The Evolution of the Preservative Efficacy Test and Where the Journey Might Lead

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Introduction – Subjects to be covered

- Short history of the
  - Antimicrobial Effectiveness Test - USP
  - Efficacy of Antimicrobial Preservation – Ph Eur (and other European pharmacopoeias)
  - (commonly referred to as the Preservative Efficacy Test or PET
- TGO 77
- Alternative Methods & Approaches
- Cosmetics and Toiletries Methods
- Future Developments of the Pharmacopoeias

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Brief History

- First appeared in the USP XVIII in 1970
  - Comprised of inoculating large numbers of different microorganisms into product and examining for survivors over a 28 day period.
  - *Candida albicans* ATCC 10231
  - *Aspergillus niger* ATCC 16404
  - *Escherichia coli* ATCC 4352 (later changed to ATCC 8739)
  - *Pseudomonas aeruginosa* ATCC 9027
  - *Staphylococcus aureus* ATCC 6538

Brief history (contd.)

- First appeared in the European pharmacopoeia in early 1990's
  - Used similar organism set, but with addition of *Zygosaccharomyces rouxii* for oral preparations containing high sugar concentration
  - Different organisms for different product types
  - Different time intervals for sampling
  - Different interpretations for results

Brief history (contd.)

- With further revisions to pharmacopoeias there were attempts to better define the procedure
- At the same time there was a desire to harmonise the USP with Ph Eur
- From the American perspective there was felt to be a number of compromises made in the interests of harmonisation
- Today there is a great deal of harmonisation in the way the test is set up but sadly there are still differences in some product groupings and interpretation of results
- Both pharmacopoeias now recommend testing plant isolates along with proscribed organisms, especially Gram negative organisms

TGO 77 and ARGPM (Australian) Guidelines for Microbial Quality

- Guidance 17: Microbial quality of prescription and over-the-counter medicines
- Previously ARGPM Appendices 16 & 17: Preservative efficacy testing and Microbial quality of medicines
- Version 1.0, July 2013
- Refers to TGO 77

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PE Criteria for Medicines for Multidose Use, Part 17.4

- Should be adequately preserved for duration of shelf-life
- Unless self-preserving, will contain preservatives
- Closed shelf-life studies
- Shall comply with the BP App XVI C or the Ph Eur 5.1.3 "Efficacy of Antimicrobial Preservation"
- Only exceptions are liquid oral antacid medicines which may comply with the USP <51> "Amticrobial Effectiveness Test"
- To be tested at beginning and end of marketed shelf-life

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PE Criteria for Medicines for Multidose Use, Part 17.4 (cont.)

- Open shelf-life studies – for sterile medicines intended for multidose use, eg multidose injectable or ophthalmic preparations
  - Recommends ISO 14730 : Ophthalmic optics – contact lens care products – antimicrobial preservative efficacy testing and guidance on determining discard dating
  - Can also use modified PET that has re-challenges over the proposed in-use period with a reduced challenge inoculum
  - Can use PET results after simulated in-use
  - Results of microbial content tests after patient use for full duration of open shelf-life

Alternative methods

- Everyone would love a simple and rapid method!
- Methods have been proposed where a D-value is determined – not proven to be reliable predictor of 28 day test, but may have a role in R & D labs (see following slide)
- Methods have been proposed with use of pooled groups of organisms – have been shown to be unreliable
- A new development is the offering of ready-to-use cultures for PET set-up (2 providers at present)

Example of use of in-house isolates in the PET and the regrowth phenomenon (Phoenix effect)

Time Point	*S. aureus* AMS 027 (ATCC6538)	*P.aeruginosa* AMS 095 (ATCC 9027)	*C.albicans* AMS 003 (ATCC 10231)	*A.niger* AMS 032 (ATCC 16404)	*Enterobacter gergoviae*
Inoculum cfu/ml	7.5×10^5	5.4×10^5	1.0×10^6	1.6×10^5	9.1×10^5
0 hr	9.3×10^5	5.4×10^5	8.7×10^5	1.6×10^5	4.9×10^5
48 hr	<10	<10	N/A	N/A	6.4×10^2
7 days	<10	<10	N/A	N/A	4.4×10^2
14 days	<10	<10	<10	1.0×10^2	1.1×10^4
28 days	<10	<10	<10	<10	$>2.5 \times 10^6$

