Test Tunnel

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UV MEASUREMENT DEVICE

Specification	Identification
Type:	UV PowerMap(Calibrated)
Manufacturer:	EIT, S/N 1004
Measurement Range:	UVA 320 to 390 nm
[mWsec/cm²]	UVB 280 to 320 nm
	UVC 250 to 260 nm
	UVV 395 to 445 nm
Temperature:	Type J thermocouple 250ºC Max.

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UV MEASUREMENT DEVICES

NOTE:

It is recommended to use the same light meter throughout the study; if the light meter is changed the results will be different since no two light meters read the same

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RESISTANCE DETERMINATION

The Limited Spearman-Karber Method (LSKM) was used to determine the BI's resistance to the UV light.

(The fractional negative method)

Based on this inactivation curve a D-value can be estimated for each specific BI preparation used.

LIMITED SPEARMAN KARBER METHOD (LSKM)

The practice involves exposing several groups of BIs to various exposure times and doses of UV light to evaluate the bacterial reduction following a standardized growth test.

The exposure times and UV doses form a consistent set of intervals that map the survival time of the BIs from reliable positives to reliable negatives.

LIMITED SPEARMAN KARBER METHOD (LSKM)

With the LSKM, the "mean time/dose to sterility" (U_{sk}), is calculated from which the D-value can be derived using the final stages of microbial inactivation.

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PARAMETERS OF RESISTANCE DETERMINATION

For each resistance determination the following parameters have to be defined:

- Number of samples per group
- Number of exposures
- Exposure time [min]
- Exposure dose [mWsec/cm²]

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STEPS USED IN DETERMINING RESISTANCE

1. The required number of BIs are exposed to the UV light using predefined exposure time/doses.
2. The BIs are evaluated using the growth test.
3. The BIs were incubated for 7 days.
4. The results of each exposed BI is evaluated based on the turbidness of the growth media.

   *No turbidity observed = no growth = kill = sterile =N

   *Turbidity observed = growth = no kill = not sterile =P

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RESISTANCE DETERMINATION
No Turbidity Observed

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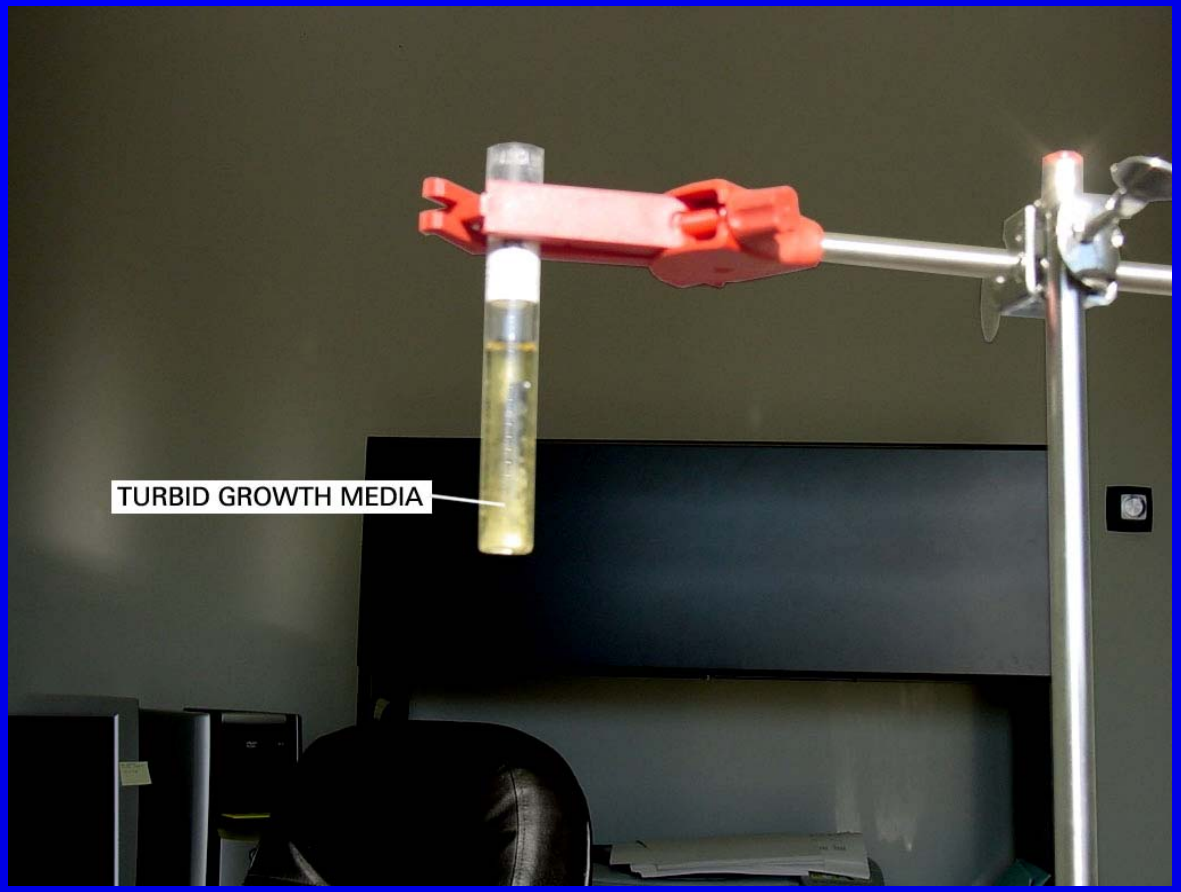
RESISTANCE DETERMINATION
Turbidity Observed



TEST DOCUMENTATION

- Spore type, population, lot number, expiration date
- Incubation time (days), temperature
- UV dose per exposure time
- Number of BIs per exposure time
- Test evaluation: P = Turbid = Positive
  N = Clear = Negative

RESISTANCE DETERMINATION

- SCREENING TEST

Screening test is used to determine the inactivation curve.

Parameters of the Screening test:

BIs:	All populations
Exposure times [min]:	.5,1.2,4,8,16,32,60
Number of BIs per exposure:	5
UV light distance:	5 cm

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RESISTANCE DETERMINATION

SCREENING TEST

Evaluation of test results:

- Exposures showing all positive BIs
- Exposure showing fractional results
- Exposures showing all negative BIs
- D-value for the test BI
- Model behavior for the test BI

