PMF NEWSLETTER

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PURPOSE: To provide a forum for discussion of microbiology issues in the pharmaceutical industry.

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President's Message

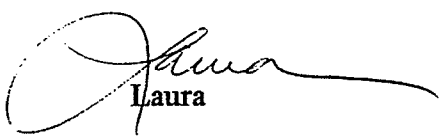
Around the holidays, we can reflect upon the achievements of our group. We thank everyone that has contributed to the PMF Newsletter. Many of our members do not give us written feedback. However, I am very pleased to inform you that as I travel throughout the United States presenting seminars in Pharmaceutical Microbiology, I find members approaching me to let me know how much they like the Newsletter. This is very crucial feedback as we need to continuously evaluate the importance of the Newsletter in your work and how well we are fulfilling the PMF mission.

The Board of Directors would like to thank all of you, who took time to fill out our questionnaires; especially the one included in this issue on Microbial Identification Methods. We are getting a low response to the benchmarking studies. The data derived from them is very valuable as it gives a picture across a wide variety of microbiology laboratories but only if a significant number of responses are received. We are not expecting 100% participation and we understand that we have multiple members from many organizations. However, taking this into consideration and comparing to benchmark studies from other organizations, we can expect 40% participation. This is not happening. In this situation then, the Board can come to the conclusion that information derived from questionnaires is not of interest to our membership and future benchmark initiatives may be canceled.

We are interested in knowing topics that you would like for us to address in future Newsletters. Please write to us using the return address in your Newsletter or e-mail me at EMSource@aol.com.

I am very pleased to announce that we received a Letter to the Editor, which is included in this issue. A letter to the editor is another method you can use to make public comment on what has been published and at the same time encourage others to think and discuss a particular issue.

I am looking forward to hearing from you.



Laura

Letter to the Editor

Madam,

In the resume of Mr. Evans' lecture on "Current Issues in Sterility Assurance Compliance", (PMF Newsletter, 1998, Volume 5, Number 3, Page 5) there are two statements on which I would like to comment:

1. "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."
2. "There is no easy way to demonstrate a sterility assurance level greater than 10^{-3} for aseptic processing. Just filling more vials ...is not the way. He suggested filling enough vials to show failures, use of open doors in the suite or ungowned personnel."

In the first statement the zero tolerance (no contaminated vials) seems to be the target. However, in the second statement it is suggested that there should be an active search, even by violating all CGMP rules, for contaminated vials. These two statements are impossible to reconcile and the FDA should clarify its present position.

It is world wide accepted that media fills should mimic as closely as possible the actual production process. Preferably, interventions, such as changing pumps, should be done during media fills. However, this is very far from brutal violations of aseptic procedures such as the use of open doors or the allowance of ungowned personnel in the suite. Failures in media fills performed under such poor conditions have no significance for the actual production process and cannot lead to the detection of possible failures during the production. On the contrary, relatively high numbers of failures, as a result of such bad practices, would rather mask any possible real failure and is therefore counterproductive.

Although it is acknowledged that the above cited statements do not represent the views of the Agency, it would be wise if FDA officials refrain from statements that are scientifically not justified and lead to confusion.

Sincerely,
Hans van Doorne, Ph.D.
Assistant Professor in Pharmaceutical Microbiology
Department of Pharmaceutical Technology
University of Groningen
The Netherlands
E-mail: H.van.Doorne@farm.rug.nl

Highlights of the USP Open Conference held in New Orleans, Louisiana, May 1998
Part 2 of 3
<1207> Integrity Evaluation (container-closure testing)

1. The biggest problem is how to simulate small holes in the containers to be used as positive controls.
2. One company does Microbial Ingress Testing using a 10^7 suspension of Serratia marcescens.
3. People would like to have information on how to do The Microbial Ingress Test. The chapter as written, offers no details on any method mentioned.
4. Limitation of the Microbial Ingress Test: As media ages, it may not be acceptable to use during stability studies.

