| ION Nutritional Labs | Standard Operating Procedure<br>Microbial Identification via Biolog Microstation | SOP Number<br>D-1016 | Revision<br>0 |
|---|---|---|---|
| | | Effective Date<br>08/14/23 | Page<br>Page 1 of 21 |

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## 1.0 Purpose

The Biolog MicroStation System is an optical reading instrument for identifying microorganisms. BioLog's patented technology uses each microbe's ability to use particular carbon sources or chemical sensitivity assays to produce a unique pattern or "Phenotypic Fingerprint" for that microbe and compares that fingerprint to their identification databases. This procedure details processes used to identify microbial organisms with the Biolog MicroStation.

## 2.0 Scope

Any samples collected for quality or environmental testing may be used on this analyzer to identify gram-negative and gram-positive aerobes, anaerobes, and yeasts.

## 3.0 Responsibility

3.1 It is the responsibility of QC Laboratory Analysts to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure it is being followed.

3.3 It is the responsibility of QC Laboratory Management to keep this procedure aligned with current practices.

## 4.0 Definitions

4.1 **Microplate** –GENIII plates purchased from Biolog with 95 carbon source utilization tests (one in each well), with A1 well as control; can be used for aerobes and anaerobes; yeast microplate has 94 tests and two control wells (A1 and D1)

4.2 **Pattern** – a unique arrangements of reactions on a microplate, which occur from the metabolism of different reagents in each well; the Microstation system reads these patterns with a lamp and optical filters, and compares them to a data library of microbes

4.3 **GEN III** – microplate used for the identification of aerobic gram negative and gram positive microbes; also refers to the process of preparing plates, and to criteria used on the Biolog analyzer

4.4 **AN** – used for ID of anaerobic microbes; also refers to the process of preparing plated and to the criteria used on the Biolog analyzer

4.5 **YT** – type of microplate used for the identification of yeasts, also refers to the process of preparing plates, and to the criteria used on the Biolog analyzer

4.6 **Culture** – growth on media, used to obtain isolate

4.7 **Culture Media** – agar used to grow and isolate microorganisms. In most cases BUG (Biolog Universal Growth), BUG+B, TSA, or CHOC will be used.

4.8 **Isolate** – pure microbiological culture, not mixed with any other species

4.9 **Inoculum** – liquid suspension of isolate and appropriate IF mixed to a specific %T

4.10 **IF-A, IF-B, IF-C, AN-IF** – Inoculation Fluid; media used to grow isolates in microplates. The letters A,B,C,& AN refer to the protocol followed for positive identification on the MicroStation.

4.11 **%T** – Percent Transmittance; the cell density measured with the turbidometer, used to make the IF at the correct concentration for cellular growth on the microplates

4.12 **Turbidometer** – instrument used to measure %T of IF before the second incubation

4.13 **Protocol** – refers to a setting in the Biolog analyzer software

4.14 **MicroLog Secure 6.3** – software used to operate the Biolog MicroStation

4.15 **Audit trail viewer** – software used to document all activity with MicroLog Secure 6.3 as required by 21 CFR part 11

4.16 **Aseptic technique** – standard laboratory procedures used to prevent contamination

4.17 **SIM** – an abbreviation for a similarity index value used to assess how well a sample is identified: a value of 1 indicates a perfect match; a value of 0 indicates no match; a larger value is a better match; a value of 0.5 is the threshold for GEN III

4.18 **DIST** – indicates the approximate number of mismatches between MicroPlate results and the database pattern aggregate for that species; a smaller value is a better match