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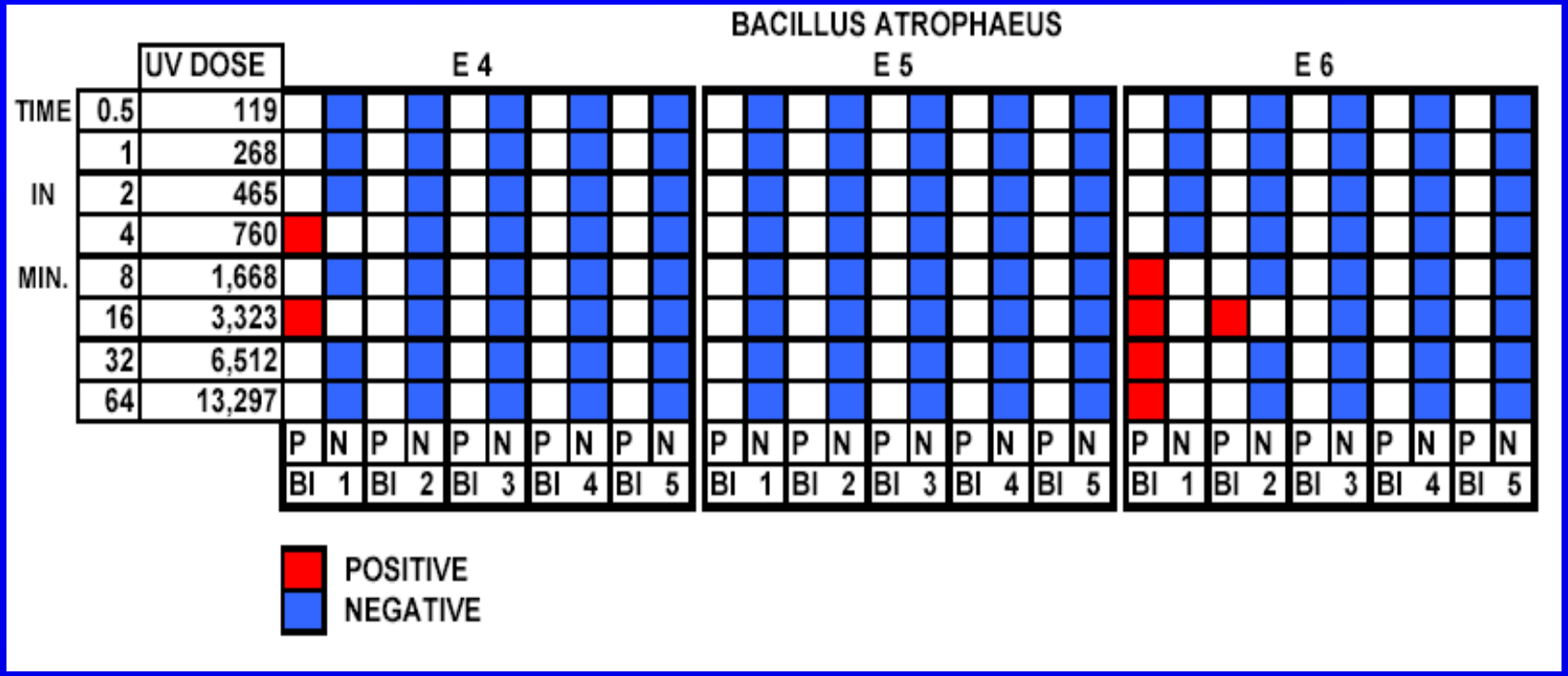
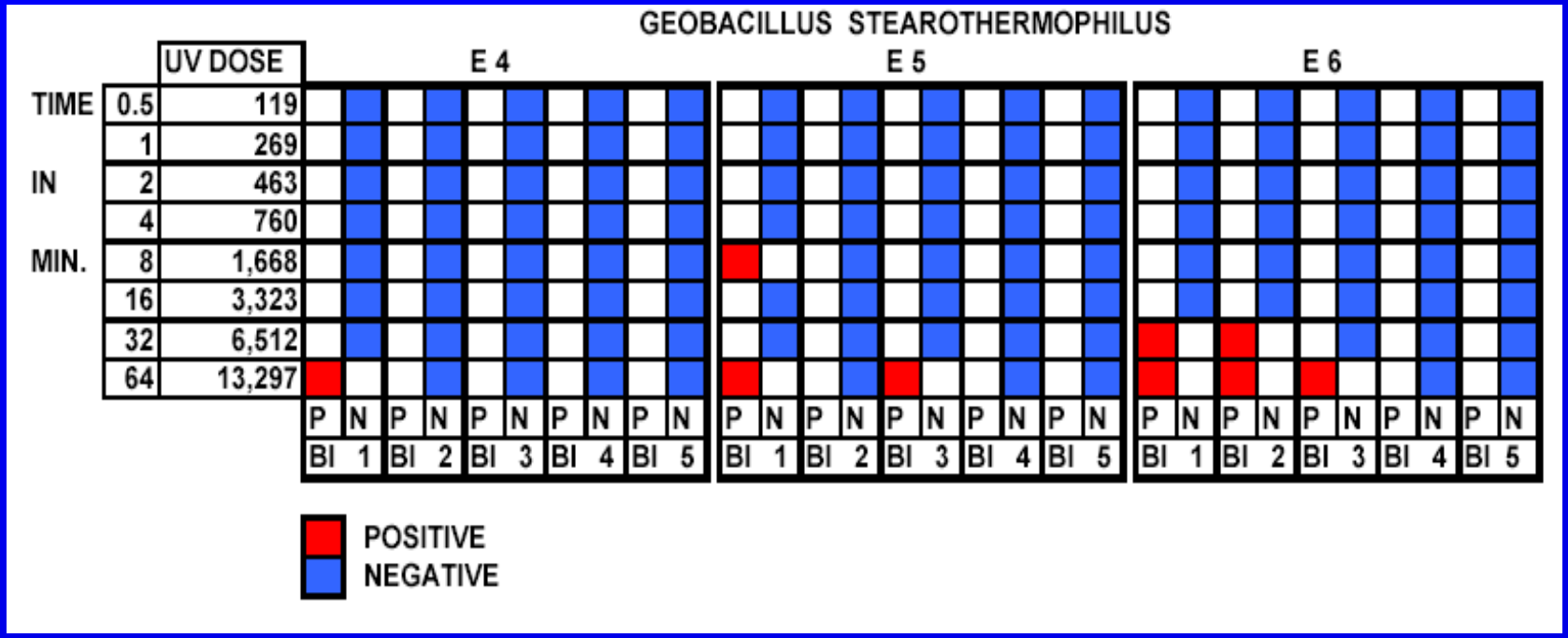
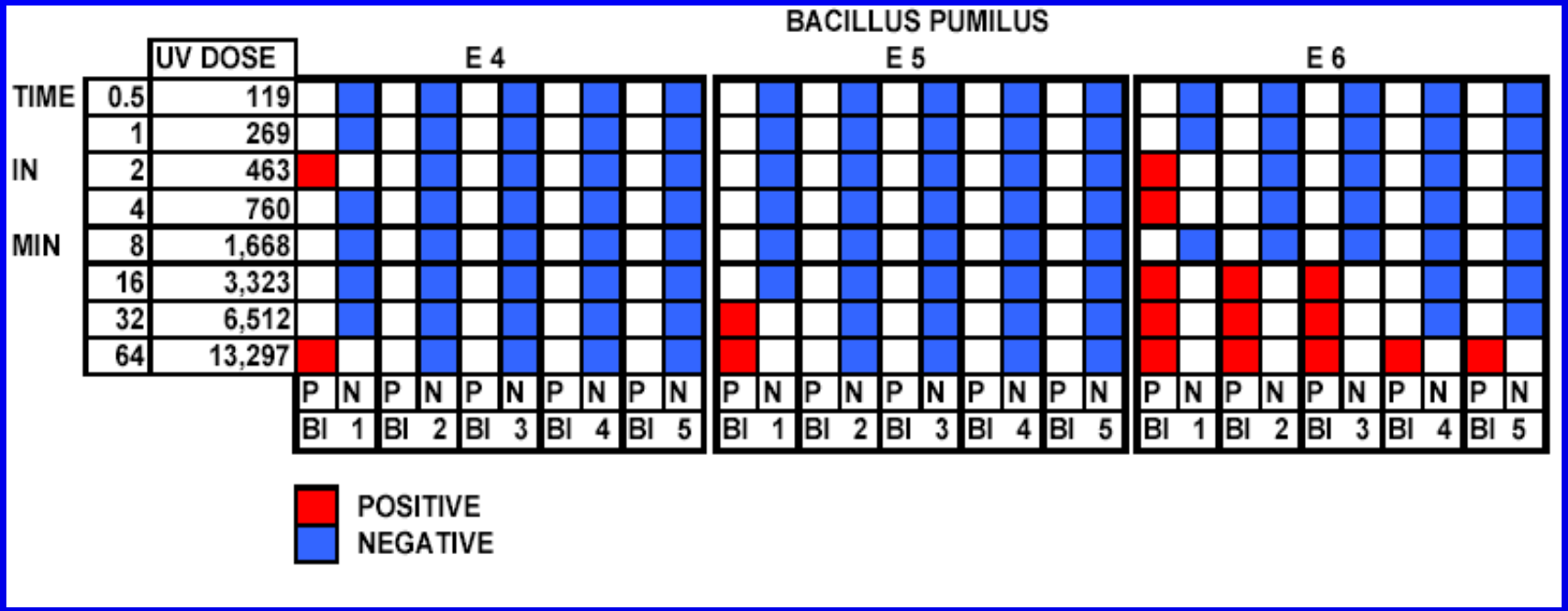
SCREENING TEST



SCREENING TEST



SCREENING TEST



INACTIVATION CURVE

Based on the screening test additional exposures were done to show an accurate transition from all positive to all negative indicators.