<61> Microbial Limit and <1111> Microbial Attributes

1. Test for coliforms should be eliminated as a general test.
2. Objectionable organism - The definition is debatable. Should be revised to match Paul Motise's CGMP notes of March 1998.
3. General guidance should be provided in chapter <1111>.

4. Re-testing should be eliminated from <61>.
5. The decision tree presented at the conference should be upgraded to include Burkholderia and Klebsiella species.
6. Fungal specifications are tighter now calling for ≤ 10 CFU/mL for all dosage forms except for oral solids. These specifications are too low.
7. It was recommended to have specifications for aerosols on a per aerosol can basis and on a per patch basis for topical patches.
8. Attendees suggested changing total yeasts and molds to ≤ 50 CFU/mL.
9. The inclusion of the coliform test will be reassessed.
10. Methods should be in <61> while requirements should be in monographs.
11. Decision tree should be incorporated into <1111>.
12. Consider deleting indicator organisms.
13. Consider re-evaluating sample size. (10 gram samples were considered excessive.)
14. Add information on other microorganisms that can be isolated by the methods.
15. Consider providing guidance on risk assessment.

<1227> Validation of Microbial Recovery

1. Purpose and rationale of this chapter needs to be clarified.
2. Define "triplicates". Is it 3 sample preparations or 3 separate experiments?
3. Statistics need to be re-evaluated.
4. Chapter needs to define "similar" (should numbers be ± 10, 20, 30% or what?).
5. 70% recovery was considered too high. Consensus opinion was that a recovery of 50% is acceptable.

PMF Microbial Identification System Survey Responses

The PMF asked you in the Spring and Summer issues for information regarding your microbial identification system practices. Your responses are as follows:

There were 17 respondents representing the following types of firms:

Conventional Pharmaceutical	8
Licensed Biopharmaceutical	3
Biotechnology	1
Medical Device	1
Contract Test Laboratories	3
OTC Drugs	1

Members reported using the following systems in their laboratories:

System	G+ cocci	Micro-cocci	G+ Non-spore rods	G+ Spore Rods	Mycobacteria	G- ferm	G- NFT	Anaerobes	Yeasts	Fungi (Molds)
API	8	3	3	3		6	7	3	4	
Biolog	4	4	3	3		4	4		2	
Fame by GC	1	1	1	1		1	1		1	
MIDI	6	7	5	5	2	6	6	2	3	2
Minitek	3	3				1	1			
PCR	1	1	1		1	1	1	1		
Spec-trum 10										
Vitek	12	4	5	10		13	11	7	13	
Other: Specify	Staphase-1		Remel Rapid CHB-1			Remel Rapid NF -1				

The following systems were not employed: Crystal, Enterotubes, IDS, Microscan, Qualicon, and Spectrum 10. Nine respondents perform fungal identifications manually either macroscopically or microscopically.

Respondents indicated their choices for the **Best** and **Worst** Systems were:

Best:	G+ cocci	Micrococci	G+ Non-spore rods	G+ Spore Rods	Mycobacteria	G- ferm	G- NFT	Anaerobes	Yeasts	Fungi (Molds)
Vitek	5	1	2	6		8	5	5	5	
Biolog		1								
API	2	1	2			1	2		1	
MIDI	2	2	2				2		1	
PCR				1	1			1		
Minitek	1	3								
Rapid CB			1							

Worst:	G+ cocci	Micrococci	G+ Non-spore rods	G+ Spore Rods	Mycobacteria	G- ferm	G- NFT	Anaerobes	Yeasts	Fungi (Molds)
Vitek		3								
API	1			1					1	
MIDI		1				2	1			1

In the area of satisfaction with the vendor, the responses were as follows:

