



# BBL CRYSTAL™ Identification Systems

## Rapid Gram-Positive ID Kit

8809921  
Revised: 2000/10

U.S. Pat. 5,182,082

U.S. Pat. 5,338,666

CLIA COMPLEXITY: HIGH

CDC IDENTIFIER CODES

ANALYTE: 0412

TEST SYSTEM: 07920

### INTENDED USE

The **BBL CRYSTAL™** Rapid Gram-Positive (RGP) Identification System (ID) is a miniaturized identification method employing modified conventional, fluorogenic and chromogenic substrates. It is intended for the identification of aerobic gram-positive bacteria.<sup>1,2,13,16</sup>

### SUMMARY AND EXPLANATION

Micromethods for the biochemical identification of microorganisms were reported as early as 1918.<sup>3</sup> Several publications reported on the use of the reagent-impregnated paper discs and micro-tube methods for differentiating enteric bacteria.<sup>3,4,7,17,19</sup> The interest in miniaturized identification systems led to the introduction of several commercial systems in the late 1960s, and they provided advantages in requiring little storage space, extended shelf life, standardized quality control and ease of use.

In general, many of the tests used in the **BBL CRYSTAL** ID Systems are modifications of classical methods. These include tests for fermentation, oxidation, degradation and hydrolysis of various substrates. In addition, there are chromogen and fluorogen linked substrates, as in the **BBL CRYSTAL** RGP ID panel, to detect enzymes that microbes use to metabolize various substrates.<sup>5,7,8,9,11,12,14,15</sup>

The **BBL CRYSTAL™** RGP ID kit is comprised of (i) **BBL CRYSTAL** RGP ID panel lids, (ii) **BBL CRYSTAL** bases and (iii) **BBL CRYSTAL™** ANR, GP, RGP, N/H ID Inoculum Fluid (IF) tubes. The lid contains 29 dehydrated substrates and a fluorescence control on tips of plastic prongs. The base has 30 reaction wells. Test inoculum is prepared with the inoculum fluid and is used to fill all 30 wells in the base. When the lid is aligned with the base and snapped in place, the test inoculum rehydrates the dried substrates and initiates test reactions.

Following an incubation period, the wells are examined for color changes or presence of fluorescence that result from metabolic activities of the microorganisms. The resulting pattern of the 29 reactions is converted into a ten-digit profile number that is used as the basis for identification.<sup>18</sup> Biochemical and enzymatic reaction patterns for the 29 **BBL CRYSTAL** RGP ID substrates for a wide variety of microorganisms are stored in the **BBL CRYSTAL** RGP ID data base. Identification is derived from a comparative analysis of the reaction pattern of the test isolate to those held in the database. A complete list of taxa that comprises the current database is provided in **Table 1** (see pg. 7).

### PRINCIPLES OF THE PROCEDURE

The **BBL CRYSTAL** RGP ID panels contain 29 dried biochemical and enzymatic substrates. A bacterial suspension in the inoculum fluid is used for rehydration of the substrates. The tests used in the system are based on microbial utilization and degradation of specific substrates detected by various indicator systems. Enzymatic hydrolysis of fluorogenic substrates containing coumarin derivatives of 4-methylumbelliferone (4MU) or 7-amino-4-methylcoumarin (7-AMC), results in increased fluorescence that is easily detected visually with a UV light source.<sup>11,12,14,15</sup> Chromogenic substrates upon hydrolysis produce color changes that can be detected visually. In addition, there are tests that detect the ability of an organism to hydrolyze, degrade, reduce or otherwise utilize a substrate in the **BBL CRYSTAL** ID Systems.

Reactions employed by various substrates and a brief explanation of the principles employed in the system are described in **Table 2** (see pg. 8). Panel location in referred tables indicates the row and column where the well is located (example: 1J refers to Row 1 in column J).

### Reagents

The **BBL CRYSTAL** RGP ID panel contains 29 enzymatic and biochemical substrates. Refer to **Table 3** (see pg. 9) for a list of active ingredients.

### Precautions: *in vitro* Diagnostic

After review by the U.S. Centers for Disease Control and Prevention (CDC), and the Food and Drug Administration (FDA) under CLIA '88, this product has been identified as high complexity. The CDC Analyte Identifier Code is 0412; the CDC Test System Identifier Code is 07920.

After use, all infectious materials including plates, cotton swabs, inoculum fluid tubes, and panels must be autoclaved prior to disposal or incineration.

### STORAGE AND HANDLING/SHELF LIFE

**Lids:** **BBL CRYSTAL** RGP lids are individually packaged and must be stored unopened in a refrigerator at 2 – 8°C. DO NOT FREEZE. Visually inspect the package for holes or cracks in the foil package. Do not use if the packaging

SIZE: 5.5" W x 8.5" L  
COLOR: Standard Black  
DRAFT: 5/16/96

appears to be damaged. Lids in the original packaging, if stored as recommended, will retain expected reactivity until the date of expiration.

**Bases:** Bases are packaged in two sets of ten, in **BBL CRYSTAL** incubation trays. The bases are stacked facing down to minimize air contamination. Store in a dust-free environment at 2 – 30°C, until ready to use. Store unused bases in the tray, in plastic bag. Empty trays should be used to incubate inoculated panels.

**Inoculum Fluid:** **BBL CRYSTAL** ANR, GP, RGP, N/H ID Inoculum Fluid (IF) is packaged in two sets of ten tubes. Visually inspect the tubes for cracks, leaks, etc. Do not use if there appears to be a leak, tube or cap damage or visual evidence of contamination (i.e., haziness, turbidity). Store tubes at 2 – 25°C. Expiration dating is shown on the tube label. Only ANR, GP, RGP, N/H Inoculum Fluid should be used with **BBL CRYSTAL** RGP ID panels.