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INACTIVATION CURVE

INACTIVATION CURVE PARAMETERS

Test sample:	BIs with all populations
Exposure time [min] Or UV dose [mWsec/cm²]:	Adjusted based on results of screening experiment
Number of samples per Exposure:	5
UV light distance:	25 centimeters

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INACTIVATION CURVE

EVALUATION OF TEST RESULTS

- D-value estimation of the test sample
- Model behavior of the test sample
- Reproducibility of the inactivation effect

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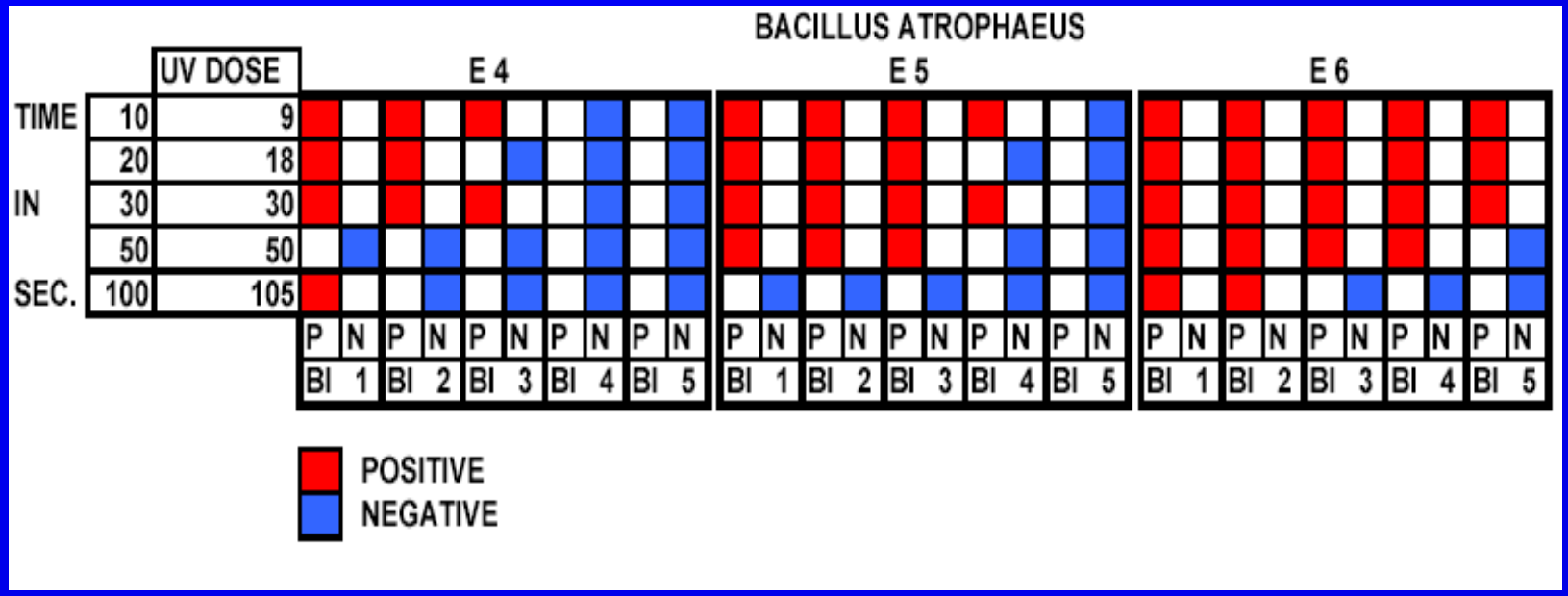
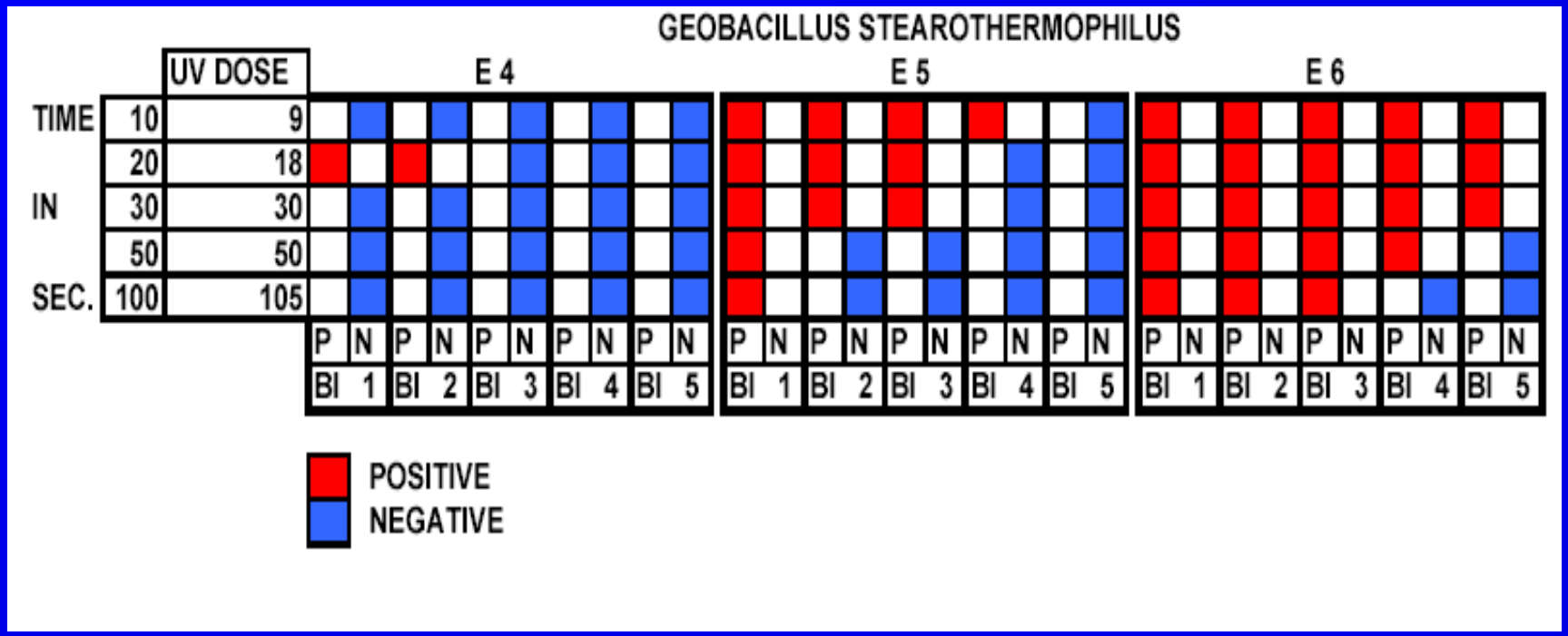
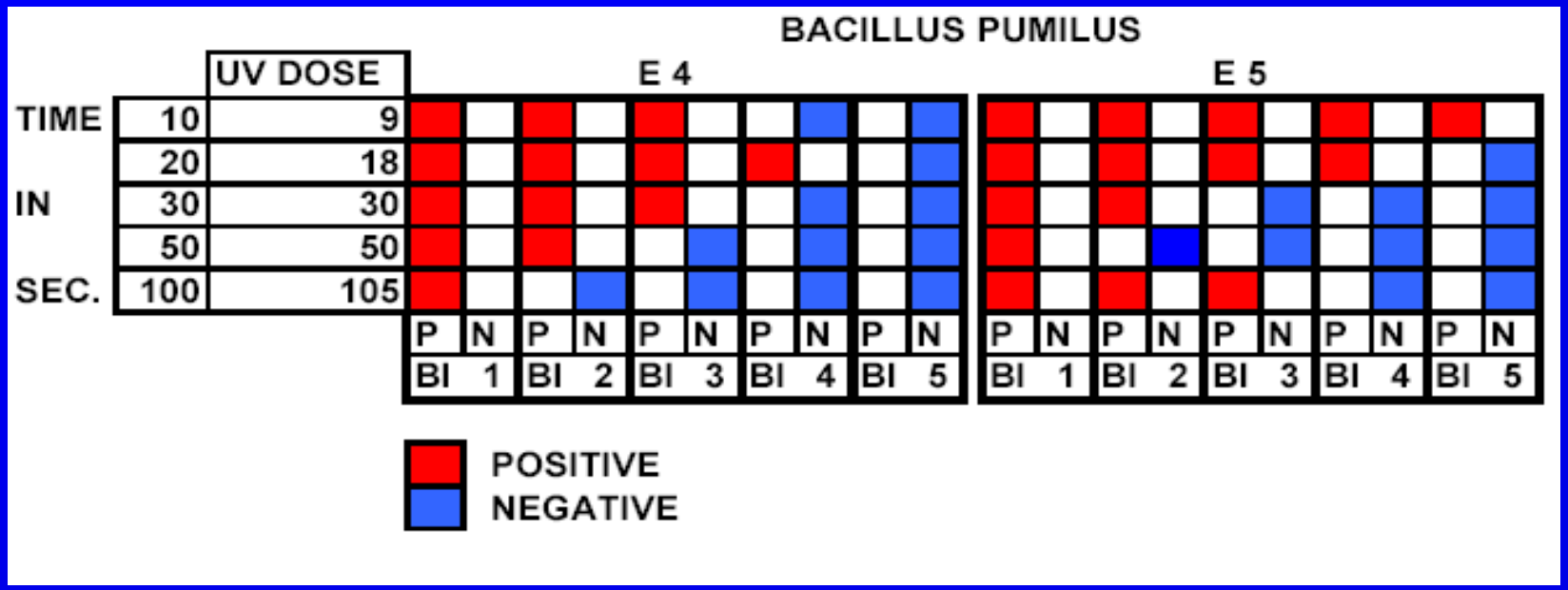
INACTIVATION CURVE