4.19 **PQV** – Process Quality Verification

## 5.0 References

5.1 Biolog Microstation Identification Validation Protocol

5.2 MicroLog Secure 6.2 User Guide

5.3 Biolog Microstation User Guide

5.4 D-113, SOP, Microbiological Media Validation

5.5 D-1016-F1, Form, Microbial Identification Log

5.6 A-106, SOP, Documentation Guidelines for cGMP Records

5.7 C-502, SOP, Record Storage, Retention, and Destruction

## 6.0 Limitations

6.1 With the exception of yeast, the instructions provided in this procedure are not for any mold or fungi.

6.2 The product is not for human in vitro diagnostic use.

6.3 The Biolog analyzer is not able to quantify or count any colonies. It is solely for identification.

6.4 Only use media purchased from Biolog or that has been validated as per SOP D-113 Microbiological Media Validation.

6.5 The microbe to be identified must be from a pure culture that is no more than 24 hours old.

6.6 The test panels will only identify members of the species in the current databases (GEN III, AN, and YT). Other species and atypical strains will usually yield a "no identification" result.

## 7.0 Procedure

7.1 These steps are to be followed in the order as written. Keep in mind, this is a two day process. Each sample must be incubated twice: once for a pure culture, second for the microplate reactions to take place. Only use fresh growth (22-24 hours for most bacteria) of isolated colonies to make the inoculum.

7.2 First Culture and Incubation - Materials Needed

7.2.1 Sample for analysis

7.2.2 Appropriate growth media

7.2.3 Sterile swabs

7.2.4 Incubator with appropriate environment for growth

7.3 First Culture and Incubation – Procedure

7.3.1 See section 11.0 "Media selection and Process Chart" to select the appropriate media for optimal growth. Label all plates prior to streaking.

7.3.2 Using aseptic technique, streak plates for isolation.

7.3.3 Incubate for at least 22 hours in the environment appropriate for the organism to grow. See charts for optimal growth environment. Record the date, time, and tech initials on form D-1016-F1 Microbial Identification Log.

7.3.4 Use the red biohazard bags for disposal of all contaminated waste generated in this process.

7.4 Visual Plate Examination - Materials Needed

7.4.1 Colony magnifier lamp

7.4.2 Gram stain kit, if anaerobes have been cultured

7.4.3 Microscope

7.5 Visual Plate Examination – Procedure

7.5.1 After at least 22 hours of incubation, examine areas of confluent growth. If the lawn is not uniform in texture and color, this indicates that the culture is not pure. **Preparing an inoculum directly from a mixed-growth plate will not ID any growth on the analyzer.**

7.5.2 Conversely, sometimes a culture may be pure, but gives the appearance of heterogeneity. This is due to a common phenomenon whereby microorganisms produce more than one colony type. To be certain of its identity, purify and test each colony type individually. Be aware that different colony types of the same strain may ID but give different phenotype profiles.

7.5.3 In instances where growth is indistinguishable between yeast and fungi, a wet prep may be performed to differentiate genus. **If the wet prep determines the growth to be a fungi, it cannot be identified on the analyzer at this time, and must be sent to a third party laboratory for analysis.**

7.5.4 In circumstances where there is not sufficient growth for suspected anaerobic

microbes, a second sub culture is necessary. Additionally, if the suspected organism is a historically weak grower, you may swab 2 plates for the first culture, and take isolates from both plates, so long as the growth on both plate does not exhibit pleomorphism between the two.

7.5.5 After identifying a pure culture on the plate, follow the instructions in the next sections to prepare the IF and Microplates.

7.6 MicroPlate Inoculation and Incubation – Materials Needed

7.6.1 Pure microbial culture

7.6.2 Inoculation Fluid

7.6.3 Sterile swabs or streakerz

7.6.4 Turbidimeter

7.6.5 8 channel pipette

7.6.6 Sterile pipette tips

7.6.7 Sterile multichannel pipette fluid reservoir

7.6.8 MicroPlate ( GEN III or YT)

7.6.9 Incubator with appropriate environment for microbial growth

7.7 MicroPlate Inoculation and Incubation – Procedure

7.7.1 Calibrate the turbidimeter.

7.7.1.1 Before powering on, make sure the dial is turned all the way left, and the display is at 0%T. If the hand in the display is not at 0, turn the screw on the front of the turbidimeter to adjust the hand to 0.

