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Povidone
A study of disinfection

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Key points

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*Validation

*Methods

*Results

*Discussion

Validation

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Assurance that intended usage is accomplished

*Robust – high success rate

*Reliable – accurate results of sample

*Reproducible – same results each sample

Why Validate Processes

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*Quality assaurance standard
*Accurate data collection
*Tool in developing best practice
*To meet accreditation standard

*We produce a product to be transplanted we can say with confidence that the product is safe and reliable

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Regulatory Bodies/Accreditation

*AATB
*EBAA
*ISO
*Health Canada
*CSA

Requirement for accreditation

Cost/Benefit

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Costs

Test materials
Standards
Quality assurance
Equipment
Analysis
Committee Work
Studies
Travel

Benefits

More efficient outputs
Reliability
Greater confidence of
*Staff
*Bank (tissues)
*Customer

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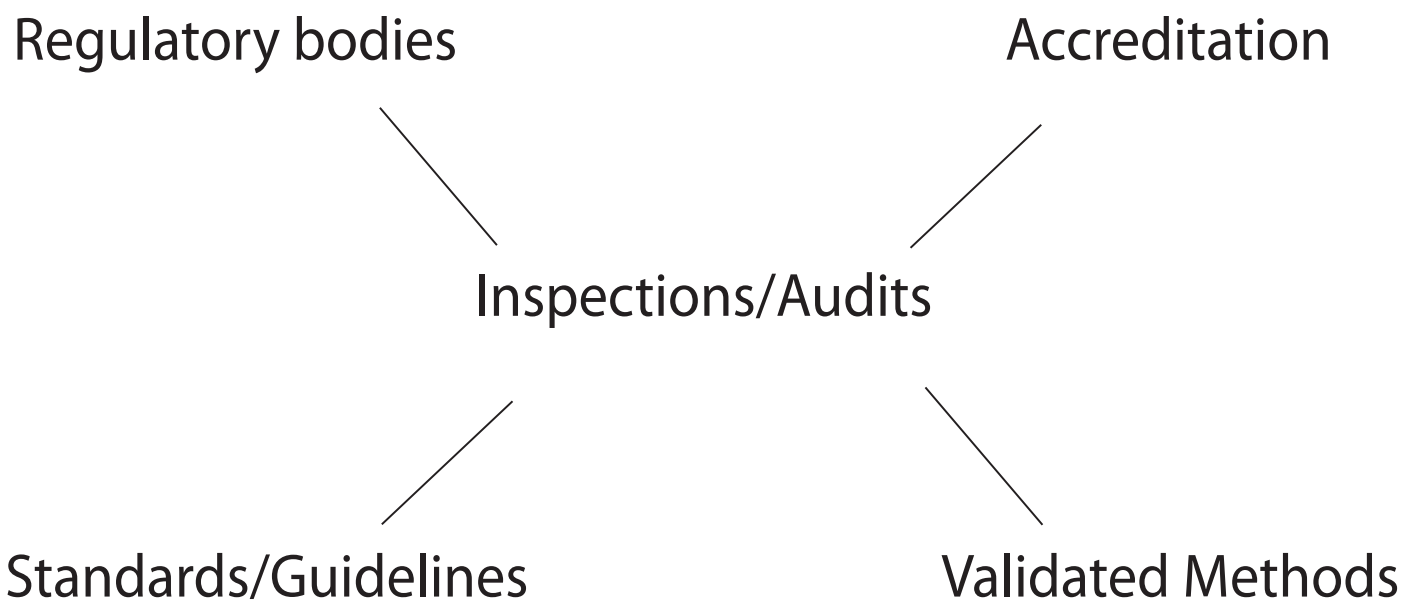

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How do you validate a process or method?