INACTIVATION CURVE



INACTIVATION CURVE



KILL TIME EXPOSURE

The kill time was selected such that all samples would show a negative result after the growth test.

Positive results were evaluated as “late positive results” and therefore not compliant with the model on microbial reduction.

KILL TIME EXPOSURE

KILL TIME EXPOSURE PARAMETERS

Test sample:	BIs with all populations
Exposure time in Minutes or UV dose In [mWsec/cm²]:	Adjusted based on the results of screening and inactivation curve experiment
Number of samples per Exposure:	10
UV light distance:	5 centimeters

KILL TIME EXPOSURE

EVALUATION

- Reproducibility of the inactivation effect
- Model behavior of the test sample

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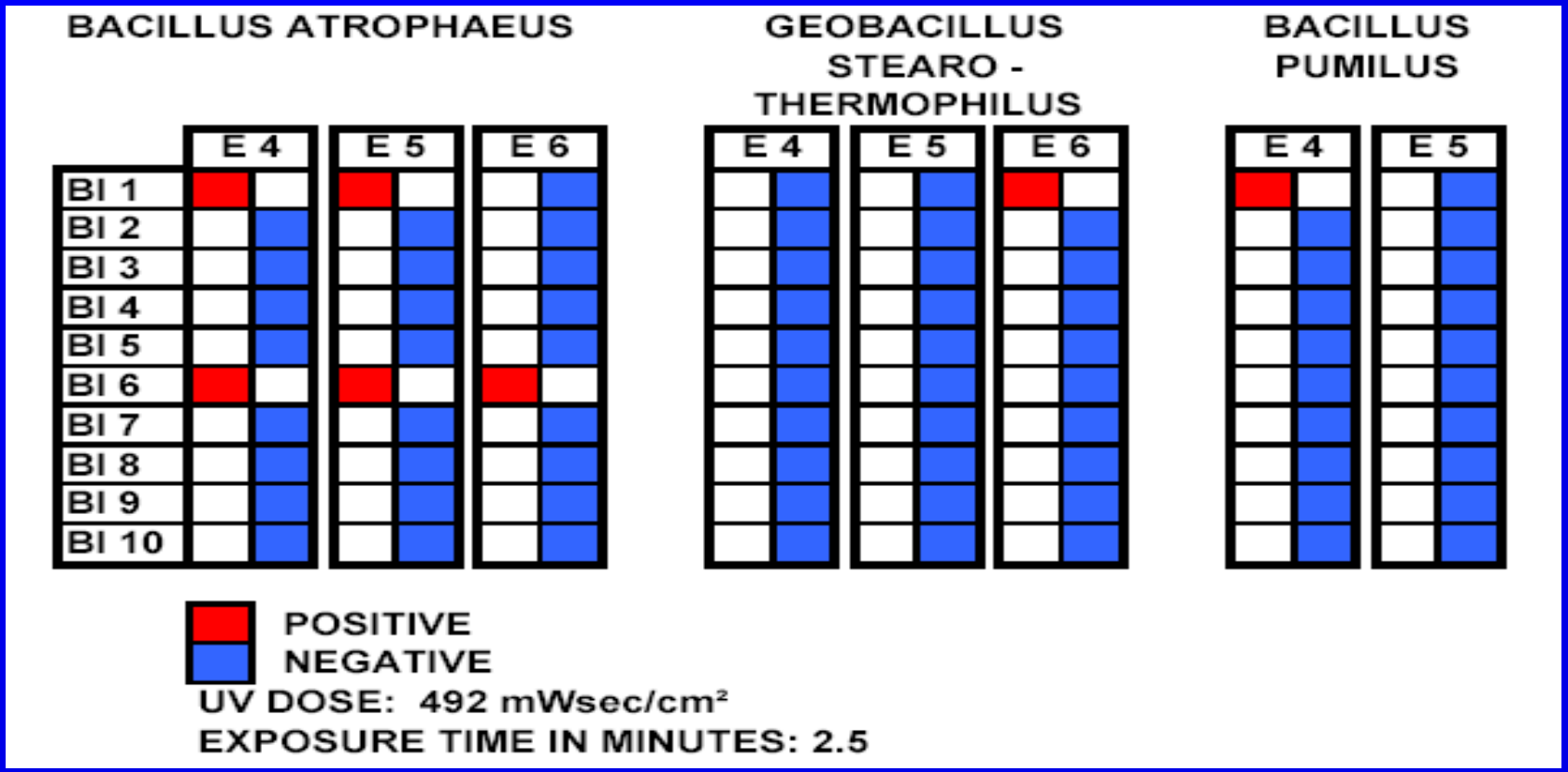
KILL TIME EXPOSURE



D-VALUES

	BACILLUS ATROPHAEUS			GEOBACILLUS STEAROTHERMOPHILUS			BACILLUS PUMILUS		
	E 4	E 5	E 6	E 4	E 5	E 6	E 4	E 5	E 6
mWsec/cm² (250-260 nm)	69.75	7.9	46.2	4.37	17.6	42.8	122.9	18.9	175.6
SECONDS	21.24	2.58	13.8	1.92	5.4	12.3	35.4	5.4	51.6

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RESULTS SUMMARY

- All dose values were estimated using the values observed with the EIT PowerMap measuring device

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"SKIP" TIME POINT POSITIVE RESULTS

In response to "Skip" time point positive results Dr. James Akers states:

"Sterilization theory tells us that at a fixed dosage conditions the D-value should be consistent for a given spore or organism preparation. Therefore, when skip time point positive results, the effect is certainly the result of factors relating to the BI preparation."

