

BIOLOG'S SOFTWARE 4.2 OFFERS 21 CFR PART 11 COMPLIANCE

INSIDE

- 1 **Biolog's Software 4.2 Offers Compliance with 21 CFR Part 11**
- 2 **Pure Cultures are Important for Good IDs**
- 3 **Handling Difficult to Grow Bacteria**
- 4 **Where Can I find Reference Information?**

21 CFR 11: FEATURES

- Assigned User Privileges
- Electronic Signatures
- Original Records Are Secure
- Audit Trails

Today's FDA regulatory compliance standards are more stringent than ever. Validating a microbial identification system can be a laborious and challenging task. Biolog understands that system validation is essential for our customers who operate in an FDA compliant environment, and has made every effort to meet these needs.

Biolog offers both MicroLog™ and OmniLog™ Validation Packages, providing all the important components you need to validate your Biolog system's performance specifications. We even offer an On-Site Validation Service. More recently, we added security measures to our software to meet 21CFR Part 11 standards.

21CFR Part 11 is intended to ensure the integrity of electronic records by:

1. Controlling access
2. Providing documentation of what was changed, by whom and when
3. Providing audit trails
4. Ensuring that original record files have not been modified or deleted.

MicroLog™ release 4.2 and OmniLog™ release 1.1 give support to full compliance with 21CFR Part 11 by offering "Restricted Access Mode". This is an added security feature designed to ensure the integrity of program use and electronic files by controlling and recording user access.

HOW RESTRICTED ACCESS MODE WORKS:

1. A Program Administrator must be chosen to install the software and oversee the MicroLog™ System. Make sure to select Restricted Access mode during installation. The Administrator can subsequently change this mode to unrestricted access at any time.
2. The Administrator creates a User List of registered users and assigns levels of program access to those users. Each user has a username and password that must be correctly entered to log in.
3. The software automatically creates a Log-In Log that documents the history of program use. This log is un-editable, and can only be accessed by the Administrator.
4. The Log-In Log records:
 - The past 100 log-in attempts, in reverse chronological order.
 - The username, date and time logged-in and logged-out, and whether the user name is registered for each log-in attempt.
 - Privileges each registered user has been assigned.
 - Whether the program is in Restricted or Unrestricted Access mode.
 - As log-in records in excess of 100 drop from the list, they are saved to a "Read Only" file. Subsequent records of 100 will be saved to new files with different date stamps.
5. The Administrator assigns levels of access privileges to each user on an individual basis.
6. Privileges include:
 - Log-In – allows the user to log in and out of the MicroLog software using a password, and gives access to all features necessary to perform an identification and print results. All data will be saved automatically by Auto Save. User cannot use Data Management functions.
 - Set-Up - allows the user to change screen colors and fonts, have access to the Detailed Reader Set-up page (for troubleshooting), and disable the Auto Save feature. User cannot use Data Management functions.
 - View/Print – allows user to view or print data files. User cannot edit data files.
 - Edit – allows user access to all Data Management features, including editing data files and compiling databases.
 - Admin – allows user complete access to all aspects of the software (just like the Program Administrator). User cannot delete or change user names.

All electronic records may be retrieved for auditing purposes by authorized personnel. An Electronic record will contain the time, date and username of the person who saved the original record under the Restricted access mode. The file directory (location) is visible and if a backed up version is edited the time, date and username of the modifier are recorded and frozen. The edited file and the original parent file are visible. Original records can never be edited.

For more detailed information on using Restricted Access mode, please consult Section 3 in the MicroLog™ 4.2 User Guide. Biolog will continue to support our customer's system validation needs in every way possible, as quickly as possible.

PURE CULTURES ARE IMPORTANT FOR GOOD IDENTIFICATIONS

As with any microbial identification system, the precision and accuracy of MicroLog™ results require proper sample preparation. This process begins with isolating a pure and healthy culture on Biolog Universal Growth media. Using pure cultures when inoculating Biolog MicroPlates™ cannot be stressed enough. Inoculum unknowingly prepared from a mixed-growth plate can produce poor identifications and result in much frustration and extra work. Here are some simple guidelines and tips to ensure your sample is a pure culture.

