BBL CRYSTAL™ IDENTIFICATION SYSTEMS

ENTERIC/NONFERMENTER ID KIT

CLIA COMPLEXITY: High
CDC IDENTIFIER CODES:
Analyte: 0412
Test System: 07485

INTENDED USE

The **BBL CRYSTAL™** Enteric/Nonfermenter (E/NF) Identification (ID) System is a miniaturized identification method employing modified conventional and chromogenic substrates. It is intended for the identification of aerobic gram-negative bacteria that belong to the family *Enterobacteriaceae* as well as some of the more frequently isolated glucose fermenting and nonfermenting gram-negative bacilli.

SUMMARY AND EXPLANATION

Micromethods for the biochemical identification of microorganisms were reported as early as 1918.[5] Several publications reported on the use of the reagent-impregnated paper discs and micro-tube methods for differentiating enteric bacteria.[1,2,5,12,13,22,25,26,28] 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 linked substrates to detect enzymes that microbes use to metabolize various substrates.[7,14,15,18,21]

The **BBL CRYSTAL** E/NF ID kit is comprised of (i) **BBL CRYSTAL** E/NF panel lids, (ii) **BBL CRYSTAL** bases and (iii) **BBL CRYSTAL™** Enteric/Stool ID Inoculum Fluid (IF) tubes. The lid contains 30 dehydrated substrates 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. Color changes result from metabolic activities of the microorganisms. The resulting pattern of the 30 reactions is converted into a ten digit profile number that is used as the basis for identification.[24] Biochemical and enzymatic reaction patterns for the 30 **BBL CRYSTAL** E/NF ID substrates with a wide variety of microorganisms are stored in the **BBL CRYSTAL** E/NF ID data base. Identification is derived from a comparative analysis of the reaction pattern of the test isolate to those held in the data base. A complete list of taxa that comprises the current E/NF data base is provided in Table 1.[3,10,11,19,27]

PRINCIPLES OF THE PROCEDURE

The **BBL CRYSTAL** ID panels contain 30 dried biochemical and enzymatic substrates. A bacterial suspension in inoculum fluid is used for rehydration of the substrates. The tests used in the **BBL CRYSTAL** E/NF Identification System are based on microbial utilization and degradation of specific substrates detected by various indicator systems. Fermentation reactions detect the ability of an isolate to metabolize carbohydrates in the absence of atmospheric oxygen, and oxidation reactions are based on the ability of an organism to metabolize the substrate with oxygen as the final electron acceptor. Both reactions are usually detected by the use of a pH indicator in the test substrate. Chromogenic substrates upon hydrolysis produce color changes that can be detected visually. In addition, there are other tests that detect the ability of an organism to hydrolyze, degrade, reduce or otherwise utilize a substrate in the **BBL CRYSTAL** ID System.

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** E/NF ID panel contains 30 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 07485.

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

STORAGE AND HANDLING/SHELF LIFE

Lids: Lids are individually packaged and must be stored unopened at 2 – 25°C. 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 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 – 25°C, 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** Enteric/Stool ID Inoculum Fluid (IF) is packaged in two sets of ten tubes. Visually inspects 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. **BBL CRYSTAL** Enteric/Stool ID Inoculum Fluid may be used with either **BBL CRYSTAL** E/NF or RS/E panels.

On receipt, store the **BBL CRYSTAL** E/NF kit 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. 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 a blood agar plate such as **Trypticase**™ Soy Agar with 5% Sheep Blood. Use of a MacConkey Agar plate is also acceptable. The test isolate must be a pure culture no more than 24 h old. 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 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. [3]

TEST PROCEDURE

Materials Provided: BBL CRYSTAL Enteric/NF kit:

20 **BBL CRYSTAL** Enteric/NF Panel Lids,
20 **BBL CRYSTAL** Bases,
20 **BBLCRYSTAL** Enteric/Stool ID Inoculum Fluid Tubes. Each tube has approximately 2.2 ± 0.1 mL of Inoculum Fluid containing: NaCl 8.50 g, 3-Morpholinopropanesulfonic acid 0.8372 g, Purified water to 1000 mL.
2 incubation trays,
1 **BBL CRYSTAL** E/NF Report Pad.