On receipt, store the **BBL CRYSTAL** RGP ID kit at 2 – 8°C. Once opened, only the lids need to be stored at 2 – 8°C. The remaining components of the kit may be stored at 2 – 25°C. If the kit or any of the components are stored refrigerated, each should be brought to room temperature prior to use.

#### SPECIMEN COLLECTION AND PROCESSING

**BBL CRYSTAL** ID Systems are **not** for use directly with clinical specimens. Use isolates from media such as **Trypticase™** Soy Agar with 5% Sheep Blood (**TSA II™**) or Columbia Agar with 5% Sheep Blood (Columbia Blood Agar). Use of selective media such as Phenylethyl Alcohol Agar with 5% Sheep Blood (PEA) or Columbia CNA Agar with 5% Sheep Blood (CNA) is also acceptable. Media containing esculin should not be used. The test isolate must be a pure culture, no more than 18 – 24 h old for most genera; for some slow growing organisms up to 48 h may be acceptable. When swabs are utilized, only cotton-tipped applicators should be used to prepare the inoculum suspensions. Some polyester swabs may cause problems with inoculation of the panels. (See "Limitations of the Procedure".)

The incubator used should be humidified to prevent evaporation of fluid from the wells during incubation. The recommended humidity level is 40 – 60%. The usefulness of **BBL CRYSTAL** ID Systems or any other diagnostic procedure performed on clinical specimens is directly influenced by the quality of the specimens themselves. It is strongly recommended that laboratories employ methods discussed in the *Manual of Clinical Microbiology* for specimen collection, transport and inoculation onto primary isolation media.<sup>1,16</sup>

#### TEST PROCEDURE

##### Materials Provided: **BBL CRYSTAL** RGP ID Kit –

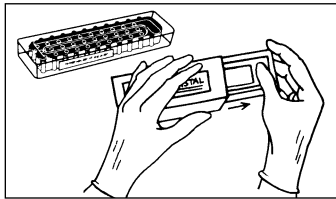
20 **BBL CRYSTAL** RGP ID Panel Lids, 20 **BBL CRYSTAL** Bases, 20 **BBL CRYSTAL** ANR, GP, RGP, N/H ID IF Tubes. Each tube has approximately 2.3 ± 0.15 mL of Inoculum Fluid containing: KCl 7.5 g, CaCl<sub>2</sub> 0.5 g, Tricine N-[2-Hydroxy-1, 1-bis (hydroxymethyl)methyl] glycine 0.895 g, purified water to 1000 mL, 2 incubation trays, 1 **BBL CRYSTAL** RGP ID Color Reaction Chart and Results Pad.

**Materials Not Provided:** Sterile cotton swabs (*do not use polyester swabs*), incubator (35 – 37°C) non-CO<sub>2</sub> (40 – 60% humidity), McFarland No. 2 standard, **BBL CRYSTAL** Panel Viewer, **BBL CRYSTAL** ID System Electronic Codebook or **BBL** Rapid Gram-Positive Manual Codebook, and appropriate culture media.

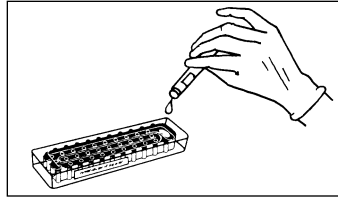
Also required are the necessary equipment and labware used for preparation, storage and handling of clinical specimens.

**Test Procedure:** **BBL CRYSTAL** RGP ID System requires a Gram stain.

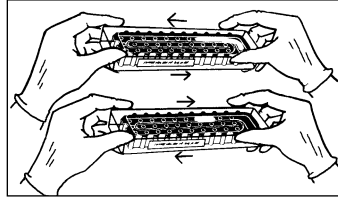
- Remove lids from pouch. Discard desiccant. Once removed from the pouch, covered lids should be used within 1 h. Do not use the panel if there is no desiccant in the pouch.
- Take an inoculum fluid tube and label with patient's specimen number. Using aseptic technique, pick colonies of the same morphology with the tip of a sterile cotton swab (*do not use a polyester swab*) or a wooden applicator stick from one of the recommended media (see section under "Specimen Collection and Processing").
- Suspend colonies in a tube of **BBL CRYSTAL** ANR, GP, RGP, N/H ID Inoculum Fluid.
- Recap tube and vortex for approximately 10 – 15 sec. The turbidity should be equivalent to a McFarland No. 2 standard. If the inoculum suspension concentration is in excess of the recommended McFarland standard, one of the following steps is recommended:
  - Use a fresh tube of inoculum fluid to prepare a new inoculum suspension equivalent to a McFarland No. 2 standard.
  - If additional colonies are unavailable for preparation of a new inoculum suspension, using aseptic techniques, dilute the inoculum by adding the minimum required volume (not to exceed 1.0 mL) of 0.85% sterile saline or inoculum fluid to bring down the turbidity equivalent to a McFarland No. 2 standard. Remove the excess amount added to the tube with a sterile pipet so that the final volume of inoculum fluid is approximately equivalent to that of the original volume in the tube (2.3 ± 0.15 mL). Failure to make this adjustment in volume will result in spilling of the inoculum suspension over the black portion of the base rendering the panel unusable.
- Take a base, and mark the patient's specimen number on the side wall.



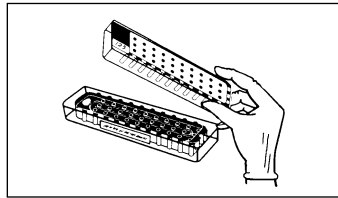
6. Pour entire contents of the inoculum fluid tube into target area of the base.



