PMF NEWSLETTER

A quarterly publication of the Pharmaceutical Microbiology Forum

DISTRIBUTED INTERNATIONALLY

Volume 5, Number 3 Fall 1998

IN THIS ISSUE:

PURPOSE: To provide a forum for discussion of microbiology issues in the pharmaceutical industry.
The information contained in this newsletter is the professional opinions of our members and does not represent the policies or operations of any corporation or government agency to which members may be associated. *PMF Newsletter* is intended to serve as an open forum and confidentiality will be maintained. The information in *PMF Newsletter* is solely for information purposes and is developed from sources believed to be reliable. Statements expressed constitute current opinions derived through analysis of available information and professional networking. Articles or opinions are for information only for PMF members to stimulate discussion and are not the views of the PMF board or regulatory agencies. The *PMF Newsletter* cannot make any representations as to the accuracy or completeness of the information presented and the publisher cannot be held liable for errors.

President's Message

The future of Microbiology looks brighter than ever. You may have seen reports of microorganisms found in the water system of the space station. In the area of water microbiology there are great advances in generation of results. New rapid methods can assist and cause a very positive impact. We have discussed ATP Bioluminescence in this newsletter in the past. Now it is time to look at a new technology that produces results in only 90 minutes. I am referring to Fluorescence Labeling with Laser Scanning currently marketed by Chemunex. This technology can detect as low as one cell and does not require the cell to divide. Only viable cells are able to incorporate a non-fluorescent stain and the esterases in the cell cytoplasm cleave the stain producing a fluorescent compound that is picked up by the laser. As this technology can cause profound changes in the way you set up and run your laboratories obtaining same day results, we will provide you with more information in a future publication. In the meantime, you can call the vendor for more details and reprints of papers related to this technology. Call Chemunex at 1-800-411-6734 or e-mail them at ChemunexUS@AOL.com.

I would also like to take this opportunity to announce that the PMF Board of Directors has decided to implement a renewal fee of $10.00 for those members who joined **before** 1998. This is the first time since we were founded in 1992 that a renewal fee will be in effect. The Board of Directors decides on April of each year if renewal fees are necessary. The Board is anticipating additional expenses in the coming year. The fees are used only to cover operational expenses of the organization and none of the directors, editors, or advisors receives compensation for his/her time and contributions.

Renewal fees must be received by the end of February, 1999 in order to receive the PMF Newsletter in 1999. Send your renewal fee to Cindy McInnis, our Assistant Treasurer, as indicated in PMF's membership form. Please direct any questions or concerns regarding this or any PMF matter to me at (850) 763-5453 or preferably via e-mail at EMSOURCE@AOL.COM. You may also contact any of the board members at their e-mail addresses published in this issue.

Laura

Internet Resources for the Pharmaceutical Worker

Part 2 of 2

Scott Sutton, Ph.D.

Specific Web Sites

Listed below are several sites of general interest to the pharmaceutical worker:

EMEA- http://www.eudra.org/emea.html
FDA CBER- http://www.fda.gov/cber/
FDA CDER- http://www.fda.gov/cder/
FDA Center for Devices and Radiological Health- http://www.fda.gov/cdrh/index.html
FDA Main Site- http:/www.fda.gov
ICH- http://www.ifpma.org/ich1.html
Microbiology Network- http://www.microbiol.org
PDA- http://www.pda.org
USP- http://www.usp.org

Newsgroups
The Internet allows a cheap means of communications for many people. This has spawned a large number of discussion groups via USENET. Many of these are of interest. The search tools below allow you to scan the messages in these groups to find those of interest.

Alta Vista- http://www.altavista.digital.com/
DejaNews Research Service- http://www.dejanews.com/
Excite- http://www.excite.com/query.html
InfoSeek- http://www2.infoseek.com/Query
Liszt-http://www.liszt.com/

Gophers
Immense amounts of very useful materials, and software, can be found on gophers or at FTP sites. Listed below are some tools for searching FTP sites for shareware.