Parameter	Most	Comments	Least	Comments
Accuracy	Vitek-7		Vitek-2	*Bacillus*-1, Environmental Isolates Confidence levels >85% not achieved-1
	Biolog-2	Larger database-more reliable for environmental isolates than Vitek-1	Biolog-1	Not good for sporeformers-1
	PCR-1		API-1	
	API-1			
	MIDI-1	Hard to find technical support-1	MIDI-1	
Local Rep	Vitek-11	Exceptional-2	Vitek-0	
	Biolog-1		Biolog-2	Only 1 visit
			MIDI-3	
Tech Support	Vitek-10	Unmatched-1 Exceptional-1	Vitek-0	
	Biolog-1		Biolog-1	
	MIDI-3		MIDI-2	
Audit	Vitek-2		Vitek-0	
	Biolog-0		Biolog-0	
Training	Vitek-8	Excellent-2	Vitek-0	
	Biolog-0		Biolog-2	Have to pay additional amount and fly to CA-2
	MIDI-2			
Warranty	Vitek-8		Vitek-0	
	Biolog-1		Biolog-0	
	MIDI-1		MIDI-2	
Service	Vitek-9	Unmatched-1; Prompt-1	Vitek-1	Calibration kit not available-1
	Biolog-1		Biolog-0	
	MIDI-2		MIDI-1	

Only two systems were audited: by one firm each: Fame system and Vitek (for an unknown reason the latter was not qualified by the auditing firm).

Qualification of systems was reported as follows:

Vendor Qualification	Number	Comments
Vitek	11	In progress-1
Biolog	4	In progress-1
API	3	
MIDI	5	In progress-1
FAME by GC	1	

Members deemed systems unacceptable for certain uses as described below:

System	Issues	Number of Respondents
Vitek	Cannot Id *Micrococcus* except manually using anaerobe card	2
	Incorrect Ids *Micrococcus*	2
	Poor job on *Bacillus*	2
	Not able to id slow growing water organisms	1
	Difficult time differentiating between coagulase negative *Staphylococcus* species	1
	Corynebacteria and G+ rods outside the *Bacillus*- incorrect identifications	1
	Corynebacteria and *Methylobacteria*	2
	Cannot use for nonfermentors due to requirement for subculture to blood agar at 35 C- not optimum for growth of same	1
	Micrococcus is identified *S. auricularis*	1
	Does not identify environmental isolates well	1
Biolog	Did not meet validation acceptance criteria for yeasts	1
	Gram + sporeformers interfere with reaction wells causing poor identifications	1
API	NE works better than Vitek NFC	1
	Does not identify yeast well	1
MIDI	Does not sufficiently discriminate molds	1
	E. coli requires an other system for confirmation	2
	Difficult to differentiate between similar gram negatives (closely related)	1
	Does not work for *Staphylococcus* and *Corynebacteria*-requires a lot of growth from relatively slow growing organisms	1
	Verifies *Staphylococcus* with Staphase and GN fermentors with biochemical tests	1

Additional comments were reported by respondents:

Comments	Number of Respondents
Biolog is great: For the money involved, personnel and training required	1
PCR- Very new-not fully validated. Too new to determine level of customer support	1
MIDI- *Corynebacteria* leave residues on the liners which you need to learn to change	1
MIDI- Very user unfriendly	1
MIDI- Very disappointed in technical support	2
Desperately desire a system to identify *Corynebacteria*	1
API Coryne strips- had a lot of success	1
Vitek identifies 90% of our organisms but equipment/supplies expensive	1
MIDI- industrial oriented database but system still in its infancy	1
Minitek- Use to identify *Micrococcus* when Vitek fails but due to manual nature subject to color interpretation errors	1
Industry needs logical, practical, consistent approaches in validation of systems	1
Training and experience are essential with the automated systems	1

Summary and Conclusions:
The most popular system employed is the Vitek. It supercedes all other systems in customer satisfaction particularly in the area of vendor support (local representative, technical support, training, and service). All users report difficulties with each system with accuracy of identifications particularly with *Cornyebacteria* and *Micrococci* and environmental isolates. Manual identifications of fungi is the normal method. With two exceptions, vendor audits are not performed. However, system qualification is a routine practice.