7.7.1.2 Turn on the turbidimeter and wait 15 minutes for warm up.

7.7.1.3 Put a blank IF vial into the tube well on the top of the turbidimeter and use the screw on the front to adjust the display to read 100%T. The turbidimeter is now ready for use.

7.7.2 Liquid Inoculum Preparation

7.7.2.1 Prior to starting, make sure the correct IF is selected and pre-label all IF tubes.

7.7.2.2 Place the IF tube into the top of the turbidimeter and remove the cap. Make sure the display is at 100%T.

7.7.2.3 Using a sterile swab, gently select a very small amount of an isolate colony from the agar plate.

7.7.2.4 Place the swab into the tube and disperse the growth into the fluid. Keep in mind, the inoculum MUST be uniformly suspended. Clumpy colonies may need additional agitation to achieve a homogenous suspension. It takes only a very small amount of colony to get to the correct %T. Start with a smaller amount than needed and work your way up to the appropriate %T. An incorrect cellular density will not ID any microbes and will need to be recultured, resulting in at least a 24 hour delay in result.

7.7.2.5 More than one inoculum can be made at a time as long as they are placed in the incubator within 20 minutes. Waiting any longer may cause inaccurate identification. Anaerobic bacteria are especially sensitive to delays. If you are running a batch of MicroPlates, set them up (from preparing the inoculum to pipetting into the MicroPlate) so you will not exceed the 20 minute deadline. The process tracking log has space for up to 20 entries at a time.

7.7.3 MicroPlate Preparation

7.7.3.1 Remove the MicroPlates from their pouches and label each MicroPlate (not the lid) with appropriate identification.

7.7.3.2 Using aseptic technique, pour the cell suspension into a sterile multichannel Pipette fluid reservoir.

7.7.3.3 Firmly attach eight sterile tips to the 8-Channel repeating Pipette. Make sure the tips are straight, they can be carefully adjusted at the head of the pipette. Do not touch any part of the tips that come in contact with the inoculum as it will contaminate the culture. If the pipette head does not audibly click when the tips are loaded, it will not aspirate or dispense properly.

7.7.3.4 Prior to filling the tips with the suspension, Make sure the pipette is on the 100 µl/multi dispense program. The electronic pipette will prime the tips automatically. Check that all the tips are filling equally/ not leaking. If there are bubbles or leaking, do not to attempt to fill the plate. Instead, carefully dispense the inoculum back into the reservoir, dispose the tips, and try again.

7.7.3.5 Fill all MicroPlate wells with 100 µl per well. Take care to position the tips inside of the wells at an angle. Avoid touching the bottom of the wells, which could transfer carbon sources and alter the reaction in the well.

7.7.3.6 If the fluid level in the tips gets low, refill and continue dispensing. The pipette will double beep, signaling you to purge and refill if necessary.

7.7.3.7 Examine the MicroPlate to make sure all wells are filled. Any missing wells can be filled with leftover inocula with a single pipette. However, if a well is missing a sample, another one may be overfilled. Do not attempt to move samples from one well to another. Most IFs will form a soft gel shortly after inoculation.

7.7.3.8 Cover the MicroPlate with its lid. Again, microplates must be placed in the incubator no later than 20 minutes after inoculation. Anaerobic plates must be incubated in a hydrogen free environment. In some circumstances when a known anaerobe's growth is scant, the plates can be placed in a candle jar sealed with a strip of parafilm stretched around the neck of the jar and lid.