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"SKIP" TIME POINT POSITIVE RESULTS

"In a review article published in Block (2003) it was reported that UV light kills microorganisms in a dose-response that follows first order of kinetics just as steam and dry heat do. Therefore, it is my view that when skip time point positives are found in UV light testing it is most likely the result of spores being layered on the surface of the BI in a manner that protects them from lethal UV light exposure."

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"SKIP" TIME POINT POSITIVE RESULTS

"In actual treatment of tubs or other materials in a UV Tunnel, layering would not be likely because of more disperse population as result of low bioburden. Therefore, I do not believe that the "Skip" or "Out of Sequence" positives observed in some studies are relevant, nor I am surprised that more consistent results are observed when using BI's of lower concentrations"

Dr. James Akers – June 4, 2004

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"SKIP" TIME POSITIVE RESULTS-ROGUE BIs

What causes Rogue Indicators

- The spores form clumps/agglomerations
- The spores are coated in debris
- There are catalytic or protective substances present
- The carrier surface contains fissures, cracks, etc.
- Effects can occur even using very clean spore suspensions and defect free carrier surfaces

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Rogue Biological Indicator

Example of a “clump”; we will kill the bugs on the surface of the clump but not the ones hiding under the dead bodies

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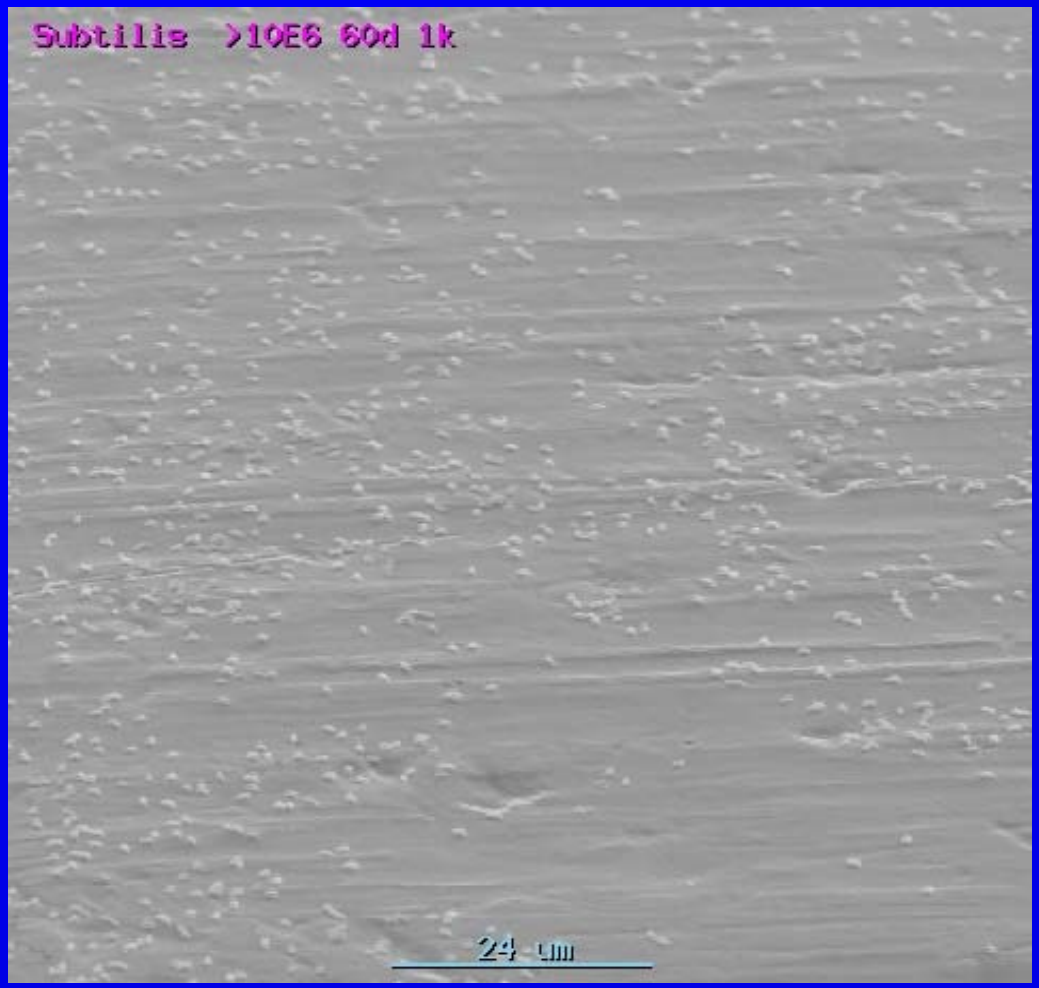
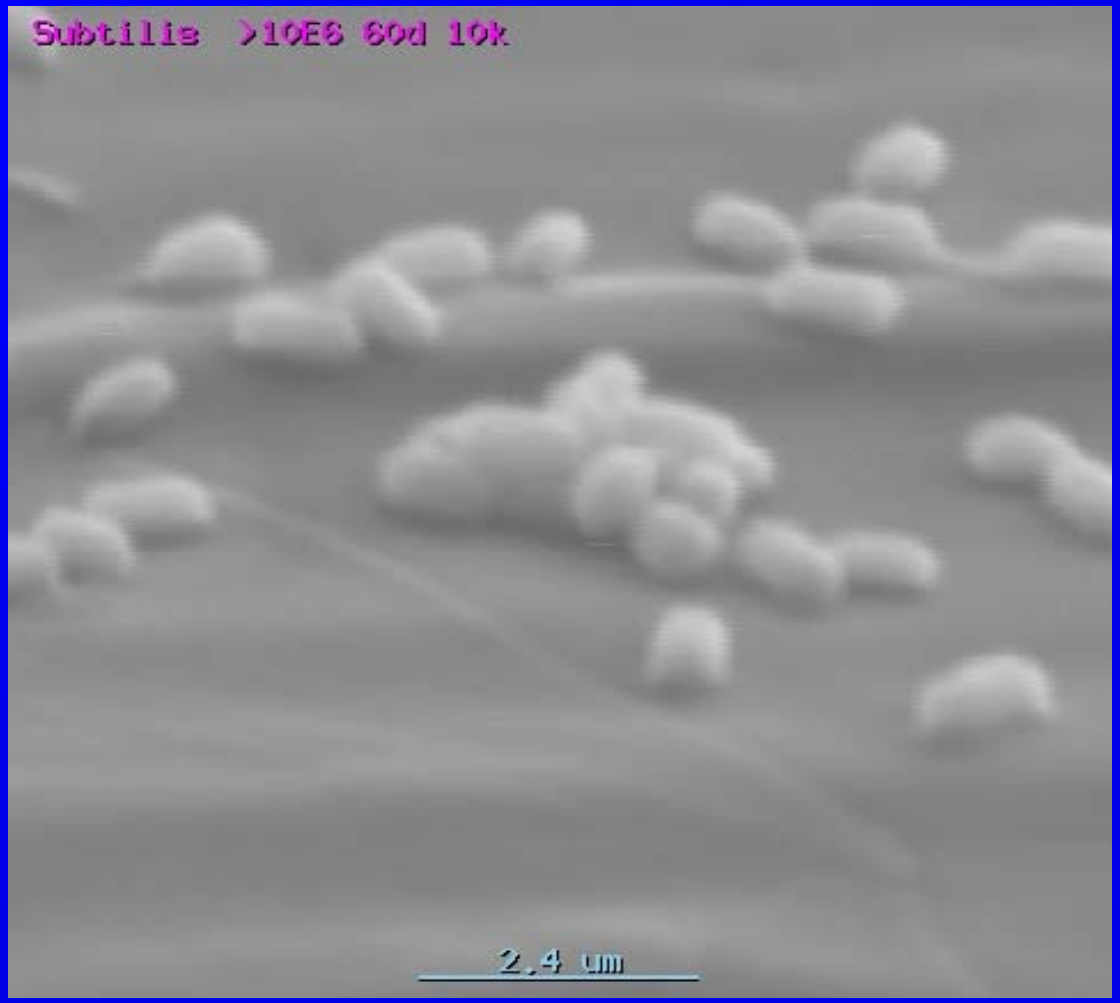
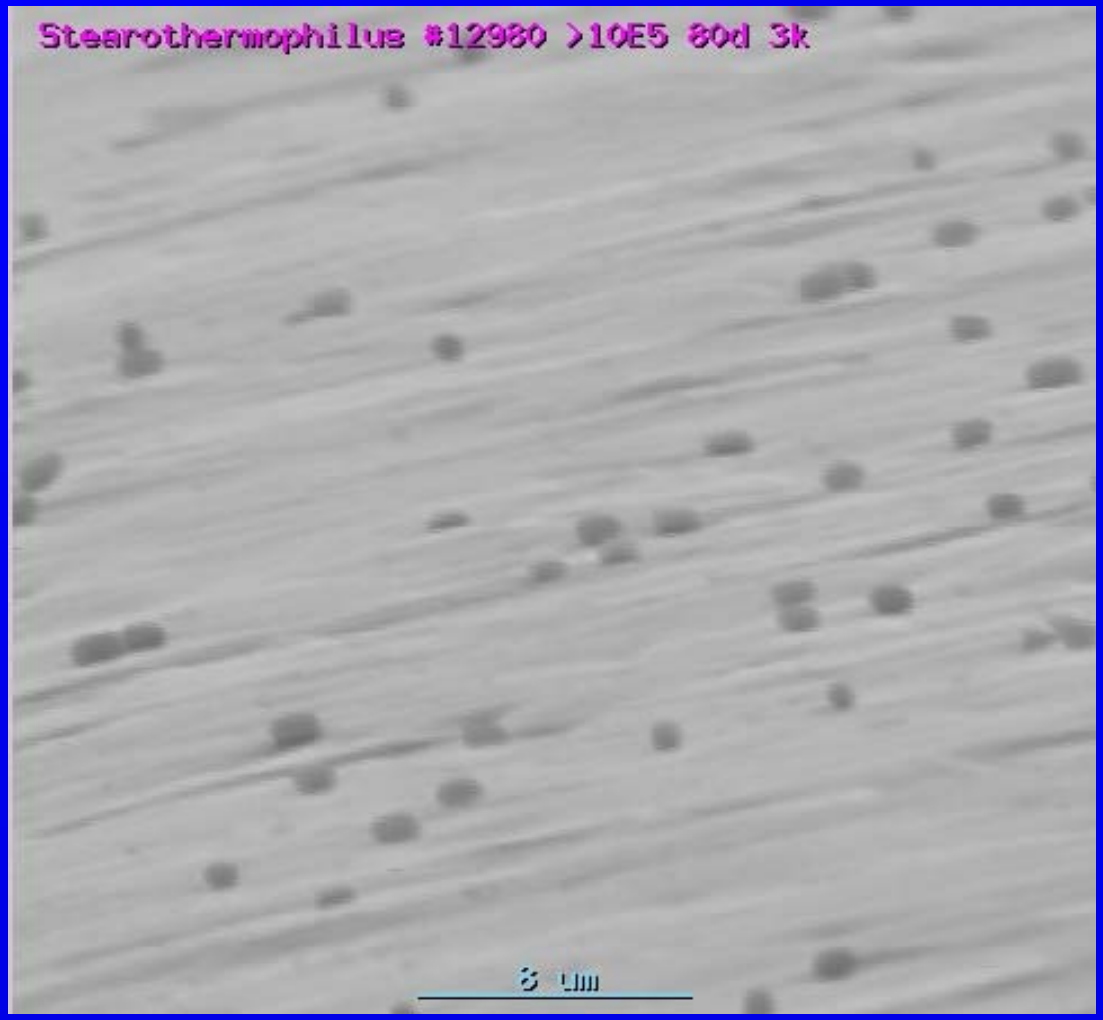
B. ATROPHAEUS E6 PICTURE TAKEN AT 60 DEGREES 1K MAGNIFICATION