Nanobacteria

Nanobacteria are coccoid bacteria (*Nanobacterium sanguineum gen. et. sp.* nov.) which were isolated from sterile, commercial Fetal Bovine Serum (FBS) by a group at the University of Kuopio, Kuopio, Finland. They can pass through 100 nM (but not 50 nM) filters although they have cell walls. In culture the cell size increases due to the production of a very thick, calcified cell envelope. They are not culturable in standard microbiological media but can be cultured under cell culture conditions (with or without mammalian cells, 5-10% CO2). Gamma irradiation and high doses of aminoglycoside antibiotics and other agents can prevent multiplication. They are highly heat-resistant. According to the investigators, they have been isolated from more than 80% of commercial FBS and newborn bovine sera and are the most common contaminant present in cell cultures. They are cytotoxic to mammalian cell culture when present in high concentrations relative to the numbers of mammalian cells. They gain entry to the host's cells by triggering phagocytosis (by cells that are normally phagocytic). It is postulated that this contamination may be the reason why only about 10% of FBS batches support cell cloning. "These novel organisms are one of the causes for cell vacuolization, poor thriving and unexpected cell lysis, problems often encountered in mammalian cell." (Brown et. al.) They are not detectable with present sterility testing methods. The organisms have been found in both human and cow blood. The authors have suggested an association between these organisms and the formation of kidney stones. It is also postulated that this organism may have originated from outer space. An ELISA detection kit is available from Abcell. For more information contact them at **abcell @ kolumbus.fi.** or FAX: +358-17-283. Because of the clinical implications and the use of serum in mammalian cell cultures, it has been reported by some of our PMF members that the issue is being raised by US and European inspectors.

Note: The existence of Nanobacteria is controversial in that many people do not believe they exist. Instead, they believe that these are artifacts resulting from the amplification technique in the PCR process. They cite that only the group from Finland has reported them and there has been no corroborating information from other researchers. In addition, many of the publications do not require peer review. People who have worked with bioburden testing on plated medium of serum report that the lipids in the serum form micelles which grow larger as the agar dries out giving the appearance of growing bacterial colonies. It is clear that more work on this topic is required.

If you'd like more information see the following references:

Akerman, K. *et. al.*, Scanning, Vol. 15, Supplement III (1993).

Brown and al. ed., *A New Potential Threat in Antigen and Antibody Products: Nanobacteria,* Vaccines 97, Cold Spring Harbor Laboratory Press, New York, 1997.

Ciftcioglu, N. *et. al.*, *Apotopic effect of Nanobacteria on cultured mammalian cells*, Mol. Biol. Cell, Suppl. Vol. 7, (1996): 517a.

Ciftcioglu, N. and E. Kajander. *Interaction of nanobacteria with cultured mammalian cells.* Pathophysiology. Vol. 4, pp.259-270, 1998.

Kajander, E. *et. al.*, *Comparison of Staphylococci and Novel Bacteria-like Particles from Blood*, Zbl. Bakt. Suppl. 26, 1994.

Kajander, E. *et. al.*, *Nanobacteria for blood, the smallest culturable autonomously replicating agent on Earth*, SPIE (Proceedings of the International Society for Optical Engineering), Vol. 3111. 1997 pp. 419-428.

Kajander, E. *et. al.*, *Fatal (fetal) bovine serum: discovery of Nanobacteria,* Mol. Biol. Cell, Suppl. Vol. 7, (1996): 517a.

Kajander, E. and N. Ciftcioglu. *Nanobacteria: An alternative mechanism for pathogenic intra- and extracellular calcification and stone formation,* Proc. Natl. Acad. Sci., USA. Vol 95, pp.8274-8279, July 1998.

USP Corner

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, via mail The United States Pharmacopoeial Convention, 12601 Twinbrook Parkway, Rockville, MD 20852 or via e-mail at RD @ USP.org. When communicating with Dr. Dabbah, let him know you are a PMF member.