All results are expressed as CFU (Colony Forming Unit) per g. < = Less than > = greater than
N/A = Not Applicable

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Use of Ready-to-use Organisms

- Are an attractive proposition because they substantially reduce organism preparation time
  - Said to be approx 10 minutes as opposed to 1 – 1.5 hours
- Are they the same as organisms prepared as per pharmacopoeial guidance?
  - Freeze dried and unlikely to be in log-phase of growth
  - McIver *et al*, 2011 claimed equivalence to conventional inocula in outcome of PET results
  - However, noted that results were more variable with ready-to-use organisms, except for *A. brasiliensis,* and possibly more difficult to kill. The reason could not be explained by the authors
- Offer possibility of substantial set-up cost reductions
  - McIver *et al*, 2011 claimed 18 PET's were invalidated due to inocula being outside of target inoculum range causing 90 hours extra time in re-tests plus supply costs
- Therefore these products offer some promise but need further evaluation to fully understand the differences to standardised inocula

Product 1 Non-sterile preserved saline spray (USP Category 2)

McIver *et al*, 2011

Figure 6. Results for *A. brasiliensis* in Product 1.

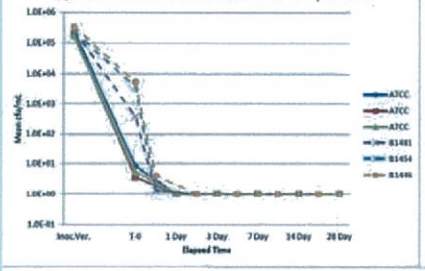


Figure 7. Results for *C. albicans* in Product 1.

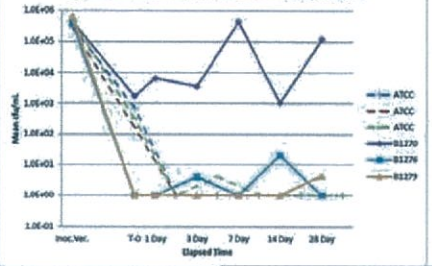


Figure 8. Results for *E. coli* in Product 1.

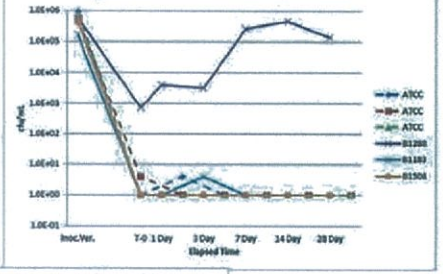


Figure 9. Results for *P. aeruginosa* in Product 1.

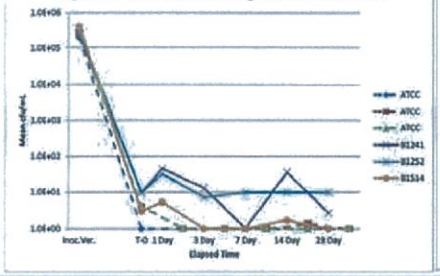
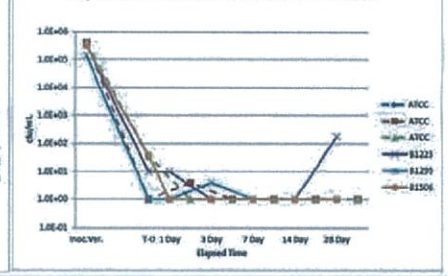


Figure 10. Results for *S. aureus* in Product 1.



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Product 2 Sterile preserved eye drops (USP Category 1)

McIver *et al*, 2011

Figure 11. Results for *A. brasiliensis* in Product 2.

