BBL CRYSTAL™ IDENTIFICATION SYSTEMS

Anaerobe ID Kit

CLIA COMPLEXITY: HIGH
CDC IDENTIFIER CODES
ANALYTE: 0412
TEST SYSTEM: 07561

INTENDED USE

The **BBL Crystal™** Anaerobe (ANR) Identification (ID) System is a miniaturized identification method employing modified conventional, fluorogenic and chromogenic substrates. It is intended for the identification of frequently isolated anaerobic bacteria.[1,2,6,9,10,11,16,20,24]

SUMMARY AND EXPLANATION

Micromethods for the biochemical identification of microorganisms were reported as early as 1918.[3] Several publications reported on the use of the reagent-impregnated paper discs and micro-tube methods for differentiating enteric bacteria.[3,4,8,21,23] 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** ANR ID panel, to detect enzymes that microbes use to metabolize various substrates.[5,8,12,13,14,15,17,18,19]

The **BBL CRYSTAL** ANR ID kit is comprised of (i) **BBL CRYSTAL** ANR 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.[22] Biochemical and enzymatic reaction patterns for the 29 **BBL CRYSTAL** ANR ID substrates for a wide variety of microorganisms are stored in the **BBL CRYSTAL** ANR ID database. 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.

PRINCIPLES OF THE PROCEDURE

The **BBL CRYSTAL** ANR 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 [5,12,13,14,15] with a UV light source.[15,17,18] 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. 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** ANR ID panel contains 29 enzymatic and biochemical substrates. Refer to Table 3 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 07561.

After use, all infectious materials including plates, cotton swabs, inoculum tubes, filter papers used for indole tests and panels must be autoclaved prior to disposal or incinerated.

STORAGE AND HANDLING/SHELF LIFE

Lids: Lids are individually packaged and must be stored unopened in a refrigerator at 2 – 8ºC. DO NOT FREEZE. Visually inspect the package for holes or cracks in the foil package. Do not use if the packaging 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 – 25ºC, until ready to use. Store unused bases in the tray, in plastic bag. Empty trays should be used to incubate 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 – 25ºC. Expiration dating is shown on the tube label. Only **BBL CRYSTAL** ANR, GP, RGP, N/H ID inoculum fluid should be used with **BBL CRYSTAL** ANR panels.

On receipt, store the **BBL Crystal** ANR kit at 2 – 8ºC. Once opened, only the lids need to be stored at 2 – 8ºC. The remaining components of the kit may be stored to 2 – 25ºC. 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 a nonselective blood agar medium such as CDC Anaerobe Blood Agar, Brucella Blood Agar, Columbia Blood Agar, or Schaedler Blood Agar. The test isolate must be a pure culture, no more than 24 – 48 h old for most genera; for some slow growing cocci (up to 72 h) and *Actinomyces* species (72 – 96 h) older cultures may be acceptable. Only cotton-tipped applicator swabs should be used to prepare inoculum as some polyester swabs may cause problems with inoculation of the panels. (See "Limitations of The Procedure".) Once lids are removed from the sealed pouches, they must be used within 1 h to ensure adequate performance. The plastic cover should remain on the lid until used.

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 placement on primary isolation media.[1] Other recommended readings relevant to anaerobic specimen handling include *Wadsworth Anaerobic Bacteriology Manual*[24] and *Principles and Practice of Clinical Anaerobic Bacteriology*.[6]

TEST PROCEDURE

Materials Provided: BBL CRYSTAL ANR ID Kit –

20 **BBL CRYSTAL** Anaerobe ID Panel Lids,

20 **BBL CRYSTAL** Bases,

20 **BBL CRYSTAL** ANR, GP, RGP, N/H ID Inoculum Fluid Tubes. Each tube has approximately 2.3 ± 0.15 ml of Inoculum Fluid containing: KCl 7.5 g, $CaCl_2$ 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** ANR ID Report Pad.