## 8.0 Using the Microstation Analyzer

8.1 Security Overview and Part 11 Compliance

8.1.1 Operation of this analyzer requires the use of a custom built Windows PC. This computer does not have any specific user as the main Windows account. Instead of having individual Windows accounts and permissions, there is one general account that all QC Lab personnel will have access to. For security and in accordance with 21 CFR part 11, all lab personnel will receive their own login and choose their own case-sensitive, alpha numeric password. Furthermore, this computer is not connected to the Ion Labs network; it does not have access to email or any of the company drives, nor does it have the ability for remote access. Additionally, all actions performed in MicroLog Secure 6.3.1 are documented and timestamped with a separate audit trail program that runs in the background. All results are printed automatically, and are also stored on the computer. Finally, all results are generated in a proprietary format and cannot be transferred, shared, or altered in any way.

8.2 Start Up the Analyzer

8.2.1 Open MicroLog Secure 6.3.1 on the computer connected to the MicroStation.

8.2.2 Log in with username and password.

8.2.3 Click the *setup* tab.

8.2.4 Power on the MicroStation and wait for the power up sequence to complete.

8.2.5 Click *initialize reader* button and wait for initialization to complete. Once complete, *Com Port: Open and Ready* is displayed with a green background. If the com port is not set to 5, it will not initialize.

8.2.6 Click on the *read* tab. The MicroStation is now ready to set up a batch and read plates.

8.3 Create a Batch List

8.3.1 On the Read screen, fill in the Plate Information for your plate.

8.3.2 Click the Add to List button – this adds the plate to the batch – but moves the selection down for adding another new plate.

8.3.3 Select the New plate in the list, fill out the Plate Information and click the Add to List button (or press the Enter key) to add a plate to the batch list. Normally plate information is cleared when starting a new plate. Un-checking the Clear Information After Save check box leaves the last plate's information as a starting point which saves a lot of work. This is useful when entering multiple plates of the same type; change the sample name for each new plate.

8.3.4 The batch can be printed as a loading manifest to keep plates in order when loading.

8.3.5 After the batch has been created, read each plate in order of the batch. Results print automatically.

8.3.6 After valid results have been called, dispose of microplates and their lids into the red biohazard waste.

8.4 Putting Plates on the Microstation

8.4.1 Make sure the reaction pattern is well developed.

8.4.2 Wipe the bottom of the MicroPlate to remove condensation and fingerprints.

8.4.3 Open the analyzer lid and place the MicroPlate on the reader tray with the A1 well at the top left hand corner, and the upper right corner has a notch missing.

8.4.4 Gently push the MicroPlate down until it snaps into a level position in the reader tray and remove the MicroPlate lid.

8.4.5 Close the analyzer lid.

8.4.6 Click *Read Plate* or double- click on the sample in the batch screen.

## 9.0 Identification Results and Criteria

9.1 Positive Identification of Genus, Species and Subspecies requirements

9.1.1 A Species ID can be called if and when the SIM value for the first choice species exceeds the ID threshold. The ID threshold is set at a higher level for early reads in order to avoid premature misidentification. The threshold starts at 1.0 and declines linearly over the first 8 hours to 0.5. From 8 hours on, any time the SIM of the first choice species exceeds 0.5, a Species ID can be called. When the SIM match is high enough, the ID Box displays with a Subspecies, a Species or a Genus level identification message. Entering the incubation hour at each read is vital because this determines the SIM threshold and other decision criteria.

9.2 No Identification

9.2.1 In the situations listed below, the analyzer will not be able to ID both genus and species. The software will generate a prompt to continue incubation or try a different protocol.

9.2.1.1 If the MicroPlate has a sufficient pattern (at least three positive reactions) and the SIM match is still too low for a Species identification, a Genus level identification or *No ID* as a final result is displayed.

9.2.1.2 If the MicroPlate has an insufficient pattern (defined as less than three positive reactions), the incubation time is extended up to 48 hours. The ID Box displays *No ID Yet - Continue Incubation* until a Species identification is obtained or until the 36 to 48 hour mark is reached.

9.2.1.3 If there is no Species identification by 36 hours, the ID box displays either a Genus level identification or a *No ID* message at the final 36 to 48 hour read.