*Decide on analytical requirements
*Plan a suite of experiments
*Carry out experiments
*Use data to assess fitness for purpose
*Produce a statement of validation

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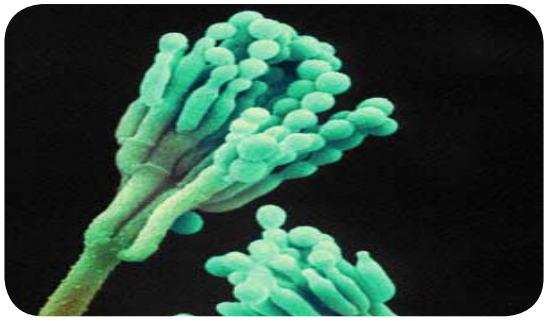
Methods

*Cell culture
*Optical density
*Serial dilution
*Spread plating
*Experimental groups
*Colony forming units (CFU)
*Tissue culture

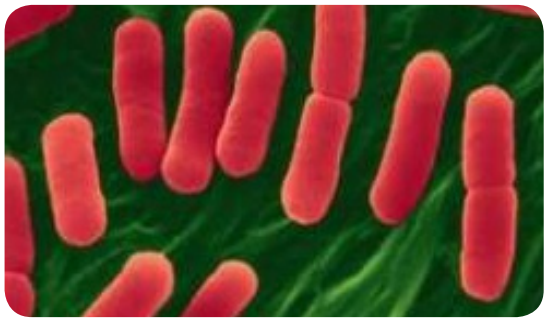
Cell culture

*SBA, 24-48hrs @37°C

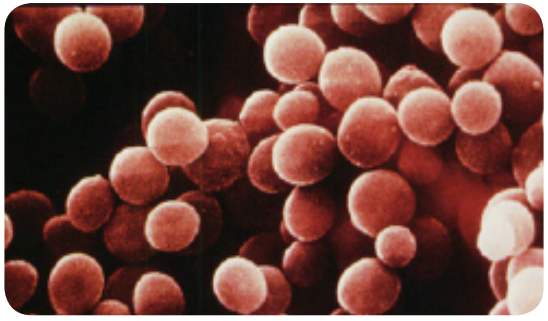
*IMA, 72hrs @25°C



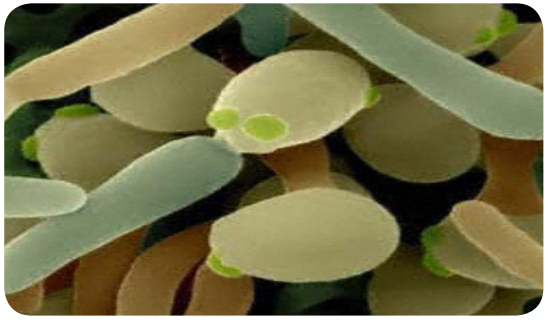
Penicillium electron micrograph, © Dennis Kunkel Microscopy Inc.



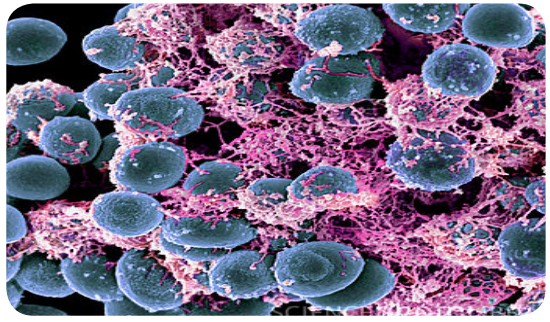
Escherichia coli, © 2004 Dennis Kunkel Microscopy Inc.



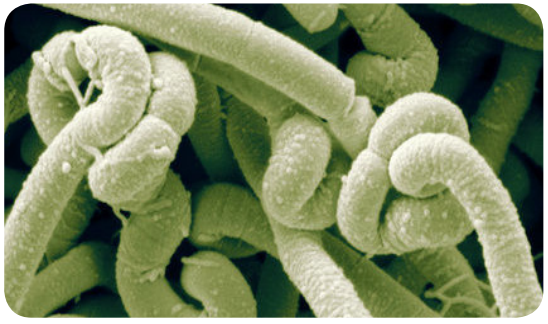
Staphylococcus aureus electron micrograph from Visuals Unlimited, http://www.visualsunlimited.com



Candida Albicans © Dennis Kunkel Microscopy Inc.



Staphylococcus epidermidis, coloured scanning electron micrograph. National Institutes of Health/Photo Library, http://www.nih.gov/about/nihphotos.htm



Bacillus Cereus, SCIMAT/SCIENCE PHOTO LIBRARY. http://www.microbiologyonline.org.uk

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Optical denisty

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*Spectrophotometer
*According to their growth conditions
*Inoculated into Hank's B SS, sterile inoculation loop
* OD of 1.0 via dilution of the stock solution @ 620nm.

