

BBL CRYSTAL™ Identification Systems

Neisseria/Haemophilus ID Kit

CLIA COMPLEXITY: HIGH
CDC IDENTIFIER CODES
ANALYTE: 0412
TEST SYSTEM: 07807

INTENDED USE

The **BBL CRYSTAL™** *Neisseria/Haemophilus* (N/H) Identification (ID) system is a miniaturized identification method employing modified conventional, fluorogenic and chromogenic substrates. It is intended for the identification of frequently isolated *Neisseria* and *Haemophilus* as well as several other fastidious bacteria.^{1,2,6,15,18}

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.^{3,4,8,19,21} 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 N/H ID panel**, to detect enzymes that microbes use to metabolize various substrates.^{5,6,8,9,10,13,14,15,16,17}

The **BBL CRYSTAL™** N/H ID kit is comprised of (i) **BBL CRYSTAL N/H 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 fluorescent 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.²⁰ Biochemical and enzymatic reaction patterns for the 29 **BBL**

CRYSTAL N/H ID substrates for a wide variety of microorganisms are stored in the **BBL CRYSTAL N/H 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 N/H 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.^{13,14,16,17} 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 N/H 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 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 07807.

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: 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 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 **BBL CRYSTAL** ANR, GP, RGP, N/H Inoculum Fluid should be used with **BBL CRYSTAL** N/H ID panels.

On receipt, store the **BBL CRYSTAL** N/H 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 Chocolate Agar, **Trypticase**[™] Soy Agar with 5% Sheep Blood (TSA), Columbia Agar with 5% Sheep Blood (Columbia) and Nutrient Agar. Use of selective media such as Martin-Lewis Agar, Thayer- Martin Modified (MTM) Agar, New York City (NYC) Medium Modified, V Agar (for *G. vaginalis*), and **GC-Lect**[™] Agar 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. Only cotton-tipped applicator swabs should be used to prepare the inoculum suspension 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 inoculation onto primary isolation media.^{1,17}

TEST PROCEDURE

Materials Provided: **BBL CRYSTAL** N/H ID Kit –

- 20 **BBL CRYSTAL** N/H 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, CaCl₂ 0.5 g, Tricine N-[2-Hydroxy-1, 1-bis (hydroxymethyl)methyl] glycine 0.895 g, purified water to 1000ml.
- 2 incubation trays
- 1 **BBL CRYSTAL** N/H ID Report Pad.

Materials Not Provided: Sterile cotton swabs (*do not use polyester swabs*), incubator (35 – 37°C) non-CO₂ (40 – 60% humidity), McFarland No. 3 standard, **BBL CRYSTAL** Panel Viewer (includes **BBL CRYSTAL** Color Reaction Charts), **BBL CRYSTAL** ID System Electronic Codebook or **BBL CRYSTAL** N/H Manual Codebook (see “Availability”), 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** N/H ID System requires a Gram stain.

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 fluid tube and label with patient’s specimen number. Using aseptic technique, with the tip of a sterile cotton swab (*do not use 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. 3 standard. If the inoculum suspension concentration is in excess of the recommended McFarland standard, one of the following steps is recommended:
 - a. Use a fresh tube of inoculum fluid to prepare a new inoculum suspension equivalent to a McFarland No. 3 standard.
 - b. 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. 3 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.

5. 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** N/H 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, or coming out of the target area towards 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 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 – 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-CO₂ 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. 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 the 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 for an interpretation of the reactions. Use the results pad to record reactions.

- a. Read columns F thru J first, using the regular (white) light source.
- b. Read columns A thru E (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, 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.

QUALITY CONTROL

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

1. Inoculate a panel with *Moraxella (Branhamella) catarrhalis* ATCC® 25240 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 1J) are positive per color reaction chart (after 1 – 2 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 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** N/H ID System is designed for the taxa provided. Taxa other than those listed in Table 1 are not intended for use in this system.