Materials Not Provided: Sterile cotton swabs *(do not use polyester swabs)*, incubator (35 – 37°C) non-CO_2 (40 – 60% humidity), McFarland No. 4 and No. 5 standards, **BBL CRYSTAL**™ Panel Viewer (includes **BBL CRYSTAL** Color Reaction Charts), **BBL CRYSTAL**™ ID System Electronic Codebook or **BBL CRYSTAL** ANR Manual Codebook, **BBL**™ DMACA Indole Reagent Droppers, nonselective culture plate and catalase reagent.

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

Test Procedure: BBL CRYSTAL ANR ID System requires Gram stain, catalase and indole test results. Prior to panel set-up, catalase and indole tests should be performed. Perform indole test per instructions provided in the package insert. For catalase test a 15.0% solution of hydrogen peroxide with 1.0% Tween 80 added is recommended.[7,24]

1. Remove lids from pouch. Discard desiccant. Once removed from the pouch, covered lids should be used within 1 h. Do not use panel if there is no desiccant in the pouch.

2. Take an inoculum fluid tube and label with patient's specimen number. Using aseptic technique, with the tip of a sterile cotton swab *(do not use a polyester swab)* or a wooden applicator stick or disposable plastic loop, pick colonies of the same morphology from one of the recommended media (see section "Specimen Collection and Processing").

3. Suspend colonies in a tube of **BBL CRYSTAL** ANR, GP, RGP, N/H ID Inoculum Fluid.

4. Recap tube and vortex for approximately 10 – 15 sec. The turbidity should be equivalent to a McFarland No.4 standard (not to exceed McFarland No. 5 standard). If the inoculum concentration is in excess of the recommended McFarland standard, one of the following steps is recommended:

    a. With a fresh tube of inoculum fluid, prepare a new inoculum equivalent to a McFarland No.4 standard.
    b. If additional colonies are unavailable for preparation of a new inoculum, using aseptic techniques, dilute the inoculum by adding the minimum required volume (not to exceed 1.0 ml) of 0.85% sterile saline to bring down the turbidity equivalent to a McFarland No.4. Remove the excess amount added to the tube with a sterile pipet, so that the final volume of inoculum is approximately equivalent to that of the original volume in tube (2.3 ± 0.15 ml). Failure to do this will result in spilling of the inoculum over the black portion of the base rendering the panel unusable.

5. Take a base, and mark the patient's specimen number on the side wall.

6. Pour entire contents of inoculum fluid 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** ANR ID panels, the inoculum should

be slowly 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 downwards simultaneously until the lid snaps into place (listen for two "clicks").

Purity Plate: Using a sterile loop, recover a small drop from the inoculum tube either before or after inoculating the base and inoculate an agar slant or plate (any nonselective medium) for purity check. Discard inoculum tube and cap in a biohazard disposal container. Incubate the slant or plate for 24 – 48 h at 35 – 37°C under anaerobic 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_2 incubator with 40 – 60% **humidity**. Trays should not be stacked more than two high during incubation. The incubation time for panels is **4 h** at 35 – 37°C. NOTE: The incubator door should not be opened repeatedly during the incubation period (preferably less than 3 times).

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 for an interpretation of the reactions. Use the **BBL CRYSTAL** ANR Report pad to record reactions.

a. Read columns G thru J first, using the regular (white) light source.

b. Read columns A thru F (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 (A4).

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 numbers (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

Select the appropriate **BBL CRYSTAL** Anaerobe database from the offered menu. The type of primary plate used to prepare the inoculum will determine the appropriate database. For use with Brucella or Columbia Blood Agar media; select alternate blood-agar database from the menu**.**

The resulting profile number and off-line test results (Gram stain, catalase and indole) should be entered on a PC in which the **BBL CRYSTAL** ID System Electronic Codebook has been installed, to obtain the identification. If a PC is not available contact Becton Dickinson Microbiology Systems Technical Services for assistance with the identification.

QUALITY CONTROL

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

1. Inoculate a panel with *Bacteroides fragilis* ATCC® 25285 per recommended procedure (refer to "Test Procedure").

2. Prior to incubation, let panel remain at room temperature for 1 min (not more than 2 min).

3. Read and record reactions with the aid of the panel viewer and color reaction chart.

4. If any of the wells, except 1F, are positive per color reaction chart (after 1 – 2 min), DO NOT USE PANELS from this lot. Contact Becton Dickinson Microbiology Systems Technical Services. (NOTE: Well 1F [Escosyl] should be positive upon rehydration).