Serial dilution

*1.0mL of stock solution was added to 9.0mL of Hanks
*Serial dilution to 10^{-10} was performed bacterial culture on each day

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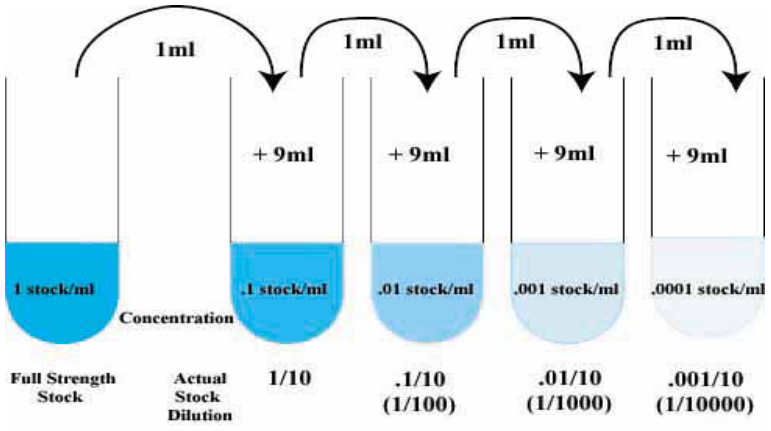

Spread plating

*Determine the disinfection efficacy of Povidone™
*100µL of the dilution were placed on agar plates
*Spread using a flame sterilized bacterial cell spreader
*Incubated for 48-72 hours at the appropriate temperature

Experimental groups

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*Povidone™ only group
*Tissue incubated w/ bacterial cells -> exposed to Povidone™
*Incubated at 37°C for

Control groups

*Hank's solution only
*After incubation in Hanks media for 1.5 hours

Colony forming units (CFU)

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*Calculated using spread plating and incubating the plates at their appropriate temperatures for 48-72 hours.
*Analyzed and the number of colonies
*Less than 330 colonies and recorded on the results sheets. Calculations-> different dilutions->overall concentration of bacteria in the stock solution using the first dilution where there more than ten colonies observed to grow on a plate.

Tissue culture

*Povidone incubation(tissue-Povidone™)->washed w/Hanks
* (TSB) culture tubes and incubated (7days)
* allow any trace bacteria to grow

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Results

*Povidone™ effective decontaminant for S. aureus, C. albicans, S. epidermidis, E. coli, B. cereus, and Penicillium
*Each bar equals one of the ten fold dilutions
*Experimental group consisted of ten dilutions to a dilution factor of 10^{-10}
* Bar furthest to the left is the 10-1 dilution and the bar on the far right for each group is the 10-10 dilution group

S. aureus

Graph 1 – A graphical representation of three experiment trials involving S. aureus being challenged by HBS, HBS Incu, Pov only, and Pov + Tiss. Each bar represents a ten-fold dilution.

