INTENDED USE
BBL Oxi/Ferm Tube II is a ready-to-use identification system for fermentative, oxidase positive and for nonfermentative Gram negative bacteria isolated from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE
Microbiological method. Fermentative, oxidase positive and nonfermentative, oxidase positive or negative Gram negative bacteria play a role as infectious agents. This group of bacteria utilizes carbohydrates principally by oxidation and, only occasionally, by fermentation. These organisms usually possess both the enzymes, catalase, which can break down hydrogen peroxide to water and oxygen, and oxidase (cytochrome c-oxidase), an enzyme which can rapidly oxidize dimethyl- or tetramethyl-paraphenylene diamine. The oxidase test is used to separate them from the Enterobacteriaceae which are, with the exception of Plesiomonas shigelloides, oxidase negative [It has been proposed recently to include P. shigelloides into the Enterobacteriaceae based on 16S rRNA sequencing and because it contains the enterobacterial common antigen.1,2 Previously, this organism which is oxidase positive, was included into the family Vibrionaceae. By means of classical biochemical tests and, eventually, a variety of confirmatory tests for selected organisms, the identification of clinically important taxa of nonfermentative, oxidase positive or negative, and fermentative, oxidase positive Gram negative bacteria may be performed with the BBL Oxi/Ferm Tube II system.

BBL Oxi/Ferm Tube II is a self-contained, compartmented plastic tube containing twelve different media that allow the determination of 14 biochemical reactions. The enclosed inoculating wire allows inoculation of all compartments in one step from one or a few single colonies of an isolate. Additionally, an off-line oxidase test is needed. After 48 hours of incubation, the results are read and all positive reactions are recorded. The Results Pad and Color Reaction Chart permit a rapid check of the positive reactions obtained. The checked positive numbers are totaled, and the composite number is then located in the BBL Oxi/Ferm Tube II Biocode Manual to identify the organisms. Where two or more organisms are listed, the confirmatory tests required to further identify them are also given.

The following flow diagram demonstrates how the oxidase test may used to differentiate members of the family Enterobacteriaceae from these fermentative, oxidase positive and nonfermentative, oxidase positive or negative Gram negative bacteria and when BBL Oxi/Ferm Tube II or BBL Enterotube™ II are used:

Pure culture on nonselective medium ↓

Positive - Oxidase test

negative

↓

Inoculate BBL Oxi/Ferm Tube II

↓

Anaerobic glucose

Positive

Negative

Aeromonas spp.,
Plesiomonas shigelloides,
Vibrio spp.
Achromobacter spp.,
Alcaligenes spp., Bordetella bronchiseptica, Moraxella spp., Pasteurella spp., etc.

Enterobacteriaceae

Positive

Acinetobacter spp.,
Stenotrophomonas maltophilia, etc.

Negative*
REAGENTS
BBL Oxi/Ferm Tube II
Substrates and other active ingredients (contained in appropriate solid base media), and coloration of the uninoculated media:

- **Medium 1 (Ana-Gluc):**
  Glucose (10.0 g/l), with bromthymol blue as a pH indicator. The medium is covered with wax to provide anaerobic conditions. Uninoculated: green.

- **Medium 2 (Arginine):**
  Arginine (10.0 g/l), with bromcresol purple as a pH indicator. The medium is covered with wax to provide anaerobic conditions. Uninoculated: yellow.

- **Medium 3 (Lysine):**
  Lysine (10.0 g/l), with bromcresol purple as a pH indicator. The medium is covered with wax to provide anaerobic conditions. Uninoculated: yellow.

- **Medium 4 (Lactose/N\textsubscript{2}):**
  Lactose (20.0 g/l), potassium nitrate (2.0 g/l) and sodium nitrite (0.5 g/l), together with phenol red as a pH indicator and covered with wax to allow detection of gas formation. Uninoculated: red.

- **Medium 5 (Sucrose/Indole):**
  Sucrose (15.0 g/l) and tryptophan (1.2 g/l), with bromthymol blue as a pH indicator. Uninoculated: green.

