1

MICROBIAL IDENTIFICATION USING THE BIOMÉRIEUX VITEK® 2 SYSTEM

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OBJECTIVE

This chapter describes the VITEK 2 automated microbiology system and its application in the identification of microorganisms.

PRINCIPLES

The VITEK 2 is an automated microbiology system utilizing growth-based technology. The system is available in three formats (VITEK 2 compact, VITEK 2, and VITEK 2 XL) that differ in increasing levels of capacity and automation. Figure 1 shows the VITEK 2 compact system. All three systems accommodate the same colorimetric reagent cards that are incubated and interpreted automatically.

Figure 1. VITEK 2 Compact Instrument and Workstation.

VITEK 2 Compact

This format focuses on the industrial microbiology-testing environment while also having application for low to middle volume clinical laboratories. Features specifically developed for industrial microbiology include 21 CFR Part 11 compliance (for electronic records and signatures) and a colorimetric reagent card (BCL) used to identify the spore-forming Gram-positive bacilli (i.e., *Bacillus* and related genera). The other colorimetric reagent cards (GN, GP, YST) apply to all system formats for both industrial and clinical laboratories.

VITEK 2 and VITEK 2 XL

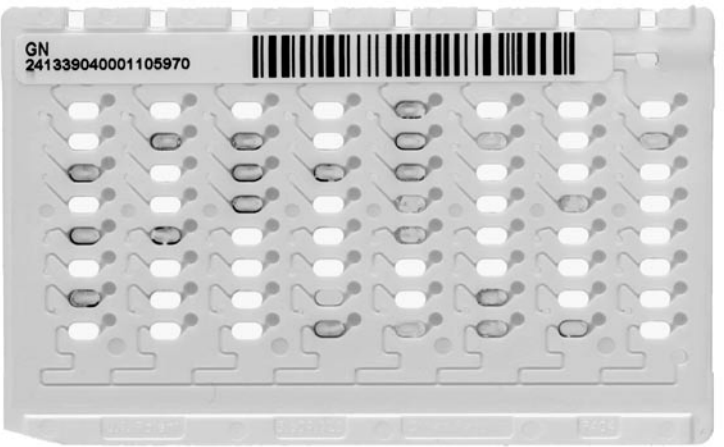
These formats are more focused on the clinical microbiology laboratory and provide increased levels of automation and capacity for higher volume laboratories. They also provide an option of automatic pipetting and dilution for antimicrobial susceptibility testing.

Reagent Cards

The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel

that prevents contact with the organism-substrate admixtures. Each card has a pre-inserted transfer tube used for inoculation (described below). Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system. Figure 2 shows the GN card.

Figure 2. VITEK 2 GN Colorimetric Identification Card.



There are currently four reagent cards available for the identification of different organism classes as follows:

1. GN - Gram-negative fermenting and non-fermenting bacilli
2. GP - Gram-positive cocci and non-spore-forming bacilli
3. YST - yeasts and yeast-like organisms
4. BCL - Gram-positive spore-forming bacilli

Product-specific details for each of the identification cards are shown below.

Culture Requirements

The on-line product information contains a culture requirements table that lists parameters for appropriate culture and inoculum preparation. These parameters include acceptable culture media, culture age, incubation conditions, and inoculum turbidity.

Suspension Preparation

A sterile swab or applicator stick is used to transfer a sufficient number of colonies of a pure culture and to suspend the microorganism in 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube. The turbidity is adjusted accordingly (see Table 1) and measured using a turbidity meter called the DensiChek™.

Table 1: Suspension Turbidities Used for Card Inoculation.

Product	McFarland Turbidity Range
GN	0.50 – 0.63
GP	0.50 – 0.63
YST	1.80 – 2.20
BCL	1.80 – 2.20

Inoculation

Identification cards are inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension is placed into a special rack (cassette) and the identification card is placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The cassette can accommodate up to 10 tests (VITEK 2 Compact; see Figure 3) or up to 15 tests (VITEK 2 and VITEK 2 XL; see Figure 4). The filled cassette is placed either manually (VITEK 2 compact) or transported automatically (VITEK 2 and VITEK 2 XL) into a vacuum chamber station. After the vacuum is applied and air is re-introduced into the station, the organism suspension is forced through the transfer tube into micro-channels that fill all the test wells.

Card Sealing and Incubation

Inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30 or up to 60 cards. All card types are

incubated on-line at 35.5 ± 1.0ºC. Each card is removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data are collected at 15-minute intervals during the entire incubation period.

