www.microbiologyforum.org

PMF NEWSLETTER

A Publication of the Pharmaceutical Microbiology Forum
DISTRIBUTED INTERNATIONALLY

Volume 12, Number 4 April, 2006

In This Issue:

Editor's Message	1
The Harmonization of MLT - Absence of Specified Microorganisms Scott Sutton	2
The Importance of Data Analysis in DNA Fingerprinting Jeff Little	9
Upcoming Events	12
RMUG - Rapid Microbiology User's Group	12
Discussion List Update	12
Book Review - QC of Microbiology Labs by Lucia Clontz	14

The PMF gratefully acknowledges the contributions of :

- Vectech Pharmaceutical Consultants, Inc, (http://www.vectech.com)
- High Peaks Associates, Inc. (http://www.highpeaks.us)

to the continued success of the *PMF Newsletter*

Microbial Identification

Scott Sutton, Ph.D
Editor, PMF Newsletter

One of the central concerns of the microbiology laboratory is the identification of microbial samples. In this month's newsletter we look at two approaches to that question.

The first approach is found in the second of three articles in a series on the harmonized microbial limits tests. This month's article is a comparison of the current USP, Pharm. Eur. and the proposed harmonized method. It is clear from this comparison that the three methods are very different, and the question left to each laboratory is whether the existing methods to demonstrate "the absence of specified microorganisms" will meet the harmonized requirements. Detailed tables are provided directly comparing the critical aspects of the test among the three versions to aid in the evaluation.

The second approach to microbial identification is found in a review article that focuses on the practical application of a popular genotypic identification system—the RiboPrinter. As the author notes, "the RiboPrinter is only as informative as the operator allows it to be." The use of genotypic methods does not eliminate all need for controls and data interpretation, it is as needed as ever and demands an understanding of the theory and operation of the equipment. The author provides real-life examples of situations and the appropriate control methods to avoid misinterpreting the data provided by this method. In addition, suggestions are provided to enhance its utility as a source of information in an investigation.

RMUG gets mention as well - the Rapid Microbiology User's Group is offering free subscriptions to their newsletter for those interested in the introduction of alternate methods into the pharmaceutical industry.

This issue also sees a brief description of a recommended book - Quality Control Systems for the Microbiology Laboratory: The Key to Successful Inspections by Lucia Clontz (DHI, 2001). This book is a good guide for both the experienced laboratory manager as well as the new-comer to the field of pharmaceutical microbiology.

I hope that you find these articles of some use. Please do not hesitate to contact me with any questions, comments or concerns that you might have. In addition, this is your publication - please consider submitting an essay on any topic of interest to the professional microbiologist.

Scott Sutton, Ph.D.
scott.sutton@microbiol.org

The Harmonization of the Microbial Limits Test - Absence of Specified Microorganisms

Scott Sutton, Ph.D.
Vectech Pharmaceutical Consultants

The last issue of the *PMF Newsletter* (vol 12, no. 3) contained a review of the harmonization status of the Microbial Limits Tests – Enumeration Chapter. In addition, the article provided a brief overview of the compendial harmonization process as agreed to by the Pharmacopeial Discussion Group (PDG). In this article, I would like to focus on the other side of the Microbial Limits Tests – the "Absence of Specified Microorganisms" component.

There is a significant controversy in the United States over the intent of this evaluation. The FDA is bound by the concern expressed in the Code of Federal Regulations (21CFR 211.113 and 21CFR 211.165) relating to the importance of "objectionable microorganisms." This is not the concern of the compendial chapters. The controversy is worthy of discussion, but not the topic of this review – it will be discussed in length in the final of three articles on the harmonization of the microbial limits tests to be published in next month's newsletter.

What follows is a tabular presentation of the existing "Microbial Limits – Absence of Specified Microorganisms" tests from the current USP and Pharm Eur, as well as the draft harmonized document (the finalized document is extremely close to this version, but not release to the industry). It is provided as an aid to evaluation, and may assist in determining whether revalidation of method suitability studies is needed. It should be noted that this harmonization draft represents a true compromise by all parties, with (at least in the author's opinion) significant changes from the current USP, Pharm Eur and JP chapters.

Stages of the PDG Process*

1. Identification of the item to be harmonized
2. Investigation into existing texts
3. Proposal for Expert Committee Review
4. Official Inquiry
   This is the version published in the *Pharmacopeial Forum* or *PharmEuropa* for public comment
5. Consensus
   5A – Provisional
   This is the coordinating pharmacopeia's proposal for consensus. If all three agree, it goes on to 5B, otherwise work continues for consensus
   5B – Draft Sign-off
6. Regional Adoption and Implementation
   6A – Adoption
   6B – Implementation
7. Inter-Regional Implementation

* from USP Chapter <1196>

References:

EP. 2006. 2.6.13 Microbiological Examination of Non-Sterile Products (Test for Specified Micro-Organisms). Pharm Eur. 5.0:156-161

USP. 2003. <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms *Pharm Forum*. 29(5):1722-1733