- **Medium 6 (Xylose):**
  Xylose (10.0 g/l), with bromthymol blue as a pH indicator. Uninoculated: green.

- **Medium 7 (Aer-Gluc):**
  Glucose (10.0 g/l), with bromthymol blue as a pH indicator. Uninoculated: green.

- **Medium 8 (Maltose):**
  Maltose (10.0 g/l), with bromthymol blue as a pH indicator. Uninoculated: green.

- **Medium 9 (Mannitol):**
  Mannitol (10.0 g/l), with bromthymol blue as a pH indicator. Uninoculated: green.

- **Medium 10 (PA):**
  Phenylalanine (1.0 g/l) and ferric ammonium citrate (1.0 g/l) in an appropriate base medium. Uninoculated: beige.

- **Medium 11 (Urea):**
  Urea (20.0 g/l), with phenol red as a pH indicator. Uninoculated: beige; may have a pale rose hue.

- **Medium 12 (Citrate):**
  Sodium citrate (2.0 g/l), with bromthymol blue as a pH indicator. Uninoculated: green.

PRECAUTIONS

**[IVD]** For professional use only.

Do not use tubes if they show evidence of microbial contamination, discoloration, wax detachment from the respective media, drying, cracking or other signs of deterioration.

Any of the following conditions may interfere with the accuracy of **BBL Oxi/Ferm Tube II**:
- Dehydration or liquefaction,
- Lifting of wax from media surfaces,
- Any change of media colors from those indicated under “Uninoculated” in the **REAGENTS** section.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store **BBL Oxi/Ferm Tube II** in the dark at 2 to 8° C, in their original box until just prior to use. Avoid freezing and overheating. The tubes may be inoculated up to the expiration date and incubated for the recommended incubation times. Tubes from opened packages can be used up to the expiration date. Opened tubes must be used immediately.

USER QUALITY CONTROL

Inoculate **BBL Oxi/Ferm Tube II** with the strains mentioned below. For inoculation, incubation, and reading, proceed as described in **Test Procedure**.
### PROCEDURE

**Materials Provided**

- **BBL Oxi/Ferm Tube II.** Microbiologically controlled.
- Results Pad, including a color reaction chart for **BBL Oxi/Ferm Tube II.**

**Materials Not Provided**

- **BBL Oxi/Ferm Tube II Biocode Manual, document number CM-212116.CE.**
- Indole Dropper Reagent (Kovacs' reagent), 50 ampoules, cat. no. 261185)
- Oxidase Dropper Reagent, 50 ampoules, cat. no. 261181; alternatively, **BD DrySlide™ Oxidase test (cat. no. 231746, 75 tests) may be used.**
- Materials and reagents to perform the supplemental tests as mentioned in the **BBL Oxi/Ferm Tube II Biocode Manual.**

**Specimen Types**

**BBL Oxi/Ferm Tube II** is a biochemical identification system and must **not** be used directly with clinical specimens. Use isolates from appropriate nonselective media (see **Test Procedure**). **BBL Oxi/Ferm Tube II** may be used to identify nonfermentative, oxidase positive or negative, and fermentative, oxidase positive Gram negative rods isolated from any specimen.

**Test Procedure**

For the inoculation of **BBL Oxi/Ferm Tube II**, growth from nonselective media such as **BD Columbia Agar with 5% Sheep Blood** or **BD Trypticase™ Soy Agar II with 5% Sheep Blood** shall be used. The culture used for the inoculation should be at least 18 hours old, but generally not be older than 48 hours. Only in very rare cases, organisms may take longer to produce sufficient growth for the inoculation.

Make sure that the isolate that shall be identified with **BBL Oxi/Ferm Tube II** is a pure culture of a Gram negative rod.