Archie (FTP via the Web)- http://pubweb.nexor.co.uk/public/archie/servers.html
ArchiePlex (FTP via the Web)- http://www.lerc.nasa.gov/archieplex/
Filez- http://www.filez.com/index.html
FTPsearch- http://129.241.190.13/ftpsearch/
Gopher Jewels (gophers)- http://galaxy.einet.net/GJ/index.html
Gophers WorldWide (gophers)- gopher://gopher.micro.umn.edu/11/
Other%20Gopher%20and%20Information%20Servers
Monster FTP Sites List- http://hoohoo.ncsa.uiuc.edu/ftp/
Veronica (gophers)-gopher://gopher.scs.unr.edu/11/veronica

E-mail Lists
Mail lists provide forums for discussion of specific topics without the distractions common to the more easily accessible newsgroups. Frequently, the mail list is moderated by someone who is motivated to keep the 'junk' out of the list, and so the discussion can be of a very high quality.

Mail lists are processed by servers. A subscriber mails his message to the server which, in turn, sends the message out to everyone on the list. Replies to the message are in their turn, sent to all on the list. In this way, the mail list supports a discussion of a topic via Email. Two very good lists for pharmaceutical workers include:
PDA's Pharmaceutical Sci-Tech Mail List-
http://www.pharmweb.net/pwmirror/pwq/pharmwebq2.html

PMFlist- http://www.microbiol.org/pmf.htm

Search engines are available for finding interesting mail lists:

Liszt-http:www.liszt.com/

Reference.com- http://www.reference.com/

Summary
The Internet is a large and constantly changing source of information. This short overview cannot possibly provide a comprehensive review of all the resources available to the pharmaceutical worker, but we have tried to present a few tools to help the interested person find a starting point

in their search. If you have any comments or suggestions, or would like to tell us about other places that you have found to be of great help, please send them to :

sysop@microbiol.org- Operator of the Microbiology Network; or
vlmicro@microbiol.org- Additions to the Virtual Library: Microbiology & Virology Section

~ **About the Author:** Dr. Sutton is the Associate Director of Microbiology at Alcon. He serves on the USP Microbiology Subcommittee and is one of the owners of the Microbiology Network.

The following is a summary of 3 of the presentations at the 7th Annual Pharmaceutical/Biotechnology Microbiology Seminar held in Wilmington, NC in April 1998

Ms. Terri Polson, Glaxo-Wellcome, *'Developing a Strategy for Package Integrity Assessment'*

- PDA Technical Report No. 27 on package integrity (replacing No.4) is due out at the end of the 2nd Qtr '98.
- All packages leak. You must therefore define your leak rate specification. The leakage rate is typically measured in cubic cm gas per second. The human eye can detect 0.1 cm^3 / second. A specification below 10^{-3} cm^3 / second is appropriate and is often set at 10^{-5} cm^3 / second.
- The dye immersion test can be 'manipulated' to be more or less sensitive by changing the dye type or for example, measuring leakage visually rather than spectrophotometrically.
- The current container closure integrity tests available are:

1. Bubble Test: Gross method whereby one immerses the package in water under vacuum and looks for bubbles.
2. Tracer gases: Most sensitive method.
3. Electrical Conductivity
4. Ultrasonic Imaging
5. Vacuum/ Pressure Decay
6. Lid Deflection
7. Liquid Tracers (e.g., dye ingress)- required by Europe (ISO 8362-2)
8. Gas Ionization
9. Microbial Challenge (aerosolization or immersion)- Problems with this method are it is costly, labor intensive, no standard method (i.e., time of exposure, which organisms to use, no defined incubation time and temperature)

- There is a new FDA draft document released in January 1998: *Guidance for Industry Container and Closure Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products*

Mr. Kristen Evans, Atlanta District, FDA, *FDA Perpectives- Current Issues in Sterility Assurance Compliance'*

Note: The statements below represent the views of the speaker and not necessarily the Agency.

- The commonly used Stumbo Murphy Cochran equation for calculation of sterilization validation (i.e., spore log reduction) is not being correctly employed by the industry and FDA will cite you. The original application of this equation was for evaluation of Biological Indicators (BI's) in Biological Indicator Evaluator Resistometer (BIER) vessels which required the following pre-requisites:

  1) The BI's are being exposed to uniform sterilization conditions. The only way to do this is to have the BI's all in the same location and not scattered throughout the vessel as you do when you validate an autoclave.