An additional confirmatory test is required for reporting an isolate identified in the system as *Neisseria gonorrhoeae* as follows: (1) when positive results are obtained from persons at low risk, (2) when positive results are obtained from patients with sociologic or medicolegal implications.¹¹

The **BBL CRYSTAL** N/H ID database was developed with **BBL**TM 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** N/H ID System: Chocolate, **TSA II**TM, Columbia and Nutrient. Use of selective media, such as Martin-Lewis, MTM, NYC Medium, V and **GC-Lect** 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** N/H ID System is based on statistical use of specially designed tests and an exclusive database.

While **BBL CRYSTAL** N/H 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. 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 Chocolate, TSA, Columbia or Nutrient plates. Use of selective media such as Martin-Lewis, MTM, NYC Medium, V and **GC-Lect** is also acceptable.

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 three clinical laboratories, (total of three evaluations), the reproducibility of **BBL CRYSTAL** N/H ID substrates’ (29) reactions was studied by replicate testing. The reproducibility of the individual substrate reactions ranged from 85.7% to 100%. The overall reproducibility of **BBL CRYSTAL** N/H ID panel was determined to be 95.9%.²²

Accuracy of Identification: The performance of **BBL CRYSTAL** N/H ID System was compared to a currently available commercial system 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 513 total isolates tested from the three studies using the **BBL CRYSTAL** N/H Identification System 459 (89.5%) were correctly identified without the use of supplemental tests, and 480 (93.6%) were correctly identified when supplemental tests were included. A total of 26 (5.1%) isolates were incorrectly identified, and a message of “No Identification” was obtained for 7 (1.4%) isolates.²²

AVAILABILITY

Cat. No.	Description
245130	BBL CRYSTAL™ <i>Neisseria/Haemophilus</i> ID Kit, containing 20 each: BBL CRYSTAL N/H ID Panel Lids, BBL CRYSTAL

Bases and **BBL CRYSTAL** ANR, GP, RGP, N/H 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, 50/60Hz.

BBL CRYSTAL[™] Panel Viewer, Longwave UV Tube.

BBL CRYSTAL[™] Panel Viewer, White Light Tube.

BBL CRYSTAL[™] ID System Electronic Codebook.

BBL CRYSTAL[™] Identification Systems *Neisseria/Haemophilus* Manual Codebook.

BBL[™] Chocolate II Agar (GC II Agar with Hemoglobin and **IsoVitaleX**[™]), pkg. of 20.

BBL[™] Chocolate II Agar (GC II Agar with Hemoglobin and **IsoVitaleX**[™]), ctn. of 100.

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

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

BBL[™] Martin-Lewis Agar, pkg. of 20.

BBL[™] Martin-Lewis Agar, ctn. of 100.

BBL[™] New York City (NYC) Medium Modified, pkg. of 20.

BBL[™] New York City (NYC) Medium Modified, ctn. of 100.

BBL[™] Nutrient Agar, pkg. of 10.

BBL[™] Thayer-Martin, Modified (MTM II) Agar, pkg. of 20.

BBL[™] Thayer-Martin, Modified (MTM II) Agar, ctn. of 100.

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II™), pkg. of 20.

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II™), ctn. of 100.

BBL™ V Agar (for *G. vaginalis*), pkg. of 10.

BBL™ V Agar (for *G. vaginalis*), pkg. of 100.

GC-Lect™ Agar, pkg. of 20.

GC-Lect™ Agar, ctn. of 100.

BBL™ 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.

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

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ATCC is a trademark of the American Type Culture Collection.