1. Prior to inoculating **BBL Oxi/Ferm Tube II**, an oxidase test must be performed. This test assists the microbiologist in deciding if the organism in question is a member of the **Enterobacteriaceae** or an oxidative-fermentative gram-negative rod and whether to inoculate a **BBL Enterotube II** or a **BBL Oxi/Ferm Tube II**. Apply one to three drops of Oxidase Dropper Reagent to a piece of white filter paper placed into the lid of a Petri dish. With a plastic loop, remove a visible amount of growth from one or several identical colonies of the isolate from the plate that is used to deliver the inoculum for inoculating the **BBL Oxi/Ferm Tube II**. Slightly mix the inoculum with the oxidase reagent spot on the filter paper and wait for the reaction.
for 15 to 30 seconds. A dark blue to black color indicates a positive oxidase reaction. Do not use results obtained after 1 minute!
Alternatively, **BD DrySlide Oxidase test** may be used. Follow the instructions accompanying this product.

2. Prepare one sheet from the coding pad for the isolate by entering patient name, specimen number, and date.
3. Enter the result of the oxidase test into this sheet.
4. Take one **BBL Oxi/Ferm Tube II** and enter patient name, specimen number, and date on the label.
5. Remove both caps. The tip of the inoculating wire is under the white cap. Without flaming the wire, pick a well-isolated colony with the tip of the wire (**Figure 1**). Do not puncture the agar.
6. Inoculate **BBL Oxi/Ferm Tube II** by first twisting the wire, then withdrawing it through all the compartments applying a turning motion (**Figure 2**).
7. Reinsert the wire (without sterilizing) into **BBL Oxi/Ferm Tube II** until the notch on the wire is aligned with the opening of the tube (**Figure 3**). The tip of the wire should be visible in the citrate compartment. Break wire at notch by bending. The portion of the wire remaining in the tube maintains anaerobic conditions which are necessary for true fermentation.
8. With the broken off part of the wire, punch holes through the foil covering the air inlets into the following compartments: sucrose/indole, xylose, aerobic glucose (Aer-Gluc), maltose, mannitol, PA, urea and citrate (**Figure 4**). Replace both caps.
9. Incubate at 35 – 37° C for 48 hours with **BBL Oxi/Ferm Tube II** lying on its flat surface or in an upright position. Allow for air circulation between incubated tubes. Note that inspection of the urea compartment is required after 18 to 24 hours incubation since this information may be needed as a confirmatory test ("rapid urease test", see **BBL Oxi/Ferm Tube II Biocode Manual**).

**Results**
Follow the instructions given below to identify the isolate. Information on the appearance of positive and negative reactions is provided in Table 1.
1. After 24 hours of incubation, only read the urea reaction and record a positive result which may be needed for this isolate as a supplemental test (rapid urease test).
2. After 48 hours of incubation, interpret all reactions. With the exception of indole, read the reactions in a sequential manner by comparing the colors of the media in the tube after incubation with those given in the color scheme on the cover of the coding pad and (eventually) with an uninoculated **BBL Oxi/Ferm Tube II** which must be brought to room temperature first (**Figure 5**).
3. Indicate each positive test result, including a positive 48 hours urea result by circling the number appearing below the appropriate compartment on the Results Pad (**Figure 6**).
4. To perform the indole test: Place **BBL Oxi/Ferm Tube II** horizontally on the bench or vertically in a rack with medium 1 pointing downward. By using a needle or the broken-off end of a **BBL Oxi/Ferm Tube II** inoculating wire or by melting with a hot inoculating wire, punch or melt a hole of about 3-4 mm in diameter in the plastic film above the Sucrose/Indole compartments.
5. Add 3 - 4 drops of Kovacs' reagent (cat. no. 261185 to the Sucrose/Indole compartment. Allow reagent to contact the surface of the medium. A positive test is indicated by development of a red color in the added reagent within 10 sec. If positive, circle the appropriate number on the prepared sheet.
6. Add up all circled digits within each bracketed section and enter this sum in the space provided below the arrows (**Figure 6**).
7. Read the five digits in these spaces as a biocode number (e.g., 32303).
8. Find this five-digit number in the **BBL Oxi/Ferm Tube II Biocode Manual**. This number is used to identify the genus and/or the species of the organism. For the code number given in the above example and in Figure 6, the identification *Pseudomonas aeruginosa* is obtained.
9. If more than one organism name appears behind a profile number, the confirmatory tests given in the Biocode Manual should be performed to differentiate these organisms.
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6: BBL Oxi/Ferm Tube II
Results Pad
Table 1: Appearance of negative and positive reactions in BBL Oxi/Ferm Tube II