9.2.1.4 A *No ID - Insufficient Pattern* result message at 36 to 48 hours displays additional suggestions for retesting the strain with a different protocol.

9.2.1.5 If the number of borderline reactions in the online MicroPlate exceed 25, the ID Box displays *Too many Borderline Reactions* as a final result.

9.2.1.6 If the color in the A1 reference well becomes too dark, the ID Box displays a False Positive message, usually followed by a suggestion for retesting the strain with a different protocol.

9.2.2 Additionally, a result of *No ID* can be from a mismatch.

9.2.2.1 After a MicroPlate is read, the View Details Window automatically displays the major mismatches between the #1 ranked species and your sample MicroPlate on the Pos/Neg Graphic tab. When this happens, check for black + and - signs in the on-screen MicroPlate. The black (+) and (-) signs show mismatches between your sample and the database record (approximately +>80% pos, -<20%pos).

9.2.2.2 If the mismatches are all positive (+) or all negative (-), you may have made a testing error and a new culture must be made.

- 'All +' means the plate pattern is giving fewer positive reactions than the species you're comparing it against. Possible causes are: under inoculation, the A1 well is overfilled/clumpy/cloudy, the

cells have been mishandled, cultured on incorrect media, wrong IF, incorrect incubation environment.

- 'All –' means the plate pattern is giving more positive reactions than the species you're comparing it to. Possible causes include: Over inoculation, A1 well is under filled, contamination from an impure culture.

## 10.0 Annual Performance Qualification

10.1 The Biolog MicroStation has no user serviceable parts.

10.2 A Performance Qualification should be done annually. Follow Biolog protocol VP-003MLS located in the Microstation user manual.

## 11.0 Media Selection and Process Chart

| | | **Protocol A** | **Protocol B** | **Protocol C1** | **Protocol C2** | **YT** | **AN** |
|---|---|---|---|---|---|---|---|
| | | Standard Protocol | | Microaerophilic Protocol | Fastidious or Methylobactrium | | |
| CULTURE FOR ISOLATION | Culture Media | BUG+B, BUG, TSA | BUG+B and BUG | BUG+B | CHOC, BUG+B & R2A | BUY | BUA+B, BAG |
| | Atmosphere | AIR | AIR | 65% CO2 | 65% CO2 | AIR | ANA |
| | Media Temperature | 33°C | 33°C | 33°-37°C | 33°-37°C | | 35°-37°C |
| | Incubation Atmosphere & Temperature | AIR 31-35°C | | | | AIR 26-28°C | AN Microplate must be incubated in a hydrogen-free anaerobic atmosphere |
| | | Media and Temperatures may vary due to requirements of low or high enrichment media and strict lower temperature requirements for some species | | | | | |
| MICROPLATE INOCULATION | Inoculating Fluid | IF-A | IF-B | IF-C | IF-C | AN-IF | AN-IF |
| | Inoculum %T | 95 - 98%T | 92 - 95%T | 90 - 95%T | 62 - 68%T | 47%T | 65%T |
| | MicroPlate - µ l per well | 100 µl | 100 µl | 100 µl | 100 µl | 100 µL | 100 µL |
| | Incubation Time in hours | Up to 22 hours ** | | | | 24, 48, 72 | 20-24 |
| | | **Incubation time may be extended to 36 hours by the system if an insufficient number of positive reactions are detected at 22 hours. | | | | | |

## 12.0 Documentation Requirements

12.1 A PQV check must be performed for each completed logbook page as outlined in SOP A-106 Documentation Guidelines for cGMP Records.

12.2 Documents will be maintained following SOP C-502 Record Storage, Retention, and Destruction.

## 13.0 Revision History

| Revision | Date | Description of Changes | CCR | By |
|---|---|---|---|---|
| 0 | 07/11/23 | New procedure. | N/A | B. Echevarria |

## 14.0 Attachments

14.1 Attachment 1 – Environmental Monitoring ID Map

14.2 Attachment 2 – Probiotic ID Map

## Attachment 1 – Environmental Monitoring ID Map

Streak onto TSA Plate/Blood Agar or SDA plate for each isolate type on each sample.

