

Recent microbiological changes implemented through TGO 77

Paul Priscott &
Ngoc Anh-Thu (Thu) Phan
ams Laboratories,
Silverwater

Presented at the ATGC conference, Sydney, 29 April 2010

Topics to be covered

- Introduction
- Why PE Testing?
- Selection of Category and Methods
- BP and USP comparison
- Environmental Isolates
- Case Study
- Other microbiological topics

The heart of the changes

- Was asked to focus on preservative efficacy testing
- Therapeutic Goods Order no 77
Microbiological standards for medicines –
effective January 2010
- New Code of GMP (based on PIC/S) –
effective July 2010

Why PE Testing?

- Required for registration of multi-dose products
- Part of the formulation development process
- Part of the stability programme
- Part of the ongoing stability programme

Pharmacopoeia Descriptions

- British Pharmacopoeia Appendix XVI C - Efficacy of Antimicrobial Preservation
- European Pharmacopoeia, Section 5.1.3 Efficacy of Antimicrobial Preservation.
- United States Pharmacopoeia - chapter 51 Antimicrobial Effectiveness Test for Category 4 products.

Why PE Testing?

- Key Factors affecting the efficacy of the antimicrobial preservative added:
 1. The active ingredient
 2. The excipients
 3. Storage conditions
 4. The container and its closure
- The BP states for a product *“it shall be demonstrated that the antimicrobial activity of the preparation as such or if necessary, with the addition of a suitable preservative or preservatives provides adequate protection from adverse effects that may arise from microbial contamination or proliferation during storage and use of the preparation”*

Selection of Category and Method

- All products sent for any PE testing after the prescribed stability storage conditions and times are recommended to be sent in the final packaged product as distributed by the sponsor.
- The BP/EP categories are for
 - Parenteral and ophthalmic preparations
 - Topical preparations
 - Oral preparations

Selection of Category and Method

- The preparation is individually challenged with a prescribed inoculum of 10^5 to 10^6 cfu/g/ml of preparation of bacteria and fungi and tested over 28 days.
- *Pseudomonas aeruginosa* ATCC 9027
- *Staphylococcus aureus* ATCC 6538
- *Candida albicans* ATCC 10231
- *Aspergillus brasiliensis* (formerly niger) ATCC 16404
- *Escherichia coli* ATCC 8739 for oral products
- *Zygosacharomyces rouxii* (NCYC 381) for high sugar oral products

Selection of Category and Method

- These are tested at the initial time points and various time intervals depending on the product category.
- These are performed by traditional plate count methods or membrane filtration.
- The test method must be qualified for the product under evaluation to ensure the correct diluent is used in assays for surviving microorganisms.

Acceptance Criteria

- Parenteral and ophthalmic preparations

	Category	6h	24h	7d	14d	28d
Bacteria	A	2	3	-	-	No recovery
	B	-	1	3	-	No increase
Fungi	A	-	-	2	-	No increase
	B	-	-	-	1	No increase

Acceptance Criteria

- Topical preparations

	Category	2d	7d	14d	28d
Bacteria	A	2	3	-	No increase
	B	-	-	3	No increase
Fungi	A	-	-	2	No increase
	B	-	-	1	No increase

Acceptance Criteria

- Oral Preparations

	14d	28d
Bacteria	3	No increase
Fungi	1	No increase

- For oral products of antacids with an aqueous base the above criteria must be met, however the initial inoculum must be between 1×10^3 and 1×10^4 cfu/ml of product tested, as per USP.

BP and USP comparison

- Parenteral and ophthalmic preparations

	Category	6h	24h	7d	14d	28d
Bacteria	A	2	3	-	-	No recovery
	B	-	1	3 (1.0)	- (3.0)	No increase (no increase from 14d result)
Fungi	A	-	-	2	-	No increase
	B	-	-	- (no increase)	1 (no increase)	No increase (no increase)

(USP requirements)

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BP and USP comparison

- Topical preparations

	Category	2d	7d	14d	28d
Bacteria	A	2	3	-	No increase
	B	-	-	3 (2)	No increase (no increase from 14d result)
Fungi	A	-	-	2	No increase
	B	-	-	1 (no increase)	No increase (no increase)

(USP requirements)