  2) The use of this equation allows survivors so the cycle you are evaluating would expect to have some positives. FDA will not accept positives in a validation exercise.

- FDA is currently working on a media fill policy. They will be looking at every positive unit- what is the organism, where did it come from, and what can you do about it. Strongly suggested that QA observe media fills to determine whether manufacturing is simulating representative activities.

  It may not be appropriate to throw away weight check vials as you would during normal fills. These might be the only vials contaminated during a simulated intervention. He is most interested in the unfilled vials remaining at the end of the media fill- these have been sitting on the turntable the longest- he suggests that you fill every vial and incubate. Some firms pull vials off the line for use in growth promotion testing. He urged firms to incubate all vials first and then perform the growth promotion testing.

- He cited numerous FDA 483's including:

  1998 Gold Sheet in which Baxter had a Class I recall after 4 molds recovered in 10,000 media fill vials (passed the 1 in 1000 criteria). Investigated and later found a filter integrity problem. FDA would like to see zero tolerance in media fills.

  FDA is expecting firms to define 'objectionable organisms'.

  Cited firms for not performing Environmental Monitoring (EM) of curtains surrounding aseptic filling core.

  Cited firms who do not standardize trend nomenclature for organisms- in one firm one species was entered into an EM trending program 14 different ways so that if you went to do a trend you'd only get a partial printout depending on the way you spelled the organism.

  FDA expects disinfectants used in aseptic filling areas to be sterilized prior to use.

  Isolator validation- Doesn't recommend using BI's on stainless coupons exclusively. You need to evaluate the substrates (such as glass vials) used within your chamber. VHP has tremendous variation in efficacy depending on the material.

  There is no easy way to demonstrate a sterility assurance level of greater than 10^{-3} for aseptic processing. Just filling more vials and showing no growth in a media fill is not the way. He suggested filling enough vials to show failures, use of open doors in the suite or ungowned personnel.

  Expects firms to perform BI spore population determinations survival-kill studies as acceptance criteria for incoming lots of indicator.

USP Supplement 8 defines sterility test allowances to repeat testing if the initial test is invalidated but 21CFR 610.12 for biologics has not changed and allows for two repeat tests. What is CBER's position? The loophole does exist for biologics manufacturers. FDA is going to be especially focused on your investigation if you repeat the test. Although not legally required, it might be wise to follow the stricter USP procedure.

FDA's position on monitoring within unclassified microbiological labs- Evans said that there is no regulatory requirement to do so and that he is concerned that you might create 'false positives' due to extra manipulations- but he still thought it was a 'good idea'.

Dr. Myron Sasser, Ph.D., President MIDI Systems, *'Taxonomy: Nomenclature and Identification'*

- Humans shed 1,000,000 skin flakes per day, all with G+ bacteria.
- Prior to identification 2 or 3 passages are needed in order for stressed organisms to recover.
- Bergey's Manual is way out of date- a new CD-ROM and on-line version is presently in the works.
- The only official source for microbial taxonomy is the *International Journal of Systematic Bacteriology.*
- Definitions:

  Species: a collection of strains having similar biochemical properties

  Strain: Offspring of a single isolate in pure culture

  Biovar: Subspecies of a strain distinguished by chemical tests.

  Serovar: Subspecies of a strain distinguished by serology.

  Pathovar: Subspecies of a strain which infects plants.

- ATCC does not have the money to validate the strain collections. Many are mixed cultures. 60% of the Corynebacteria are not correctly identified. Not all 'Type' reference strains are correct. However, ATCC Preceptrol cultures less than Number 35,000 are correctly identified.

Highlights of the USP Open Conference held in New Orleans, LA, May 1998
Part 1 of 3

Comments regarding Harmonization:

The goal is to have identical standards and test methods. However, differences in products, cultures, ingredients, etc. make this effort difficult.
The fundamental difference is the use of the test. In Antimicrobial Effectiveness Test (AET), the USP has it as a shelf-life, while in Europe it is a release test only. Currently, you can pass one and fail the other which is a major disharmony.