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Negative</th>
<th>Positive</th>
<th>Special Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COMPARTMENT 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaerobic Glucose</td>
<td>Green, blue</td>
<td>Yellow</td>
<td>Positive fermentation is shown by a change in color from green (neutral) to yellow (acid). Most oxidative-fermentative gram-negative rods are negative. Green and blue are considered negative.</td>
</tr>
<tr>
<td><strong>COMPARTMENT 2</strong></td>
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<tr>
<td>Arginine Dihydrolase</td>
<td>Yellow, gray</td>
<td>Purple</td>
<td>Decarboxylation of arginine results in the formation of alkaline end products. This is indicated by a change in the color of the bromcresol purple indicator from yellow (acid) to purple (alkaline). Gray is negative.</td>
</tr>
<tr>
<td><strong>COMPARTMENT 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine Decarboxylase</td>
<td>Yellow</td>
<td>Purple</td>
<td>Decarboxylation of lysine results in the formation of alkaline end products. This is indicated by a change in the color of the bromcresol purple in the medium from yellow (acid) to purple (alkaline). Gray is negative.</td>
</tr>
<tr>
<td><strong>COMPARTMENT 4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>Red</td>
<td>Yellow</td>
<td>Fermentation of Lactose is shown by a change in color from red (neutral) to yellow (acid). Most oxidative-fermentative gram-negative rods are negative.</td>
</tr>
<tr>
<td>N₂ gas production</td>
<td>Lifting or separation of wax overlay</td>
<td></td>
<td>Nitrogen gas production is evidenced by a lifting or separation of the wax overlay from the agar surface. Occasionally, nitrogen gas production will also be detected by separation of the agar from the compartment wall.</td>
</tr>
<tr>
<td><strong>COMPARTMENT 5</strong></td>
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<tr>
<td>Sucrose</td>
<td>Green, blue-green, or blue</td>
<td>Yellow</td>
<td>Oxidation of this carbohydrate is evidenced by a change in color from green (neutral) to yellow (acid). Green and blue are considered negative.</td>
</tr>
<tr>
<td>Indole</td>
<td>Colorless</td>
<td>Red</td>
<td>All reactions of the BBL Oxi/Ferm Tube II should be recorded before the addition of Kovacs' indole reagent. The production of indole from the metabolism of tryptophan by the bacterial enzyme tryptophanase is detected by the development of a pink to red color after the addition of Kovacs' reagent. The reagent is added to the compartment after 48 hours incubation of the tube.*</td>
</tr>
<tr>
<td>Xylose, Aerobic Glucose, Maltose, Mannitol</td>
<td>Green, blue-green, or blue</td>
<td>Yellow</td>
<td>Oxidation of xylose, glucose, maltose, and mannitol are evidenced by a change in color from green (neutral) to yellow (acid). Green and blue are considered negative.</td>
</tr>
<tr>
<td><strong>COMPARTMENT 10</strong></td>
<td></td>
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<tr>
<td>PA</td>
<td>Beige</td>
<td>Light brown</td>
<td>Phenylalanine is degraded to phenylpyruvic acid which produces a brownish complex with ferric salts. Any brown tinge is to be rated as positive.</td>
</tr>
<tr>
<td><strong>COMPARTMENT 11</strong></td>
<td></td>
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</tr>
<tr>
<td>Urea</td>
<td>Beige</td>
<td>Purple</td>
<td>Urease, an enzyme produced by various microorganisms, hydrolyzes urea to ammonia causing the color of the phenol red indicator in the medium to shift from beige (acid) to pink or purple (alkaline). Pale rose should be considered negative.</td>
</tr>
<tr>
<td><strong>COMPARTMENT 12</strong></td>
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<tr>
<td>Citrate</td>
<td>Green</td>
<td>Blue</td>
<td>This test detects those organisms which are capable of utilizing citrate in the form of its sodium salt as the sole source of carbon. Organisms capable of utilizing citrate produce alkaline metabolites which change the pH of the medium resulting in a change in the color of the bromthymol blue indicator from green (neutral) to blue (alkaline). Any blue tinge is to be rated as positive.</td>
</tr>
</tbody>
</table>