BP and USP comparison

Oral Preparations

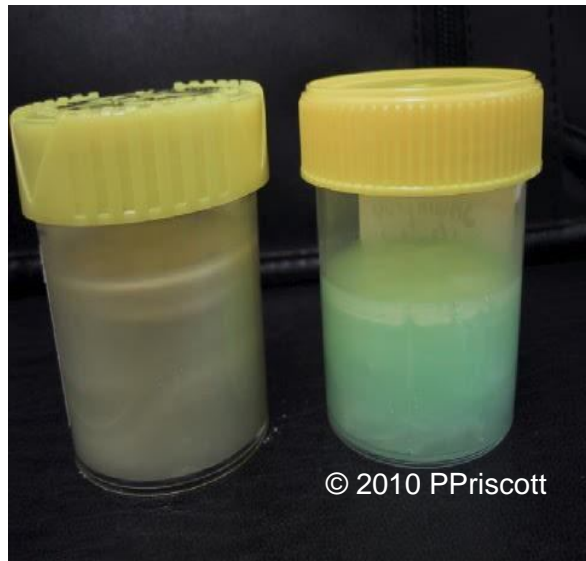
	14d	28d
Bacteria	3 (1.0)	No increase (no increase from 14d result)
Fungi	1 (no increase)	No increase (no increase)

(USP requirements)

Environmental Isolates

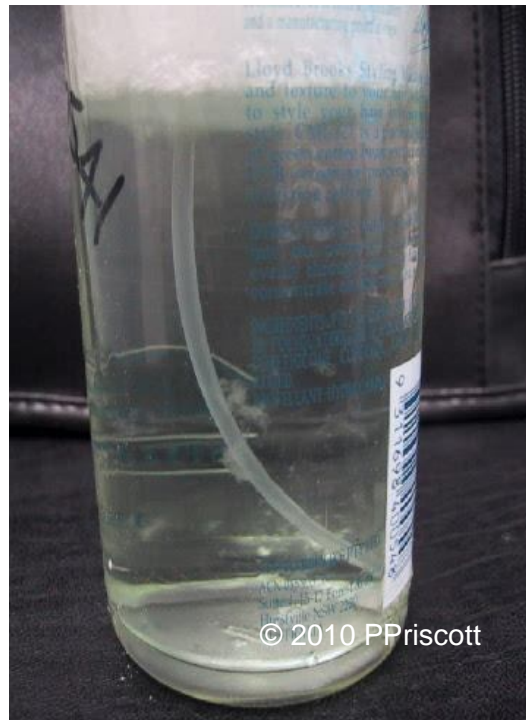
- Products may be more susceptible to environmental contamination during manufacturing due to the nature of the product and preservative system such as low pH or high water content.
- Although *Ps. aeruginosa* is included as a test organism, the reference culture strain may not be as resistant to the preservative system as various strains of *Enterobacter*, *Klebsiella* or *Burkholderia*.
- Manufacturing facilities with known environmental contaminants are recommended to isolate and include these strains in the PE testing.

What happens if the product gets contaminated



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P.aeruginosa

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Case Study

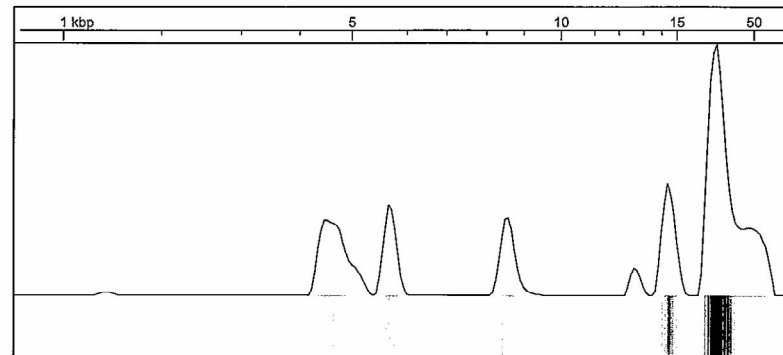
- The product was found to have high levels Total Aerobic Plate count levels exceeding the client specification.
- Identification of the isolated contaminant by both API and Riboprinting found it to be *Enterobacter gergoviae*.
- Comparison of the strain through the Riboprinter database found the strain to be unique for that facility.