5. If all wells are negative, then incubate panel for 4 h at 35 – 37°C.

6. Read panel with the panel viewer and color reaction chart; record reactions using the Report Pad.

7. Compare recorded reactions with those listed in Table 4. If discrepant results are obtained, confirm purity of quality control strain before contacting Becton Dickinson Microbiology Systems Technical Services.

8. 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.

LIMITATIONS OF THE PROCEDURE

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

All **BBL CRYSTAL** Anaerobe ID databases were developed with **BBL**™ media. Reactivity of some substrates in rapid identification systems may be dependent upon the source media used in inoculum preparations. We recommend the use of the following **BBL** media for use with the **BBL CRYSTAL** ANR ID System: CDC Anaerobe Blood Agar, Schaedler Agar with Vitamin K_1 and 5% Sheep Blood, Columbia Agar with 5% Sheep Blood and Brucella Blood Agar with Hemin and Vitamin K_1 (see "Availability").

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** ANR ID System is based on statistical use of specially designed tests and an exclusive database.

While **BBL CRYSTAL** ANR 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, or wooden applicator sticks, or disposable plastic loops 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. Once lids are removed from the sealed pouches, they must be used within 1 h to ensure adequate performance. The plastic cover should remain on the lid until used.

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 **face down** (larger windows facing up; label facing down) to maximize the effectiveness of substrates.

Colonies should be taken from **nonselective** blood agar plates such as **BBL**™ CDC Anaerobe, Brucella, Columbia, and Schaedler (see "Availability").

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.

PERFORMANCE CHARACTERISTICS

Reproducibility: In an external study involving four clinical laboratories (total of five evaluations), the reproducibility of **BBL CRYSTAL** ANR ID substrate (29) reactions was studied by replicate testing. The reproducibility of the individual substrate reactions ranged from

96.2% to 100%. The overall reproducibility of **BBL CRYSTAL** ANR panel was determined to be 99.1% .[25]

Accuracy of Identification: The performance of **BBL CRYSTAL** ANR ID System was compared to a currently available commercial system, as well as to conventional reference identification methods based on VA Wadsworth Laboratory recommendations, using clinical isolates and stock cultures. A total of five studies were conducted in four 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 633 total isolates tested from the five studies, 588 (93%) were correctly identified (including isolates that required supplemental testing) by the **BBL CRYSTAL** ANR Identification System. A total of 36 (6%) isolates were incorrectly identified, and a message of "No Identification" was obtained for 9 (1%) isolates.[25]

AVAILABILITY

Cat. No.	Description
245010	**BBL CRYSTAL**™ Anaerobe ID Kit, containing 20 each: **BBL CRYSTAL** Anaerobe ID Panel Lids, **BBL CRYSTAL** Bases and **BBL CRYSTAL** Anaerobe ID Inoculum Fluid.
	BBL CRYSTAL™ ANR, GP, RGP, N/H ID Inoculum Fluid, ctn of 10.
	BBL CRYSTAL™ Panel Viewer, Domestic model, 110 V, 60 Hz.
	BBL CRYSTAL™ Panel Viewer, European model, 220 V, 50 Hz.
	BBL CRYSTAL ™ Panel Viewer, Japanese model, 100 V, 50/60 Hz.
	BBL CRYSTAL™ Panel Viewer Longwave UV Tube.
	BBL CRYSTAL™ Panel Viewer White Light Tube
	BBL CRYSTAL™ ID System Electronic Codebook.
	BBL CRYSTAL™ Identification Systems Anaerobe Manual Codebook.
	BBL™ CDC Anaerobe Blood Agar with 5% Sheep Blood, pkg of 20 plates.
	BBL™ CDC Anaerobe Blood Agar with 5% Sheep Blood, ctn of 100 plates.
	BBL™ Schaedler Agar with Vitamin K_1 and 5% Sheep Blood, pkg of 20.

BBL™ Schaedler Agar with Vitamin K_1 and 5% Sheep Blood, ctn of 100.

BBL™ Columbia Agar with 5% Sheep Blood, pkg of 20.