For LAL, now there is a global reference standard.

Definitions regarding methods:

- Adoption: you choose one over another.
- Mutual Recognition: Two different methods that yield the same result.

- Concordance: Process designed to provide a way to show that using another method, the outcome is similar. You will have to 'apply' (fill out a form) to USP for concordance approval of a method.

Aseptic Processing and Process Validation:

- Non-viable particulates are described in IES and Federal Standard 209E.
- Environmental Monitoring- Purpose is to give a general broad spectrum assessment of the environment. It is only a snapshot in time.
- Current air testing methods cannot detect below 2 or 3 CFU per cubic meter.
- Chapter <1116> should clarify that the limits listed for Class 10,000 and 100,000 areas apply to areas used in aseptic manufacturing processes or testing only.
- The chapter implies that cleanrooms used for sterility testing are included.
- The Monograph does not clearly address the need to monitor product contact surfaces.
- Critical areas need to be tested at the end of the run. Most companies are not monitoring this way because the critical areas are sterilized (e.g., filling needles) prior to the run.
- Areas that are not sterilized but which are wiped down with a sanitizer need to be addressed.
- There may be a confusion as to what critical is. Definition of 'critical' was taken from FDA but does not really address surfaces.
- If sterility test passes but the environmental monitoring exceeds action levels, do you reject the lot? Most companies do an investigation and some companies as a result, do reject the lot.
- The title of Chapter <1116> does not match the contents or intention.
- The chapter needs to focus on aseptic processing and needs to delete reference to other controlled environments to clearly exclude terminally sterilized products.
- The chapter needs to explain how to use the numbers in the context of release/rejection of lots. Are the numbers averages? Are the numbers alert/action limits?
- The numbers need to be harmonized with the EP because the USP uses single points while the EP uses averages.
- The USP should determine if total yeasts and molds needs to be evaluated and, if so, which medium should be used.

Isolators <1206>:

- Calling an isolator Class 100 does not imply anything regarding airflow, air velocity, or pressures. Class 100 conditions should be met under static conditions.

- The isolator used for sterility testing does not require a HEPA filter as Class 100 conditions can be met without it. Some isolators have 0.22 μM filters in the vent vs. having a HEPA.

- Sterility testing could 'possibly' be done in a glove box. How the sealed glove box should be set-up, needs to be described.

- The use of 10^{-6} Sterility Assurance Level is debatable for isolators. Words such as decontamination or sanitization may be preferable. Also, there is a consideration to use a 10^{4} challenge instead of a 10^{6}.

- Some people felt that isolators need to be placed in a controlled access area but unclassified area. Controlled Access Area wording should be changed to Limited Access Area because the word 'control' implies that monitoring and other activities be required.

Harmonization:

The USP Open Conference attendees discussed the following chapters:

<71> Sterility Tests
<51> Antimicrobial Effectiveness Testing
<52> Antimicrobial Preservative Effectiveness Testing for Vaccines
<85> Bacterial Endotoxins Test
<13> Concordance of Foreign Pharmacopeial Tests and Assays

The comments were on <71>, <85>, and <51>.

Sterility <71>

- There is confusion on Table 3 as there is a footnote indicating use of powder products as liquids even though Table 4 deals with solid products.

- Growth promotion is to be performed on dehydrated media on a lot basis not on a per batch basis.

- EP does not recognize Alternative Thioglycollate Medium because this medium is for medical devices and the EP does not cover medical devices.

- Inconsistencies in USP philosophy were noted as terminally sterilized products are described as having a 7 day incubation period for sterility yet there were discussions to make it a 14 day test as the other sterility tests, in case some organisms are injured. However, there is the proposal for parametric release of a product without testing.

- The cut-off for a Bacteriostasis/Fungistasis volume for direct transfer as 2,000 mL is not clear.

- Antibiotics are not well-addressed in the Monograph.