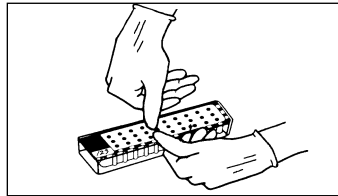
7. Hold base in both hands and roll inoculum gently along the tracks until all of the wells are filled. Roll back any excess fluid to the target area and place the base on a bench top. Due to the high cell concentrations used in BBL CRYSTAL RGP ID panels, the inoculum should be carefully rolled across the tracks to ensure a proper fill of all wells. Make sure there is no excess fluid between the wells before the lid is aligned.



8. Align the lid so that the labeled end of the lid is on top of the target area of the base.

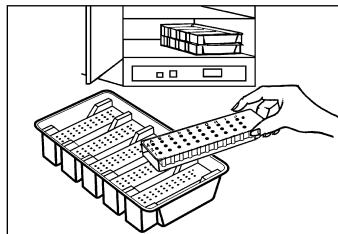


9. Push down until a slight resistance is felt. Place thumb on edge of lid towards middle of panel on each side and push downward simultaneously until the lid snaps into place (listen for two "clicks").

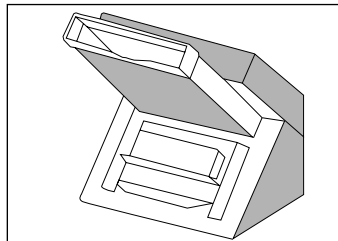


**Purity Plate:** Using a sterile loop, recover a small drop from the inoculum fluid tube either before or after inoculating the base and inoculate an agar slant or plate (any appropriate medium) for purity check. Discard inoculum fluid tube and cap in a biohazard disposal container. Incubate the slant or plate for 24 – 48 h at 35 to 37°C under appropriate conditions. The purity plate or slant may also be used for any supplementary tests or serology, if required.

**Incubation:** Place inoculated panels in incubation trays. Ten panels can fit in one tray (5 rows of 2 panels). All panels should be incubated **face down** (larger windows facing up; label facing down) in a non-CO<sub>2</sub> incubator with 40 to 60% **humidity**. Trays should not be stacked more than two high during incubation. The incubation time for panels is 4 h at 35 to 37°C. **NOTE:** The incubator door should not be opened repeatedly during the incubation period (preferably less than 3 times). Panels should be read within 30 min after removing from incubator.



**Reading:** After the recommended period of incubation, remove the panels from the incubator. All panels should be read **face down** (larger windows up; label facing down) using the BBL CRYSTAL Panel Viewer. Refer to the color reaction chart and/or **Table 3** (see pg. 9) for an interpretation of the reactions. Use the results pad to record reactions.



- Read columns E thru J first, using the regular (white) light source.
- Read columns A thru D (fluorescent substrates) using the UV light source in the panel viewer. A fluorescent substrate well is considered positive *only if* the intensity of the fluorescence observed in the well is *greater* than the Negative Control well (4A).

**Calculation of BBL CRYSTAL™ Profile Number:** Each test result (except 4A, which is used as a fluorescence negative control) scored positive is given a value of 4, 2, or 1, corresponding to the row where the test is located. A value of 0 (zero) is given to any negative result. The values resulting from each positive reaction in each column are then added together. A 10-digit number is generated; this is the profile number.

Example:	A	B	C	D	E	F	G	H	I	J
4	*	+	-	-	+	+	+	-	+	-
2	-	+	+	+	-	+	-	+	+	-
1	+	-	+	-	+	-	-	+	+	-
Profile	1	6	3	2	5	6	4	3	7	0

\*(4A) = fluorescent negative control

The resulting profile number and cell morphology, if known should be entered on a PC in which the **BBL CRYSTAL** ID System Electronic Codebook has been installed to obtain the identification. A Manual Codebook is also available. If a PC is not available contact Becton Dickinson Microbiology Systems Technical Services for assistance with the identification.

**User Quality Control:** Quality control testing is recommended for each lot of panels as follows –

- Inoculate a panel with *Streptococcus pyogenes* ATCC® 19615 per recommended procedure (refer to “Test Procedure”).
- Prior to incubation, let panel remain at room temperature for 1 min (not more than 2 min).
- Read and record reactions with the aid of the panel viewer and color reaction chart.
- If any of the wells (except 1J) are positive per color reaction chart (after 1 – 2 min), DO NOT USE PANELS from this lot. Contact Becton Dickinson Technical Services.  
*NOTE:* Well 1J can appear positive upon rehydration; this is not indicative of a problem.
- If all wells are negative, then incubate panel for 4 h at 35 – 37°C.
- Read panel with the panel viewer and color reaction chart; record reactions using the results pad.
- Compare recorded reactions with those listed in **Table 4** (see pg. 10). If discrepant results are obtained, confirm purity of quality control strain before contacting Becton Dickinson Technical Services.
- The incubator door should not be opened repeatedly during the incubation period (preferably less than 3 times). Expected test results for additional quality control test strains are listed in **Table 5** (see pg. 10).

#### LIMITATIONS OF THE PROCEDURE

The **BBL CRYSTAL** RGP Identification System is designed for the taxa provided. Taxa other than those listed in Table 1 are not intended for use in this system.

The **BBL CRYSTAL** RGP ID System database includes some species that are rarely isolated from human clinical specimens and were not encountered in the clinical studies of this product. It also includes some species that were encountered less than 10 times in the clinical studies. Refer to **Table 1** (see pg. 7) for a breakdown of the number of strains per species tested in clinical trials. The laboratorian should determine if additional testing is required to confirm identity of those species for which performance has not been established (i.e., those where less than 10 isolates were evaluated in the clinical trials for this product).