USP. 2006. <61> Microbial Limits Tests USP 29:2503-2508

USP. 2006. <1196> Pharmacopeial Harmonization. USP 29:3031-3035

Table 1: Harmonized Chapter Numbering Scheme

USP	EP
<61> Microbiological Examination Of Nonsterile Products: Microbial Enumeration Tests	2.6.12 Microbiological Examination Of Nonsterile Products: Microbial Enumeration Tests
<62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms	2.6.13 Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms
<1111> Microbiological Quality of Nonsterile Pharmaceutical Products	5.1.4 Microbiological Quality of Nonsterile Pharmaceutical Products

Continued from page 2

Table 2. Absence of Specified Details			
Issue	USP <61> (current)	EP Chapter 2.6.13 (current)	Harmonized
Media Growth Promotion – Organisms for Trypticase Soy	• *Staphylococcus aureus* • *Escherichia coli* • *Pseudomonas aeruginosa* • *Salmonella*	• *Staphylococcus aureus* ATCC 6538 (NCIMB 9518, CIP 4.83) • *Escherichia coli* ATCC 8739 (NCIMB 8545, CIP 53.126) • *Salmonella typhimurium* (a strain not pathogenic for man is to be used) *Pseudomonas aeruginosa* ATCC 9027 (NCIMB 8626, CIP 82.118)	• *Staphylococcus aureus* ATCC 6538 (NCIMB 9518, CIP 4.83, NBRC 13276) • *Pseudomonas aeruginosa* ATCC 9027 (NCIMB 8626, CIP 82.118, NBRC 13275) • *Escherichia coli* ATCC 8739 (NCIMB 8545, CIP 53.126. NBRC 3972) • *Salmonella enterica ssp typhimurium* ATCC 14028 or *Salmonella enterica ssp abony* NCTC 6017 of CIP 80.39 *Candida albicans* ATCC 10231 (NCPF 3179, IP 48.72, NBRC 1594) (for SDA) **Note** – detail provided on method of preparation and culture
Media Growth Promotion – Methodology	Not detailed	Use less than 100 CFU per media in mixture.	• Detailed instructions for confirmation of nutritive, selective and indicative properties of media (see table below). Colonies are comparable in appearance and indication reactions to a previously used batch of medium • Use less than 100 CFU.
Method Suitability		• Use less than 100 CFU per media in mixture. • All tests must work in presence and absence of the product	Detailed instructions for confirmation of nutritive, selective and indicative properties of media in the presence of the product. (see table below)
Sample Volume	10 grams	10 grams	10 grams

Continued on page 2

Continued from page 3

Absence of Specified (Table 2 – cont.)			
Issue	USP <61> (current)	EP Chapter 2.6.13 (current)	Harmonized
Media Sterility Check	Not detailed	Combined with Negative Product Control	Recommended

Test for *Staphylococcus aureus*	• Bring specimen up to 100 mL with TSB
	• Incubate
	• If growth, streak on Vogel-Johnson Agar Medium (or Baird Parker Agar, or Mannitol-Salt Agar
	• Compare colonies for characteristics given – if absent, meets specification
	• If suspect colonies present, conduct coagulase test. Must be coagulase negative to meet specifications.
		• Bring specimen up to 100 mL with TSB and incubate at 35-37C for 18-48 hours
		• Streak onto Baird-Parker Agar and incubate at 35-37C for 18-72 hours.
		• Examine for black colonies of gram-positive cocci.
		• Confirm by biochemical tests.
		  • Coagulase
		  • Deoxyribonuclease test
			• Sample Preparation
			• Inoculate a suitable amount of TSB with 1 g of sample. Incubate at 30-35°C for 18 to 24 hours
			• Selection and Subculture
			• If growth, streak onto Mannitol Salt Agar and incubate at 30-35°C for 18-72 hours.
			• Examine colonies for distinctive morphology.
			• Confirm identity of suspect colonies.

Test for *Pseudomonas aeruginosa*	• Bring specimen up to 100 mL with TSB • Incubate • If growth, streak on Cetrimide Agar Medium • Compare colonies for characteristics given – if absent, meets specification • If suspect colonies present, streak colonies onto Pseudomonas Agar Medium for the Detection of Fluorescein and Pseudomonas Agar for the Detection of Pyocyanin. • Compare colonies for characteristics given on these additional agars– if absent, meets specification • Confirm suspect colonies with oxidase test. Must be oxidase negative to meet specifications.	• Bring specimen up to 100 mL with TSB. Incubate at 35-37°C for 18-48 hours • If growth, streak onto Cetrimide Agar and incubate at 35-37°C for 18-72 hours. Product passes if no growth. • If growth, gram stain. If gram-negative rods seen, transfer colonies to TSB and incubate at 41-43°C for 18-24 hours. If no growth the product passes.	• Sample Preparation • Inoculate a suitable amount of TSB with 1 g of sample. Incubate at 30-35°C for 18 to 24 hours • Selection and Subculture • If growth, streak onto Cetrimide Agar and incubate at 30-35°C for 18-72 hours. • Examine colonies for distinctive morphology. • Confirm identity of suspect colonies.