Figure 3. VITEK 2 Compact Cassette Loaded with 10 Cards and Suspension Tubes and Bar Code Scanner for Data Entry.

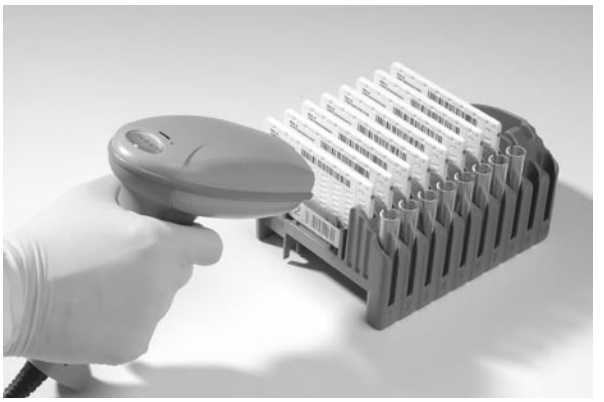


Figure 4. VITEK 2 Cassette Loaded with Cards and Suspension Tubes Being Loaded Into the Automatic Transport System.

Optical System

A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction is read every 15 minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm is used to eliminate false readings due to small bubbles that may be present.

Test Reactions

Calculations are performed on raw data and compared to thresholds to determine reactions for each test. On the VITEK 2 Compact, test reaction results appear as "+"," –", "(–)" or "(+)". Reactions that appear in parentheses are indicative of weak reactions that are too close to the test threshold. With the VITEK 2 or VITEK 2 XL, these weak reactions appear as "?".

Database Development

The databases of the VITEK 2 identification products are constructed with large strain sets of well-characterized microorganisms tested under various culture conditions. These strains are derived from a variety of clinical and industrial sources as well as from public (e.g., ATCC) and university culture collections.

Analytical Techniques

Test data from an unknown organism are compared to the respective database to determine a quantitative value for proximity to each of the database taxa. Each of the composite values is compared to the others to determine if the data are sufficiently unique or close to one or more of the other database taxa. If a unique identification pattern is not recognized, a list of possible organisms is given, or the strain is determined to be outside the scope of the database.

Identification Levels

An unknown biopattern is compared to the database of reactions for each taxon, and a numerical probability calculation is performed. Various qualitative levels of identification are assigned based on the numerical probability calculation. The different levels and associated information are shown in Table 2.

Mixed Taxa Identifications

This occurs when the biopattern is representative of a collective taxon and generates a genus-level, group-level, or slashline identification. In rare cases, species-level identification can be a mixed taxon comprised of two subspecies. Supplemental tests may be used to delineate representative species or subspecies of these collective taxa.

Supplemental Testing

In the case of low discrimination identifications, two or three choices are listed in the order of their probability calculations. However, all taxa appearing in a low discrimination identification are viable choices and should only be ruled out after additional testing and/or observation. The lab report contains recommended supplemental tests that allow for differentiation of choices in low discrimination identifications. If the supplemental tests listed are insufficient to complete the identification, then standard microbiology references should be consulted.

Table 2. Identification Levels.

ID Message Confidence Level	Choices	% Probability	Comments
Excellent	1	96 to 99	N/A
Very Good	1	93 to 95	N/A
Good	1	89 to 92	N/A
Acceptable	1	85 to 88	N/A
Low Discrimination	2 to 3	Sum of choices = 100; after resolution to one choice, percent probability reflects the number associated with the selected choice.	2 to 3 taxa exhibit the same biopattern. Separate by supplemental testing.
Unidentified Organism	> 3 or 0	N/A	Either > 3 taxa exhibit the same biopattern or Very atypical biopattern. Does not correspond to any taxon in the database. Check Gram stain and purity.

Non-Reactive Biopattern

When a biopattern is calculated for an unknown organism that is either completely negative or consists of both negative tests and tests with reactions that lie too close to the test thresholds, the identification result will be "Non-reactive biopattern." If one encounters a non-reactive biopattern, a note will appear that states: "Organism with low reactivity biopattern – please check viability." The species that can potentially trigger this note are shown in separate tables for each of the respective products described below.