The **BBL CRYSTAL** RGP ID database was developed with **BBL™** brand media. Reactivity of some substrates in miniaturized identification systems may be dependent upon the source media used in inoculum preparations. We recommend the use of the following media for use with the **BBL CRYSTAL** RGP ID System: **TSA II** or Columbia Blood Agar. Use of selective media, such as PEA or CNA is also acceptable. Media containing esculin should not be used.

**BBL CRYSTAL** Identification Systems use a modified microenvironment; therefore, expected values for its individual tests may differ from information previously established with conventional test reactions. The accuracy of the **BBL CRYSTAL** RGP ID System is based on statistical use of specially designed tests and an exclusive database.

While **BBL CRYSTAL** RGP ID System aids in microbial differentiation, it should be recognized that minor variations may exist in strains within species. Use of panels and interpretation of results require a competent microbiologist. The final identification of the isolate should take into consideration the source of the specimen, aerotolerance, cell morphology, colonial characteristics on various media as well as metabolic end products as determined by gas-liquid chromatography, when warranted.

Only cotton-tipped applicator swabs should be used to prepare the inoculum suspension as some polyester swabs may cause the inoculum fluid to become viscous. This may result in insufficient inoculum fluid to fill the wells. Covered lids once removed from the sealed pouches must be used within 1 h to ensure adequate performance.

The incubator where panels are placed should be humidified to prevent evaporation of inoculum fluid from the wells during incubation. The recommended humidity level is 40 – 60%.

The panels, after inoculation, should only be incubated upside-down (larger windows facing up; label facing down) to maximize the effectiveness of substrates.

If the **BBL CRYSTAL** test profile yields a “No identification” result and culture purity has been confirmed, then it is likely that (i) the test isolate is producing *atypical BBL CRYSTAL reactions* (which may also be caused by procedural errors), (ii) the test species is not part of the intended taxa or (iii) the system is unable to identify the test isolate with the required level of confidence. Conventional test methods are recommended when user error has been ruled out.

#### EXPECTED VALUES

The expected substrate reactions for the species of organisms most frequently encountered in the clinical study of **BBL CRYSTAL** RGP ID System are shown in **Table 6** (see pg. 11). The provided percentages were generated from reactions given by the organisms used in generating the database. **Table 1** (see pg. 7) shows all the taxa tested during database generation.

## PERFORMANCE CHARACTERISTICS

**Reproducibility:** In an external study involving three clinical laboratories, (total of three evaluations), the reproducibility of **BBL Crystal** RGP ID substrate (29) reactions was studied by replicate testing. The reproducibility of the individual substrate reactions for the three sites performing the testing ranged from 88.9% – 100%. In the Reproducibility Phase, one of the sites encountered false negative results with Mannitol and Maltose when testing a specific strain of *S. aureus*. However, in the Clinical Phase of the study, for this site, 100% correct identifications were obtained for the nineteen (19) clinical isolates of *S. aureus* tested. In addition, the overall reproducibility for Mannitol and Maltose, for all three clinical trial sites combined, was determined to be 94.7% and 96.3% respectively. The overall reproducibility of the **BBL CRYSTAL** RGP ID panel was determined to be 99.4%.<sup>20</sup>

**Accuracy of Identification:** The performance of **BBL CRYSTAL** RGP ID System was compared to currently available commercial systems using clinical isolates and stock cultures. A total of three studies were conducted in three independent laboratories. Fresh, routine isolates arriving in the clinical laboratory, as well as previously identified isolates of the clinical trial sites' choice were utilized to establish performance characteristics.

Out of 604 total isolates tested from the three studies using **BBL CRYSTAL** RGP Identification System, 537 (88.9%) were correctly identified without the use of supplemental tests, and 550 (91.1%) were correctly identified when supplement tests were included. A total of 53 (8.8%) isolates were incorrectly identified, and a message of "No Identification" was obtained for 1 (0.2%) isolate.<sup>20</sup> **Table 7** (see pg. 12) shows the accuracy of identification for the species most frequently encountered (i.e., 10 or more isolates) in the clinical trial as well as for the remaining group of species where less than 10 isolates were tested.

## AVAILABILITY

Cat. No.	Description	Description
245250	<b>BBL CRYSTAL™</b> Rapid Gram-Positive ID Kit, containing 20 each: <b>BBL CRYSTAL</b> RGP ID Panel Lids, <b>BBL CRYSTAL</b> Bases and <b>BBL CRYSTAL</b> ANR, GP, RGP, N/H ID Inoculum Fluid.	<b>BBL™</b> Columbia Agar with 5% Sheep Blood, pkg. of 20.
	<b>BBL CRYSTAL™</b> ANR, GP, RGP, N/H ID Inoculum Fluid, ctn. of 10.	<b>BBL™</b> Columbia Agar with 5% Sheep Blood, ctn. of 100.
	<b>BBL CRYSTAL™</b> Panel Viewer, Domestic model, 110 V, 60 Hz.	<b>BBL™</b> Columbia CNA Agar with 5% Sheep Blood, pkg. of 20.
	<b>BBL CRYSTAL™</b> Panel Viewer, European model, 220 V, 50 Hz.	<b>BBL™</b> Columbia CNA Agar with 5% Sheep Blood, ctn. of 100.
	<b>BBL CRYSTAL™</b> Panel Viewer, Japanese model, 100 V, 50/60 Hz.	<b>BBL™</b> Phenylethyl Alcohol Agar with 5% Sheep Blood, pkg. of 20.
	<b>BBL CRYSTAL™</b> Panel Viewer, Longwave UV Tube.	<b>BBL™</b> Phenylethyl Alcohol Agar with 5% Sheep Blood, ctn. of 100.
	<b>BBL CRYSTAL™</b> Panel Viewer, White Light Tube.	<b>BBL™</b> Trypticase™ Soy Agar with 5% Sheep Blood (TSA II™), pkg. of 20.
	<b>BBL CRYSTAL™</b> ID System Electronic Codebook.	<b>BBL™</b> Trypticase™ Soy Agar with 5% Sheep Blood (TSA II™), ctn. of 100.
	<b>BBL CRYSTAL™</b> Identification Systems Rapid Gram-Positive Manual Codebook.	<b>BBL™</b> Gram Stain Kit, pkg. of 4 x 250 ml bottles.