Record sample information plus ABC designation based on number of isolates sample and the date in the Biolog ID Log Book.

Place streaked plates into the 30-35°C incubator if bacteria and 20-25 °C incubator if yeast

After 24 hours remove streaked plates from incubators, verify that there are no mixed colonies. If there are, streak each isolate onto fresh TSA or SDA and label as 1, 2, or 3 along with sample number and place back into appropriate incubator for 24hours

If the plates are not mixed, gather supplies and let come to room temp:
Protocol A (bacteria)- Reservoirs, pipet, pipette tips, If-A, Gen III microplates
Protocol YT (yeast)- Reservoirs, pipette tips, IF-YT, YT microplates

Inoculate fluids to correct turbidity:
Protocol A- 95-98%
Protocol YT-47%

Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.

Place microplate into incubator for 20 hours. Same incubators as samples was in originally. Place date on ID log under correct Protocol.

Remove microplates from incubator and read on Biolog.
If an ID is given, the microplate and the original isolate may be discarded, record date on ID Log under ID complete.
If "NO ID Yet", place microplate back into incubator for another 24 hours, Yeast may be returned to incubator for a further 24 hours if necessary. Re-read on Biolog.
If "NO ID" or "False Positive" place original isolate into next phase of testing.
IF YT NO ID must be sent out for ID as they is no further testing in-house.

## Attachment 1 – Environmental Monitoring ID Map (continued)

| Protocol B |
|---|

| Repeat original steps except:<br>Inoculate in IF-B<br>Turbidity should be 92-95%<br>Microplate is Gen III<br>Record in ID logbook the date under protocol B column for the specific isolate |
|---|

| After 24 hours read on Biolog, ensure that the protocol is changed to "B"<br>If an ID is given, the microplate and the original isolate may be discarded, record date on IDLog under ID complete.<br>If "NO ID Yet", place microplate back into incubator for another 24 hours. Re-read on Biolog.<br>If "NO ID" place isolate into next phase of testing. |
|---|

| Protocol C1/C2 |
|---|

| Repeat original steps except:<br>Inoculate in IF-C<br>Turbidity should be 90-92%<br>Microplate is Gen III<br>Record in ID log book the date under the Protocol C1 column for the specific isolate |
|---|

| After 24 hours read on Biolog, ensure that the protocol is changed to "C1"<br>If an ID is given, the microplate and the original isolate may be discarded, record date on IDLog under ID complete.<br>If "NO ID" isolate may be tried on C2 protocol<br>Turbidity – changes to 65%<br>Microplate is Gen III, incubate for 24 hours, read on Biolog if still "NO ID"<br>Send to Charles River for genotype ID (ACCUPRO-ID-5).<br>Record in Comments in ID log book that ID was sent out. |
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## Attachment 2 – Probiotic ID Map

**Bacillus Species**

**B. subtilis**

- Record sample information and the date in the Biolog ID Log Book
- Streak onto Blood Agar and incubate in 30-35°C for 24 hours
- Gather supplies for Protocol A-reservoirs, pipet, pipette tips, IF-A, and Gen III Microplate and allow to come to room temp.
- Inoculate fluid to correct turbidity: 95-98%
- Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.
- Place microplate into 30-35°C incubator for 20 hours. Check protocol column and/or hour on the ID log.
- Remove microplates from incubator and read on Biolog.
- If an ID is given, the microplate and the original isolate may be discarded, record date on IDLog under ID complete.
- If "NO ID Yet", place microplate back into incubator for another 24 hours.
- If still "NO ID yet", re-streak on Blood Agar and utilize Profile B, IF-B and gen microplate.