Materials Required But Not Provided: Sterile cotton swabs (*do not use polyester swabs*), Incubator (35 – 37°C) non - CO_2 (40 – 60% humidity), **BBL CRYSTAL** Light Box/Panel Viewer (includes **BBL CRYSTAL** Color Reaction Charts), **BBL CRYSTAL** ID System Electronic Codebook or **BBL CRYSTAL** E/NF Manual Codebook (see "Availability"), nonselective culture plate (e.g., **Trypticase** Soy Agar with 5% Sheep Blood, **BBL™** DMACA Indole Reagent Droppers, **BBL™** Oxidase Reagent Droppers (see "Availability").

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

Test Procedure: BBL CRYSTAL E/NF ID System requires oxidase and indole test results. Prior to **BBL CRYSTAL** E/NF panel set-up, oxidase and indole tests should be performed from a nonselective isolation plate no more than 24 h old. Perform oxidase and indole tests per instructions provided in the package insert for the reagents.

1. 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.

2. Take an inoculum 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 one well isolated large (2 – 3 mm or larger in diameter) colony (or 4 – 5 smaller colonies of the same morphology) from a blood plate such as **Trypticase** Soy Agar with 5% Sheep Blood. Use of a MacConkey Agar plate is also acceptable.

3. Suspend colonies in a tube of **BBL CRYSTAL** Enteric/Stool Inoculum Fluid.

4. Recap tube and vortex for approximately 10 – 15 sec.

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 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.

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 fluid tube either before or after inoculating the base and inoculate an agar slant or plate (any appropriate media) for purity check. Discard inoculum fluid tube and cap in a biohazard disposal container. Incubate the slant or plate for 18 – 24 h at 35 – 37°C in a non-CO_2 incubator. 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 E/NF panels is **18 – 20 h** at 35 – 37°C.

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™** light box or Panel Viewer. Refer to the color reaction chart and/or Table 3 for an interpretation of the reactions. Use the **BBL CRYSTAL** E/NF Report Pad to record reactions.

Calculation of BBL CRYSTAL Profile Number: Each test result that is 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	5	4	6	0	2	4	4	3	7	1

The resulting profile number and off-line test results (indole and oxidase) 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.

QUALITY CONTROL

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

1. Set up a **BBL CRYSTAL** E/NF panel with *Klebsiella pneumoniae* ATCC® 33495 per recommended procedure (refer to "Test Procedure").

2. Prior to incubation, let panel remain at room temperature optimally for 3 min (but less than 5 min).

3. Read and record reactions with the aid of the light box or panel viewer and **BBL CRYSTAL** E/NF Color Reaction Chart.

4. If any of the wells are positive per color chart (after 3 – 5 min), DO NOT USE PANELS from this lot. Contact Becton Dickinson Microbiology Systems Technical Services.

5. If all wells are negative, then incubate panel for 18 – 20 h at 35 – 37°C.

6. Read panel with **BBL CRYSTAL** Light Box or Panel Viewer and **BBL CRYSTAL** E/NF Color Reaction Chart; record reactions using the **BBL CRYSTAL** 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.

Expected test results for additional quality control test strains are listed in Table 5.

LIMITATIONS OF THE PROCEDURE

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

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** E/NF Identification System is based on statistical use of specially designed test and an exclusive database.

When antisera are available, the biochemical identification of selected organisms, such as *Salmonella*, *Salmonella* subgroup 3, *Shigella*, enteropathogenic *Escherichia coli* A-D, and *Vibrio cholerae*, should be extended by antigenic analysis. [3,8]

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. 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 a blood agar plate such as **Trypticase** Soy Agar with 5% Sheep Blood. Use of a MacConkey Agar Plate is also acceptable.

BBL CRYSTAL Identification Systems are NOT for use directly with clinical specimens.

PERFORMANCE CHARACTERISTICS

Reproducibility: In an external study involving three (3) clinical laboratories, the reproducibility of E/NF substrates' (30) reactions was studied by replicate testing. The reproducibility of individual substrate reactions ranged from 96.3 –100%. The overall reproducibility of **BBL CRYSTAL** E/NF panel was determined to be 99.6%.

Accuracy of Identification: The performance of **BBL CRYSTAL** E/NF ID System was compared to currently available commercial systems using clinical isolates and stock cultures.