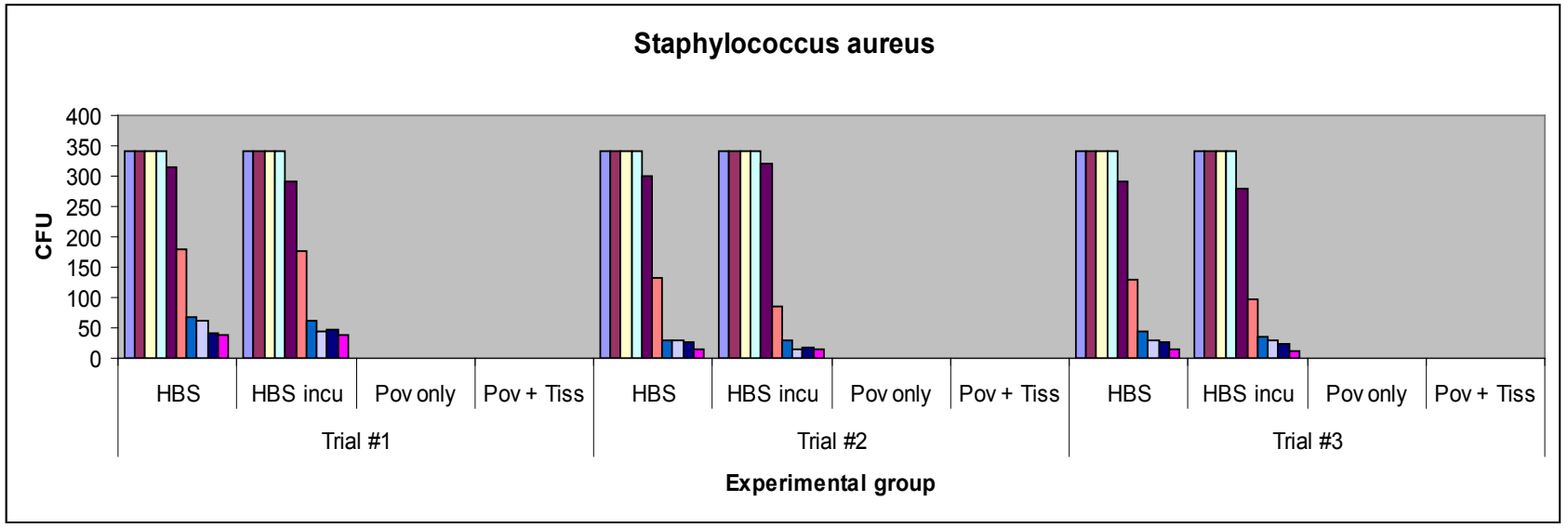

Table 1 – This table represents whether or not there was growth of S. aureus in TSB broth after 5 days incubation at 37°C for each dilution for each of the three trials.

Growth -/+	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8	10-9	10-10
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-

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C. albicans

Graph 2– A graphical representation of three experiment trials involving C. albicans being challenged by HBS, HBS Incu, Pov only, and Pov + Tiss. Each bar represents a ten-fold dilution.

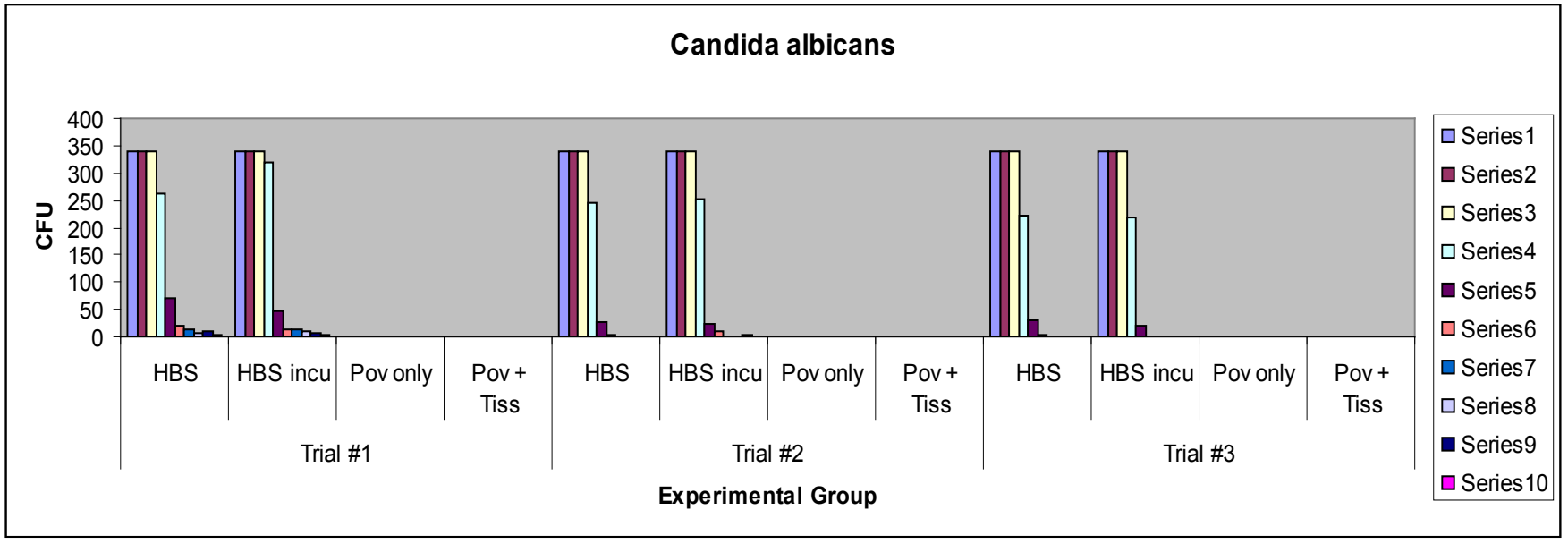

Table 2 – This table represents whether there was growth of C. albicans in TSB broth after the tissue was incubated for 5 days at 37°C.