LOOK FOR SUPPLEMENT 10 AROUND MARCH, 1999. IT BECOMES OFFICIAL ON MAY 15, 1999.

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.

-February 3, 1999. Microbiology Updates. Natick, MA. Elite MicroSource.
-February 4, 1999. Microbiology Updates. Cary, NC. Elite MicroSource.
-February 11, 1999. SIMS. Schering-Plough Kenilworth, NJ.
-March 4-5, 1999. PDA Spring Conference. Omni Rosen Hotel. Orlando, Florida.
-March 23, 24 1999. AAI Seminar Series. Laboratory Efficiency and Compliance: Meeting the Challenges of both in the Pharmaceutical Industry. Somerset, NJ.
-March 24, 1999. Microbiology Updates. Miami, FL. Elite MicroSource.
-March 25, 1999. Microbiology Updates. Atlanta, GA. Elite MicroSource.
-April 20-21, 1999. AAI Seminar Series. 8th Annual Pharmaceutical Microbiology Seminar: Current Issues and Practical Applications. Newark Airport, NJ.
-April 22, 1999. Microbiology Updates. Commack, New York. Elite MicroSource.

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.

Message from the Treasurer

Dear Members:

This notification is for all members who joined the PMF before 1998. To cover the costs of providing a quality newsletter consistently each quarter, the Board of Directors of the PMF has voted that dues of $10.00 each will be charged for the year 1999. Dues statements will be sent in February, to all members who joined before 1998. Payment has to be received by April 30, 1999 to guarantee uninterrupted receipt of your copy of the newsletter.

Sincerely,
E.A. Darner
Treasurer, PMF

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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.

POSITIONS OPEN

Microbiologist I/II

Perform routine microbiological procedures including microbial limits testing in raw materials and finished products, as well as monitor environmental/equipment/water quality, endotoxin levels and preservative effectiveness. Responsibilities may also include sterility testing and other general laboratory work.

Requirements: A minimum of three years laboratory experience in pharmaceuticals industry with a BS/BA in Microbiology/Biology or equivalent. Working experience with personal computers and general laboratory equipment is essential. Good oral and written communication skills, and familiarity with cGMP and aseptic technology.

Microbiologist III

Provide experienced analytical support to pre-clinical development and manufacturing groups including timely response to emergency situations. Conduct endotoxin testing, validate different test procedures and equipment, develop special testing procedures, and participate in environmental monitoring and routine laboratory testing as required.

Requirements: Seven or more years of relevant laboratory experience in pharmaceutical industry with a BS/BA in Microbiology/Biology or equivalent. Strong technical ability in microbiology testing of various products. Extensive knowledge in industrial microbiology and expertise in troubleshooting microbiological problems. Proficient in oral and written communication, computer skills and thorough knowledge of cGMP.

Please fax or send resume to:
Ms. Geri Filippini
Human Resources
Oread
3401 Hillview Avenue
Palo Alto, CA 94304-1351
Fax Number: (650) 813-4600

Current Compendia

US Pharmacopoeia (USP) 23 Supplement 9 (November 15, 1998)

European Pharmacopoeia (EP) 1997 / Supplement 1999

Japanese Pharmacopoeia (JP) XIII 1996 / Supplement 1998

* 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.fda.gov/ora/science_ref/lpm/lpmtc.html	FDA Laboratory Procedures Manual
http://129.109.136.65/microbook	Sam Baron, MD. Medical Microbiology Textbook 4th ed. Complete book online
http://home.earthlink.net/~bajwa/news.htm	Sample SOP and Qualification Method for Excel Spreadsheets

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.