In an internal study, the performance of the **BBL CRYSTAL** E/NF was evaluated. Results from 169 enteric and nonenteric isolates (representing 45 species) tested were analyzed. Discrepant identifications were resolved by the use of other commercial systems. These results are shown below:

N = 169	ID Without Supplemental Testing	ID With Supplemental Testing	No ID or Misidentified
BBL CRYSTAL E/NF	163 (96.4%)	167 (98.8%)	2 (1.2%)

The performance of the **BBL CRYSTAL** Enteric/Nonfermenter ID test was evaluated in three independent clinical laboratories.[29] Both 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 the 299 fresh clinical isolates tested by the laboratories' current identification methods**,** the **BBL CRYSTAL** ID System correctly reported 96.7% (289) including 16 instances where two or three organisms were reported and required supplemental testing to resolve.

Out of the 291 previously identified challenge strains confirmed by the laboratories' current identification methods, the **BBL CRYSTAL** ID System correctly reported 96.9% (282) including 8 instances where two or three organisms were reported and required supplemental testing to resolve.[29]

AVAILABILITY

Cat. No.	Description
245000	**BBL CRYSTAL**™ Enteric/Nonfermenter ID Kit, containing 20 each: **BBL CRYSTAL**™ Enteric/NF Panel Lids, **BBL CRYSTAL** Bases, **BBL CRYSTAL**™ Enteric/Stool ID Inoculum Fluid Tubes.
	BBL CRYSTAL™ Light Box, Domestic model, 110V, 60 Hz.
	BBL CRYSTAL™ Light Box, European model, 20 V, 50 Hz.
	BBL CRYSTAL™ Light Bulb.
	BBL CRYSTAL™ Panel Viewer, Domestic model, 10 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 Enteric/Nonfermenter Manual Codebook.

Trypticase™ Soy Agar with 5% Sheep Blood, pkg of 20 plates.

Trypticase™ Soy Agar with 5% Sheep Blood, ctn of 100 plates.

BBL™ DMACA Indole Reagent Droppers, 50s.

BBL™ Oxidase Reagent Droppers, 50s.

BBL CRYSTAL™ Enteric/Stool ID Inoculum Fluid, ctn of 10.

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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29. Data on file at Bection Dickinson Microbiology Systems.

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

Rev. 12/02 (PI 07/01)

Approved by:

Date effective:

_____ _____

Supervisor Date

_____ _____

Director Date

Reviewed by:

Table 1
Taxa in BBL CRYSTAL E/NF System*

Acinetobacter baumanii	*Flavobacterium indologenes*	*Salmonella choleraesuis*
	Flavobacterium meningosepticum	*Salmonella paratyphi* A
Acinetobacter lwoffi	*Flavobacterium odoratum*	*Salmonella* species
		Salmonella typhi
Aeromonas caviae	*Hafnia alvei*	
Aeromonas hydrophila		*Serratia ficaria*
Aeromonas sobria	*Klebsiella ornithinolytica*	*Serratia fonticola*
Aeromonas veronii	*Klebsiella oxytoca*	*Serratia liquefaciens*
	Klebsiella ozaenae	*Serratia marcescens*
Agrobacterium tumefaciens	*Klebsiella pneumoniae*	*Serratia odorifera* 1
	Klebsiella rhinoscleromatis	*Serratia odorifera* 2
Burkholderia (Pseudomonas) cepacia		*Serratia plymuthica*
Burkholderia (Pseudomonas) pseudomallei	*Kluyvera ascorbata*	*Serratia rubidaea*
	Kluyvera cryocrescens	
Cedecea davisae		*Shewanella putrefaciens*
Cedecea lapagei	*Leclercia adecarboxylata*	
Cedecea neteri		*Shigella dysenteriae*
	Moellerella wisconsensis	*Shigella* species (includes *S. boydii* and
Chromobacterium violaceum		*S. flexneri*)
	Morganella morganii	*Shigella sonnei*
Chryseomonas luteola		
	Pantoea (Enterobacter) agglomerans	*Sphingobacterium multivorum*
Citrobacter amalonaticus		
Citrobacter freundii	*Pasteurella aerogenes*	*Stenotrophomonas maltophilia*
Citrobacter (diversus) koseri	*Pasteurella haemolytica*	
	Pasteurella multocida	*Tatumella ptyseos*
Edwardsiella hoshinae		
Edwardsiella tarda	*Plesiomonas shigelloides*	*Vibrio alginolyticus*
		Vibrio cholerae
Enterobacter aerogenes	*Proteus mirabilis*	*Vibrio damsela*
Enterobacter asburiae	*Proteus penneri*	*Vibrio fluvialis*
Enterobacter cloacae	*Proteus vulgaris*	*Vibrio hollisae*
Enterobacter gergoviae		*Vibrio metschnikovii*
Enterobacter sakazakii	*Providencia alcalifaciens*	*Vibrio mimicus*
Enterobacter taylorae	*Providencia rettgeri*	*Vibrio parahaemolyticus*
	Providencia rustigianii	*Vibrio vulnificus*
Escherichia coli	*Providencia stuartii*	
Escherichia coli serogroup 0111		*Weeksella virosa/zoohelcum*
Escherichia coli serogroup 0157	*Pseudomonas aeruginosa*	
Escherichia coli AD	*Pseudomonas diminuta*	*Yersinia enterocolitica* group (includes *Y.*
Escherichia fergusonii	*Pseudomonas fluorescens*	*enterocolitica. Y. frederiksenii, Y. intermedia*
Escherichia hermanii	*Pseudomonas gladioli*	and
Escherichis vulneris	*Pseudomonas paucimobilis*	*Y. kirstensenii)*
	Pseudomonas putida	*Yersinia pseudotuberculosis*
Ewingella americana	*Pseudomonas stutzeri*	
	Pseudomonas vesicularis	*Yokenella regensburgei (Koserella trabulsii)*
Flavimonas oryzihabitans		
	Rahnella aquatilis	Miscellaneous Gram-Negative Bacilli
Flavobacterium breve		(delineated below)[1]
Flavobacterium gleum	*Salmonella arizonae*	