APPLICATIONS

GN Card

The GN card is used for the automated identification of 135 taxa of the most significant fermenting and non-fermenting Gram-negative bacilli. The list of claimed species is shown in Table 3. Of the 135 taxa, 12 are grouped taxa in either genus (3), group (6), or slashline (3) designations. When representative taxa of these collective designations are included, the total number of taxa claimed by GN is over 160. As with low discrimination identifications, grouped taxa can be separated into their component taxa by supplemental testing and/or observation. A list of the grouped taxa is shown in Table 4. There are informational notes that appear with certain taxa and these are shown in Table 5. When no reactivity is encountered, one is referred to a list of species that may be associated with a non-reactive biopattern (see Table 6).

The GN card is based on established biochemical methods and newly developed substrates measuring carbon source utilization, enzymatic activities, and resistance (ASM 1998, Atlas 1993, Brenner et al. 1993, Chang et al. 2002, Coenye et al. 2001a, Coenye et al. 2001b, De Baere et al. 2001, Freney et al. 2000, Gavini et al. 1989, Holt et al. 1994, Krieg and Holt 1984, Murray et al. 1999, Richard and Kiredjian 1992, Smith et al. 1991, Vandamme et al. 1999). There are 47 biochemical tests and one negative control well. Final identification results are available in approximately 10 hours or less. The list of test substrates is shown in Table 7.

In a recent multi-site study, the performance of the VITEK 2 GN was evaluated using 562 isolates of both commonly and rarely observed species of

Gram-negative bacilli, including 153 non-fermentative strains. The reference identification was determined with api® 20 E and api 20 NE identification kits. Overall, the VITEK 2 GN correctly identified 96.8% of the isolates, including 6.4% low discrimination with the correct species listed. Misidentifications occurred at 3.0% and no identifications occurred at 0.2%.

GP Card

The GP card is used for the automated identification of 115 taxa of the most significant non-spore-forming Gram-positive bacteria (primarily cocci). The list of claimed species is shown in Table 8. Of the 115 taxa, seven are grouped taxa in either slashline (4) or species (3) designations. When representative species or subspecies of these collective designations are included, the total number of taxa claimed by GP is over 120. As with low discrimination identifications, grouped taxa can be separated into their component taxa by supplemental testing and/or observation. A list of the grouped taxa is shown in Table 9. There are informational notes that appear with certain taxa and these are shown in Table 10. When no reactivity is encountered, one is referred to a list of species that may be associated with a non-reactive biopattern (see Table 11).

The GP identification card is based on established biochemical methods and newly developed substrates (Atlas 1993, Barros et al. 2001, Bille et al. 1992, Collins et al. 1984a, Collins et al. 1984b, Collins and Lawson 2000, Collins et al. 2001, Coykendall 1989, Devriese et al. 1988, Farrow et al. 1989, Freney et al. 2000, Holt et al. 1994, Kilpper-Bälz and Schleifer 1987, Krieg and Holt 1984, Murray et al. 1999, Poyart et al. 2002, Schlegel et al. 2000, Viera et al. 1998, Whiley et al. 1999). There are 43 biochemical tests measuring carbon source utilization, enzymatic activities and resistance. Final identification results are available in approximately eight hours or less. The list of test substrates is shown in Table 12.

In a recent multi-site study, the performance of the VITEK 2 GP was evaluated using 457 isolates of both commonly and rarely observed species of Gram-positive cocci. The reference identification was determined with api STAPH and api 20 STREP identification kits. Overall, the VITEK 2 GP correctly identified 96.5% of the isolates, including 2.2% low discrimination with the correct species listed. Misidentifications occurred at 3.3% and no identifications occurred at 0.2%.

Table 3. Organisms Identified by the GN Card.