Approved by:

Date effective:

Supervisor

Date

Director

Date

Reviewed by:

Table 1

Taxa in BBL CRYSTAL™ N/H ID System		
<i>Actinobacillus actinomycetemcomitans</i>	<i>Moraxella atlantae</i>	<i>Neisseria mucosa</i>
<i>Cardiobacterium hominus</i> ¹	<i>Moraxella (Branhamella) catarrhalis</i>	<i>Neisseria sicca</i>
<i>Eikenella corrodens</i>	<i>Moraxella lacunata</i> ¹	<i>Neisseria subflava</i> (includes <i>N. subflava</i> biovar <i>flava</i> ,
<i>Gardnerella vaginalis</i>	<i>Moraxella nonliquefaciens</i>	<i>N. subflava</i> biovar <i>perflava</i> and <i>N. subflava</i> biovar <i>subflava</i>)
<i>Haemophilus aphrophilus/paraphrophilus</i>	<i>Moraxella osloensis</i>	
<i>Haemophilus ducreyi</i>	<i>Moraxella phenylpyruvica</i> ¹	<i>Neisseria weaverii</i> ¹
<i>Haemophilus haemoglobinophilus</i> ¹	<i>Moraxella</i> species (includes <i>M. atlantae</i> , <i>M. lacunata</i> , <i>M. nonliquefaciens</i> ,	<i>Oligella</i> species (includes <i>O. urethralis</i> and <i>O. ureolytica</i>)
<i>Haemophilus haemolyticus</i>	<i>M. osloensis</i> and <i>M. phenylpyruvica</i>)	<i>Oligella ureolytica</i> ¹
<i>Haemophilus influenzae</i>	<i>Neisseria cinerea</i> ¹	<i>Oligella urethralis</i>
<i>Haemophilus parahaemolyticus</i> ¹	<i>Neisseria elongata</i> (includes <i>N. elongata</i> ssp <i>elongata</i> , <i>N. elongata</i> ssp <i>glycolytica</i> and <i>N. elongata</i> ssp <i>nitroreducens</i>)	<i>Pasteurella multocida</i>
<i>Haemophilus parainfluenzae</i>		<i>Suttonella indologenes</i>
<i>Haemophilus segnis</i> ¹	<i>Neisseria flavescens</i> ¹	
<i>Kingella denitrificans</i>	<i>Neisseria gonorrhoeae</i>	
<i>Kingella kingae</i>	<i>Neisseria lactamica</i>	
<i>Kingella</i> species (includes <i>K. denitrificans</i> and <i>K. kingae</i>)	<i>Neisseria meningitidis</i>	

Key: 1 = These taxa have <10 unique **BBL CRYSTAL** profiles in the current database.

Table 2
Principles of Tests Employed in the BBL CRYSTAL™ N/H ID System

Panel Location	Test Feature	Code	Principle (Reference)
4A	Fluorescent negative control	FCT	Control to standardize fluorescent substrate results
2A	4MU-phosphate	FHO	Enzymatic hydrolysis of the amide or glycosidic bond results in the release of a fluorescent coumarin derivative. ^{5,9,13,14,16,17}
1A	L-proline-AMC	FPR	
4B	L-serine-AMC	FSE	
2B	LYS-ALA-AMC	FLA	
1B	L-tryptophan-AMC	FTR	
4C	L-phenylalanine-AMC	FPH	
2C	N-succinyl-ALA-PRO-ALA-AMC	FNS	
1C	ALA-ALA-PHE-AMC	FAA	
4D	L-glutamic acid-AMC	FTA	
2D	L-arginine-AMC	FAR	
1D	Ornithine-AMC	FOR	
4E	Glycine-AMC	FGL	
2E	GLY-PRO-AMC	FGP	
1E	4MU-β-D-galactoside	FBG	
4F	Saccharose	SAC	Utilization of carbohydrate results in lower pH and change in indicator (Phenol red). ^{1,2,3,4,8,18}
2F	Maltotriose	MTT	
1F	Carubinose	CAR	
4G	Pyranose	PYO	
2G	Maltobiose	MTB	
1G	Dissacharide	DIS	
4H	Riberol	RBL	
2H	Levulose	LEV	
1H	p-nitrophenyl-phosphorylcholine	PHC	Enzymatic hydrolysis of the colorless aryl substituted glycoside releases yellow p-nitrophenol. ^{5,10,14}
4I	γ-L-glutamyl-p-nitroanilide	GGL	Enzymatic hydrolysis of the colorless amide substrate releases yellow p-nitroaniline. ^{5,10,14}
2I	p-nitrophenyl-phosphate	PHO	Enzymatic hydrolysis of the colorless aryl substituted glycoside releases yellow p-nitrophenol. ^{5,10,14}
1I	o-nitrophenyl-β-D-galactoside (ONPG)	OPG	
4J	Urea	URE	Hydrolysis of urea and the resulting ammonia change the pH indicator color (Bromothymol blue). ^{2,7,12}
2J	Resazurin	REZ	Reduction of resazurin to resorufin results in a color change. ⁶
1J	Ornithine	ORN	Utilization of ornithine results in pH rise and change in the color of the indicator (Bromocresol purple). ²