**B sporogenes/ L. coagulans**

- Send out for testing

**Lactobacillus Species**

**L. acidophilus** (MAY NEED TO SEND OUT FOR TESTING)

- Record sample information and the date in the Biolog ID Log Book
- Streak onto Blood Agar, place into a candle jar and incubate in 30-35°C for 24 hours
- Gather supplies for Protocol C1-reservoirs, pipet, pipette tips, IF-X, and Microplate XX
- Inoculate fluid to correct turbidity:
- Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.
- Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.
- Place microplate into candle jar and incubator for 20-24 hours. Same incubators as samples was in originally. Place check on ID log under correct Protocol.
- Remove microplates from incubator and read on Biolog.
- If an ID is given, the microplate and the original isolate may be discarded, record date on IDLog under ID complete.
- If "NO ID Yet", place microplate back into incubator for another 24 hours.

## Attachment 2 – Probiotic ID Map (continued)

**Lactobacillus Species**

**L. gassieri**

- Record sample information and the date in the Biolog ID Log Book
- Streak onto Blood Agar and incubate in 30-35°C for 24 hours
- Gather supplies for Protocol XXX-reservoirs, pipet, pipette tips, IF-X, and Microplate XX
- Inoculate fluid to correct turbidity:
- Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.
- Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.
- Place microplate into incubator for 20 hours. Same incubators as samples was in originally. Place date on ID log under correct Protocol.
- Remove microplates from incubator and read on Biolog.
- If an ID is given, the microplate and the original isolate may be discarded, record date on IDLog under ID complete.
- If "NO ID Yet", place microplate back into incubator for another 24 hours

**L. paracasei**

- Record sample information and the date in the Biolog ID Log Book
- Streak onto BUA agar, place into anaerobic container and incubate in 35-39°C for 24 hours.
- Streak the 24 hour sample onto another BUA agar, place into anaerobic container and incubate in 35-37°C for another 24 hrs.
- Gather supplies for Protocol AN-reservoirs, pipet, pipette tips, IF-AN, and AN Microplate
- Inoculate fluid to correct turbidity: 6-65%
- Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.
- Place microplate into anaerobic container and incubate at 35-39°C for 20 hours. Place date on ID log under correct Protocol.
- Remove microplates from incubator and read on Biolog.
- If an ID is given, the microplate and the original isolate may be discarded, record date on IDLog under ID complete.
- If "NO ID Yet", place microplate back into incubator for another 24 hours

## Attachment 2 – Probiotic ID Map (continued)

**L. plantarum**

- Record sample information and the date in the Biolog ID Log Book
- Streak onto Blood Agar and incubate in 30-35°C for 24 hours
- Gather supplies for Protocol C1 -reservoirs, pipet, pipette tips, IF-C, and Gen III Microplate
- Inoculate fluid to correct turbidity 90-92%
- Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.
- Place microplate into incubator 30-35°C for 20 hours. Same incubators as samples was in originally. Place date on ID log under correct Protocol.
- Remove microplates from incubator and read on Biolog.
- If an ID is given, the microplate and the original isolate may be discarded, record date on IDLog under ID complete.
- If "NO ID Yet", place microplate back into incubator for another 24 hours

**L. rhamnosus**

- Record sample information and the date in the Biolog ID Log Book
- Streak onto Blood Agar and place into candle jar, incubate in 30-35°C for 24 hours
- Gather supplies for Protocol C2-reservoirs, pipet, pipette tips, IF-C, and Gen III Microplate
- Inoculate fluid to correct turbidity: 60-65%
- Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.
- Place microplate into a candle jar and incubate 30-35°C for 20 hours. Place check on ID log under correct Protocol.
- Remove microplates from incubator and read on Biolog.
- If an ID is given, the microplate and the original isolate may be discarded, record date on ID Log under ID complete.