Growth -/+	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8	10-9	10-10
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-

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E. coli

Graph 3– A graphical representation of the three experimental trials using E. coli where each bar represents a ten-fold dilution.

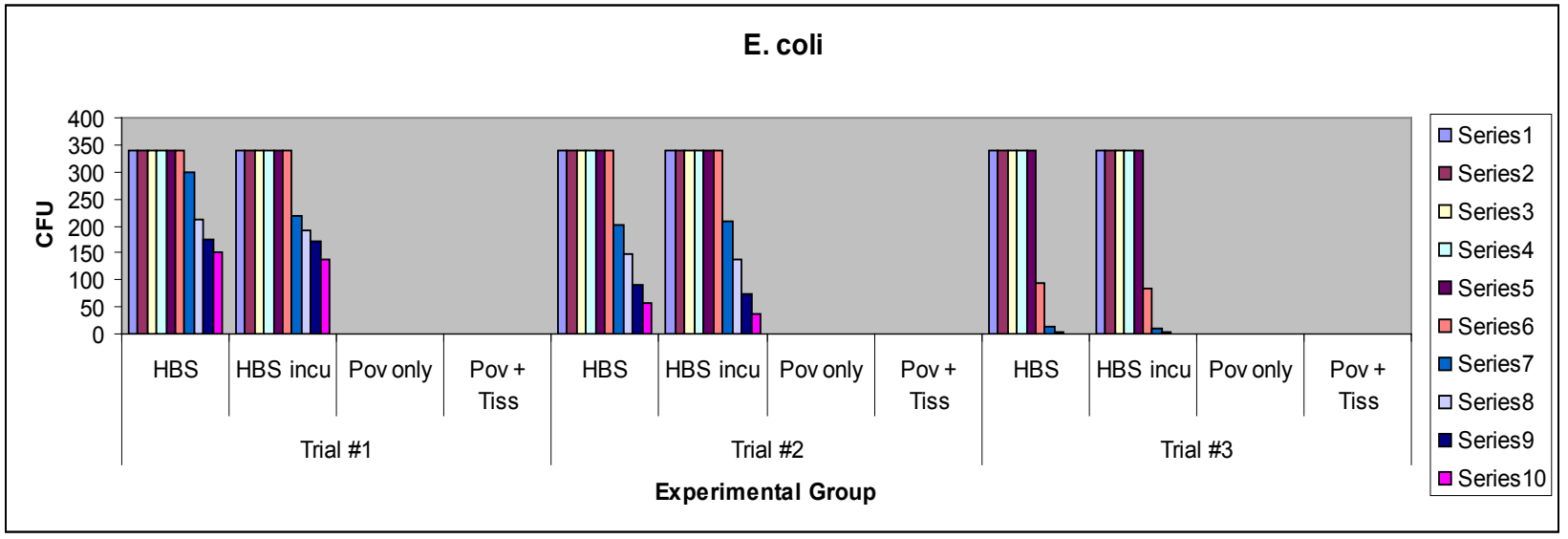

Table 3 - This table represents whether there was growth of E. coli in TSB broth after the tissue was incubated for 5 days at 37ºC.

Growth -/+	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8	10-9	10-10
	+	-	-	-	-	-	-	-	-	-
	+	+	+	-	-	-	-	-	-	-
	+	+	-	-	-	-	-	-	-	-

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S. epidermidis

Graph 4 – This is a graphical representation of the growth of S. epidermidis for the experimental groups.