- Some attendees requested a change in wording of temperatures for storage of medium currently between 2 and 25° C. The request calls for increasing the upper limit to 30° C in order to allow staging of media in isolators.

- Subculture of turbid products is different in USP and EP. USP requires transfer in 3-7 days whereas EP requires transfer in 14 days. The comment here is that 3 days is too short while 14 days is too long.

- The five, 500 mL rinses for membrane filtration were requested to be changed to read 'the equivalent of 5, 500 mL rinses'. The EP has no limit for rinsing membranes.

- The use of ≤ 100 CFU for inoculum in the USP was criticized by the European participants. It is argued that when inoculum is down to 1 or 2 CFU, there is a statistical possibility of having no organisms in an inoculated aliquot.

- Growth Promotion differences in EP and USP: EP requires growth of bacteria in 3 days, yeasts and fungi in 5 days; USP requires growth within 7 days for all organisms.

- Bacteriostasis/Fungistasis (B/F) Test: EP and USP have the same growth requirements as described above in growth promotion.

- Firms are expected to re-validate growth promotion and B/F testing for the new organisms in USP 23.

- The Monograph needs to clarify the number of containers needed for membrane filtration via classical method of culturing membranes. The number of samples needs to be doubled because each half of the membrane only sees half the volume of sample.

Antimicrobial Effectiveness Test (AET) <51>

- Category 2 for anhydrous products should not be necessary.

- Ointments should not have preservatives.

- The 70% population recovery of the validation chapter will be re-evaluated. People are having trouble with this.

- Preparation of inocula is different in USP and EP. Agar and broth are allowed per USP while EP only allows agar.

- Testing of solids in not needed if product/material has a low water activity.

- Deletion of non-aqueous products with a proper explanation may be in order. Preservatives should not be included in non-aqueous products.

- It is expected that 40% of US products will fail to meet the EP criteria.

- The AET has a major difference in philosophy. EP uses the test during product development stage whereas, the USP test covers marketed products and it is a requirement to be met for the life of the product.

Bacterial Endotoxins Test <85>

- USP will consider monographs for the alternate methods.
- The pH referred to in the Monograph is the pH of the reaction mixture and not the sample. This should be clarified.
- Users confirm label claim; the word determine in reference to label claim should be changed.

USP

The PMF recommends that you *write directly to the USP with your comments on all proposals.* You can write representing your company, or as an individual scientist.

Any questions concerning USP documents should be sent to Dr. Roger Dabbah. You can reach Dr. Dabbah at (301) 816-8336, or via e-mail at RD @ USP.org. When communicating with Dr. Dabbah, let him know you are a PMF member.

CALENDAR OF EVENTS

The Calendar of Events is provided as a service to PMF Newsletter readers. Submission of complete and accurate information will be published on a space-available basis.

- October 21-22, 1998. Elite MicroSource Seminars. USP Microbiology Updates. San Francisco, CA (Oct 21), San Diego, CA (Oct 22). Contact Elite MicroSource at (850) 763-5453 or emsource @ aol.com. [There are seminars in November in New Jersey and Pennsylvania, and December seminars in North Carolina and Massachusetts].
- November 9-13, 1998. Parenteral Drug Association Annual Meeting, Courses and Exhibitions. Washington, DC. Contact PDA at (301) 986-0293 or info@pda.org
- November 10-12, 1998. Microbiological Control Conference. Islamorada, FL. Contact *The Microbiological Update* at (305) 664-8513.

Future Topics

The purpose of the Newsletter is a sharing of information among Microbiologists. Your contributions to the *PMF Newsletter* are needed in the form of short articles, letters to the Editor, job openings, comments, or suggestions. Please direct your correspondence to *PMF Newsletter*, c/o L. Valdes-Mora, 3166 Wood Valley Road, Panama City, FL 32405 [Tel (850) 763-5453]. Submit any articles with your name and phone number in case we need to contact you. Your name and company will not appear without prior written authorization.

Advertisements:

The PMF newsletter will accept advertisements for both those seeking employment, as well as those with current job openings. We also encourage any advertisements for products or items that are new and of interest to microbiologists. There is no fee for the placements. All material is subject to approval by the Board of Directors. Please submit copy to Laura Valdes-Mora.