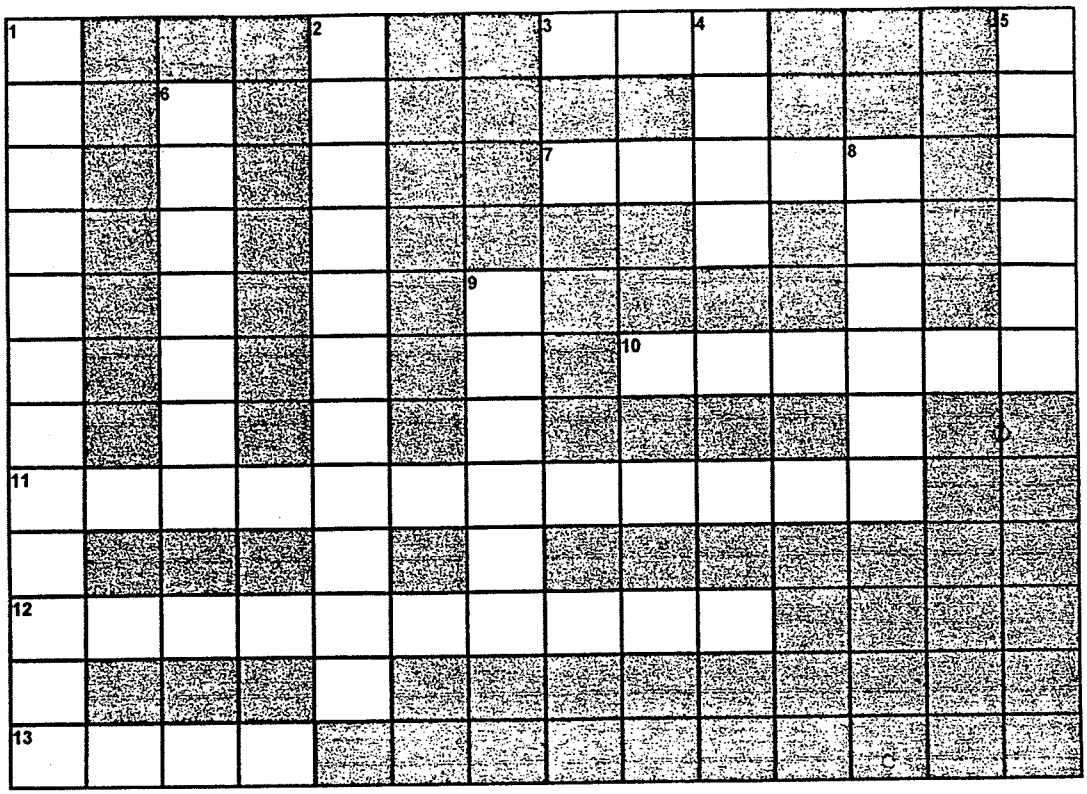
REGULATORY CORNER

Actual FDA 483 Citations:

1. **During a raw material sampling demonstration in the receiving warehouse, the sampler was observed bending down maintaining his uncovered neck and uncovered sideburns under laminar flow directly over an opened container of drug substance for a duration of approximately 25 seconds.**
2. **The sampling booth is located in an uncontrolled room in the warehouse.**
3. **Debris was found on the grates immediately below the filters during a walk through of the warehouse.**

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

Crossword Puzzle



Across

4. Host who harbors and spreads but shows no sign of a disease
7. Cold-loving organism
8. Bacteriocin produced by *P. aeruginosa*
10. Another name for bacterial sexual recombination
11. Current edition is 23

Down

1. Pasteur developed a vaccine against this virus
2. Flagella which surround the cell surface
3. Frozen carbon dioxide
5. Non-malignant
6. Infection transmitted to a patient by an attending physician
7. Flagella located at one or both ends
8. Mycoplasma (abbr.)
9. Ethanol (abbr.)

Crossword Puzzle Solution

P[1]									C[2]				E[13]
S				P					O		C[6]		L
Y				L	O	P[7]			N		A		I[12]
C				O					J		R		H
H						D[9]			U		R		P[11]
R	G	I	N	E	B[10]	R			G		I		O
O		E				Y			A		E		R
P[5]				P[4]	S	U[3]			T	I	R	E	H
													I[12]
													L
													E[13]