*Includes recent name changes: older names are in parentheses. (Holt, J.G., N.R. Kreig, P.H.A. Sneath, J.T. Staley, and S.T. Williams [ed.]. 1994. Bergey's manual of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.)

[1] "Miscellaneous Gram-Negative Bacilli" refers to a group of oxidase positive species that are relatively inactive and indistinguishable from each other in **BBL CRYSTAL** Enteric/Nonfermenter ID System. Refer to tables 1, 2 and 3 provided in the package insert for further identification when the first choice identification is "Miscellaneous Gram-Negative Bacilli".

"Miscellaneous Gram-Negative Bacilli" include:

Alcaligenes faecalis	*Methylobacterium species*
Alcaligenes piechaudii	*Moraxella lacunata*
Alcaligenes xylosoxidans subsp. *denitrificans*	*Moraxella osloensis*
Alcaligenes xylosoxidans subsp. *xylosoxidans*	*Ochrobactrum anthropi*
Bordetella bronichiseptica	*Oligella urethralis*
Burkholderia (Pseudomonas) pickettii	*Pseudomonas alcaligenes*
CDC Group IV C-2	*Pseudomonas fluorescens*[2]
Comamonas acidovorans	*Pseudomonas mendocina*
Comamonas testosteroni	*Pseudomonas pseudoalcaligenes*
Eikenella corrodens	*Pseudomonas putida*[2]

[2] May also be identified separately in database.

