

AN MicroPlate™

A1 Water	A2 N-Acetyl-D- Galactosamine	A3 N-Acetyl-D- Glucosamine	A4 N-Acetyl-β-D- Mannosamine	A5 Adonitol	A6 Amygdalin	A7 D-Arabitol	A8 Arbutin	A9 D-Cellobiose	A10 α-Cyclodextrin	A11 β-Cyclodextrin	A12 Dextrin
B1 Dulcitol	B2 D-Erythritol	B3 D-Fructose	B4 L-Fucose	B5 D-Galactose	B6 D-Galacturonic Acid	B7 Gentiobiose	B8 D-Gluconic Acid	B9 D-Glucosaminic Acid	B10 α-D-Glucose	B11 α-D-Glucose- 1-Phosphate	B12 D-Glucose- 6-Phosphate
C1 Glycerol	C2 D,L-α-Glycerol Phosphate	C3 m-Inositol	C4 α-D-Lactose	C5 Lactulose	C6 Maltose	C7 Maltotriose	C8 D-Mannitol	C9 D-Mannose	C10 D-Melezitose	C11 D-Melibiose	C12 3-Methyl-D- Glucose
D1 α-Methyl-D- Galactoside	D2 β-Methyl-D- Galactoside	D3 α-Methyl-D- Glucoside	D4 β-Methyl-D- Glucoside	D5 Palatinose	D6 D-Raffinose	D7 L-Rhamnose	D8 Salicin	D9 D-Sorbitol	D10 Stachyose	D11 Sucrose	D12 D-Trehalose
E1 Turannose	E2 Acetic Acid	E3 Formic Acid	E4 Fumaric Acid	E5 Glyoxylic Acid	E6 α- Hydroxybutyric Acid	E7 β- Hydroxybutyric Acid	E8 Itaconic Acid	E9 α-Ketobutyric Acid	E10 α-Ketovaleric Acid	E11 D,L-Lactic Acid	E12 L-Lactic Acid
F1 D-Lactic Acid Methyl Ester	F2 D-Malic Acid	F3 L-Malic Acid	F4 Propionic Acid	F5 Pyruvic Acid	F6 Pyruvic Acid Methyl Ester	F7 D-Saccharic Acid	F8 Succinamic Acid	F9 Succinic Acid	F10 Succinic Acid Mono-Methyl Ester	F11 m-Tartaric Acid	F12 Urocanic Acid
G1 Alaninamide	G2 L-Alanine	G3 L-Alanyl-L- Glutamine	G4 L-Alanyl-L- Histidine	G5 L-Alanyl-L- Threonine	G6 L-Asparagine	G7 L-Glutamic Acid	G8 L-Glutamine	G9 Glycyl-L- Aspartic Acid	G10 Glycyl-L- Glutamine	G11 Glycyl-L- Methionine	G12 Glycyl-L- Proline
H1 L-Methionine	H2 L-Phenylalanine	H3 L-Serine	H4 L-Threonine	H5 L-Valine	H6 L-Valine plus L-Aspartic Acid	H7 2'-Deoxy Adenosine	H8 Inosine	H9 Thymidine	H10 Uridine	H11 Thymidine-5'- Mono-phosphate	H12 Uridine-5'- Mono-phosphate

FIGURE 1. Carbon Source in AN MicroPlate

INTRODUCTION

The Biolog AN MicroPlate (Figure 1) is designed for identification of a very wide range of anaerobic bacteria, including the genera *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Fusobacterium*, *Lactobacillus*, *Lactococcus*, *Megasphaera*, *Pectinatus*, *Pediococcus*, *Peptostreptococcus*, *Propionibacterium* and *Weissella*. These genera are important in industrial and environmental applications, especially the food industry where they are responsible for both food production and food spoilage. Some clinical species are not currently in our database. These will be added when they meet our performance criteria. The AN MicroPlate employs the same redox chemistry used in the Biolog GP2 and GN2 MicroPlates. This chemistry, based on reduction of tetrazolium, responds to the process of metabolism (oxidation of substrates) rather than to metabolic by-products (e.g. acid). Biolog's universal chemistry works with any carbon source and greatly simplifies the testing process, as no color developing chemicals need to be added after incubation.

AN MICROPLATE

The Anaerobe Database contains over 350 taxa of anaerobic bacteria including over 70 species of lactic acid bacteria.