Table 3
Reagents used in the **BBL CRYSTAL N/H 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-phosphate	FHO	blue fluorescence >FCT well	blue fluorescence ≤FCT well	4MU-phosphate	≤1
1A	L-proline-AMC	FPR	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-proline-AMC	≤1
4B	L-serine-AMC	FSE	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-serine-AMC	≤1
2B	LYS-ALA-AMC	FLA	blue fluorescence >FCT well	blue fluorescence ≤FCT well	LYS-ALA-AMC	≤1
1B	L-tryptophan-AMC	FTR	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-tryptophan-AMC	≤1
4C	L-phenylalanine-AMC	FPH	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-phenylalanine-AMC	≤1
2C	N-succinyl-ALA-PRO-ALA-AMC	FNS	blue fluorescence >FCT well	blue fluorescence ≤FCT well	N-succinyl-ALA-PRO-ALA-AMC	≤1
1C	ALA-ALA-PHE-AMC	FAA	blue fluorescence >FCT well	blue fluorescence ≤FCT well	ALA-ALA-PHE-AMC	≤1
4D	L-glutamic acid-AMC	FTA	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-glutamic acid-AMC	≤1
2D	L-arginine-AMC	FAR	blue fluorescence >FCT well	blue fluorescence ≤FCT well	L-arginine-AMC	≤1
1D	Ornithine-AMC	FOR	blue fluorescence >FCT well	blue fluorescence ≤FCT well	Ornithine-AMC	≤1
4E	Glycine-AMC	FGL	blue fluorescence >FCT well	blue fluorescence ≤FCT well	Glycine-AMC	≤1
2E	GLY-PRO-AMC	FGP	blue fluorescence >FCT well	blue fluorescence ≤FCT well	GLY-PRO-AMC	≤1
1E	4MU-β-D-galactoside	FBG	blue fluorescence >FCT well	blue fluorescence ≤FCT well	4MU-β-D-galactoside	≤1
4F	Saccharose	SAC	Gold/Yellow	Orange/Red	Saccharose	≤300
2F	Maltotriose	MTT	Gold/Yellow	Orange/Red	Maltotriose	≤300
1F	Carubinose	CAR	Gold/Yellow	Orange/Red	Carubinose	≤300
4G	Pyranose	PYO	Gold/Yellow	Orange/Red	Pyranose	≤300
2G	Maltobiose	MTB	Gold/Yellow	Orange/Red	Maltobiose	≤300
1G	Disaccharide	DIS	Gold/Yellow	Orange/Red	Disaccharide	≤300
4H	Riberol	RBL	Gold/Yellow	Orange/Red	Riberol	≤300
2H	Levulose	LEV	Gold/Yellow	Orange/Red	Levulose	≤300
1H	p-n-p-phosphorylcholine	PHC	Yellow	Colorless	p-n-p-phosphorylcholine	≤10
4I	γ-L-glutamyl-p-nitroanilide	GGL	Yellow	Colorless	γ-L-glutamyl-p-nitroanilide	≤10
2I	p-n-p-phosphate	PHO	Yellow	Colorless	p-n-p-phosphate	≤10
1I	ONPG	OPG	Yellow	Colorless	ONPG	≤10
4J	Urea	URE	Aqua/Blue	Yellow/Green	Urea	≤50
2J	Resazurin	REZ	Pink	Blue/Purple	Resazurin	≤1
1J	Ornithine	ORN	Purple	Yellow/Gray	Ornithine	≤200