## Attachment 2 – Probiotic ID Map (continued)

**Bifidobacterium Species**

**B. bifidum**

- Record sample information and the date in the Biolog ID Log Book
- Streak onto BUA Agar and incubate in 35-37°C for 24 hours.
- After 24 hours re-streak onto another BUA agar and incubate in 35-37°C for 24 hours.
- Gather supplies for Protocol AN-reservoirs, pipet, pipette tips, IF-AN, and AN Microplate
- Inoculate fluid to correct turbidity: 60-65%
- Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.
- Place microplate into anaerobic container and incubator at 35-37°C for 20 hours. Place date on ID log under correct Protocol.
- Remove microplates from incubator and read on Biolog.
- If an ID is given, the microplate and the original isolate may be discarded, record date on IDLog under ID complete.
- If "NO ID Yet", place microplate back into incubator for another 24 hours

**B. breve**

- Record sample information and the date in the Biolog ID Log Book
- Streak onto BUA Agar and incubate in 35-37°C for 24 hours.
- After 24 hours re-streak onto another BUA agar and incubate in 35-37°C for 24 hours.
- Gather supplies for Protocol AN-reservoirs, pipet, pipette tips, IF-AN, and AN Microplate
- Inoculate fluid to correct turbidity: 60-65%
- Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.
- Place microplate into anaerobic container and incubator at 35-37°C for 20 hours. Place date on ID log under correct Protocol.
- Remove microplates from incubator and read on Biolog.
- If an ID is given, the microplate and the original isolate may be discarded, record date on IDLog under ID complete.
- If "NO ID Yet", place microplate back into incubator for another 24 hours

## Attachment 2 – Probiotic ID Map (continued)

**B. lactis**

- Record sample information and the date in the Biolog ID Log Book
- Streak onto BUA Agar and incubate in 35-37°C for 24 hours.
- After 24 hours re-streak onto another BUA agar and incubate in 35-37°C for 24 hours.
- Gather supplies for Protocol AN-reservoirs, pipet, pipette tips, IF-AN, and AN Microplate
- Inoculate fluid to correct turbidity: 60-65%
- Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.
- Place microplate into anaerobic container and incubator at 35-37°C for 20 hours. Place date on ID log under correct Protocol.
- Remove microplates from incubator and read on Biolog.
- If an ID is given, the microplate and the original isolate may be discarded, record date on IDLog under ID complete.
- If "NO ID Yet", place microplate back into incubator for another 24 hours

**B. longum**

- Record sample information and the date in the Biolog ID Log Book
- Streak onto BUA Agar and incubate in 35-37°C for 24 hours.
- After 24 hours re-streak onto another BUA agar and incubate in 35-37°C for 24 hours.
- Gather supplies for Protocol AN-reservoirs, pipet, pipette tips, IF-AN, and AN Microplate
- Inoculate fluid to correct turbidity: 60-65%
- Inoculate Microplate with 100ul of Inoculating fluid, use 8 channel pipet. Label Microplate with sample information.
- Place microplate into anaerobic container and incubator at 35-37°C for 20 hours. Place date on ID log under correct Protocol.
- Remove microplates from incubator and read on Biolog.
- If an ID is given, the microplate and the original isolate may be discarded, record date on ID Log under ID complete.
- If "NO ID Yet", place microplate back into incubator for another 24 hours

| ION Nutritional Labs | Microbial Identification Log | | |
|---|---|---|---|
| | Form: D-1016-F1 | CCR No. N/A | Revision: 0 |

Logbook Number: _____

| Received Date | Sample ID | Initial Culture Date | Status Check column when initiated and N/A if not applicable | | | | | | | | | | | Date Identification Completed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Protocol A | | Protocol B | | Protocol C1 | Protocol C2 | Protocol YT | | | Protocol AN | | |
| | | | 24 hr | 48 hr | 24 hr | 48 hr | | | 24 hr | 48 hr | 72 hr | 24 hr | 48 hr | |
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