The Biolog AN MicroPlate performs 95 discrete tests simultaneously and gives a characteristic reaction pattern called a "metabolic fingerprint". These fingerprint reaction patterns provide a vast amount of information conveniently contained on a single Biolog MicroPlate. The patterns are compared using Biolog MicroLog™ database software to give an identification.

Other anaerobic kit-based identification methods rely on fewer tests to perform identifications. Therefore an anaerobic organism that was previously characterized by another method may yield an identification result that differs from the Biolog AN MicroPlate & Database. This difference may be due to several factors. When determining the validity of an anaerobic identification result from the Biolog AN MicroPlate and another method, consider the following:

- The MicroLog System bases its identification on a larger number of tests
- The MicroLog System covers more species
- The taxonomy of many anaerobic genera is poorly defined

Various methods have different numbers and types of organisms within their database. Figure 2 presents several popular kit-based methods. The Biolog AN MicroPlate has a much larger number of tests, which provides greater fingerprint discrimination and a larger database.

Manufacturer	Number of Anaerobic Species in Database	Number of Tests Used for Identification
Biolog, Inc MicroLog™	359	95
bioMérieux Vitek®	84	28
bioMérieux API 20A®	77	20
BBL® Crystal™	107	28
Innovative Diagnostic Systems, Inc. RapID ANA II	96	18

FIGURE 2. Comparison of Commercial Test Kits for Anaerobes

Beyond the number of tests provided to detect an unknown, some methods rely primarily on fermentation of sugars. This approach to identification does not provide the necessary environment for every organism of interest. Many bacteria cannot utilize sugars via a fermentative process and are missed or react weakly with these methods.

The state of anaerobic taxonomy further exacerbates the limitations of other methods. The taxonomy of anaerobic bacteria is based on much less information and is not as developed as aerobic taxonomy. For example, the classification of *Clostridium botulinum* is based on the organism's ability to produce one or more forms of botulinum neurotoxin. Five morphologically distinct organisms have been shown to produce this neurotoxin. According to current anaerobic taxonomy all five of these organisms should be classified (identified) as *C. botulinum*. Strains of these species which do not produce the toxin, are not classified as non-toxicogenic *C. botulinum*, but would be called, for example *C. sporogenes*. If this same criterion were applied to aerobic taxonomy then all Shiga toxin producing species of enteric bacteria would be assigned to the genus *Shigella*.

PROCEDURE FOR USING AN MICROPLATES

The procedure is fast and simple, involving only 5 steps, and requiring only 2 to 3 minutes hands-on time per sample.

- 1) A pure culture of a bacterium is grown on a Biolog Universal Anaerobe agar plate (Biolog Catalog # 70007 for a 500g jar or 71007 for pre-poured plates) in either an anaerobic chamber or anaerobic jar until enough growth is present to prepare a suspension.
- 2) The bacteria are swabbed from the surface of the agar plate, and suspended to a specified density in AN Inoculating Fluid (Biolog Catalog # 72007). This step may be performed in an anaerobic chamber.
- 3) 100 µl of bacterial suspension is pipetted into each well of the AN MicroPlate (Biolog Catalog # 1007) under aerobic conditions.
- 4) The MicroPlate is incubated in an anaerobic jar with a hydrogen free anaerobic atmosphere at 35° C for 20-24 hours. (A hydrogen free anaerobic atmosphere can be produced by either the Oxoid AnaeroGen™ or the Mitsubishi AnaeroPak™ System, Pack-Anaero.)
- 5) The MicroPlates are removed from the anaerobic container and read either visually or with the Biolog MicroStation™ Reader, compared to the AN Database (Biolog catalog # 22607D), and a result is determined.

ANAEROBE DATABASE

The Biolog AN database has the largest database of any kit-based method. This superior product will be an invaluable addition to your anaerobic laboratory. Some highlights are:

- *Bifidobacterium*, usually called *Bifidus*, which could not formerly be speciated by ID kits, can now be identified with this product.
- The genera *Pectinatus* and *Megasphaera* are not covered in other anaerobic test kits but can be identified with the MicroLog AN database.
- The MicroLog database has 43 *Lactobacillus spp.*, and over 70 species of lactic acid bacteria. Other kit based methods cover approximately 7 species.

For more information, contact us using the information below.