For specific catalog number information, visit our website <http://www.bd.com/microbiology>, or contact the nearest Becton Dickinson Microbiology Systems office.

## REFERENCES

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20. Data on file at Becton Dickinson Microbiology Systems.

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ATCC is a trademark of the American Type Culture Collection.

Becton, Dickinson and Company.  
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(800) 638-8663

Table 1  
Taxa in BBL CRYSTAL™ RGP ID System

<i>Actinomyces pyogenes</i>	<i>Erysipelothrix rhusiopathiae</i>	(includes <i>P. damnosus</i> , <i>P. parvulus</i> ,* <i>P. pentosaceus</i> )	<i>Streptococcus cricetus</i>
<i>Aerococcus viridans</i> (2)	<i>Gardnerella vaginalis</i>	<i>Rhodococcus equi</i>	<i>Streptococcus crista</i>
<i>Bacillus brevis</i> (2)	<i>Gemella haemolysans</i>	<b><i>Staphylococcus aureus</i> (55)</b>	<i>Streptococcus equi</i> (includes <i>S. equi</i> ssp <i>equi</i> and <i>S. equi</i> ssp <i>zooepidemicus</i> )
<i>Bacillus cereus</i> (4)	<i>Gemella morbillorum</i>	<b><i>Staphylococcus epidermidis</i> (59)</b>	<i>Streptococcus equinus</i>
<i>Bacillus licheniformis</i> (1)	<i>Gemella species</i> (includes <i>G. haemolysans</i> , <i>G. morbillorum</i> )	<i>Staphylococcus gallinarum</i>	<i>Streptococcus gordonii</i> (1)
<i>Bacillus megaterium</i>	<i>Lactococcus garvieae</i>	<b><i>Staphylococcus haemolyticus</i> (50)</b>	<i>Streptococcus group CG</i> (7)
<i>Bacillus pumilus</i>	<i>Lactococcus lactis</i> ssp <i>cremoris</i>	<b><i>Staphylococcus hominis</i> (28)</b>	<i>Streptococcus intermedius</i> (3)
<i>Bacillus species</i> (includes <i>B. brevis</i> , <i>B. licheniformis</i> , <i>B. megaterium</i> , <i>B. pumilus</i> , <i>B. alvei</i> )	<i>Lactococcus lactis</i> ssp <i>hordniae</i>	<i>Staphylococcus intermedius</i> (2)	<i>Streptococcus milleri</i> group (includes <i>S.</i> <i>anginosus</i> , <i>S. constellatus</i> and <i>S. intermedius</i> ) (3)
<i>Bacillus subtilis</i> (1)	<i>Lactococcus lactis</i> ssp <i>lactis</i> (1)	<i>Staphylococcus lentus</i>	<i>Streptococcus mitis</i> (2)
<i>Corynebacterium bovis</i>	<i>Lactococcus species</i> (includes <i>L. garvieae</i> , <i>L. lactis</i> ssp <i>cremoris</i> , <i>L. lactis</i> ssp <i>hordniae</i> , <i>L. lactis</i> ssp <i>lactis</i> )	<i>Staphylococcus lugdunensis</i> (4)	<b><i>Streptococcus mitis</i> group</b> (includes <i>S. mitis</i> and <i>S. oralis</i> ) (12)
<i>Corynebacterium diphtheriae</i> (includes <i>C. diphtheriae</i> ssp <i>gravis</i> , <i>C. diphtheriae</i> ssp <i>intermedius</i> , <i>C. diphtheriae</i> ssp <i>mitis</i> )	<i>Leuconostoc citreum</i>	<i>Staphylococcus saccharolyticus</i>	<i>Streptococcus mutans</i>
<i>Corynebacterium jeikeium</i> (5)	<i>Leuconostoc lactis</i>	<b><i>Staphylococcus saprophyticus</i> (39)</b>	<i>Streptococcus mutans</i> group (includes <i>S. cricetus</i> , <i>S. mutans</i> and <i>S. sobrinus</i> ) (1)
<i>Corynebacterium pseudodiphtheriticum</i> (2)	<i>Leuconostoc mesenteroides</i> ssp <i>mesenteroides</i> (1)	<i>Staphylococcus sciuri</i>	<i>Streptococcus oralis</i>
<i>Corynebacterium pseudotuberculosis</i>	<i>Leuconostoc species</i> (includes <i>L. citreum</i> , <i>L. lactis</i> , <i>L. mesenteroides</i> ssp <i>mesenteroides</i> )	<b><i>Staphylococcus simulans</i> (19)</b>	<b><i>Streptococcus pneumoniae</i> (54)</b>
<i>Corynebacterium renale</i> group	<i>Listeria monocytogenes</i> (2)	<b><i>Staphylococcus warneri</i> (12)</b>	<b><i>Streptococcus pyogenes</i> (49)</b>
<i>Corynebacterium species</i> (includes <i>C. bovis</i> , <i>C. pseudodiphtheriticum</i> , <i>C. pseudotuberculosis</i> , <i>C. renale</i> group <i>C. ulcerans</i> ) (1)	<i>Listeria murrayi</i> /grayi	<i>Staphylococcus xylosum</i> (2)	<i>Streptococcus salivarius</i>
<i>Corynebacterium ulcerans</i>	<i>Micrococcus kristinae</i>	<i>Stomatococcus mucilaginosus</i> *(1)	<i>Streptococcus salivarius</i> group (includes <i>S. salivarius</i> and <i>S. vestibularis</i> ) (2)
<i>Enterococcus avium</i>	<i>Micrococcus luteus</i> (1)	<b><i>Streptococcus agalactiae</i> (52)</b>	<i>Streptococcus sanguis</i> (3)
<i>Enterococcus casseliflavus/gallinarum</i> (4)	<i>Micrococcus roseus</i>	<i>Streptococcus anginosus</i> (3)	<i>Streptococcus sanguis</i> group (includes <i>S. crista</i> , <i>S. sanguis</i> , and <i>S. gordonii</i> ) (5)
<i>Enterococcus durans</i> (2)	<i>Micrococcus species</i> (includes <i>M. kristinae</i> , <i>M. luteus</i> , <i>M. roseus</i> ) (6)	<i>Streptococcus bovis</i> (includes <i>S. bovis</i> I and <i>S. bovis</i> II) (3)	<i>Streptococcus sobrinus</i>
<b><i>Enterococcus faecalis</i> (55)</b>	<i>Oerskovia species</i> (includes <i>O. turbata</i> , <i>O. xanthineolytica</i> )	<i>Streptococcus constellatus</i>	<i>Streptococcus uberis</i>
<b><i>Enterococcus faecium</i> (25)</b>	<i>Paenibacillus alvei</i>		<i>Streptococcus vestibularis</i>
<i>Enterococcus raffinosus</i>	<i>Pediococcus species</i>		