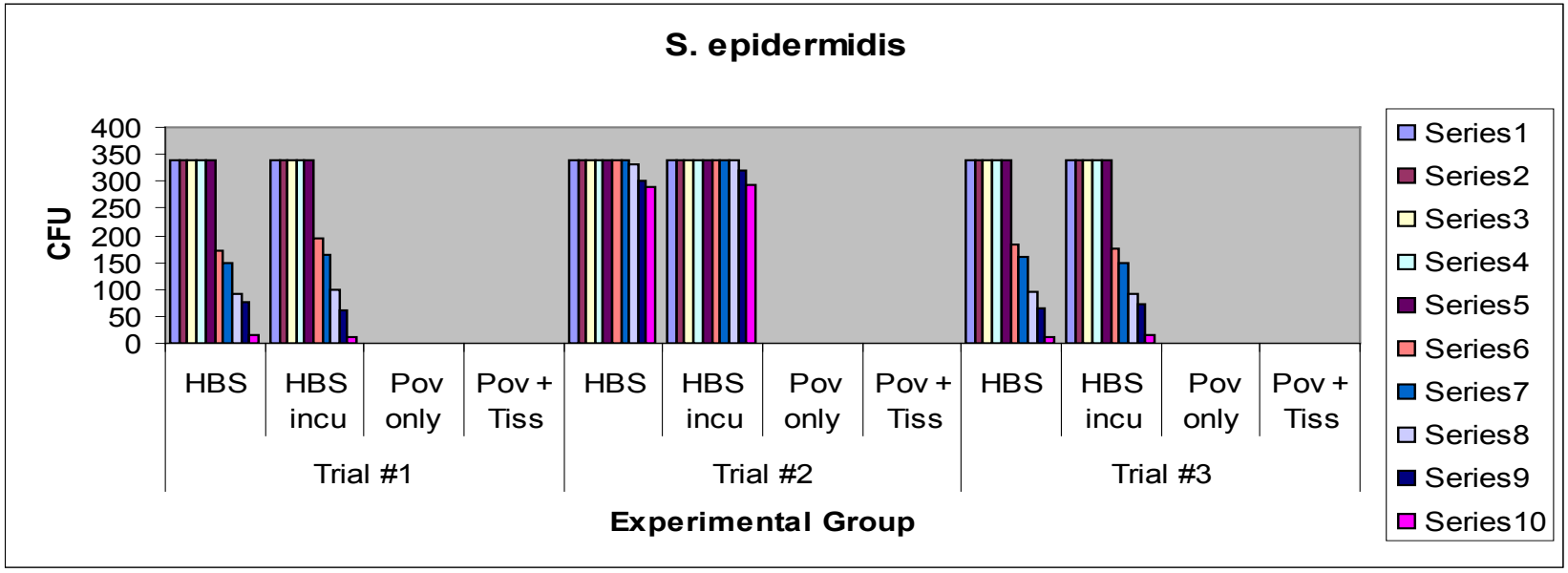

Table 4 – Shows whether there was growth of S. epidermidis in TSB broth after the tissue samples were incubated at 37°C for five days.

Growth -/+	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8	10-9	10-10
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-

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B. cereus

Graph 5 – This is a graphical representation of the growth of B. cereus for each experiment group and all three trials.