Case Study

RiboPrinter® Microbial Characterization System
Sample 216-359-S-7 Report

 DuPont Qualicon

Process	KHA
Enzyme	EcoRI
Label	PU0801820
Sample Comment	
Event Log	



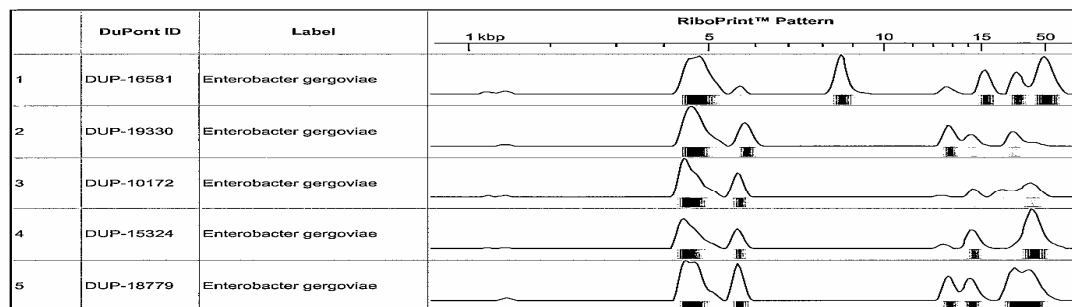
	Type	Number	Similarity	Label	RiboPrint™ Pattern	
					1 kbp	5 10 15 50
1	DuPont Neighbor	DUP-18779	0.80	<i>Enterobacter gergoviae</i>		
2	DuPont Neighbor	DUP-16581	0.77	<i>Enterobacter gergoviae</i>		
3	DuPont Neighbor	DUP-10172	0.74	<i>Enterobacter gergoviae</i>		
4	DuPont Neighbor	DUP-15324	0.72	<i>Enterobacter gergoviae</i>		
5	DuPont Neighbor	DUP-6617	0.68	<i>Vibrio parahaemolyticus</i>		
6	RiboGroup	ECORI 216-359-S-7	1.00			

Case Study

RiboPrinter® Microbial Characterization System
DuPont ID Report



DuPont Qualicon



Case Study

- AMS Labs performed PE testing with the addition of the isolated *E. gergoviae* strain.
- Results from the PE test shows no recovery of all four test organisms, however, the *E. gergoviae* counts far exceeded those of the reference cultures.
- This was found to be attributed to the lower pH of the product and the natural parabens resistance *E. gergoviae*.

Case Study

Time Point	<i>S. aureus</i> AMS 027 (ATCC6538)	<i>P.aeruginosa</i> AMS 095 (ATCC 9027)	<i>C.albicans</i> AMS 003 (ATCC 10231)	<i>A.niger</i> AMS 032 (ATCC 16404)	<i>Enterobacter</i> <i>gergoviae</i>
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.
N/A = Not Applicable

< = Less than

> = greater than

Case Study

- 3 other products – all of them topical preparations were found to show increased growth in *E. gergoviae* over the 28 day test.
- All results showed slight reductions in counts after 48 hours from the preservative system, however, resistance was demonstrated by increases in counts after 14 days with only one out of the four products tested showing a reduction in counts at day 28 from the 14 day count.



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Other microbiology-related changes

- see Karen Longstaff, OLSS, TGA - <http://www.tga.gov.au/lab/presmicrobiology.pdf>

Other microbiology-related changes

- **Sterile products**
- **Non-sterile products, must comply with harmonised pharmacopoeial requirements**
 - Aqueous oral
 - Non-aqueous oral
 - Rectal
 - Oromucosal, gingival, cutaneous, nasal & auricular
 - Vaginal
 - Transdermal patches
 - Inhalants
- **Complementary medicines**
 - Oral dosage form containing animal, vegetable or mineral ingredients
 - + / - boiling water to be added (“special” TGA requirements)

Other microbiology-related changes

- **Enterobacteria test replaced by harmonised method for Bile Tolerant Gram Negative (BTGN) organisms**
- **Greater emphasis on “objectionable” organisms**

Objectionable microorganisms

- The list (specified organisms) is not necessarily exhaustive and for a given preparation it may be necessary to test for other microorganisms depending on the nature of the starting materials and the manufacturing process.
- In addition to specified organisms the significance of other microorganisms recovered is evaluated in terms of:
 - Route of administration (hazard varies)
 - Formulation (ability to support growth, preservation, aw etc)
 - Method of application
 - Intended recipient (neonates, elderly, compromised)
 - Use of immunosuppressive agents, corticosteroids
 - Presence of disease, wounds, organ damage

from K Longstaff, 2009

Objectionable microorganisms

- Where warranted, a risk-based assessment of relevant factors is conducted by personnel with specialised training in microbiology and in the interpretation of microbiological data.

from K Longstaff, 2009

Thank you for your attention!

Please don't beat the messengers!