TABLE 2

Principles of Tests Employed In The BBL CRYSTAL E/NF System

Panel Location	Test Feature	Code	Principle (Reference)
4A	Arabinose	ARA	Utilization of carbohydrate results in lower pH and change in indicator (Phenol Red). [4, 6, 8, 16]
4B	Mannose	MNS	
4C	Sucrose	SUC	
4D	Melibiose	MEL	
4E	Rhamnose	RHA	
4F	Sorbitol	SOR	
4G	Mannitol	MNT	
4H	Adonitol	ADO	
4I	Galactose	GAL	
4J	Inositol	INO	
2A	p-nitrophenyl phosphate	PHO	Enzymatic hydrolysis of the colorless aryl substituted glycoside or phosphate ester releases yellow p-nitrophenol. [7, 14, 15, 18, 21]
2B	p-nitrophenyl α-β-glucoside	BGL	
2C	p-nitrophenyl β-galactoside	NPG	
2D	Proline nitroanilide	PRO	Enzymatic hydrolysis of colorless amide substrate releases yellow p-nitroaniline. [7, 14, 15, 18, 21]
2E	p-nitrophenyl bis-phosphate	BPH	Enzymatic hydrolysis of the colorless aryl substituted glycoside or phosphate ester releases yellow p-nitrophenol. [7, 14, 15, 18, 21]
2F	p-nitrophenyl xyloside	BXY	
2G	p-nitrophenyl α-arabinoside	AAR	
2H	p-nitrophenyl phosphorylcholine	PHC	
2I	p-nitrophenly β-glucuronide	GLR	
2J	p-nitrophenyl-N-acetyl glucosaminide	NAG	
1A	γ-L-glutamyl p-nitroanilide	GGL	Enzymatic hydrolysis of the colorless amide substrate releases yellow p-nitroaniline. [7, 14, 15, 18, 21]
1B	Esculin	ESC	Hydrolysis of esculin results in a black precipitate in the presence of ferric ion. [17]
1C	p-nitro-DL-phenylalanine	PHE	Oxidative deamination of phenylalanine results in a brown color in the presence of ferric ion. [4,17]
1D	Urea	URE	Hydrolysis of urea and the resulting ammonia changes the pH indicator color (Bromthymol blue). [4, 9, 17]
1E	Glycine	GLY	Degration of glycine results in alkaline metabolites that change color of the pH incidator (Bromthymol blue). [29]
1F	Citrate	CIT	Utilization of citrate results in alkaline metabolites that change color of the pH indicator (Bromthymol blue). [4, 23]
1G	Malonate	MLO	Utilization of malonate results in alkaline metabolites that change the color of the pH indicator (Bromthymol blue). [17]
1H	Tetrazolium	TTC	Reduction of the tetrazolium compound results in formation of a red formazan. [29]
1I	Arginine	ARG	Anaerobic catabolism results in pH rise and change in the color of the indicator (Bromcresol purple). [4, 20]
1J	Lysine	LYS	

TABLE 3

Reagents used in the BBL CRYSTAL E/NF ID System

LOCATION	SUBSTRATE	CODE	POS	NEG	ACTIVE INGREDIENTS	Approx. AMT (g/10 mL)
4A	Arabinose	ARA	Gold/Yellow	Orange/Red	Arabinose	3.5
4B	Mannose	MNS	Gold/Yellow	Orange/Red	Mannose	3.0
4C	Sucrose	SUC	Gold/Yellow	Orange/Red	Sucrose	2.8
4D	Melibiose	MEL	Gold/Yellow	Orange/Red	Melibiose	1.0
4E	Rhamnose	RHA	Gold/Yellow	Orange/Red	Rhamnose	3.0
4F	Sorbitol	SOR	Gold/Yellow	Orange/Red	Sorbitol	3.5
4G	Mannitol	MNT	Gold/Yellow	Orange/Red	Mannitol	1.8
4H	Adonitol	ADO	Gold/Yellow	Orange/Red	Adonitol	2.5
4I	Galactose	GAL	Gold/Yellow	Orange/Red	Galactose	1.5
4J	Inositol	INO	Gold/Yellow	Orange/Red	Inositol	1.3
2A	p-n-p-phosphate	PHO	Yellow	Colorless	p-n-p-phosphate	0.025
2B	p-n-p-α-β-glucoside	BGL	Yellow	Colorless	p-n-p-α-β-glucoside	0.025
2C	p-n-p-β-galactoside	NPG	Yellow	Colorless	p-n-p-β-galactoside	0.06
2D	Proline nitroanilide	PRO	Yellow	Colorless	Proline nitroanilide	0.07
2E	p-n-p bis phosphate	BPH	Yellow	Colorless	p-n-p bis phosphate	0.02
2F	p-n-p-xyloside	BXY	Yellow	Colorless	p-n-p-xyloside	0.03
2G	p-n-p-α-arabinoside	AAR	Yellow	Colorless	p-n-p-α-arabinoside	0.03
2H	p-n-p-phosphorycholine	PHC	Yellow	Colorless	p-n-p-phosphorycholine	0.03
2I	p-n-p-β-glucuronide	GLR	Yellow	Colorless	p-n-p-β-glucuronide	0.02
2J	p-n-p-N-acetyl glucosaminide	NAG	Yellow	Colorless	p-n-p-N-acetyl glucosaminide	0.04
1A	γ -L-glutamyl p-nitroanilide	GGL	Yellow	Colorless	γ -L-glutamyl p-nitroanilide	0.03
1B	Esculin	ESC	Brown/Maroon	Clear/Straw	Esculin	0.14
1C	Phenylalanine	PHE	Gold/Dk. Orange	Yellow	p-nitro-DL-phenylalanine	0.1
1D	Urea	URE	Aqua/Blue	Yellow/Green	Urea	0.2
1E	Glycine	GLY	Aqua/Blue	Yellow/Green	Glycine	0.7
1F	Citrate	CIT	Aqua/Blue	Yellow/Green	Citrate	0.8
1G	Malonate	MLO	Aqua/Blue	Yellow/Green	Malonic acid	1.5
1H	Tetrazolium	TTC	Pink/red*	Clear	Triphenyl Tetrazolium chloride	0.15
1I	Arginine	ARG	Red/Purple	Yellow/Brown	Arginine	1.5
1J	Lysine	LYS	Red/Purple	Yellow/Brown	Lysine	0.5