Enterobacteriaceae	Non-Enterobacteriaceae
Buttiauxella agrestis	*Achromobacter xylosoxidans* ssp. *dentrificans*
Cedecea davisae	*Achromobacter xylosoxidans* ssp. *xylosoxidans*
Cedecea lapagei	*Acinetobacter baumannii*
Citrobacter amalonaticus	*Acinetobacter haemolyticus*
Citrobacter braakii	*Acinetobacter junii*
Citrobacter farmeri	*Acinetobacter lwoffii*
Citrobacter freundii	*Actinobacillus ureae*
Citrobacter koseri	*Aeromonas hydrophila/Aeromonas caviae*
Citrobacter sedlakii	*Aeromonas salmonicida*
Citrobacter youngae	*Aeromonas sobria*
Edwardsiella hoshinae	*Aeromonas veronii*
Edwardsiella tarda	*Alcaligenes faecalis* ssp. *faecalis*
Enterobacter aerogenes	*Bordetella bronchiseptica*
Enterobacter amnigenus 1	*Bordetella trematum*
Enterobacter amnigenus 2	*Brevundimonas diminuta/vesicularis*
Enterobacter asburiae	*Brucella melitensis*
Enterobacter cancerogenus	*Budvicia aquatica*
Enterobacter cloacae	*Burkholderia cepacia* group
Enterobacter gergoviae	*Burkholderia gladioli*
Enterobacter intermedius	*Burkholderia mallei*
Enterobacter sakazakii	*Burkholderia pseudomallei*
Escherichia coli	CDC group EF-4 *(Pasteurella)*
Escherichia coli O157	CDC group EO-2 *(Psychrobacter)*
Escherichia fergusonii	*Chromobacterium violaceum*
Escherichia hermannii	*Chryseobacterium gleum*
Escherichia vulneris	*Chryseobacterium indologenes*
Ewingella americana	*Chryseobacterium meningosepticum*
Hafnia alvei	*Comamonas testosteroni*
Klebsiella oxytoca	*Delftia acidovorans*
Klebsiella pneumoniae ssp. *ozaenae*	*Francisella tularensis*
Klebsiella pneumoniae ssp. *pneumoniae*	*Mannheimia haemolytica*
Klebsiella pneumoniae ssp.*rhinoscleromatis*	*Methylobacterium* spp.
Kluyvera ascorbata	*Moraxella* group
Kluyvera cryocrescens	*Myroides* spp.
Leclercia adecarboxylata	*Ochrobactrum anthropi*
Moellerella wisconsensis	*Oligella ureolytica*
Morganella morganii ssp. *morganii*	*Pasteurella aerogenes*
Morganella morganii ssp. *sibonii*	*Pasteurella multocida*
Pantoea agglomerans	*Pasteurella pneumotropica*
Pantoea spp.	*Photobacterium damselae*
Proteus mirabilis	*Plesiomonas shigelloides*
Proteus vulgaris group/*Proteus penneri*	*Pseudomonas aeruginosa*
Providencia alcalifaciens	*Pseudomonas alcaligenes*
Providencia rettgeri	*Pseudomonas fluorescens*
Providencia rustigianii	*Pseudomonas luteola*

Table 3. Organisms Identified by the GN Card (continued).

Enterobacteriaceae	Non-Enterobacteriaceae
Providencia stuartii	*Pseudomonas mendocina*
Rahnella aquatilis	*Pseudomonas oryzihabitans*
Raoultella ornithinolytica	*Pseudomonas pseudoalcaligenes*
Salmonella choleraesuis ssp. *arizonae*	*Pseudomonas putida*
Salmonella group	*Pseudomonas stutzeri*
Salmonella ser. Gallinarum	*Ralstonia mannitolilytica*
Salmonella ser. Paratyphi A	*Ralstonia paucula*
Salmonella typhi	*Ralstonia pickettii*
Serratia ficaria	*Rhizobium radiobacter*
Serratia fonticola	*Shewanella putrefaciens*
Serratia liquefaciens group	*Sphingobacterium multivorum*
Serratia marcescens	*Sphingobacterium spiritivorum*
Serratia odorifera	*Sphingobacterium thalpophilum*
Serratia plymuthica	*Sphingomonas paucimobilis*
Serratia rubidaea	*Stenotrophomonas maltophilia*
Shigella group	*Vibrio alginolyticus*
Shigella sonnei	*Vibrio cholerae*
Yersinia enterocolitica group	*Vibrio fluvialis*
Yersinia pestis	*Vibrio hollisae*
Yersinia pseudotuberculosis	*Vibrio metschnikovii*
Yersinia ruckeri	*Vibrio mimicus*
Yokenella regensburgei	*Vibrio parahaemolyticus*
	Vibrio vulnificus

Table 4. GN Grouped Taxa.