BBL™ Columbia Agar with 5% Sheep Blood, ctn of 100.

BBL™ Brucella Blood Agar with Hemin and Vitamin K_1, pkg of 20.

BBL™ Brucella Blood Agar with Hemin and Vitamin K_1, pkg of 100.

BBL™ DMACA Indole Reagent Droppers, ctn of 50.

BBL™ Gram Stain Kit, pkg 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.**

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25. Data on file at Becton Dickinson Microbiology Systems.

TECHNICAL INFORMATION: In the United States, telephone Technical Services, toll free (800) 638-8663, selection 2.

Rev. 1/99 (PI 7/99)

Approved by:

Date effective:

Supervisor: _____ Date: _____

Director: _____ Date: _____

Reviewed by:

Table 1

Taxa in BBL CRYSTAL™ ANR ID System

Gram- Negative Bacilli		
Bile Tolerant	**Bile Sensitive**	**Non-Pigmented, Pitting**
Bacteroides fragilis **group**	**Non Pigmented**	Bacteroides
B. caccae	Prevotella	*B. ureolyticus*
B. distasonis group[3]	*P. bivia*	Campylobacter
B. eggerthii	*P. buccae*	*C. gracilis*
B. fragilis	*P. buccalis*	Fusobacterium
B. ovatus	*P. disiens*	*F. gonidiaformans*[1,4]
B. stercoris	*P. oralis*	*F. mortiferum*
B. thetaiotaomicron	*P. oris*	*F. necrophorum*
B. uniformis	*P. veroralis*[4]	*F. nucleatum*
B. vulgatus	**Non Pigmented, Non Pitting**	*F. russii*
Other:	Bacteroides	*F. varium*
B. splanchnicus	*B. capillosus*	Leptotrichia
Porphyromonas levii[4]	Tissierella	*L. buccalis*
Bile Sensitive Pigmented	*T. praeacuta*	
Capnocytophaga species	**Bile Tolerant**	
Prevotella	**Non Pigmented**	
P. corporis	***Bilophila***	
P. denticola	*B. wadsworthia*	
P. intermedia	Desulfomonas	
P. loescheii	*D. pigra*	
P. melaninogenica	Desulfovibrio	
Porphyromonas	**species**	
P. asaccharolytica	Campylobacter	
P. endodontalis	*C. curvus/rectus*	
P. gingivalis		

Clostridia	Non-Spore Forming GramPositiveBacilli	Gram-Positive Cocci
Clostridium	Actinomyces	Gemella
C. baratii	*A. bovis*	*G. morbillorum*
C. beijerinckii	*A. israelii*	Peptostreptococcus
C. bifermentans	*A. meyeri*	*P. anaerobius*
C. botulinum	*A. naeslundii*	*P. asaccharolyticus*
C. butyricum	*A. odontolyticus*	*P. indolicus*
C. cadaveris	*A. pyogenes*	*P. magnus*
C. clostridioforme	*A. viscosus*	*P. micros*
C. difficile	Atopobium	*P. prevotii*
C. glycolicum	*A. minutum*	*P. tetradius*
C. hastiforme	Bifidobacterium	Ruminococcus
C. histolyticum	*B. adolescentis*	*R. productus*[4]
C. innocuum	*B. dentium*	Staphylococcus
C. limosum	*B.* species	*S. saccharolyticus*
C. novyi A	Eubacterium	Streptococcus
C. paraputrificum[4]	*E. aerofaciens*	*S. constellatus*
C. perfringens	*E. lentum*	*S. intermedius*
C. putrificum[1]	*E. limosum*	**Gram-Negative Cocci**
C. ramosum	Mobiluncus	Veillonella **species**
C. septicum	*M. curtisii*	
C. sordellii	*M. mulieris*	
C. sphenoides	*M.* species[2,4]	
C. sporogenes	Propionibacterium	
C. subterminale	*P. acnes*	
C. tertium	*P. avidum*	
C. tetani[4]	*P. granulosum*[4]	
	P. granulosum[4]	
	P. propionicum	
	Lactobacillus	
	L. acidophilus	
	L. casei	
	L. catenaforme	
	L. fermentum	
	L. jensenii	
	L. johnsonii	
	L. rhamnosus	

Key: 1= Taxon in **BBL CRYSTAL**, **BBL**™ Schaedler database only.