KEY: \* = These taxa have fewer than 10 unique BBL CRYSTAL profiles in the current database.  
("x") = Number of isolates (i.e., "x") encountered in the clinical trial. If no number in parenthesis is shown after an organism name or group description, these species were not encountered in the clinical trial

Note #1: There were 17 additional isolates encountered in the clinical trial that are not shown above. Four (4) (i.e., 3 *Staphylococcus* species and 1 viridans streptococci were identified only to the genus level by the reference system against which BBL CRYSTAL RGP was compared, although BBL CRYSTAL RGP identified organisms to the species level. Thirteen (13) were identified by the reference system, but were not included in the BBL CRYSTAL RGP database taxa

Note #2: The organisms shown in bold face type were encountered 10 or more times in the clinical study for this product.

Note #3: The organisms not shown in bold face type are either species which are rarely isolated from human clinical specimens or species that were infrequently (less than 10) encountered in the clinical study for this product. The laboratorian should determine if additional testing is required to confirm their identity.

**Table 2**  
**Principles of Tests Employed in the BBL Crystal™ RGP ID System**

Panel Location	Test Feature	Code	Principle (Reference)
4A	Fluorescent negative control	FCT	Control to standardize fluorescent substrate results.
2A	4MU- $\beta$ -D-glucoside	FGC	Enzymatic hydrolysis of the amide or glycosidic bond results in the release of a fluorescent coumarin derivative. <sup>5,8,11,12,14,15</sup>
1A	L-proline-AMC	FPR	
4B	L-arginine-AMC	FAR	
2B	L-methionine-AMC	FME	
1B	4MU- $\beta$ -D-cellobioside	FCE	
4C	4MU-phosphate	FHO	
2C	L-pyroglutamic acid-AMC	FPY	
1C	L-tryptophan-AMC	FTR	
4D	L-valine-AMC	FVA	
2D	L-phenylalanine-AMC	FPH	
1D	4MU- $\alpha$ -D-glucoside	FGS	
4E	Arabinose	ARA	Utilization of carbohydrate results in lower pH and change in indicator (Phenol red). <sup>1,2,3,4,7,16</sup>
2E	Maltose	MAL	
1E	Dextrin	DXT	
4F	Mannitol	MNT	
2F	Galactose	GAL	
1F	N-acetyl-D-glucosamine	AGN	
4G	Trehalose	TRE	
2G	Mannose	MNS	
1G	Maltotriose	MTT	
4H	o-nitrophenyl- $\beta$ -D-galactoside (ONPG) & p-nitrophenyl- $\beta$ -D-glucoside	POG	Enzymatic hydrolysis of the colorless aryl substituted glycoside releases yellow p-nitrophenol. <sup>5,9,12</sup>
2H	p-nitrophenyl- $\alpha$ -D-glucoside	AGL	
1H	p-nitrophenyl- $\beta$ -D-cellobioside	PCE	
4I	p-nitrophenyl- $\beta$ -D-glucoside	BGL	
2I	p-nitrophenyl-phosphate	PHO	
1I	p-nitrophenyl- $\beta$ -D-galactoside & p-nitrophenyl- $\alpha$ -D-galactoside	PPG	
4J	Urea	URE	Hydrolysis of urea and the resulting ammonia change the pH indicator color (Bromthymol blue). <sup>2,6,10</sup>
2J	Esculin	ESC	Hydrolysis of esculin results in a black precipitate in the presence of ferric ion. <sup>10</sup>
1J	Ornithine	ORN	Utilization of ornithine results in pH rise and change in the color of the indicator (Bromcresol purple). <sup>2</sup>