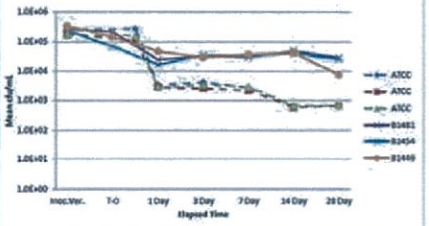


Figure 12. Results for *C. albicans* in Product 2.

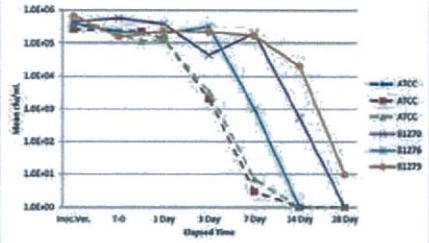


Figure 13. Results for *E. coli* in Product 2.

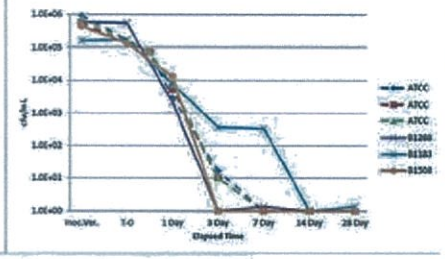


Figure 14. Results for P. aeruginosa in Product 2.

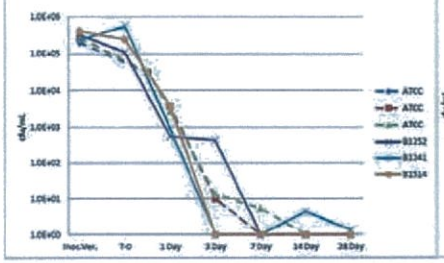
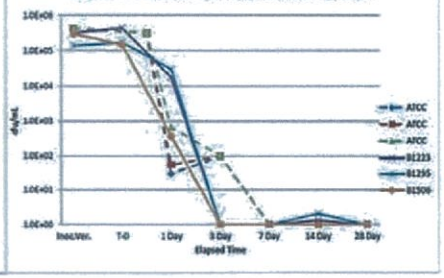


Figure 15. Results for S.aureus in Product 2.



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Cosmetics PET's

- USP and Ph Eur test methods commonly used
- Other methods published by CTFA: M-3, M-4, M-5 and M-6
- Several in-house methods have been developed by preservative supplier companies
  - Often have pooled mixtures of organisms and use multiple challenges

Cosmetics Regulation

- In Australia mostly regulated by NICNAS & ACCC
- In Europe a new regulation has been introduced to replace previous ones
  - EC 1223 / 2009, effective from 1/7/2013
  - Requires product safety report which must include a preservation challenge test to prove the microbial stability
- In the USA, the two most important laws pertaining to cosmetics marketed in the United States are the Federal Food, Drug, and Cosmetic Act(FD&C Act) and the Fair Packaging and Labeling Act (FPLA). FDA regulates cosmetics under the authority of these laws.

Future Revisions of Pharmacopoeias

- USP Forum, 40, 1, 2014 proposes to revise the Chapter <51> ANTIMICROBIAL EFFECTIVENESS TESTING
  - Greater clarity in preparation & use of test organisms
  - Use of standardized microbial cultures
  - Growth promotion of the media
  - Method suitability
  - Clarifies the definition of "no increase"
  - Clarifies method execution
  - Editorial changes to update chapter to current USP style

  - It is unlikely that full harmonization will happen any time soon!

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Useful References

- Scott VW Sutton & David Porter, PDA J Pharm Sci & Tech, 2002
- Cheryl L Moser & Brian K Meyer, Amer Assoc Pharm Scientists, 2011
- Ngoc Anh-Thu Phan, CAPSIG Seminar, 28 April 2010
- Dawn McIver *et al*, PDA Meeting poster presentation, 2011
- Wolfgang Siegert, Household & Personal Care Today, 8, 2013
- USP Forum, 40, 1, 2014
- USP <51>
- Ph Eur, EP, <5.1.3>

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Thank you

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