Continued on page 5

Continued from page 4

Absence of Specified (Table 2 – cont.)			
Issue	USP <61> (current)	EP Chapter 2.6.13 (current)	Harmonized

Test for *Salmonella* spp	• Bring specimen up to 100 mL with Fluid Lactose Medium
	• Incubate
	• If growth, pipet 1 mL into 10 mL:
	  • Fluid Selenite Medium
	  • Fluid Tetrathionate Medium
	• Incubate 12-24 hours
	• Streak growth of both Fluid Selenite and Fluid Tetrathionate onto:
	  • Brilliant Green Agar
	  • Xylose-Lysine-Deoxycholate Agar
	  • Bismuth Sulfite Agar
	• Incubate for growth – examine colonies for characteristic morphology.
	• If colonies with characteristic morphology seen, gram stain and examine for gram-negative rods.
	• Stab-Streak colonies with gram-negative rods into a butt-slant of Triple Sugar-Iron-Agar. Incubate the slants and examine for red slants with yellow butts. If seen, product fails specification
		• Bring specimen up to 100 mL with TSB and incubate at 35-37C for 18-48 hours
		• Transfer 1 mL to 100 mL Tetrathionate Bile Brilliant Green Broth and incubate at 41-43°C for 18-24 hour.
		• Subculture on at least two agars:
		  • Deoxycholate Citrate Agar
		  • Xylose, Lysine, Deoxycholate Agar
		  • Brilliant Green, Phenol Red, Lactose Monohydrate, Sucrose Agar
		• Examine colonies for distinctive morphology
		• Stab-Streak colonies with gram-negative rods into a butt-slant of Triple Sugar-Iron-Agar. Incubate the slants and examine for red slants with yellow butts.
		• Confirmation may be done using biochemical and serological tests.
			• Sample Preparation
			• Inoculate a suitable amount of TSB with 1 g of sample. Incubate at 30-35°C for 18 to 24 hours
			• Selection and Subculture
			• If growth, transfer 1 mL to 10 mL Rappaport Vassiliadis Salmonella Enrichment Broth. Incubate at 30-35C for 18-24 hours.
			• If growth, streak onto Xylose-Lysine-Deoxycholate Agar
			• Examine colonies for distinctive morphology.
			• Confirm identity of suspect colonies.

Continued on page 6

Continued from page 5

Absence of Specified (Table 2 – cont.)			
Issue	USP <61> (current)	EP Chapter 2.6.13 (current)	Harmonized
Test for *Escherichia coli*	• Bring specimen up to 100 mL with Fluid Lactose Medium • Incubate • If growth, streak onto MacConkey Agar Medium. • Incubate, then examine for distinctive colony morphology. • If colony morphology seen, transfer suspect colonies to Levine Eosine-Methylene Blue Agar Medium and streak for single colonies. • Incubate, and examine for distinctive colony morphology. If seen, product fails specification	• Bring specimen up to 100 mL with TSB and incubate at 35-37°C for 18-48 hours • Transfer 1 mL to 100 mL MacConkey Broth and incubate at 35-37°C for 18-72 hours. • Streak onto MacConkey Agar and incubate at 35-37°C for 18-48 hours. • If distinctive colony morphology seen, confirm with suitable tests, such as indole production.	• Inoculate a suitable amount of TSB with 1 g of sample. Incubate at 30-35°C for 18 to 24 hours • If growth, transfer 1 mL to 100 mL MacConkey Medium. Incubate at 42-44°C for 24-48 hours. • If growth, streak onto MacConkey Agar and incubate at 30-35°C for 18-72 hours. • If no growth, or if confirmatory tests show absence of *E coli*, product passes
Test for Bile-tolerant Gram-negative Bacteria	• None	• None	Sample preparation • Suspend not less than 1 gram sample in TSB, incubate at 20-25C for 2-5 hours Test for Absence • Use the volume corresponding to the limit required to inoculate a suitable amount of Mossel Enterobacteriaceae Enrichment Broth. Incubate at 30-35°C for 24-48 hours. • If growth, streak onto Violet Red Bile Glucose Agar Medium and incubate at between 30-35°C for 18 to 24 hours. • Product passes if no red colonies surrounded by a reddish precipitate. • Quantitative Test • Use the sample prepared above under "Sample Preparation" corresponding to 10^{-1}, 10^{-2}, 10^{-3} dilutions into Mossel Enterobacteriaceae Enrichment Broth. Incubate at 30-35°C for 24 to 48 hours. • Plate samples onto Violet Red Bile Glucose Agar Medium. Incubate for growth. • Note the smallest quantity of the product that gives growth.

Continued on page 7

Continued from page 6

Absence of Specified (Table 2 – cont.)			
Issue	USP <61> (current)	EP Chapter 2.6.13 (current)	Harmonized
Test for Enterobacteria and Certain Other Gram-Negative Bacteria	None	Detection Suspend sample in Lactose monohydrate broth, incubate at 35-37C for 2-5 hours Transfer contents equivalent to 1g product to 100 mL Enterobacteria Enrichment Broth – Mossel. Incubate for 18-24 hours. Passes if no growth Quantitative Evaluation Dilute the Lactose monohydrate broth suspension 10^{-1}, 10^{-2}, 10^{-3} into Enterobacteria Enrichment Broth – Mossel. Incubate for 24—48 hours. Plate samples onto Crystal violet, neutral red, bile agar with glucose media. Incubate for growth. • Note the smallest quantity of the product that gives growth.	None
Test for Clostridia	None	Specific tests for the presence of Clostridia, and for the enumeration of *Clostridia perfringens*	Specific tests for the presence of Clostridia
Test for *Candida albicans*	None	None	• Inoculate a suitable amount of SDB with 1 g of sample. Incubate at 20-25°C for 5-7 days • If growth, streak onto SDA Incubate at 20-25°C for 2 days • Examine colonies for distinctive morphology. • Confirm identity of suspect colonies. • If no growth, or if confirmatory tests show absence of *C. albicans*, product passes
Additional Controls	None	Use sterile sodium chloride-peptone solution pH 7.0 as test preparation to test: • Sterility of Medium • Sterility of Diluent • Aseptic Performance of the test	Use Sterile Diluent as the test preparation for each batch of diluent to verify testing conditions.
Interpretation of Results	• Must meet specs • Retest allowed using 25 gram sample	• Must meet specs • No retest	• Must meet specs • No retest