Group or Slashline Name	Species Represented
Aeromonas hydrophila/caviae	*Aeromonas caviae* *Aeromonas hydrophila*
Brevundimonas diminuta/vesicularis	*Brevundimonas diminuta* *Brevundimonas vesicularis*
Burkholderia cepacia group	*Burkholderia cepacia* *Burkholderia multivorans* *Burkholderia stabilis* *Burkholderia vietnamiensis*
Moraxella group	*Moraxella lacunata* *Moraxella nonliquefaciens* *Moraxella osloensis*
Proteus vulgaris group/*Proteus penneri*	*Proteus penneri* *Proteus vulgaris* group
Salmonella group	*Salmonella choleraesuis* ssp. *choleraesuis* *Salmonella enteritidis* *Salmonella ser.* Paratyphi B *Salmonella ser.* Paratyphi C *Salmonella* spp. *Salmonella typhimurium*
Serratia liquefaciens group	*Serratia grimesii* *Serratia liquefaciens* *Serratia proteamaculans*
Shigella group	*Shigella boydii* *Shigella dysenteriae* *Shigella flexneri*
Yersinia enterocolitica group	*Yersinia aldovae* *Yersinia enterocolitica* *Yersinia frederiksenii* *Yersinia intermedia* *Yersinia kristensenii*

Table 5. Notes Associated with Certain GN Taxa.

Note	Taxa
Confirm by serological tests	*Escherichia coli* O157 *Francisella tularensis* *Salmonella* group *Salmonella* ser. *Gallinarum* *Salmonella* ser. Paratyphi A *Salmonella typhi* *Shigella* group *Shigella sonnei*
Highly pathogenic organism	*Brucella melitensis* *Burkholderia mallei* *Burkholderia pseudomallei* *Escherichia coli* O157 *Francisella tularensis* *Vibrio cholerae* *Yersinia pestis*

Table 6. Species that May Be Non-reactive on the GN Card.

Acinetobacter lwoffii	*Moraxella nonliquefaciens*
Actinobacillus ureae	*Moraxella osloensis*
Aeromonas salmonicida	*Pasteurella multocida*
Brucella melitensis	*Pseudomonas alcaligenes*
Francisella tularensis	*Pseudomonas fluorescens*
Methylobacterium spp.	*Pseudomonas stutzeri*
Moraxella lacunata	

Table 7. Test Substrates on GN Card.

Well	Test	Mnemonic	Amount/Well
2	Ala-Phe-Pro-ARYLAMIDASE	APPA	0.0384 mg
3	ADONITOL	ADO	0.1875 mg
4	L-Pyrrolydonyl-ARYLAMIDASE	PyrA	0.018 mg
5	L-ARABITOL	lARL	0.3 mg
7	D-CELLOBIOSE	dCEL	0.3 mg
9	BETA-GALACTOSIDASE	BGAL	0.036 mg
10	H2S PRODUCTION	H2S	0.0024 mg
11	BETA-N-ACETYL-GLUCOSAMINIDASE	BNAG	0.0408 mg
12	Glutamyl Arylamidase pNA	AGLTp	0.0324 mg
13	D-GLUCOSE	dGLU	0.3 mg
14	GAMMA-GLUTAMYL-TRANSFERASE	GGT	0.0228 mg
15	FERMENTATION/ GLUCOSE	OFF	0.45 mg
17	BETA-GLUCOSIDASE	BGLU	0.036 mg
18	D-MALTOSE	dMAL	0.3 mg
19	D-MANNITOL	dMAN	0.1875 mg
20	D-MANNOSE	dMNE	0.3 mg
21	BETA-XYLOSIDASE	BXYL	0.0324 mg
22	BETA-Alanine arylamidase pNA	BAlap	0.0174 mg
23	L-Proline ARYLAMIDASE	ProA	0.0234 mg
26	LIPASE	LIP	0.0192 mg
27	PALATINOSE	PLE	0.3 mg
29	Tyrosine ARYLAMIDASE	TyrA	0.0276 mg
31	UREASE	URE	0.15 mg
32	D-SORBITOL	dSOR	0.1875 mg
33	SACCHAROSE/SUCROSE	SAC	0.3 mg
34	D-TAGATOSE	dTAG	0.3 mg
35	D-TREHALOSE	dTRE	0.3 mg
36	CITRATE (SODIUM)	CIT	0.054 mg
37	MALONATE	MNT	0.15 mg
39	5-KETO-D-GLUCONATE	5KG	0.3 mg
40	L-LACTATE alkalinisation	lLATk	0.15 mg
41	ALPHA-GLUCOSIDASE	AGLU	0.036 mg
42	SUCCINATE alkalinisation	SUCT	0.15 mg
43	Beta-N-ACETYL-GALACTOSAMINIDASE	NAGA	0.0306 mg
44	ALPHA-GALACTOSIDASE	AGAL	0.036 mg
45	PHOSPHATASE	PHOS	0.0504 mg
46	Glycine ARYLAMIDASE	GlyA	0.012 mg
47	ORNITHINE DECARBOXYLASE	ODC	0.3 mg
48	LYSINE DECARBOXYLASE	LDC	0.15 mg
52	DECARBOXYLASE BASE	0DEC	N/A
53	L-HISTIDINE assimilation	lHISa	0.087 mg
56	COUMARATE	CMT	0.126 mg
57	BETA-GLUCORONIDASE	BGUR	0.0378 mg
58	O/129 RESISTANCE (comp.vibrio.)	O129R	0.0105 mg
59	Glu-Gly-Arg-ARYLAMIDASE	GGAA	0.0576 mg
61	L-MALATE assimilation	lMLTa	0.042 mg
62	ELLMAN	ELLM	0.03 mg
64	L-LACTATE assimilation	lLATa	0.186 mg