CHECKING TO SEE IF YOUR CULTURE IS PURE

- Visual Inspection -Visually inspect the plate with the colony magnifier lamp that was provided with your Biolog System. Look for variations in colony morphologies, such as colonies growing within the colony in question, or differences in colors, sizes, colony edges, or textures. Carefully examine areas of confluent growth. If the lawn is not uniform in texture and color, this may indicate that the culture is not pure.
- Gram stain - Perform a Gram stain. Be alert for Gram variables, which can indicate that different microbes are present.
- Characterization Tests -Perform additional tests required by the manufacturer in order to classify the organism in the appropriate category.

WHAT SHOULD I DO IF MY CULTURE IS MIXED?

- Select a young, isolated colony and transfer it into 5 ml of sterile water or saline solution.
- Mix well, then place a small drop of the suspension onto BUG w/ blood media. Streak in all 4 quadrants.
- Incubate streaked plate at the appropriate temperature for the genus and species as indicated in the MicroLog™ 4.2 User Guide (see Appendix 4). If you are doing unknowns, incubating the sample at variable temperatures may be necessary.
- Following the guidelines listed above, check the plate for purity after 24 hours of incubation. Do not allow the culture to grow for too long. Repeat the procedure as many times as necessary to obtain a pure culture.

AFTER YOUR CULTURE IS PURE

- Select colonies from the last (third or fourth) quadrants of the agar plate to inoculate the MicroPlate™. This will minimize the chance of a contaminant being carried over in the final step of inoculation. Occasionally, colonies from the second quadrant must be used for slow growing organisms. If this is the case, and the organism is growing poorly, consider the suggestions in the following article on difficult to grow bacteria.
- Practice aseptic technique when processing a sample. Everything that touches the culture should be sterile. Respiratory isolates are common contaminants, so try not to talk when the lid is taken off the agar plate.

COMMON IDENTIFICATION PROBLEMS SEEN WITH MIXED CULTURES

- Excess positive reactions - your pattern is giving more positive reactions than the species you are comparing it to.
- High DIST - DIST tells you the number of mismatches between your MicroPlate™ results and the database pattern for that species. A very high DIST (such as 15 or more) may indicate contamination.

OF SPECIAL NOTE

- Mucoïd forming bacteria - Mucoïd forming Gram-negative enterics, such as *Klebsiella* sp., merit special attention. Contaminants may appear in extracellular mucus and are difficult to differentiate.
- Environmental organisms - Although the plate may appear pure, several different species of bacteria are often present in initial samples of environmental organisms. It is highly advisable to sub-culture an isolated colony three times to increase the odds of a pure culture.

Good identifications start with careful sterile technique, pure cultures, and using the correct media. If you have questions regarding these areas, please review your MicroLog™ 4.2 User Guide (Section 4, Pages 1-2; Section 6, Pages 2-4). Please do not hesitate to contact your Biolog Technical Service representative if you have additional questions.

HANDLING DIFFICULT TO GROW BACTERIA

Working with environmental isolates can represent a special challenge for the microbiologist. Since these samples often contain one or a number of unknowns, a variety of growth conditions often have to be experimented with to achieve optimal growth. Media type, temperature, and atmospheric conditions are all important to growth. If growth is very slow, it can be difficult to collect enough of the bacteria to prepare an inoculum for identification.

Initial preparation of an environmental isolate for MicroLog™ identification should be approached like any other bacterial sample. Streak Biolog Universal Growth media plates (BUG w/ blood, w/ maltose, or BUA), preparing media according to package insert. Give the organism time to acclimate to the agar plates, and try to determine the optimal temperature for growth. Once initial growth has been achieved, subculture the isolate for both purity and growth on the proper media for MicroLog™ identification. Please refer to Section 4 of the MicroLog™ 4.2 User Guide and the Instructions for Use booklets that accompany each MicroPlate™ type for complete information on preparing samples.

Occasionally, the organism will not grow as expected on that first subculture; Steps you can try:

CHANGE THE INITIAL ISOLATION MEDIA.