Continued on page 8

Continued from page 7

Table 3. Nutritive, Selective and Indicative Properties of Media (Harmonized Draft)

Medium	Property	Test Strains
Test for Bile-tolerant Gram-negative Bacteria		
Mossel Enterobacteriaceae Enrichment Broth	Nutritive	*Escherichia coli, Pseudomonas aeruginosa*
	Selective	*Staphylococcus aureus*
Violet Red Bile Glucose Agar Medium	Nutritive & Indicative	*Escherichia coli, Pseudomonas aeruginosa*
Test for E. coli		
MacConkey Medium	Nutritive	*Escherichia coli*
	Selective	*Staphylococcus aureus*
MacConkey Agar Medium	Nutritive & Indicative	*Escherichia coli*
Test for Salmonella		
Rappaport Vassiliadis Salmonella Enrichment Broth	Nutritive	*Salmonella enterica ssp.typhimurium* (or *S. enterica ssp. abony*), *E. coli*
Xylose-Lysine-Deoxycholate Agar	Nutritive & Indicative	*Salmonella enterica ssp.typhimurium* (or *S. enterica ssp. abony*), *E. coli*
Test for Pseudomonas aeruginosa		
Cetrimide Agar Medium	Nutritive	*Pseudomonas aeruginosa*
	Selective	*Escherichia coli*
Test for Staphylococcus aureus		
Mannitol Salt Agar Medium	Nutritive	*Staphylococcus aureus*
	Selective	*Escherichia coli*
Test for Clostridia		
Reinforced Medium for Clostridia	Nutritive	*C. sporogenes*
Columbia Agar Medium	Nutritive	*C. sporogenes*
Test for Candida albicans		
Sabouraud Dextrose Medium	Nutritive	*C. albicans*
Sabouraud Dextrose Agar Medium	Nutritive & Indicative	*C. albicans*

The Importance of Data Analysis in DNA Fingerprinting

Jeff Little
Vectech Pharmaceutical Consultants

Standard tests used for decades in microbiology laboratories are becoming obsolete with the innovative equipment now being introduced into the industry. The introduction of the new equipment is being driven by the need for the QC microbiology laboratory to be accurate, efficient, and cost-conscious. Any capital acquisition involves the initial start-up cost, the cost for validation, and training cost for its users. This gives the potential buyer an overabundance of information that should be carefully analyzed before spending capital on equipment that may not be as functional without the appropriate planning and training infrastructure in place.

One such system that provides numerous benefits in the field of microbial identification is the RiboPrinter*, manufactured by DuPont Qualicon. This product has been extremely well-received in the marketplace as an automated, genotypic microbial identification system. In addition, the RiboPrinter has a great deal of literature support to provide valid evidence of its usefulness for desired applications (see below).

Through the RiboPrinter's patented design, with but a few simple manual manipulations by the operator, the operator "picks" a sample from a pure, well-isolated colony (typically from a plate where the initial colony was streaked for isolation) The RiboPrinter then performs an automated series of assays on the "picked" colony sample. The cells are lysed, and the chromosomal DNA is digested with a user-selected restriction endonuclease. The DNA cleavage fragments are then run through a Southern Blot, using a probe homologous to the 16S ribosomal RNA (rRNA) codon region. The RiboPrinter then generates a digital photo, expressed as a RiboPrint pattern for automated data analysis against a validated database of bacterial species RiboPrint patterns allowing identification of the microorganism (Blanc, 1993). The automated design of the RiboPrinter is beneficial by reducing the possibility of operator error, while maintaining consistency between each sample and batch run, and provides visual results that can, if needed, be recreated within an internal laboratory.

This system compares favorably with far more extensive systems that require a much higher level of technological sophistication (Gilespie and Oliver 2004). Not every QC microbiology lab can afford to hire and maintain PCR or DNA sequencing experts, and the RiboPrinter provides an automated genotypic method to these facilities (Grif *et al* 2003, Jeffrey 2004, Andolina *et al* 2004, Sarwari *et al* 2004, Sutton and Cundell 2003).

However, like all innovative products, the RiboPrinter is only as informative as the operator allows it to be. Even with the potential for reduction of overhead costs associated with the RiboPrinter, one key factor still remains – data interpretation! The benefits of this automated genotypic identification system are clear, but even the most reliable information is useless if the generated data is misinterpreted. This article focuses on some of the potential issues that might interfere with using the equipment to its full potential, and how to resolve them. It must be noted that some of the solutions suggested may have an impact on the validation (qualification) status of the equipment or the software on existing systems. As with all changes to validated systems, care should be taken to fully evaluate the risk inherent to any change in procedure. However, many of the recommendations discussed will dramatically enhance the operation and accuracy of the RiboPrinter results and should also be evaluated from that perspective.