2= Taxon in **BBL CRYSTAL**, **BBL**™ Schaedler and **BBLCRYSTAL** alternate Blood Agar databases only.

3 = Includes *B. distasonis* and *B. merdae.*

4 = These taxa have < 10 unique **BBL CRYSTAL** profiles in the current database.

Table 2
Principles of Tests Employed in the BBL CRYSTAL ANR ID Sysytem

Panel Location	Test Feature	Code	Principle (Reference)
4A	Fluorescent negative control	FCT	Control to standardize fluorescent substrate results
2A	L-arginine-AMC	FAR	Enzymatic hydrolysis of the amide or glycosidic bond results in the release of a fluorescent coumarin derivative.[15,17,18]
1A	L-histidine-AMC	FHI	
4B	4MU-α-D-mannoside	FAM	
2B	L-serine-AMC	FSE	
1B	L-isoleucine-AMC	FIS	
4C	4MU-β-D-mannoside	FBM	
2C	Glycine-AMC	FGL	
1C	L-alanine-AMC	FAL	
4D	4MU-N-acetyl-β-D-galactosaminide	FGA	
2D	L-pyroglutamic acid-AMC	FPY	
1D	L-lysine-AMC	FLY	
4E	L-methionine-AMC	FME	
2E	4MU-β-D-cellobiopyranoside	FCE	
1E	4MU-β-D-xyloside	FXY	
4F	L-phenylalanine-AMC	FPH	
2F	L-leucine-AMC	FLE	
1F	Escosyl	FSC	Hydrolysis of the glycosidic bond results in the release of nonfluorescent esculetin.[19]
4G	Disaccharide	DIS	Utilization of carbohydrate results in lower pH and change in indicator (Phenol Red).[1,2,4,8]
2G	Furanose	FUR	
1G	Pyranose	PYO	
4H	p-nitrophenyl-α-D-galactoside	AGA	Enzymatic hydrolysis of the colorless aryl substituted glycoside releases yellow p-nitrophenol.[5,12,13,14,15]
2H	p-nitrophenyl-β-D-galactoside	NPG	
1H	p-nitrophenyl-phosphate	PHO	
4I	p-nitrophenyl-α-D-glucoside	AGL	
2I	p-nitrophenyl-N-acetyl-glucosaminide	NAG	
1I	L-proline-p-nitroanilide	PRO	Enzymatic hydrolysis of the colorless amide substrate releases yellow p-nitroaniline.[5,12,13,14,15]
4J	p-nitrophenyl-α-L-fucoside	AFU	Enzymatic hydrolysis of the colorless aryl substituted glycoside releases yellow p-nitrophenol.[5,12,13,14,15]
2J	p-nitrophenyl-β-D-glucoside	BGL	
1J	L-alanyl-L-alanine-p-nitroanilide	ALA	Enzymatic hydrolysis of the colorless amide substrate releases yellow p-nitroaniline.[5,12,13,14,15]