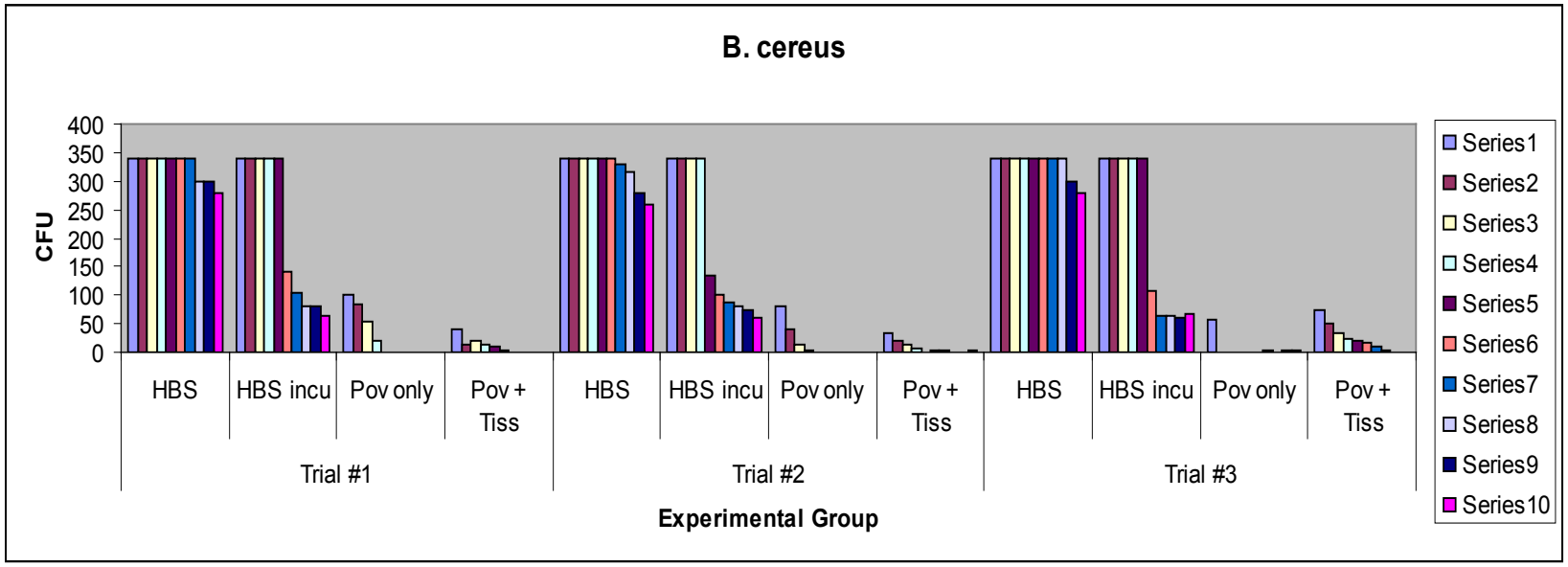

Table 5 – This table represents whether there was growth B. cereus from tissue samples incubated at 37°C for five days in TSB broth.

Growth -/+	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8	10-9	10-10
	+	+	-	-	-	-	-	-	-	-
	+	+	-	-	-	-	-	-	-	-
	+	+	+	-	-	-	-	-	-	-

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Penicillium

Graph 6 – This is a graphical representation of the experimental groups and three trials involving Penicillium.

Trial #1	HBS	+	+	+	+	+	+	+	+	+	+
	HBSincu	+	+	+	+	+	+	+	+	+	+
	Pov only	-	-	-	-	-	-	-	-	-	-
	Pov tiss	-	-	-	-	-	-	-	-	-	-
Trial #2	HBS	+	+	+	+	+	+	+	+	+	+
	HBSincu	+	+	+	+	+	+	+	+	+	+
	Pov only	-	-	-	-	-	-	-	-	-	-
	Pov tiss	-	-	-	-	-	-	-	-	-	-
Trial #3	HBS	+	+	+	+	+	+	+	+	+	+
	HBSincu	+	+	+	+	+	+	+	+	+	+
	Pov only	-	-	-	-	-	-	-	-	-	-
	Pov tiss	-	-	-	-	-	-	-	-	-	-

Table 6 – This table shows whether or not there was positive growth in TSB broth after tissue was incubated for seven days at 25°C.

Growth -/+	10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8	10-9	10-10
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-

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Discussion

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*Contamination is a reality in tissue banking
*Normal skin flora vs pathogenic growth
*Pathogenic growth is discarded
*Acceptable tissue -> processed
* Antibiotic decontamination & Betadine Povidone™
* 1% @ 37°C for 1.5 hours, rinsed -> antibiotics, rinsed

* Validate the efficacy of povidone's bactericidal & fungicidal activity

Discussion

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* It has been established that povidone concentrations of below 10%, but especially below 1% result in the most widespread bactericidal and fungicidal activity.[1,3]
* **S. aureus and S. epidermidis**, Povidone™ reduced the bioburden by a ten-log reduction
* Reduced contamination to effectively zero, or undetectable
* **C. albicans** reducing the bioburden by a ten-log reduction
*Note only a six log reduction due to being an insufficient amount valid growth on the remaining plates
* **E. coli (gram-),** no growth after Povidone™ incub.
*Note after TSB incubation, 10^{-1}, 10^{-1} and 10^{-2}, 10^{-1} - 10^{-3}
*At high concentrations, survival inside the bone matrix of cancellous, or cross contamination occurred. Too many cells present in the dilutions

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*__E. coli cont...__ five, seven and ten log reduction. Five log is a result 10^{-7} being the last dilution where ten colonies or less were found

*Five log reduction was the result of less growth for that specific trial

*__B. cereus(spore)__ eight log reduction exposure to Povidone™, indicating susceptibility

*Povidone™is an excellent disinfection method was validated
*Susceptibilties with a greater than five log reduction were shown
*Acceptable limit, Povidone™-> 1% @ 37^{o}C for 1.5hrs is adequate decontaminate for processing

References

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3. Soyer, J. 2002. The effect of 10% povidone-iodine solution on contaminated bone allografts. J. Hospital Infection. 50:183-187.