B. ATROPHAEUS E6 PICTURE TAKEN AT 60 DEGREES 10K MAGNIFICATION



GEOBACILLUS STEAROTHERMOPHILUS E5 PICTURE TAKEN AT 80 DEGREES - 3K MAGNIFICATION



GEOBACILLUS STEAROTHERMOPHILUS E5 SMALL CLUMP - 8K MAGNIFICATION

B. PUMILUS E6 - 4K MAGNIFICATION

Surface Disinfection Of Becton-Dickinson Hypak Tubs

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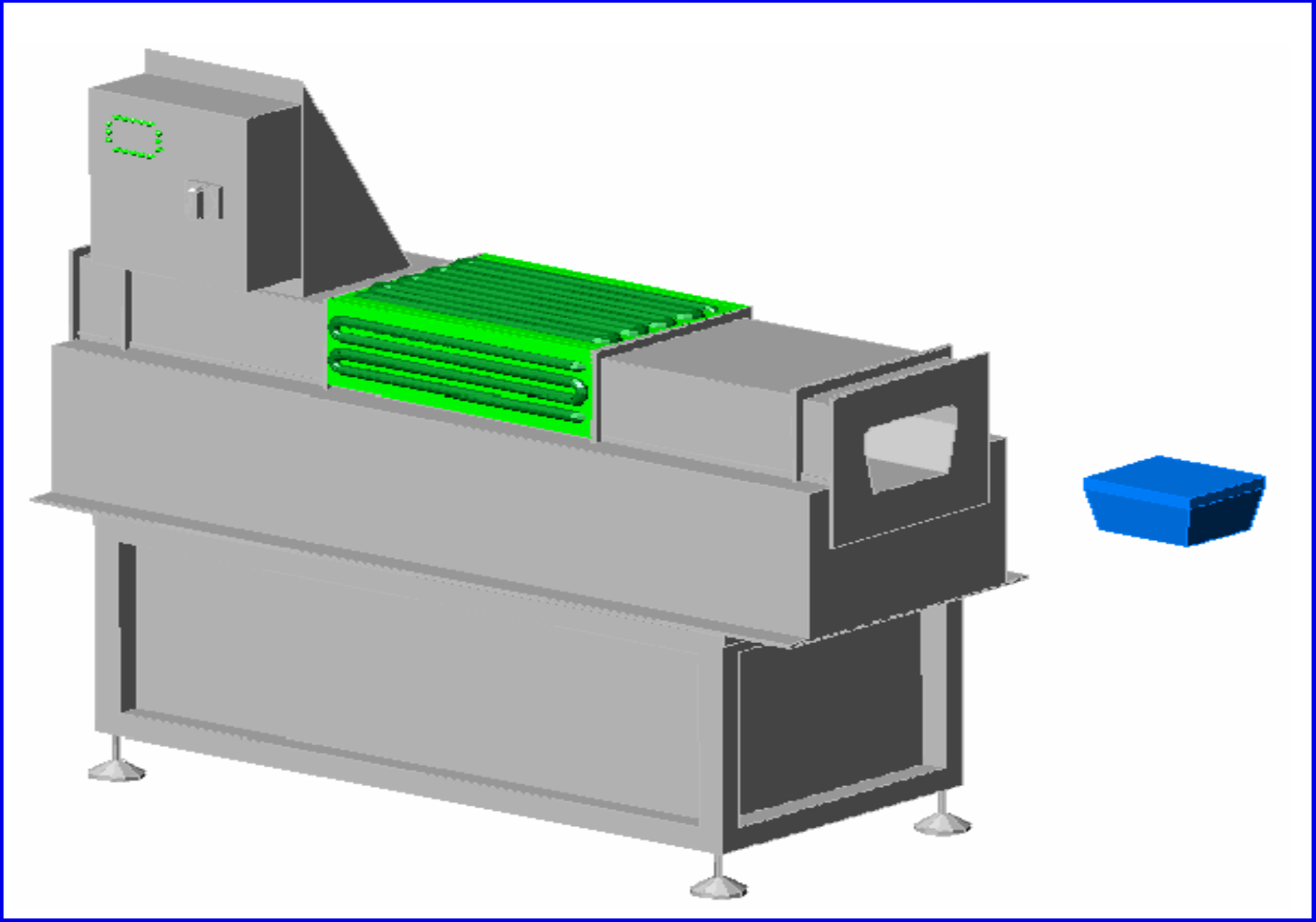
PURPOSE OF THE STUDY

