Biolog, Inc.
3938 Trust Way
Hayward, California 94545
510-785-2564 Telephone
800-284-4949 Orders
510-782-4639 Fax
www.biolog.com

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Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Ref: Docket No. 2003D-0382

Dear Gentlemen:

I am writing this letter to disagree strongly with proposed wording in a DRAFT Guidance. The relevant section is:

Guidance for Industry
Sterile Drug Products Produced by
Aseptic Processing — Current Good
Manufacturing Practice
DRAFT GUIDANCE

From Page 36,

1296
1297 Rapid genotypic methods are recommended for purposes of identification, as these methods have
1298 been shown to be more accurate and precise than biochemical and phenotypic techniques.
1299

The inclusion of this statement in the document by the FDA is over-reaching and would not be prudent for a number of reasons.

To say that genotypic methods are more accurate and precise than other methods that microbiologists use is a sweeping over-generalization. There are problems with this statement at many levels. I list examples that I am aware of below.

1. Microbial identification has always been a challenge and in spite of great progress, it remains a challenge. Grouping and assigning strains to discrete species, where there are no hard and fast rules or boundaries requires multiple, independent approaches. The majority of experts on this subject are aware that ALL current methods have limitations, drawbacks, and occasional inaccuracies, including genotypic methods such as 16s-RNA sequencing (1,2). Experts in

microbial taxonomy insist that the only truly accurate gold standard approach is a combination of these methods, termed polyphasic taxonomy (3). Species descriptions always require biochemical and phenotypic testing and characterization. One reason is that identification based on 16s-RNA sequence analysis, relies on only one gene in a cell and ignores the remaining 2000-6000 genes. Phenotypic methods can look at large number and wide range of cellular properties coded for by the genes.

2. In this statement, is not at all clear which genotypic methods are being referred to. The term "genotypic methods" covers quite a range of methods with different advantages and shortcomings. The best genotypic methods, genome sequencing, followed by 23s-RNA sequencing, followed by 16s-RNA sequencing, require experts in genome annotation and sequence alignment to make species level identifications. This is still a manual method requiring substantial expertise to do it properly. There are no automatic computerized methods that are as good as human experts in doing sequence alignment and interpretation.
3. Genotypic technologies have been slow to gain in popularity for a number of reasons. Most genotypic methods are not rapid and the best genotypic methods are typically very expensive. The issue of doing valid comparisons and rating the accuracy of a method is discussed in points that follow.
4. The accuracy of 16s-based identifications depends in part on the length of the 16s fragment that is sequenced. The commercially-available method uses a short cut 500 base pair sequence instead of a complete sequence and is less accurate.
5. 16s-RNA methods fail to make some very important species/taxa differentiations such as harmless *E. coli* compared with *E. coli* O157 and *Shigella* species. These identifications are easily and accurately made by phenotypic methods.
6. According to experts at CDC (4) it is extremely difficult to compare and generate valid numbers to compare the accuracy of different methods. It is always possible to make any system look better or worse than another system by simply biasing the selection of microorganisms that are tested. Furthermore the accuracy will depend on the skill and knowledge of the user in using any particular system, including genotypic testing systems. A lot of the confusion and disagreement over species identification is due to the fact that current taxonomy still has errors and often a "misidentified" strain belongs to a new species that is not yet described and is therefore not present in any database.
7. Phenotypic and biochemical methods are still used as the primary method in hospital laboratories and CDC has made no recommendation to change to genotypic methods for a number of reasons. Standard methods have acceptable accuracy for identification of most human pathogens.
8. Just as it is inaccurate and unfair to lump together all genotypic methods, it is also inaccurate and unfair to lump together all phenotypic and biochemical methods. Our company, Biolog, Inc. continues to develop accurate and precise phenotypic identification systems. I am aware of only one published study comparing the accuracy of Biolog against 16s-RNA sequencing (5). The accuracies reported (84.6% vs. 89.2%) were very close. This study was sponsored by the developer of the 16s sequence database. We believe that the user made some errors in doing this study and that in fact the Biolog accuracy was higher than reported. We are already at work (funded by NIH) on a new generation of our phenotypic

technology that will have even greater accuracy and will be much simpler for laboratory microbiologists to use. On our website we have posted a Bibliography listing nearly a thousand publications by scientists in diverse fields that have used our technology to get accurate characterization and identification of bacteria. We are also committed to supporting our customers' efforts in meeting the regulatory requirements of FDA and other agencies by offering an abundance of validation resources as well as modifying our software to be 21 CFR Part 11 compliant.

9. Finally, the draft statement, which appears to globally recommend or endorse genotypic methods, may give the impression that some company has lobbied to try to get their technology recommended over all others. Users of microbiological identification systems are fully capable of weighing all the advantages and disadvantages of the technologies and systems available and making the best choice to fit their needs.

I hope that my comments are helpful to the authors and that the FDA takes a balanced approach with its Guidance. I know that the FDA is always interested in having newer technologies pursued and yet this draft Guidance states a bias about which technologies should be favored. The end result would be a constraint of future beneficial technology development to the detriment of microbiologists working to meet FDA guidelines. In our view the Guidance should be objectively based on science that allows the various technologies and companies to continue to evolve and to openly compete in the market.

Sincerely,

Barry Bochner, Ph.D.
Vice President of Research & Development
Biolog, Inc.

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(5) Y.-W. Tang et al. Comparison of phenotypic and genotypic techniques for identification of unusual aerobic pathogenic gram-negative bacilli. J. Clin. Microbiol. (1998) 36:3674.