* Kovacs' indole reagent can dissolve pyorubin, this causing the development of a reddish-brown discoloration which may be incorrectly interpreted as a positive indole reaction. This source of error is eliminated by applying the Kovacs' reagent under the plastic foil and not directly onto the agar.
PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

The **BBL Oxi/Ferm Tube II** is designed specifically for the identification of non-fastidious, nonfermentative, oxidase positive or negative, and fermentative, oxidase positive Gram negative bacteria isolated from clinical specimens. Several publications on the performance of the system indicate an accuracy of identification of 87 to 93%.\(^3\)\(^4\)

In order to obtain a proper identification, the procedures given under **Test Procedure** must be followed strictly. If code numbers are obtained that require additional, confirmatory tests, these tests should be performed as indicated in APPENDIX 2 and 3 of the Biocode Manual.

**Performance Characteristics**

Accuracy: In a performance study, 220 previously identified strains (including known ATCC cultures) were analyzed using the **BBL Oxi/Ferm Tube II** identification system. The results obtained were as follows: three of the samples failed to give an identification with a competitor method, ten of the samples failed to give an identification with the **BBL Oxi/Ferm Tube II**, six of the samples gave results of identical genus but different species compared to the competitor method and five of the samples gave results of different genus compared to the competitor method. These data indicate a 97% agreement between the two methods on both a genus and a species basis.

The study samples are summarized in Table 2. Provided is a listing of the genus, species, number of samples tested and the percent of organisms giving positive biochemical reactions in each compartment of the **BBL Oxi/Ferm Tube II**.

**Table 2: Results of the performance evaluation**

<table>
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<tr>
<th>Acinetobacter</th>
<th>No. Tested</th>
<th>ANA-GLU</th>
<th>ARG</th>
<th>LYS</th>
<th>LAC</th>
<th>N2</th>
<th>SUC</th>
<th>IND</th>
<th>XYL</th>
<th>AER-GLU</th>
<th>MAL</th>
<th>MAN</th>
<th>PA</th>
<th>URE</th>
<th>CIT</th>
<th>OXI</th>
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<td>A. calco-actecus</td>
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<td>Plesiomonas</td>
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Abbreviations: A. = Achromobacter; Aer. = Aeromonas; Al. = Alcaligenes; B. = Bordetella; F. = Flavobacterium; M. = Moraxella; P. = Pasteurella; Pl. = Plesiomonas; Burk. = Burkholderia; Ps. = Pseudomonas; St. = Stenotrophomonas; V. = Vibrio.

Limitations of the Procedure
BBL Oxi/Ferm Tube II is designed for the taxa (genera and species) provided. Taxa other than those listed in Table 3 are not intended for use in this system.
Profile numbers that are marked with “L” in the Biocode Manual have been obtained with a limited number of strains only. The likelihood of these numbers has not been determined.
BBL Oxi/Ferm Tube II biocodes cannot be used to establish phenotypic identity between isolates from the same or different specimens.
Biochemical results obtained with the BBL Oxi/Ferm Tube II may differ from other methods and published material.
BBL Oxi/Ferm Tube II biocodes cannot be used to establish phenotypic identity between isolates from the same or different specimens.
Identification of Gram negative bacteria should be made with the consideration of additional characteristics such as source of specimen, history of the patient, colonial and microscopic morphology, serology and antimicrobial susceptibility patterns.
Identification of rare isolates should be repeated or additional testing performed to verify the identification of such organisms.
Some strains of organisms may exhibit atypical biochemical reactions due to unusual nutritional requirements or mutations and may be difficult to identify.
Some organisms may require longer than 48 hours incubation for proper identification.
Although it has been proposed recently to include Plesiomonas shigelloides into the Enterobacteriaceae based on 16S rRNA sequencing and because it contains the enterobacterial common antigen, this organism is included into the database of BBL Oxi/Ferm Tube II.1,2