Defined terms:

RiboPrint Pattern: After completion of the Southern Blot process, a digital picture is taken of the entire batch sample run. Each sample's banding pattern is separated from the overall batch sample run, and each independent sample band pattern is displayed horizontally with a molecular weight scale range. This banding pattern allows the operator to visually observe the banding pattern of each sample of interest.

Number: Each independent sample, within each batch sample run, is identified by the RiboPrinter by using eight digit numerators. The first, three digit number set is the unique number used to identify the specific RiboPrinter (000-001-S-1) that the sample was run on. Each RiboPrinter has its own unique three number ID. The second, three digit number set corresponds to the batch run number (000-001-S-1: where the "001" denotes the first batch ran on system "000"). The third digit is an "S,", "M," or "C" denoting whether a Sample, Marker (Standard) or Control was run in the specified lane. The final digit corresponds to the sample position within the well/lane of the batch run (000-001-S-1). The sample/control number always ranges between the numbers "1-8", allowing the operator an easy and efficient way to track and sort samples.

RiboGroup: Each RiboPrint Pattern is given a unique corresponding number based on the percent of similarity between the new sample pattern and each pre-existing sample pattern previously identified by the RiboPrinter algo-

(Continued on page 10)

(Continued from page 9)

rithm. The generated RiboGroup typically corresponds to the very first time the unique sample pattern was identified by the RiboPrinter. Therefore, if this new sample is identified as a pre-existing RiboPrint® Pattern, the original Number would be used as the new RiboGroup number. When the RiboPrint Pattern is not identified as a pre-existing pattern, the new Number (see above, Defined terms: #2) is assigned to the sample.

DuPont ID Label: The organism name (associated with a unique RiboPrint) is based on the percent of similarity between the new sample pattern and each pre-existing RiboPrint pattern present in the DuPont Identification database or the user populated Custom Identification databse. This DuPont ID Label provides an easy tool for the operator to use when interpreting the generated RiboPrint Pattern.

Possible areas to be considered:

Unidentified organism(s): Not even the known banding patterns of over 6400 patterns can accommodate each and every possible microorganism that might be identified. For this very reason, there are times when no DuPont ID Label will be provided, even though a unique RiboGroup is provided. When a new RiboPrint Pattern is identified, if the pattern shows at least 85% similarity with a pre-existing RiboPrint Pattern, the corresponding RiboGroup, and DuPont ID Label associated with the RiboGroup, will be displayed. This seems straightforward, but there are instances when the percent of accuracy falls below 85%. Between 82-84%, a pre-existing RiboGroup may be identified; however, the corresponding DuPont ID Label is not displayed. Although this is not necessarily a concern, how the operator proceeds once this situation presents itself needs to be expressed and understood within internal polices and documentation guidelines of the company (also referred to as Standard Operating Procedures – SOP). Does the existing SOP direct the operator on how to handle this situation?

Band pattern(s): Band patterns are groupings of DNA fragments that have been cut with a restriction endonuclease at specific locations along the DNA strand. Using gel electrophoresis, the fragments are separated primarily by size and charge along the gel matrix. The double-stranded DNA is then melted and hybridized to the specific chemiluminescent probe. The more homologous DNA of a given size, the more intense certain bands will appear on the image. The light emission produced by the chemiluminescent "tagged" fragments can be visually observed and recorded. Each item listed below is a separate issue that should be addressed prior to completion of a batch run.

Band(s) shifting: Caused from any variety of issues; not limited to, change of electrical charge used during gel separation, varying thickness of gel / improper pouring of gel, inappropriate gel concentration used (.1% instead of .2%), air bubbles within the gel complex. These may cause discrepancies between the exact distances the band(s) migrated through the gel. Should this be the case, the percent of similarity between new and predetermined RiboPrint Pattern(s) may differ significantly. Although the visual results may not be observable by the unaided eye, the RiboPrinter algorithm software is extremely sensitive and may recognize a subtle differential of band signal (peak intensity) and corresponding orientation of the RiboPrint Pattern(s) in question. Therefore, the operator needs to have a predetermined procedure on how to interpret significant changes based on the expected and actual placement of the bands. Running the sample on a gel with molecular weight markers decreases the number of samples that can be run, but is enormously helpful in the standardization of the data.. Does the existing SOP direct the operator on how to handle this situation?

Relative peak intensity: Peak intensity corresponds to the amount of fluorescence emitted from a RiboPrint Pattern band, and detected by the RiboPrinter. Relative peak intensity compares a corresponding band fluorescence of one RiboPrint Pattern band, with the same band fluorescence of another RiboPrint Pattern. Although the peak intensity itself should not interfere directly with the identification process used by the RiboPrinter, when combined with band shifting (see above) the relative peak intensities may vary greatly from the individual peak intensities analyzed by the RiboPrinter. Differences in peak intensity may also be caused due to the concentration of probe used during the preparation stage of the sample run, relating to the overall florescence identified by the RiboPrinter. The main concern with relative peak intensities is part fluorescence and part band placement. Since the algorithm of the RiboPrinter determines the RiboGroup and percent of similarity from both new and pre-existing RiboPrint Patterns, the operator needs to have a clear understanding in identifying how to recognize all of the variations possible between banding regions, and why some RiboPrint patterns may seem completely different, yet resemble the same percentage of similarity.