To evaluate the effectiveness of DDK's Ultraviolet (UV) Tunnel in obtaining:

A reliable surface sterilization of Becton – Dickinson Hypak syringe tubs prior to entering a Barrier Isolator or a Clean Room for syringe filling.

UV TUNNEL



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UV TUNNEL

The UV Tunnel used for this investigation had the UV lights set at an equal distance from four faces of the BD Tub.

The front and back of the BD Tub received only reflected light.

The UV Tunnel has variable speed.

SCOPE

The scope of this investigation was to:

- Establish the inactivation effect of the UV light technology on different spores of bacillus species on the surface of BD Tubs
- Test the inactivation performance of DDK's UV Tunnel under realistic conditions

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MATERIALS AND METHODS

During this investigation the following biological indicator compositions were used as microbiological systems:

- Spores of Geobacillus stearothermophilus ATCC 12980
- Spores of Bacillus atrophaeus ATCC 9372
- Inoculated onto stainless steel (as before)
- UV exposure was monitored as before

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BIOLOGICAL INDICATORS

INITIAL POPULATION

- $\geq 1.0 \times 10^3$ [cfu/carrier]
- $\geq 1.0 \times 10^4$ [cfu/carrier]
- $\geq 1.0 \times 10^5$ [cfu/carrier]
- $\geq 1.0 \times 10^6$ [cfu/carrier]

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TEST DOCUMENTATION

- Spore type, population, lot number, expiration date
- Incubation time (days), temperature
- UV dose per exposure time
- Number of BIs per exposure time
- Test evaluation: P = Turbid = Positive
  N =Clear = Negative

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RESISTANCE DETERMINATION

For all inactivation runs the test samples were placed on a pre-sterilized BD Hypak Tub in a way that the inoculated side of the carrier was exposed to the UV light without any protection by shadowing.

RESISTANCE DETERMINATION

For all inactivation runs concerning the resistance determination of test samples, the BIs were placed on the BD tub surface as follows:

- Top of BD tub: 3 locations
- Bottom of BD Tub: 3 locations
- Front of BD Tub: 3 locations
- Front corners of BD Tub: 2 locations
- Back of BD Tub; 3 locations
- Back corners of BD Tub: 2 locations
- Left side of BD Tub: 3 locations
- Right side of BD tub: 3 locations

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BD TUB WITH BIs

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RESISTANCE DETERMINATION

- Test 1: distance between the BD Tubs at four inches
- Test 2: distance between the BD Tubs at zero inches
- Conveyor speed at two feet per minute
- UV lights at two inches from the surfaces of the BD Tubs
- Both B. atropheus and G. stearothermophilus tested

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RESISTANCE DETERMINATION

Test # 1

- UV dose in mWsec/cm²
- Wave length: 250-260 nm
- Distance between the BD Tubs: 4 inches
- UV measurement instrument: EIT PowerMap

RESULTS

Test 1

BACILLUS ATROPHAEUS

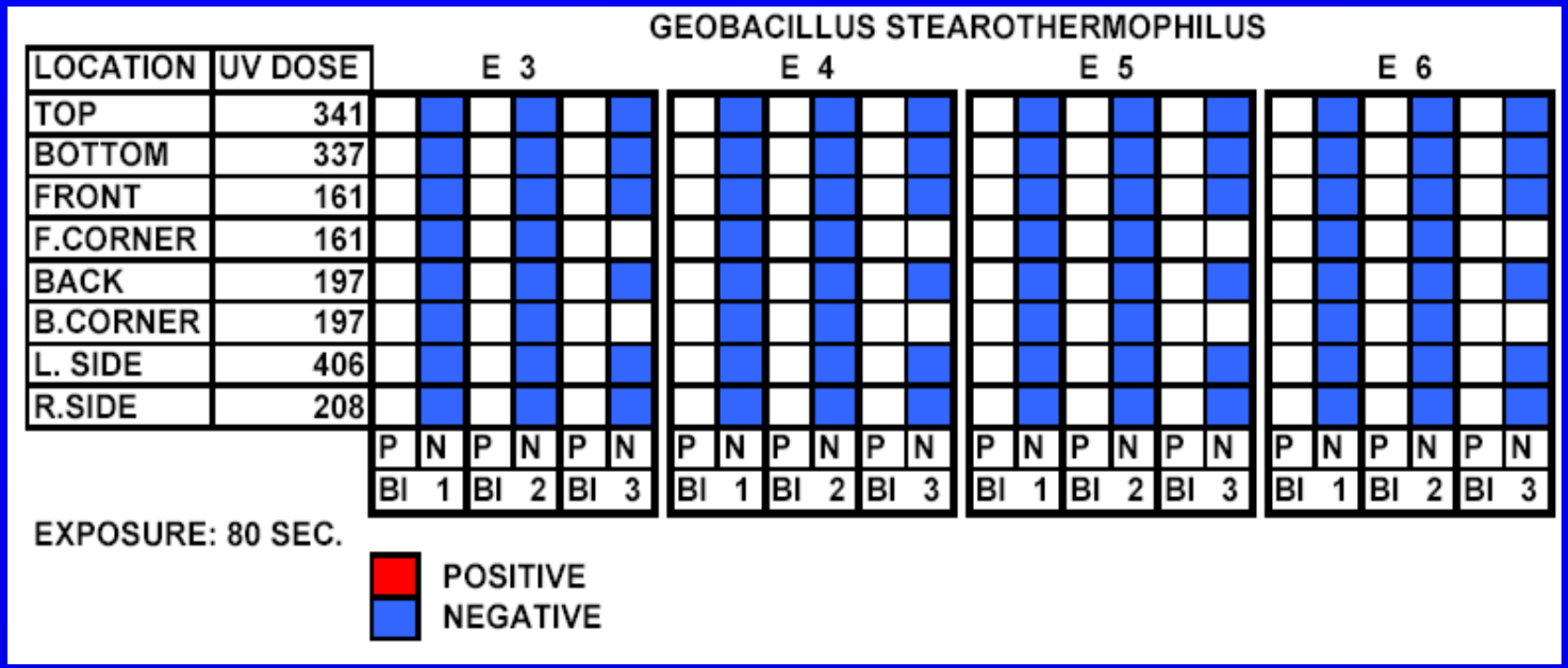
LOCATION	UV DOSE	E 3						E 4						E 5						E 6					
TOP	432																								
BOTTOM	428																								
FRONT	274																								
F. CORNER	274																								
BACK	234																								
B. CORNER	234																								
L. SIDE	383																								
R. SIDE	268																								
		P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N
		BI	1	BI	2	BI	3	BI	1	BI	2	BI	3	BI	1	BI	2	BI	3	BI	1	BI	2	BI	3

EXPOSURE: 80 SEC.