**Table 3**  
**Reagents used in the BBL CRYSTAL™ RGP ID System**

Panel Location	Substrate	Code	Pos.	Neg.	Active Ingredients	Approx. Amt. (g/L)
4A	Fluorescent negative control	FCT	n/a	n/a	Fluorescent coumarin derivative	≤1
2A	4MU-β-D-glucoside	FGC	blue fluorescence >FCT well	blue fluorescence ≤FCT well	4MU-β-D-glucoside	≤1
1A	L-proline-AMC	FPR	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-proline-AMC	≤1
4B	L-arginine-AMC	FAR	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-arginine-AMC	≤1
2B	L-methionine-AMC	FME	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-methionine-AMC	≤1
1B	4MU-β-D-cellobioside	FCE	blue fluorescence >FCT well	blue fluorescence ≤FCT well	4MU-β-D-cellobioside	≤1
4C	4MU-phosphate	FHO	blue fluorescence >FCT well	blue fluorescence ≤FCT well	4MU-phosphate	≤1
2C	L-pyroglyutamic acid-AMC	FPY	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-pyroglyutamic acid-AMC	≤1
1C	L-tryptophan-AMC	FTR	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-tryptophan-AMC	≤1
4D	L-valine-AMC	FVA	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-valine-AMC	≤1
2D	L-phenylalanine-AMC	FPH	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-phenylalanine-AMC	≤1
1D	4MU-α-D-glucoside	FGS	blue fluorescence >FCT well	blue fluorescence ≤FCT well	4MU-α-D-glucoside	≤1
4E	Arabinose	ARA	Gold/Yellow	Orange/Red	Arabinose	≤300
2E	Maltose	MAL	Gold/Yellow	Orange/Red	Maltose	≤300
1E	Dextrin	DXT	Gold/Yellow	Orange/Red	Dextrin	≤300
4F	Mannitol	MNT	Gold/Yellow	Orange/Red	Mannitol	≤300
2F	Galactose	GAL	Gold/Yellow	Orange/Red	Galactose	≤300
1F	N-acetyl-D-glucosamine	AGN	Gold/Yellow	Orange/Red	N-acetyl-D-glucosamine	≤300
4G	Trehalose	TRE	Gold/Yellow	Orange/Red	Trehalose	≤300
2G	Mannose	MNS	Gold/Yellow	Orange/Red	Mannose	≤300
1G	Maltotriose	MTT	Gold/Yellow	Orange/Red	Maltotriose	≤300
4H	ONPG & p-n-p-β-D-glucoside	POG	Yellow	Colorless	ONPG & p-n-p-β-D-glucoside	≤10
2H	p-n-p-α-D-glucoside	AGL	Yellow	Colorless	p-n-p-α-D-glucoside	≤10
1H	p-n-p-β-D-cellobioside	PCE	Yellow	Colorless	p-n-p-β-D-cellobioside	≤10
4I	p-n-p-β-D-glucoside	BGL	Yellow	Colorless	p-n-p-β-D-glucoside	≤10
2I	p-n-p-phosphate	PHO	Yellow	Colorless	p-n-p-phosphate	≤10
1I	p-n-p-β-D-galactoside & p-n-p-α-D-galactoside	PPG	Yellow	Colorless	p-n-p-β-D-galactoside & p-n-p-α-D-galactoside	≤10
4J	Urea	URE	Aqua/Blue	Yellow/Green	Urea	≤50
2J	Esculin	ESC	Brown/Maroon	Clear/Tan	Esculin	≤25
1J	Ornithine	ORN	Purple	Yellow/Gray	Ornithine	≤200

Table 5  
Additional Quality Control Strains for BBL CRYSTAL™ RGP ID System  
After 4 Hours Incubation from TSA II™ or Columbia Blood Agar

Panel Location	Substrate	Code	Staphylococcus epidermidis ATCC 12228	Bacillus brevis ATCC 8246	Enterococcus faecalis ATCC 19433	Streptococcus pneumoniae ATCC 6305
4A	Fluorescent Negative Control	FCT	-	-	-	-
2A	4MU-β-D-glucoside	FGC	-	-	+	-
1A	L-proline-AMC	FPR	-	+	-	+
4B	L-arginine-AMC	FAR	v	v	-	+
2B	L-methionine-AMC	FME	-	+	v	+
1B	4MU-β-D-cellobioside	FCE	-	*	+	-
4C	4MU-phosphate	FHO	+	-	v	-
2C	L-pyroglyutamic acid-AMC	FPY	-	+	v	-
1C	L-tryptophan-AMC	FTR	-	+	+	+
4D	L-valine-AMC	FVA	-	v	-	+
2D	L-phenylalanine-AMC	FPH	-	+	+	+
1D	4MU-α-D-glucoside	FGS	-	-	+	+
4E	Arabinose	ARA	-	-	-	-
2E	Maltose	MAL	v	-	+	v
1E	Dextrin	DXT	-	-	+	v
4F	Mannitol	MNT	-	-	+	-
2F	Galactose	GAL	-	-	+	-
1F	N-acetyl-D-glucosamine	AGN	-	-	+	v
4G	Trehalose	TRE	-	-	+	v
2G	Mannose	MNS	-	-	+	v
1G	Maltotriose	MIT	-	-	+	v
4H	ONPG & p-n-p-β-D-glucoside	POG	-*	-	+	+
2H	p-n-p-α-D-glucoside	AGL	v	-	+	v
1H	p-n-p-β-D-cellobioside	PCE	-	-	+	-
4I	p-n-p-β-D-glucoside	BGL	-	-	+	v
2I	p-n-p-phosphate	PHO	v	-	-	-
1I	p-n-p-β-D-galactoside & p-n-p-α-D-galactoside	PPG	v	-	-	+
4J	Urea	URE	-	v	-	-
2J	Esculin	ESC	v	v	+	-
1J	Ornithine	ORN	v	+	-	v