1. Perform first subculture on regular BUG or TSA.
2. Take the second subculture to BUG w/ blood.

IF THE ORGANISM GROWS AFTER FIRST SUBCULTURE TO BUG W/ BLOOD, BUT THE GROWTH IS POOR, LET IT GROW ENOUGH TO MAKE SURE YOU HAVE A PURE CULTURE.

1. Grow the organism at different temperatures to find optimum growth temperature.
 - Incubate at 26°C, 30°C or 35-37°C.
 - Remember that the optimum temperature used for growing the organism is the same temperature that should be used for incubating the MicroPlate™.

THE ORGANISM MIGHT BE FASTIDIOUS. ORGANISMS SUCH AS STREPTOCOCCI AND SOME GRAM NEGATIVES REQUIRE CO₂ FOR GROWTH.

1. Try sub-culturing Gram-positive organisms on BUG w/ blood and growing them at 35-37°C with 6.5% CO₂ atmosphere, especially if they are catalase negative.
2. Try sub-culturing Gram-negative organism that grow poorly on BUG w/ blood agar to chocolate agar, incubating under the fastidious conditions listed above.
 - Organisms that are in our GN-FAS group were all grown on chocolate agar when building our database.

IF THE ORGANISM STILL GROWS SLOWLY, ESPECIALLY IF IT IS FASTIDIOUS, LOOK FOR SMALL, PINPOINT COLONIES ON THE AGAR PLATE.

1. Use these tiny colonies to streak one or more plates to obtain additional growth.
 - Subculture a single colony using a swab that has been dipped in sterile saline.
 - Spread the colony over the entire surface of a fresh agar plate producing a “lawn” of growth.
2. Multiple growth plates may be needed to obtain the appropriate inoculum density.

IF THE ORGANISM DOES NOT GROW ON BUG OR BUG W/ BLOOD IT MAY BE AN ORGANISM THAT IS NOT IN THE MICROLOG™ DATABASE.

1. Try growing the organism on R2A.
2. Organisms that grow well on R2A such as *Methylobacterium* and *Rhizobium* are not in the current MicroLog database. However, they can be added as a User Database (see Section 7 in MicroLog™ User Guide 4.2 for more about compiling your own user database).

Note: See Appendix 4 in the MicroLog™ User Guide for a summary of the growth conditions used to set up all of the organisms in the MicroLog™ databases.

WHERE CAN I FIND REFERENCE INFORMATION?

Many of the references we use at Biolog can be found directly through our website: www.biolog.com. Select the Microbiology Links page. Listed below are some helpful websites, as well as several reference books that are useful in any microbiology laboratory.

Links:

1. www.dsmz.de/bactnom - current bacterial nomenclature. You can also link to this site through Biolog's web page.
2. www.cbs.knaw.nl/yeast.webc.asp - information on yeast. Select: List of reference and associated specimen.

Reference Books:

1. *Bergey's Manual of Systematic Bacteriology*, 4 volumes, Noel R. Krieg & John H. Holt, 1984, Williams & Wilkins, Baltimore, MD.
2. *Bergey's Manual of Determinative Bacteriology*, Ninth Edition, J. Holt, N. Krieg, P. Sneath, J. Staley, S. Williams, 1994, Williams & Wilkins.
3. *Manual of Clinical Microbiology*, Seventh Edition, P. Murray, E.J. Barron, M. P Faller, F. Tenover, R. Tenover, 1999, ASM Press, Washington D.C.
4. *Medical Tests for the Identification of Medical Bacteria*, Third Edition, Jean MacFaddin, 2000, Lippincott, Williams & Wilkins.
5. *Dictionary of Microbiology and Molecular Biology*, Third Edition, P. Singleton & D. Sainsbury, 2002, Wiley and Sons.

We hope you found this newsletter useful and informative. We would appreciate any feedback. If you have any suggestions or ideas for future topics, please e-mail us at tech@biolog.com

BIOLOG

Biolog, Inc.
3938 Trust Way
Hayward, CA 94545
Phone: (510)785-2564
Fax: (510)782-4639
www.biolog.com