Table 8. Organisms Identified by the GP Card.

Abiotrophia defectiva	*Listeria seeligeri*
Aerococcus urinae	*Listeria welshimeri*
Aerococcus viridans	*Micrococcus luteus/lylae*
Alloiococcus otitis	*Pediococcus acidilactici*
Dermacoccus nishinomiyaensis/ Kytococcus sedentarius	*Pediococcus pentosaceus*
	Rothia mucilaginosa
Enterococcus avium	*Staphylococcus arlettae*
Enterococcus casseliflavus	*Staphylococcus aureus*
Enterococcus cecorum	*Staphylococcus auricularis*
Enterococcus columbae	*Staphylococcus cohnii* ssp. *cohnii*
Enterococcus durans	*Staphylococcus cohnii* ssp. *urealyticum*
Enterococcus faecalis	*Staphylococcus epidermidis*
Enterococcus faecium	*Staphylococcus equorum*
Enterococcus gallinarum	*Staphylococcus gallinarum*
Enterococcus hirae	*Staphylococcus haemolyticus*
Enterococcus raffinosus	*Staphylococcus hominis*
Enterococcus saccharolyticus	*Staphylococcus hyicus*
Erysipelothrix rhusiopahiae	*Staphylococcus intermedius*
Facklamia hominis	*Staphylococcus kloosii*
Gardnerella vaginalis	*Staphylococcus lentus*
Gemella bergeri	*Staphylococcus lugdunensis*
Gemella haemolysans	*Staphylococcus saprophyticus*
Gemella morbillorum	*Staphylococcus schleiferi*
Gemella sanguinis	*Staphylococcus sciuri*
Globicatella sanguinis	*Staphylococcus simulans*
Globicatella sulfidifaciens	*Staphylococcus vitulinus*
Granulicatella adiacens	*Staphylococcus warneri*
Granulicatella elegans	*Staphylococcus xylosus*
Helcococcus kunziii	*Streptococcus agalactiae*
Kocuria kristinae	*Streptococcus alactolyticus*
Kocuria rosea	*Streptococcus anginosus*
Kocuria varians	*Streptococcus canis*
Lactococcus garvieae	*Streptococcus constellatus* ssp. *constellatus*
Lactococcus lactis ssp. *cremoris*	*Streptococcus constellatus* ssp. *pharyngis*
Lactococcus lactis ssp. *lactis*	*Streptococcus cristatus*
Lactococcus raffinolactis	*Streptococcus downei*
Leuconostoc citreum	*Streptococcus dysgalactiae* ssp. *dysgalactiae*
Leuconostoc lactis	*Streptococcus dysgalactiae* ssp. *equisimilis*
Leuconostoc mesenteroides ssp. *cremoris*	*Streptococcus equi* ssp. *equi*
Leuconostoc mesenteroides ssp. *dextranicum*	*Streptococcus equi* ssp. *zooepidemicus*
	Streptococcus equinus
Leuconostoc mesenteroides ssp. *mesenteroides*	*Streptococcus gallolyticus*
	Streptococcus gordonii
Leuconostoc pseudomesenteroides	*Streptococcus hyointestinalis*
Listeria grayi	*Streptococcus infantarius*
Listeria innocua	*Streptococcus intermedius*
Listeria ivanovii	*Streptococcus lutetiensis/Streptococcus bovis*
Listeria monocytogenes	*Streptococcus mitis/Streptococcus oralis*

Table 8. Organisms Identified by the GP Card (continued).