\* = variable when tested from Columbia Blood Agar

Table 4  
Quality Control Chart for BBL Crystal™ RGP ID System  
After 4 Hours Incubation from TSA II™ or Columbia Blood Agar

Panel Location	Substrate	Code	Streptococcus pyogenes ATCC 19615
4A	Fluorescent Negative Control	FCT	-
2A	4MU-β-D-glucoside	FGC	-
1A	L-proline-AMC	FPR	v
4B	L-arginine-AMC	FAR	+
2B	L-methionine-AMC	FME	+
1B	4MU-β-D-cellobioside	FCE	-
4C	4MU-phosphate	FHO	+
2C	L-pyroglyutamic acid-AMC	FPY	+
1C	L-tryptophan-AMC	FTR	+
4D	L-valine-AMC	FVA	+
2D	L-phenylalanine-AMC	FPH	+
1D	4MU-α-D-glucoside	FGS	+
4E	Arabinose	ARA	-
2E	Maltose	MAL	+
1E	Dextrin	DXT	+
4F	Mannitol	MNT	-
2F	Galactose	GAL	v
1F	N-acetyl-D-glucosamine	AGN	+
4G	Trehalose	TRE	+
2G	Mannose	MNS	+
1G	Maltotriose	MIT	+
4H	ONPG & p-n-p-β-D-glucoside	POG	v
2H	p-n-p-α-D-glucoside	AGL	v
1H	p-n-p-β-D-cellobioside	PCE	-
4I	p-n-p-β-D-glucoside	BGL	v
2I	p-n-p-phosphate	PHO	+
1I	p-n-p-β-D-galactoside & p-n-p-α-D-galactoside	PPG	v
4J	Urea	URE	-
2J	Esculin	ESC	-
1J	Ornithine	ORN	-

**Table 6**  
**Expected Reactions for Species Most Frequently Encountered in BBL CRYSTAL™ RGP ID System Clinical Trials**

Organisms	FCT	FGC	FPR	FAR	FME	FCE	FHO	FPY	FTR	FVA	FPH	FGS	ARA	MAL	DXT	MNT	GAL	AGN	TRE	MNS	MTT	POG	AGL	PCE	BGL	PHO	PPG	URE	ESC	ORN
<i>E. faecalis</i>	-	+	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	V	V	-	+	-
<i>E. faecium</i>	-	+	-	(-)	+	+	-	(+)	+	-	+	(-)	+	+	+	+	+	+	(+)	+	+	(-)	+	+	+	(-)	+	-	+	-
<i>S. aureus</i>	-	-	-	+	-	-	+	-	(-)	-	-	-	-	+	+	V	-	-	V	V	V	V	V	V	V	+	(-)	-	-	-
<i>S. epidermidis</i>	-	-	-	(-)	-	-	+	-	-	-	-	-	-	V	-	-	-	-	-	-	-	(-)	-	-	(-)	V	(-)	+	-	-
<i>S. haemolyticus</i>	-	-	-	(-)	-	-	-	-	-	-	-	-	-	(-)	-	-	-	-	-	-	-	V	V	V	V	(-)	-	-	-	-
<i>S. saprophyticus</i>	-	-	-	-	-	-	-	-	V	-	-	-	-	V	-	-	-	-	-	-	-	V	(-)	-	(-)	V	V	+	-	(-)
<i>S. simulans</i>	-	-	-	-	-	-	(-)	(+)	-	-	-	-	-	-	-	-	-	-	V	V	-	V	-	-	(-)	V	V	-	-	-
<i>S. warneri</i>	-	-	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	V	-	-	V	-	-	(-)	-	-
<i>S. agalactiae</i>	-	-	-	+	+	-	+	-	V	-	(+)	V	-	+	+	-	-	+	+	+	+	(+)	+	-	(+)	+	(-)	-	-	-
<i>S. pneumoniae</i>	-	(-)	(+)	+	+	-	-	-	+	+	+	+	-	(-)	-	-	-	-	-	-	-	+	+	-	V	-	+	-	-	-
<i>S. pyogenes</i>	-	-	-	+	+	-	+	+	+	+	+	V	-	+	+	-	V	+	(+)	+	(+)	(+)	+	-	V	+	(-)	-	(-)	-

KEY: + = ≥ 90% positive; (+) = 75 – 89% positive; V = 26 – 74% positive; (-) = 11 – 25% positive; - = ≤ 10% positive.

**Table 7**  
**Accuracy of Identification for Species Most Frequently Encountered in BBL CRYSTAL™ RGP ID System Clinical Trial**

Organism	Number tested	BBL CRYSTAL correct ID	BBL CRYSTAL correct w/supplemental tests	Total correct
<i>Enterococcus faecalis</i>	55	55	0	55
<i>Enterococcus faecium</i>	25	16	0	16
<i>Staphylococcus aureus</i>	55	54	0	54
<i>Staphylococcus epidermidis</i>	59	58	0	58
<i>Staphylococcus haemolyticus</i>	50	50	0	50
<i>Staphylococcus hominis</i>	28	22	3	25
<i>Staphylococcus saprophyticus</i>	39	36	3	39
<i>Staphylococcus simulans</i>	19	18	0	18
<i>Staphylococcus warneri</i>	12	8	4	12
<i>Streptococcus agalactiae</i>	52	50	0	50
<i>Streptococcus mitis</i> group	14	8	0	8
<i>Streptococcus pneumoniae</i>	54	54	0	54
<i>Streptococcus pyogenes</i>	49	48	0	48
Other *	93	60	3	63
Grand Total	604	537	13	550

Key: \* = This category comprises all isolates where less than 10 were encountered in clinical trials.