Table 3
Reagents used in the BBL CRYSTAL ANR 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	L-arginine-AMC	FAR	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-arginine-AMC	≤1
1A	L-histidine-AMC	FHI	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-histidine-AMC	≤1
4B	4MU-α-D-mannoside	FAM	blue fluorescence >FCT well	blue fluorescence ≤FCT well	4MU-α-D-mannoside	≤1
2B	L-serine-AMC	FSE	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-serine-AMC	≤1
1B	L-isoleucine-AMC	FIS	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-isoleucine-AMC	≤1
4C	4MU-β-D-mannoside	FBM	blue fluorescence >FCT well	blue fluorescence ≤FCT well	4MU-β-D-mannoside	≤1
2C	Glycine-AMC	FGL	blue fluorescence >FCT well	blue fluorescence ≤FCT well	glycine-AMC	≤1
1C	L-alanine-AMC	FAL	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-alanine-AMC	≤1
4D	4MU-N-acetyl-β-D-galactosaminide	FGA	blue fluorescence >FCT well	blue fluorescence ≤FCT well	4MU-N-acetyl-β-D-galactosaminide	≤1
2D	L-pyroglutamic acid-AMC	FPY	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-pyroglutamic acid-AMC	≤1
1D	L-lysine-AMC	FLY	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-lysine-AMC	≤1
4E	L-methionine-AMC	FME	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-methionine-AMC	≤1
2E	4MU-β-D-cellobiopyranoside	FCE	blue fluorescence >FCT well	blue fluorescence ≤FCT well	4MU-β-D-cellobiopyranoside	≤1
1E	4MU-β-D-xyloside	FXY	blue fluorescence >FCT well	blue fluorescence ≤FCT well	4MU-β-D-xyloside	≤1
4F	L-phenylalanine-AMC	FPH	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-phenylalanine-AMC	≤1
2F	L-leucine-AMC	FLE	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-leucine-AMC	≤1
1F	Escosyl*	FSC	blue/green fluorescence >FCT well	blue/green fluorescence ≤FCT well	Escosyl	≤1
4G	Disaccharide	DIS	Gold/Yellow	Orange/Red	Disaccharide	≤300
2G	Furanose	FUR	Gold/Yellow	Orange/Red	Furanose	≤300
1G	Pyranose	PYO	Gold/Yellow	Orange/Red	Pyranose	≤300
4H	p-n-p-α-D-galactoside	AGA	Yellow	Colorless	p-n-p-α-D-galactoside	≤7
2H	p-n-p-β-D-galactoside	NPG	Yellow	Colorless	p-n-p-β-D-galactoside	≤7
1H	p-n-p-phosphate	PHO	Yellow	Colorless	p-n-p-phosphate	≤7
4I	p-n-p-α-D-glucoside	AGL	Yellow	Colorless	p-n-p-α-D-glucoside	≤7
2I	p-n-p-N-acetyl-glucosaminide	NAG	Yellow	Colorless	p-n-p-N-acetyl-glucosaminide	≤7
1I	L-proline-p-nitroanilide	PRO	Yellow	Colorless	L-proline-p-nitroanilide	≤7
4J	p-n-p-α-L-fucoside	AFU	Yellow	Colorless	p-n-p-α-L-fucoside	≤7
2J	p-n-p-β-D-glucoside	BGL	Yellow	Colorless	p-n-p-β-D-glucoside	≤7
1J	L-alanyl-L-alanine-p-nitroanilide	ALA	Yellow	Colorless	L-alanyl-L-alanine-p-nitroanilide	≤7

* The Ecosyl substrate is fluorescent unhydrolized. Fluorescence will decrease when the enzyme is present.

Table 4
Quality Control Chart for BBL CRYSTAL ANR ID System*

Panel Location	Substrate	Code	*Bacteroides fragilis* ATCC® 25285
4A	Fluorescent negative control	FCT	–
2A	L-arginine-AMC	FAR	V
1A	L-histidine-AMC	FHI	–
4B	4MU-α-D-mannoside	FAM	V[1]
2B	L-serine-AMC	FSE	–
1B	L-isoleucine-AMC	FIS	–
4C	4MU-β-D-mannoside	FBM	+
2C	L-serine-AMC	FGL	–
1C	L-alanine-AMC	FAL	V
4D	4MU-N-acetyl-β-D-galactosaminide	FGA	+
2D	L-pyroglutamic acid-AMC	FPY	V[1,4]
1D	L-lysine-AMC	FLY	V
4E	L-methionine-AMC	FME	V
2E	4MU-β-D-cellobiopyranoside	FCE	+
1E	4MU-β-D-xyloside	FXY	V[1]
4F	L-phenylalanine-AMC	FPH	V
2F	L-leucine-AMC	FLE	+
1F	Escosyl	FSC	– [3,6,9]
4G	Disaccharide	DIS	+
2G	Furanose	FUR	+
1G	Pyranose	PYO	+[1]
4H	p-n-p-α-D-galactoside	AGA	+
2H	p-n-p-β-D-galactoside	NPG	+
1H	p-n-p-phosphate	PHO	+
4I	p-n-p-α-D-glucoside	AGL	+
2I	p-n-p-N-acetyl-glucosaminide	NAG	+
1I	L-proline-p-nitroanilide	PRO	–
4J	p-n-p-α-L-fucoside	AFU	+
2J	p-n-p-β-D-glucoside	BGL	+
1J	L-alanyl-L-alanine-p-nitroanilide	ALA	+