Table 4
Quality Control Chart for BBL CRYSTAL™ N/H ID System
After 4 Hours Incubation from Chocolate Agar

Panel Location	Substrate	Code	Moraxella (Branhamella) catarrhalis ATCC 25240
4A	Fluorescent negative control	FCT	–
2A	4MU-phosphate	FHO	–
1A	L-proline-AMC	FPR	–
4B	L-serine-AMC	FSE	+
2B	LYS-ALA-AMC	FLA	V
1B	L-tryptophan-AMC	FTR	V
4C	L-phenylalanine-AMC	FPH	+
2C	N-succinyl-ALA-PRO-ALA-AMC	FNS	+
1C	ALA-ALA-PHE-AMC	FAA	+
4D	L-glutamic acid-AMC	FTA	–
2D	L-arginine-AMC	FAR	V
1D	Ornithine-AMC	FOR	V
4E	Glycine-AMC	FGL	+
2E	GLY-PRO-AMC	FGP	–
1E	4MU-β-D-galactoside	FBG	–
4F	Saccharose	SAC	–
2F	Maltotriose	MTT	–
1F	Carubinose	CAR	–
4G	Pyranose	PYO	–
2G	Maltobiose	MTB	–
1G	Disaccharide	DIS	–
4H	Riberol	RBL	–
2H	Levulose	LEV	–
1H	p-n-p-phosphorylcholine	PHC	–
4I	γ-L-glutamyl-p-nitroanilide	GGL	–
2I	p-n-p-phosphate	PHO	–
1I	ONPG	OPG	–
4J	Urea	URE	–
2J	Resazurin	REZ	+
1J	Ornithine	ORN	V

Table 5
Additional Quality Control Strains for BBL CRYSTAL™ N/H ID System
After 4 Hours Incubation from Chocolate Agar

Panel Location	Substrate	Code	Haemophilus aphrophilus ATCC 19415	Neisseria lactamica ATCC 49142	Kingella denitrificans ATCC 33394	Haemophilus influenzae ATCC 35056
4A	Fluorescent negative control	FCT	–	–	–	–
2A	4MU-phosphate	FHO	+	–	–	+
1A	L-proline-AMC	FPR	–	+	+	–
4B	L-serine-AMC	FSE	V	+	+	V
2B	LYS-ALA-AMC	FLA	–	V	V	V
1B	L-tryptophan-AMC	FTR	V	+	+	V
4C	L-phenylalanine-AMC	FPH	+	+	+	V
2C	N-succinyl-ALA-PRO-ALA-AMC	FNS	–	–	–	–
1C	ALA-ALA-PHE-AMC	FAA	V	+	V	V
4D	L-glutamic acid-AMC	FTA	+	–	–	–
2D	L-arginine-AMC	FAR	V	+	V	+
1D	Ornithine-AMC	FOR	–	+	V	–
4E	Glycine-AMC	FGL	+	+	+	+
2E	GLY-PRO-AMC	FGP	–	V	+	–
1E	4MU-β-D-galactoside	FBG	+	+	–	–
4F	Saccharose	SAC	+	–	–	–
2F	Maltotriose	MTT	+	–	–	–
1F	Carubiose	CAR	V	–	–	–
4G	Pyranose	PYO	+	V	–	V
2G	Maltobiose	MTB	+	V	–	–
1G	Disaccharide	DIS	+	–	–	–
4H	Riberol	RBL	V	–	–	–
2H	Levulose	LEV	+	–	–	–
1H	p-n-p-phosphorylcholine	PHC	V	–	–	+
4I	γ-L-glutamyl-p-nitroanilide	GGL	+	–	–	–
2I	p-n-p-phosphate	PHO	+	–	–	+
1I	ONPG	OPG	+	+	–	–
4J	Urea	URE	–	–	–	+
2J	Resazurin	REZ	V	–	V	–
1J	Ornithine	ORN	V	V	V	+