Empty lane(s): On rare occasions a lane expected to display a banding pattern with have no image. This could be due to technician error or other factors. The sample needs to be run fresh as this is an invalid result.

Smearing / "Junk" banding: Regardless of technique used, there may be situations that arise where band smears and or "junk" banding occurs. Smears are due to the unclear separation of the DNA during the gel electrophoresis process (typically caused by change in electrical charge of the gradient being used); whereas, "junk" is DNA that was either not properly cut or where an overabundance of DNA (or limited amount of enzyme added) is loaded into a well. Smearing complicates the algorithm recognition completed by the RiboPrinter due to the

(Continued on page 11)

(Continued from page 10)

unclear light emission picked up by the digital camera. "Junk" decreases the accuracy of the algorithm by creating an additional amount of uncut strains of DNA that tend to form one large banding mass. Although smearing is easier for an operator to identify, "junk" becomes problematic for the operator due to its difficulty in identifying a valid band from a band mass created from the collection of uncut DNA. Although the cost may seem excessive on a routine basis, during an investigation, it may be worthwhile for the operator to rerun the sample, with either a different enzyme, or run alongside a positive control, molecular weight marker, or some other sample that may assist the operator in identifying true placement of the banding pattern.

Percent similarity of nearest neighbors: There may be instances were the RiboPrint Pattern displayed may have multiple listings of similar organisms within the same percentile range, yet seem completely different. This percentage is determined by the algorithm within the RiboPrinter. As with all algorithms, the correct information must be provided for the algorithm to be calculated properly. Even though all operator conditions may remain consistent, due to the above points ("a" thru "d" above), these variations will provide the RiboPrinter algorithm with slight discrepancies. Each time a completed report is generated, the operator needs to review the results and verify that the percent of similarity, generated by the algorithm, seems reasonable. Having a way to deal with such issues helps provide a fast and efficient way to uncover not only a reliable banding pattern, but also a better understanding of how the algorithm is calculated.

The RiboPrinter is a user-friendly fully automated, genotypic identification system for the QC microbiology laboratory. The distinct advantages of the RiboPrinter design allows the operator to interface directly with the enzymes needed by the specific testing requirements. This is particularly useful if the RiboPrinter is used during an investigation, as the use of multiple enzymes will frequently allow the identification of the unknown bacteria down to the strain level (Brisse 2004).

Although Dupont offers two standard restriction enzymes, EcoRI and PvuII, the operator has the option of using alternate enzymes independent of the RiboPrinter system's standards. The use of these alternate enzymes can be very useful during investigations in an attempt to distinguish between two strains of microorganisms.

USP Corner

Any questions concerning USP documents should be sent to Radhakrishna (Radha) Tirumalai, Ph.D. You can reach Dr. Tirumalai at: (706) 353-4514, via mail at United States Pharmacopeia, 126 Twinbrook Parkway, Rockville, MD 20852 or via e-mail at RST@USP.org. You can write representing your company, or as an individual scientist.

A few additional tips:

Use of Molecular Weight Controls: When starting a batch sample run, it may be extremely useful to include a positive control along with the other sample lanes. The positive control can be of any type of sample with known characteristics previously identified by the RiboPrinter. The two types commonly used are a set of molecular weight markers used as standards, or a controlled microorganism used routine to check the RiboPrint batch operation. Although the addition of a set of molecular weight controls to a batch run means one less sample can be run per batch, it provides a positive control for the operator and the entire electrophoresis/blotting process. Running this control within the same batch as the other seven samples allows the operator to easily determine if a noticed discrepancy in the banding pattern(s) is due to partial digestion, hybridization issues, smearing, signal strength, or some other concern. In addition, using two controls allows the operator to interpret the RiboPrint Pattern with even more efficiency, although at the cost of two less samples per batch run.

Manually changing "DuPont ID Labels": Another useful tool allows the operator to manually add/change a "DuPont ID Label" name that was originally assigned by the validated database of the RiboPrinter, to a specific RiboPrint Pattern. This might be done because the sample's RiboPrint pattern fell below

(Continued on page 13)

Internet Address	Description
http://dept.kent.edu/biosim/	This virtual lab is designed to serve as a study guide for the identification of unknown bacteria. The object is for you, the microbiology student, to identify a variety of unknown isolation cultures. By performing a series of simulated tests like the ones actually used in the lab, you will improve your ability to identify microorganisms.
http://www.microbes.info/	Microbes.info is an internet web site designed to list useful and interesting microbiology informational resources. to you. This web site attempts to help filter through the available sites in an organized manner.
If you have found an Internet site that contains information of relevance to pharmaceutical microbiology, please let us know.	