Streptococcus mutans	*Streptococcus suis* I
Streptococcus ovis	*Streptococcus suis* II
Streptococcus parasanguinis	*Streptococcus thermophilus*
Streptococcus pasteurianus	*Streptococcus thoraltensis*
Streptococcus pluranimalium	*Staphylococcus capitis*
Streptococcus pneumoniae	*Staphylococcus caprae*
Streptococcus porcinus	*Staphylococcus carnosus* ssp. *carnosus*
Streptococcus pyogenes	*Staphylococcus chromogenes*
Streptococcus salivarius	*Streptococcus uberis*
Streptococcus sanguinis	*Streptococcus vestibularis*
Streptococcus sobrinus	*Vagococcus fluvialis*

Table 9. GP Grouped Taxa.

Slashline or Species Name	Species or Subspecies Represented
Dermacoccus nishinomiyaensis/ Kytococcus sedentarius	*Dermacoccus nishinomiyaensis* *Kytococcus sedentarius*
Listeria ivanovii	*Listeria ivanovii* ssp. *ivanovii* *Listeria ivanovii* ssp. *londoniensis*
Micrococcus luteus/lylae	*Micrococcus luteus* *Micrococcus lylae*
Staphylococcus capitis	*Staphylococcus capitis* ssp. *capitis* *Staphylococcus capitis* ssp. *ureolyticus*
Staphylococcus hominis	*Staphylococcus hominis* ssp. *hominis* *Staphylococcus hominis* ssp. *novobiosepticus*
Streptococcus lutetiensis/ Streptococcus bovis	*Streptococcus bovis* *Streptococcus lutetiensis*
Streptococcus mitis/ Streptococcus oralis	*Streptococcus mitis* *Streptococcus oralis*

Table 10. Notes Associated with Certain GP Taxa.

Note	Taxon
Highly pathogenic organism, check Camp test and beta hemolysis	*Listeria monocytogenes*
Possibility of *Staphylococcus pasteuri* if yellow pigmented	*Staphylococcus warneri*
Possibility of *Enterococcus villorum* if veterinary	*Enterococcus durans*

Table 11. Species That May Be Non-Reactive on the GP Card.

Alloiococcus otitis	*Kytococcus sedentarius*
Dermacoccus nishinomiyaensis	*Leuconostoc mesenteroides* ssp. *cremoris*
Dolosigranulum pigrum	*Micrococcus lylae*
Gemella bergeri	*Staphylococcus auricularis*
Kocuria rosea	*Streptococcus pluranimalium*
Kocuria varians	

Table 12. Test Substrates on GP Card.

Well	Test	Mnemonic	Amount/Well
2	D-AMYGDALIN	AMY	0.1875 mg
4	PHOSPHATIDYLINOSITOL PHOSPHOLIPASE C	PIPLC	0.015 mg
5	D-XYLOSE	dXYL	0.3 mg
8	ARGININE DIHYDROLASE 1	ADH1	0.111 mg
9	BETA-GALACTOSIDASE	BGAL	0.036 mg
11	ALPHA-GLUCOSIDASE	AGLU	0.036 mg
13	Ala-Phe-Pro ARYLAMIDASE	APPA	0.0384 mg
14	CYCLODEXTRIN	CDEX	0.3 mg
15	L-Aspartate ARYLAMIDASE	AspA	0.024 mg
16	BETA GALACTOPYRANOSIDASE	BGAR	0.00204 mg
17	ALPHA-MANNOSIDASE	AMAN	0.036 mg
19	PHOSPHATASE	PHOS	0.0504 mg
20	Leucine ARYLAMIDASE	LeuA	0.0234 mg
23	L-Proline ARYLAMIDASE	ProA	0.0234 mg
24	BETA GLUCURONIDASE	BGURr	0.0018 mg
25	ALPHA-GALACTOSIDASE	AGAL	0.036 mg
26	L-Pyrrolidonyl-ARYLAMIDASE	PyrA	0.018 mg
27	BETA-GLUCURONIDASE	BGUR	0.0378 mg
28	Alanine ARYLAMIDASE	AlaA	0.0216 mg
29	Tyrosine ARYLAMIDASE	TyrA	0.0276 mg
30	D-SORBITOL	dSOR	0.1875 mg
31	UREASE	URE	0.15 mg
32	POLYMIXIN B RESISTANCE	POLYB	0.00093 mg
37	D-GALACTOSE	dGAL	0.3 mg
38	D-RIBOSE	dRIB	0.3 mg
39	L-LACTATE alkalinization	lLATk	0.15 mg
42	LACTOSE	LAC	0.96 mg
44	N-ACETYL-D-GLUCOSAMINE	NAG	0.3 mg
45	D-MALTOSE	dMAL	0.3 mg
46	BACITRACIN RESISTANCE	BACI	0.0006 mg
47	NOVOBIOCIN RESISTANCE	NOVO	0.000075 mg
50	GROWTH IN 6.5% NaCl	NC6.5	1.68 mg
52	D-MANNITOL	dMAN	0.1875 mg
53	D-MANNOSE	dMNE	0.3 mg
54	METHYL-B-D-GLUCOPYRANOSIDE	MBdG	0.3 mg
56	PULLULAN	PUL	0.3 mg
57	D-RAFFINOSE	dRAF	0.3 mg
58	O/129 RESISTANCE (comp.vibrio.)	O129R	0.0084 mg
59	SALICIN	SAL	0.3 mg
60	SACCHAROSE/SUCROSE	SAC	0.3 mg
62	D-TREHALOSE	dTRE	0.3 mg
63	ARGININE DIHYDROLASE 2	ADH2s	0.27 mg
64	OPTOCHIN RESISTANCE	OPTO	0.000399 mg