Table 3: Overview on the genera and species contained in the BBL Oxi/Ferm Tube II database (revision: July 2003)
| **Bordetella bronchiseptica** |  |
| **Brevundimonas diminuta** | **Pseudomonas diminuta** |
| **Brevundimonas vesicularis** | **Pseudomonas vesicularis** |
| **Burkholderia cepacia complex** | **Burkholderia cepacia, Pseudomonas cepacia** |
| **Burkholderia pseudomallei** | **Pseudomonas pseudomallei** |
| **Chromobacterium violaceum** |  |
| **Chryseobacterium indologenes** | **Flavobacterium indologenes** |
| **Chryseobacterium meningosepticum** | **Flavobacterium meningosepticum** |
| **Comamonas testosteroni/terrigena** | **Pseudomonas testosteroni** |
| **Delftia acidovorans** | **Comamonas acidovorans, Pseudomonas acidovorans** |
| **Empedobacter brevis** | **Flavobacterium breve** |
| **Flavobacterium spp** | May include species with new genus designation, e.g., Myroides, Chryseobacterium, Sphingobacterium etc. |
| **Kingella denitrificans** |  |
| **Moraxella spp.** | May include Psychrobacter phenylpyruvicus (formerly Moraxella phenylpyruvica) |
| **Myroides odoratus** | **Flavobacterium odoratum** |
| **Ochrobactrum anthropi** | Group Vd |
| **Oligella urethralis** | **Moraxella urethralis** |
| **Pasteurella multocida** |  |
| **Plesiomonas shigelloides** |  |
| **Pseudomonas aeruginosa** |  |
| **Pseudomonas alcaligenes** |  |
| **Pseudomonas fluorescens** |  |
| **Pseudomonas mendocina** |  |
| **Pseudomonas oryzihabitans** |  |
| **Pseudomonas putida** |  |
| **Pseudomonas spp.** |  |
| **Pseudomonas stutzeri** |  |
| **Ralstonia picketti** | **Pseudomonas pickettii** |
| **Rhizobium (Agrobacterium) radiobacter** | **Agrobacterium tumefaciens** |
| **Shewanella putrefaciens** | **Pseudomonas putrefaciens** |
| **Sphingobacterium multivorum** | **Flavobacterium multivorum** |
| **Sphingomonas paucimobilis** | **Pseudomonas paucimobilis** |
| **Stenotrophomonas maltophilia** | **Pseudomonas maltophilia, Xanthomonas maltophilia** |
| **Vibrio alginolyticus** |  |
| **Vibrio cholerae** |  |
| **Vibrio parahaemolyticus** |  |
| **Vibrio vulnificus** |  |

* The genus Acinetobacter consists of 23 genomospecies. Most clinical glucose oxidizing strains of *Acinetobacter* are *A. baumannii*. Most hemolytic strains are *A. haemolyticus*, and most glucose negative, nonhemolytic strains are *A. lwoffii*.6
* Burkholderia cepacia consists of nine genomospecies.6
* Plesiomonas shigelloides has been recently transferred to the family Enterobacteriaceae, although it is oxidase positive.6
  
6 For details, consult the respective chapters in reference 6.

**REFERENCES**

Packaging/Availability
BBL Oxi/Ferm Tube II
Cat. No. 212116 25 tubes

FURTHER INFORMATION
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