1 = Negative from **BBL**™ Schaedler
2 = Positive from **BBL**™ Schaedler
3 = Variable from **BBL**™ Schaedler
4 = Negative from **BBL**™ Brucella
5 = Positive from **BBL**™ Brucella
6 = Variable from **BBL**™ Brucella
7 = Negative from **BBL**™ Columbia
8 = Positive from **BBL**™ Columbia
9 = Variable from **BBL**™ Columbia

* Results shown are expected when **BBL**™ CDC Anaerobic Agar with 5% sheep blood is used.

Table 5
Additional Quality Control Strains for BBL CRYSTAL ANR ID System

Panel Location	Substrate	Code	*Bacteroides distasonis* ATCC® 8503	*Peptostreptococcus asaccharolyticus* ATCC® 29743	*Lactobacillus acidophilus* ATCC® 314	*Fusobacterium varium* ATCC® 27725
4A	Fluorescent negative control	FCT	–	–	–	–
2A	L-arginine-AMC	FAR	+	+	+	–[3,9]
1A	L-histidine-AMC	FHI	V	+	+[6]	–
4B	4MU-α-D-mannoside	FAM	+	–	–	–
2B	L-serine-AMC	FSE	–	–	+[6]	–
1B	L-isoleucine-AMC	FIS	–[9]	–	+	–
4C	4MU-β-D-mannoside	FBM	+[3]	–	–	–
2C	Glycine-AMC	FGL	V[1,8]	V[1]	V[2]	–
1C	L-alanine-AMC	FAL	+	V[1]	+	–
4D	4MU-N-acetyl-β-D-galactosaminide	FGA	+	–	–	–
2D	L-pyroglutamic acid-AMC	FPY	V[1,8]	–	V[4,7]	+
1D	L-lysine-AMC	FLY	V[2,5,8]	+	+	–
4E	L-methionine-AMC	FME	+	+[3,9]	+	V
2E	4MU-β-D-cellobiopyranoside	FCE	V[8]	–	+	–
1E	4MU-β-D-xyloside	FXY	+[3]	–	–	–
4F	L-phenylalanine-AMC	FPH	V[8]	V	+	–
2F	L-leucine-AMC	FLE	+	+[3]	+	V
1F	Escosyl	FSC	V	V[2,5]	–[3,6,9]	V[5]
4G	Disaccharide	DIS	+	–	+[3,6,7]	–
2G	Furanose	FUR	+	–	+	V
1G	Pyranose	PYO	+	–	+[3]	+
4H	p-n-p-α-D-galactoside	AGA	+	–	+[3,6,9]	–
2H	p-n-p-β-D-galactoside	NPG	+	–	+[3,6,9]	–
1H	p-n-p-phosphate	PHO	+	–	–	–
4I	p-n-p-α-D-glucoside	AGL	+	–	V[1]	–
2I	p-n-p-N-acetyl-glucosaminide	NAG	+	–	V[5,8]	–
1I	L-proline-p-nitroanilide	PRO	–	–	V	–
4J	p-n-p-α-L-fucoside	AFU	–	–	–	–
2J	p-n-p-β-D-glucoside	BGL	+	–	+	–
1J	L-alanyl-L-alanine-p-nitroanilide	ALA	+	–	V	–

1 = Negative from **BBL**™ Schaedler
2 = Positive from **BBL**™ Schaedler
3 = Variable from **BBL**™ Schaedler
4 = Negative from **BBL**™ Brucella
5 = Positive from **BBL**™ Brucella
6 = Variable from **BBL**™ Brucella
7 = Negative from **BBL**™ Columbia
8 = Positive from **BBL**™ Columbia
9 = Variable from **BBL**™ Columbia

* Results shown are expected when **BBL**™ CDC Anaerobic Agar with 5% sheep blood is used.