Upcoming Events

April

- 18th **Delaware Chapter Meeting of PDA**
  Location: Desmond Hotel, Malvern, PA
  Web Site: http://www.pdadelval.org
- 24th –29th **PDA Annual Meeting**
  Location: Anaheim Marriott, Anaheim, CA.
  Web Site: http://www.pda.org/annual2006

May

- 3rd - 5th **Environmental Monitoring and Stability Technical Seminar & Workshop**
  Location: New York, NY
  WebSite: http://www.novaseminars.com/
- 18th - 19th **Microrite's Training in Contamination Monitoring and Control**
  Location: Crowne Plaza, Minneapolis North
  Email Contact: info@microrite.com
- 21st - 25th **ASM Annual Meeting**
  Location: Orange County Convention Center, Orlando, FL
  Web Site: http://gm.asm.org/

June

- 5th - 6th **PMF Microbiology GMP Conference**
  Location: Philadelphia, PA
  Web Site: http://www.highpeaks.us/2006/GMP/
- 26th - 29th **Aseptic Processes and Sterile Processes**
  Location: Amsterdam, The Netherlands
  Web Site: http://www.ivthome.com/shop/Scripts/prodList.asp?idcategory=2&sortField=STARTDATE

RMUG

Sign up for the latest information on Rapid Microbiology in the Pharmaceutical Industry

The mission of the Rapid Microbiology Users Group® is to advance the field of Rapid Microbiology by producing a forum to educate, encourage, support, and challenge the pharmaceutical, cosmetic, and food industries. We fulfill this mission by publishing and distributing a monthly newsletter, hosting an annual conference, and fostering an environment for debate, discussion, and interaction with peers and regulatory agencies.

The RMUG mail list needs to be confirmed - please sign up by sending an Email to rmug@vectech.com to receive the free newsletter on developments in the field of Rapid Microbiological Methods (RMM) in the pharmaceutical industry.

Offering In-House Courses on Microbiology/Aseptic Processing:

- The Microbiology Network/High Peaks Associates
  http://www.highpeaks.us/in-house.htm
- USP
  Contact Steven Paul (stp@usp.org) for information on the course "Fundamentals of Microbiological Testing"

Discussion List Update

PMFList:
Number of Subscribers: 1,482
Number of Countries: 62
Number of Messages Last Month: 236

PSDGList (Pharma Stability Discussion Group):
Number of Subscribers: 780
Number of Countries: 19

Membership is FREE. To **join the PMFList**, visit http://microbiol.org/pmflist.htm and register.

A sister Email is devoted to topics in the **stability testing** of pharmaceuticals, medical devices and personal products. To **join the PSDGList**, visit http://microbiol.org/psdglist.htm and register.

You can ask, answer, or read questions and comments from your colleagues. Archives of the lists are available at:

- http://lists.microbiol.org/archives/PMFLIST.html
- http://lists.microbiol.org/archives/PSDGLIST.html

(Continued from page 11)

the 0.85 cut-off value for similarity in the database, or for reasons particular to that lab. This operator-initiated change is captured in the software's audit trail, and is denoted in the reports with brackets "{}" surrounding the modified entry in the DuPont ID Label field of the report once it is modified (example – *Bacillus subtilis* could be changed to {*Bacillus pumilus*}). Tight document control must be in place on this function, so that an audit trail can be easily followed especially when this change is prompted by microbial identification work conducted independently of the RiboPrinter. It may simplify cost and reference resources to list where the source of modification was found along with the modified entry (example: {*Staphylococcus aureus* – latex aggl. test}).

Creating an Internal Reference Library: Since every sample run through the RiboPrinter is assigned a specific RiboGroup, each sample can be stored and retrieved as part of an Internal Reference Library (IRL). DuPont Qualicon offers upgrades for the validated database, allowing the user to download new RiboPrint Patterns and add them to the current systems IRL database as an additional resource. In addition, the IRL can be expanded by testing a sample with different enzymes, and the eventual identification of the sample will then be associated with the different RiboPrint banding patterns. This allows for a more extensive identification of the microorganism.

Outsourcing samples to another testing facility: Should it be necessary to outsource samples, whether due to limited funding, time constraints, or other considerations, it is of great importance to make certain the outsourced system is validated, and it provides information that is compatible with the results generated by the RiboPrinter.

Summary

There are many key features which the RiboPrinter brings to a QC microbiology laboratory. Being a fully automated genotypic identification system enables reproducible, accurate results that free-up time for technicians to complete other essential tasks in parallel. Furthermore, with the ability to use any enzyme of choice, the RiboPrinter provides accurate results that can be stored and created into a fast and efficient IRL, reducing the time needed to identify unknown samples to within minutes. Although the technicians operating the RiboPrinter are just as essential as the reviewer of the generated reports, and the individual lead delegating the duties for a given project, each person involved with the project needs to be familiar with the extensive capabilities of the RiboPrinter to provide the laboratory with the most crucial information available in the marketplace today.

References

Andollina, A *et al*. 2004. Identification and Genetic Characterisation of Orthopaedic Staphylococcus Isolates Collected In Italy By Automated EcoRI Ribotyping. *FEMS Microbiol Lett*. 234:275-280.

Blanc, DS *et al.* 1993. Ribotyping of *Pseudomonas aeruginosa*: Discriminatory Power and Usefulness As a Tool for Epidemiological Studies. *J Clin Microbiol*. 31(1):71-77

Brisse, S *et al*. 2004. Species Distribution and Ribotype Diversity of Burkholderia cepacia Complex Isolates from French Patients with Cystic Fibrosis. *J Clin Microbiol*. 42 (10):4824-4827

Gillespie, BE and S.P. Oliver. 2004. Comparison of an Automated Ribotyping System, Pulsed-Field Gel Electrophoresis and Randomly Amplified Polymorphic DNA Fingerprinting for Differentiation of *Streptococcus uberis* Strains. *Biotechnol*. 3(2):165-172.