Current Compendia

US Pharmacopoeia (USP) 23 Supplement 8 (May 15, 1998)

European Pharmacopoeia (EP) 1997 / Supplement 1998

Japanese Pharmacopoeia (JP) XIII 1996 / Supplement 1998

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* If you use any other compendia, let us know for inclusion in this corner

The following Internet Sites may be of interest to you:

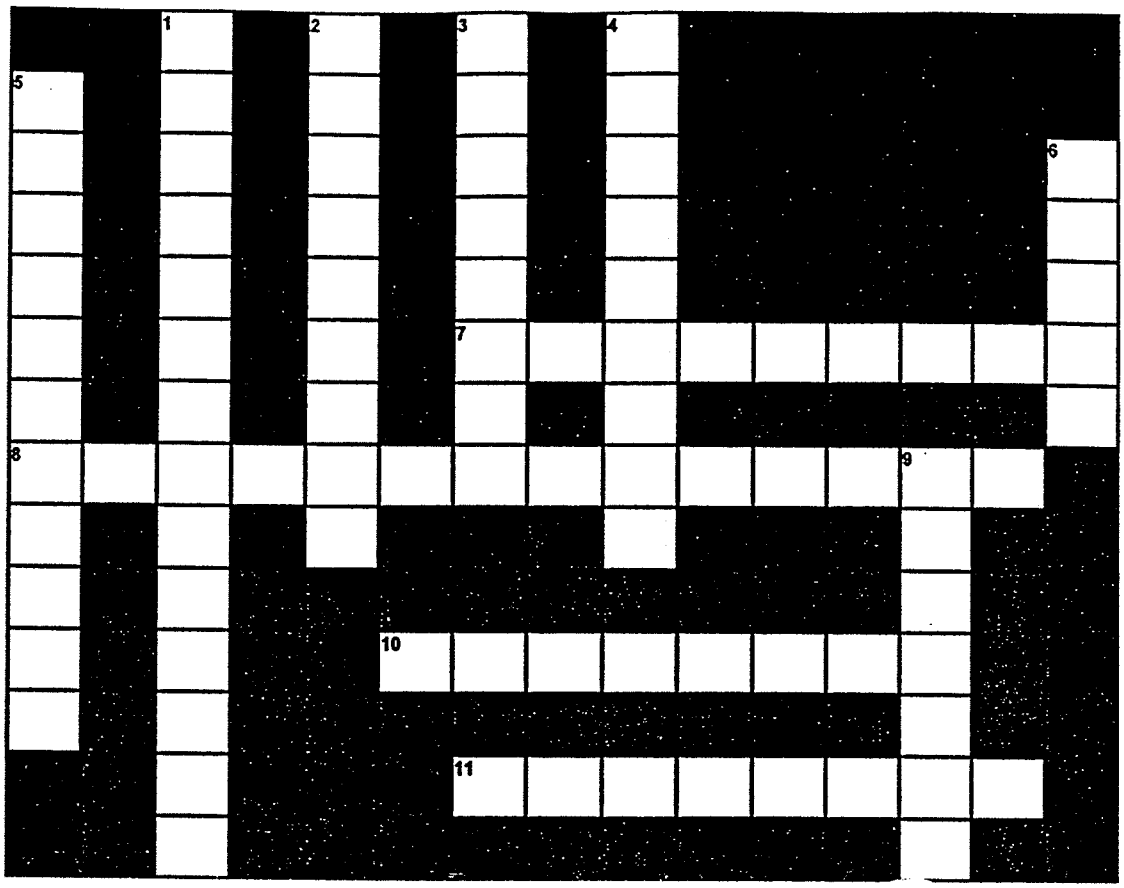
Internet Address	Description
http://www.biofind.com	Biotechnology rumor mill, positions, and more
http://bordeaux.uwaterloo.ca/biol446/appendix1.htm	Comparison of old vs. new taxonomic changes
http://members.aol.com/enigl	Microbiology consultant's newsletter
http://www.fda.gov/ora/science_ref/lpm/lpmtc.htmL	FDA Laboratory Procedures Manual
http://home.earthlink.net/~bajwa/news.htm	Sample SOP and Qualification Method for Excel Spreadsheets
http://129.109.136.65/microbook	Sam Baron, MD. Medical Microbiology Textbook 4th ed. Complete book on-line
http://www.incadinc.com	European Document Research- Official Agents for the Office of Official Publications of the European Community

If you have found an Internet site that contains information of relevance to pharmaceutical microbiology, please let us know.