*Precipitate may of may not be visible.

TABLE 4

Quality Control Chart for BBL CRYSTAL E/NF ID System

Panel Location	Substrate	Code	Klebsiella pneumoniae **ATCC® 33495**
4A	Arabinose	ARA	+
4B	Mannose	MNS	+
4C	Sucrose	SUC	+
4D	Melibiose	MEL	V
4E	Rhamnose	RHA	+
4F	Sorbitol	SOR	+
4G	Mannitol	MNT	V
4H	Adonitol	ADO	+
4I	Galactose	GAL	+
4J	Inositol	INO	+
2A	p-n-p-phosphate	PHO	V
2B	p-n-p-α-β -glucoside	BGL	+
2C	p-n-p-β -galactoside	NPG	+
2D	Proline p-nitroanilide	PRO	V
2E	p-n-p bis phosphate	BPH	V
2F	p-n-p-xyloside	BXY	+
2G	p-n-p-α-arabinoside	AAR	(+)
2H	p-n-p-phosphorylcholine	PHC	-
2I	p-n-p-β-glucuronide	GLR	-
2J	p-n-p-N-acetyl glucosaminide	NAG	-
1A	γ -L-glutamyl p-nitroanilide	GGL	+
1B	Esculin	ESC	+
1C	p-nitro-DL-phenylalanine	PHE	-
1D	Urea	URE	V
1E	Glycine	GLY	-
1F	Citrate	CIT	+
1G	Malonate	MLO	+
1H	Tetrazolium	TTC	+
1I	Arginine	ARG	V
1J	Lysine	LYS	+

+ = positive reaction - = negative reaction V = variable reaction (+) = Usually positive, but occasionally negative.

TABLE 5

Additional Quality Control Strains for CRYSTAL E/NF System

LOCATION	**CODE**	Escherichia coli **ATCC 25922**	Acinetobacter lwoffi **ATCC 17925**	Proteus vulgaris **ATCC 8427**	Enterobacter cloacae **ATCC 35030**	Pseudomonas aeru*ginosa* **ATCC 35032**
4A	ARA	V	-	-	+	-
4B	MNS	+	-	-	+	V
4C	SUC	-	-	+	+	-
4D	MEL	+	-	-	V	-
4E	RHA	+	-	-	+	-
4F	SOR	+	-	-	+	-
4G	MNT	+	-	-	+	-
4H	ADO	-	-	-	+	-
4I	GAL	+	-	+	+	+
4J	INO	-	-	-	-	-
2A	PHO	V	-	+	V	V
2B	BGL	-	-	+	V	-
2C	NPG	+	-	-	+	-
2D	PRO	-	-	-	-	+
2E	BPH	V	-	+	V	-
2F	BXY	-	-	-	+	-
2G	AAR	(-)	-	-	(+)	-
2H	PHC	-	-	+	-	V
2I	GLR	+	-	-	-	-
2J	NAG	-	-	-	+	-
1A	GGL	-	-	V	+	+
1B	ESC	-	-	+	V	-
1C	PHE	-	-	+	-	-
1D	URE	-	V	+	V	+
1E	GLY	-	V	V	-	+
1F	CIT	-	-	(+)	+	+
1G	MLO	-	-	-	+	+
1H	TTC	(+)	-	V	+	+
1I	ARG	V	-	V	(+)	+
1J	LYS	+	-	-	V	V

+ = positive reaction – = negative reaction V = variable reaction (+) = Usually positive, but occasionally negative