Grif, K et al. 2003. Identifying And Subtyping Species Of Dangerous Pathogens By Automated Ribotyping. *Diagn Microbiol Infect Dis*. 47(1):313-320.

Jeffrey, M *et al*. 2004. Validation of an Enhanced Method of Bacterial Ribotyping for Improved Efficiency and Identification of Stressed Isolates. *Pharm Technol*. 28(3):156-166.

Sarwari A, *et al*. 2004. PCR Identification and Automated Ribotyping Of Pseudomonas aeruginosa Clinical Isolates From Intensive Care Patients. *Scand J Infect Dis*. 35 (5):342-349.

Sutton, SVW and AM Cundell. 2004. Microbial Identification in the Pharmaceutical Industry. *Pharm Forum* 30(5):1884-1894.

Quality Control Systems for the Microbiology Laboratory: The Key to Successful Inspections

Lucia Clontz (DHI Publications, 2001)

Scott Sutton, Ph.D.
Vectech Pharmaceutical Consultants

Unlike many in the excellent PDA/DHI collection of resources for the pharmaceutical scientist, this book has the virtue of being less than 3 inches thick. The author (a founding leader and past president of the PMF) succinctly summarizes the critical areas of concern for a QC Microbiology in the area of GMP compliance in less than 200 pages. These areas can be summarized as:

- Chemical and Reference Standards (Chapter 2)
- Laboratory Equipment and Facilities (Chapter 3)
- Preparation of Media, Buffers and Reagents (Chapter 4)
- Environmental Monitoring and Trending (Chapters 5 and 7)
- Water Systems for Laboratory Use (Chapter 6)
- Use of Disinfectants and Sanitizers (Chapter 8)
- Training (Chapter 9)
- QA for the Laboratory (Chapter 10)

Each of the topics is handled from both a theoretical/ historical perspective and from a practical one. There are several issues in GMP compliance that will not necessarily make sense to an individual lab, these are in large part identified and explained by the author in the discussion of compliance issues surrounding each topic.

One difficulty with this topic is due to the rapidly changing nature of cGMP. An assumption of the book is that the FDA will adopt an adversarial stance in audits, a situation that the Agency has acknowledged as a concern and has been addressing. The recent release of the "cGMP for the 21st Century" initiative with PAT, dispute resolution, comparability protocols, *etc* underscore the Agency's desire to move to a more cooperative stance. As all of these have occurred since the 2001 publication of this book, it is no surprise that some of the authors concerns seem overstated in today's climate. However, even if overstated, the specific recommendations that recur throughout the book (use good science, keep up with guidance documents, have a reason for your procedures) are every bit as valid. The author does a good job of highlighting the need for good basic science in the QC laboratory.

I would have liked to see a bit more coverage of some topics. For example, Chapter 2 on reference standards provides an excellent discussion of antibiotic potency testing. This discussion goes into detail on the theory and the mathematics behind the biological assay, but only from a US perspective. The Latin Square design and issues specific to the Pharm Eur test are not mentioned. Similarly, issues surrounding finished product and release tests (the bacterial endotoxin test, antimicrobial efficacy test, sterility tests *etc*) are barely mentioned. The author has written excellent treatments of the topics in other books. The current focus on lab controls and assays is another example of the changing focus of cGMP in the lab, and one not as prevalent when the book was written.

The reader should not interpret the last two paragraphs as a strong criticism of the book. It is always possible review a book and identify issues that could be handled in a different manner. What is remarkable about this book is the overall treatment of the subject matter. The treatment of the microbiology function as a separate GMP topic worthy of in-depth review is refreshing and a much needed focus on this area of the pharmaceutical industry. This is an important book for the microbiologist in the pharmaceutical, medical device, or personal products industries.

A copy of this book will be provided to registrants at the PMF Microbiology GMP Conference June 5 - 6, 2006

The discussion of water systems is excellent, as is the review of disinfectants and sanitizers. It is an interesting exercise to compare the author's strong treatment of training in the QC microbiology lab with the PDA's technical report on the subject—the author's treatment doe not come up lacking with its focus on the practical aspects of a training program. The overall emphasis on the microbiology laboratory is a much-needed contribution to the discussion of the "Quality" of the QC organization.

The need for this discussion is growing. Recent FDA inspection trends, and guidance documents, have highlighted the microbiology laboratory as a critical operation to the overall quality production of product. USP has recently issued a draft informational chapter on microbiological laboratory practices that will be implemented by year's end and included in the 2007 USP. ISO has released document 17025 which addresses concerns for contract testing laboratories. All these documents focus on the need for good quality microbiology work. Quality Control Systems for the Microbiology Laboratory: The Key to Successful Inspections is a treatment of the subject that goes beyond the regulatory injunctions to provide some of the background as to why and how the particular concerns apply to the quality of the data from a microbiology lab. This book will be of great value to any whose responsibilities include either conducting audits of microbiology laboratories, those preparing for the audits and those whose responsibilities include the accurate generation, reporting or review of microbiological information.