Come Visit Our Website at http://www.microbiol.org/PMF.htm

Are you aware of our on-line discussion group? Membership is FREE. To join, send an e-mail to Listserv@ microbiol.org. Write ['Subscribe PMFlist' Firstname Lastname] as the first line of text (message). You can ask, answer, or read questions of and comments from your colleagues.

Crossword Puzzle



Across

7. Self-destruction of a cell usually by its own enzymes

8. Period needed to double the cell population (2 words)

10. Able to grow in the absence of air

11. Pus-producing

Down

1. Ability to distinguish microscopically between 2 neighboring objects or points (2 words)

2. Heat-resistant resting form of bacteria produced within the vegetative cell

3. Staining characteristic of Mycobacteria, for example

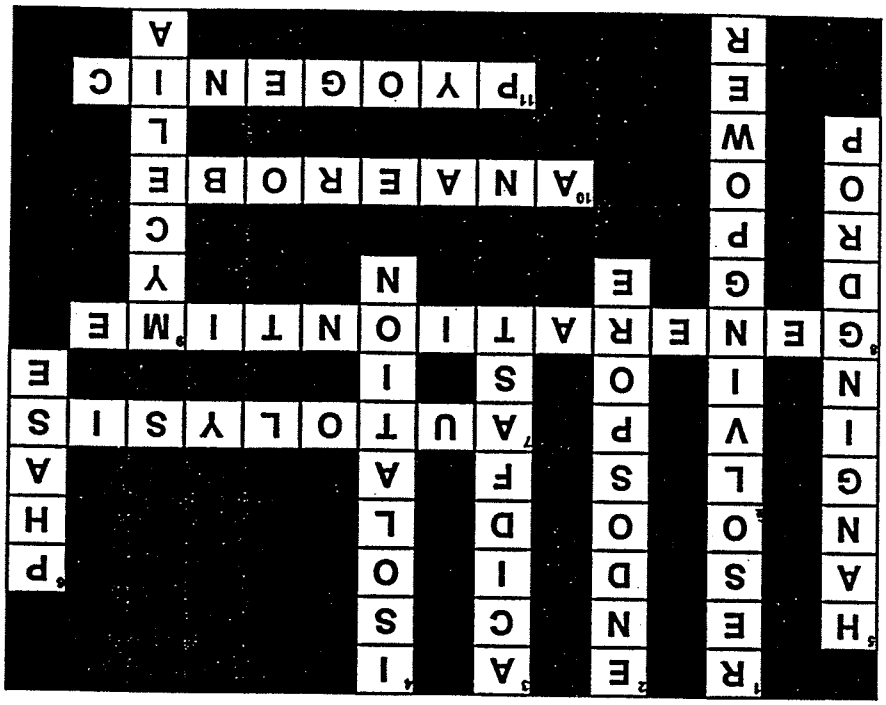
4. Separation of colonies into pure culture by subculture

5. Microscopic preparation of living cells to determine motility (2 words)

6. Type of microscopy used to view live organisms

9. Masses of hyphae comprising mold colonies

Crossword Puzzle Solution



REGULATORY CORNER

Actual FDA 483 Citations:

- Original test records have not been reviewed in a timely fashion for accuracy, completeness, and compliance with established standards.
- Notebook checkers are making changes to the final calculations without verification by another person or the concurrence of the person who had performed the measurement.

The Pharmaceutical Microbiology Forum is proud to have members in the following countries: United States, Canada, The Netherlands, Belgium, Germany, Israel, Puerto Rico and Japan.