POSITIVE
NEGATIVE

RESULTS

Test 1



RESISTANCE DETERMINATION

Test # 2

- UV dose in mWsec/cm²
- Wave length: 250-260 nm
- Distance between the BD Tubs: 0 inches
- UV measurement instrument: EIT PowerMap
- Only the front and back of the BD Tub were studied
- Only G. stearothermopholis was tested

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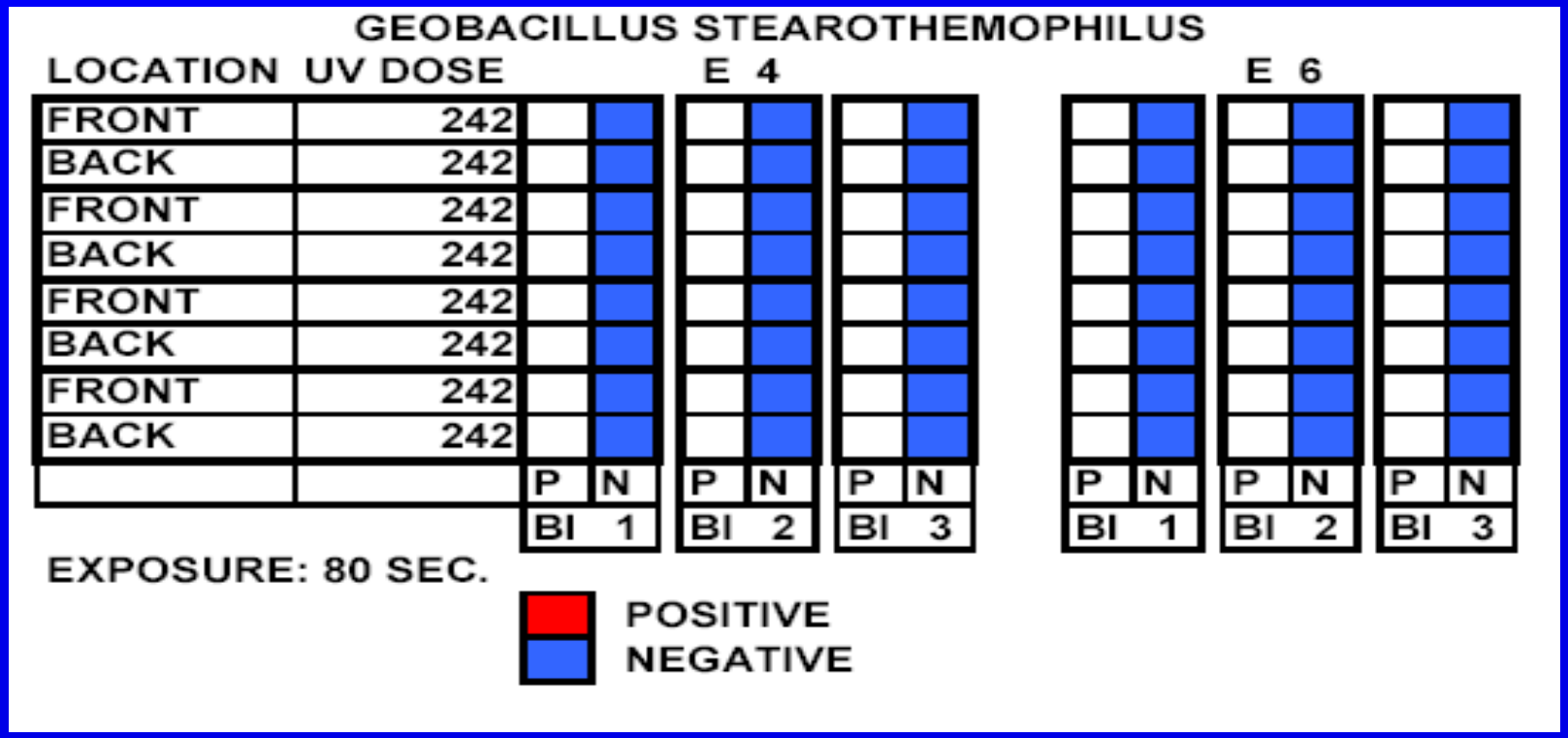
RESULTS
Test 2



VALIDATION APPROACH

Dr James Akers states:

"Other firms using UV light for the inactivation of BD Tubs have also reported that consistent kills with BIs with populations of 10^6 are not consistently possible at exposure times useful for production even when great care is taken in the manufacture of the BIs. This is because it is not possible to manufacture BIs at high spore concentrations that do not demonstrate some layering effect."

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VALIDATION APPROACH

" Since the UV dosages delivered by commercial tunnels, including the ones produced by DDK Scientific are extremely high, *the only explanation for survivors is protection as a result of layering*."

VALIDATION APPROACH

"Therefore, I do not recommend the use of BIs with a 10^6 spore population because it is clear from the scientific point of view that you are not testing organism resistance but rather BI manufacturing. A far more practical and effective approach is to use 10^3 BIs, calculate an accurate D value and provide enough dosage to achieve a 6D or greater process. This approach has already been used and in my view will not be challenged by FDA microbiology reviewers if the scientific facts are clearly and correctly presented."

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VALIDATION APPROACH

"Since the relevant industry and regulatory guidance documents call for a 4-6 spore log reduction, a 6D process based upon calculated D value and dosage would easily comply with this requirement. Although this point is often misunderstood, one does not require the use of spores with the population of 10^6 to demonstrate a six log reduction."

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VALIDATION APPROACH

"The UV Spore kill data can be further supported by the demonstration of low and dispersed incoming bioburden on tubs or materials to be treated into the tunnel. This approach confirms the margin of safety inherent in the BIs chosen because the population of even a 3 log BI will be significantly greater than the bioburden on a tub."

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VALIDATION APPROACH

"Generally, I have observed bioburden substantially lower than 10 cfu/site on the tub and less than 30 cfu on the entire tub surface when the microbial recovery is extrapolated to the entire surface area of the tub.

A low concentration of organisms spread widely over the entire surface of the tub contrasts greatly with a thousand or more highly resistant spores concentrated onto a small BI coupon."

Dr. James Akers
June 5, 2004

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CONCLUSIONS

- DDK's UV Tunnel performance is able to be validated.
- Geobacillus stearothermophilus is the recommended microorganism to be used for validation.
- 200 mWsec/cm² (UV PowerMap) can achieve up to 6 log reduction of Geobacillus stearothermophilus.
- BIs from Apex Laboratories should be used.

Comparison with other Technologies

	UV	VHP	E-Beam
Cost	Inexpensive	Very expensive	Very expensive
Use	Simple	Complicated	Complicated
Disinfection cycle time	Seconds	Hours	Minutes
Type of disinfection	Continuous or Batch	Batch only	Continuous or Batch
Environmental requirements	None	40% RH req. Permeates, should not be allow to condense	Generates a lot of Ozone, needs venting
Safety	Safest	Hazardous	Hazardous
Applications	Surface, air, water	Surface only	Surface only

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Thank You

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