YST Card

The YST card is used for the automated identification of 49 taxa of the most significant yeasts and yeast-like organisms. The list of claimed species is shown in Table 13. Of the 49 taxa, three are grouped taxa in either slashline (2) or genus (1) designations. When representative species of these collective designations are included, the total number of taxa claimed by YST is 54. As with low discrimination identifications, grouped taxa can be separated into their component taxa by supplemental testing and/or observation. A list of the grouped taxa is shown in Table 14. When no reactivity is encountered, one is referred to a list of species that may be associated with a non-reactive biopattern (see Table 15).

The YST identification card is based on established biochemical methods and newly developed substrates (Atlas 1993, Barnett et al. 2000, Kreger-van Rij 1984, Larone 1995, Lodder 1971, McGinnis 1980, Murray et al. 1999). There are 46 biochemical tests measuring carbon source utilization, enzymatic activities and resistance. Final identification results are available in approximately 18 hours. The list of test substrates is shown in Table 16.

In a recent multi-site study, the performance of the VITEK 2 YST was evaluated using 623 isolates of both commonly and rarely observed species of yeast and yeast-like organisms. The reference identification was determined with api 20C AUX identification kits. Overall, the VITEK 2 YST correctly identified 98.9% of the isolates, including 11.7% low discrimination with the correct species listed. Misidentifications occurred at 0.6% and no identifications occurred at 0.5%.

Table 13. Organisms Identified by the YST Card.

Candida albicans	*Candida zeylanoides*
Candida boidinii	*Cryptococcus albidus*
Candida catenulata	*Cryptococcus laurentii*
Candida colliculosa	*Cryptococcus neoformans*
Candida dubliniensis	*Cryptococcus terreus*
Candida famata	*Cryptococcus uniguttulatus*
Candida freyschussii	*Geotrichum capitatum*
Candida glabrata	*Geotrichum klebahnii*
Candida guilliermondii	*Kloeckera* spp.
Candida haemulonii	*Kodamaea ohmeri*
Candida intermedia	*Malassezia furfur*
Candida kefyr	*Malassezia pachydermatis*
Candida krusei/C. inconspicua/C. lambica	*Pichia farinosa*
Candida lipolytica	*Prototheca wickerhamii*
Candida lusitaniae	*Prototheca zopfii*
Candida magnoliae	*Rhodotorula glutinis/mucilaginosa*
Candida norvegensis	*Rhodotorula minuta*
Candida parapsilosis	*Saccharomyces cerevisiae*
Candida pelliculosa	*Sporobolomyces salmonicolor*
Candida pulcherrima	*Stephanoascus ciferrii*
Candida rugosa	*Trichosporon asahii*
Candida sake	*Trichosporon inkin*
Candida sphaerica	*Trichosporon mucoides*
Candida tropicalis	*Zygosaccharomyces bailii*
Candida utilis	

Table 14. YST Grouped Taxa.

Slashline or Genus Name	Species Represented
Candida krusei/C. inconspicua/C. lambica	*Candida krusei* *Candida inconspicua* *Candida lambica*
Kloeckera spp.	*Kloeckera apiculata* *Kloeckera apis* *Kloeckera japonica*
Rhodotorula glutinis/mucilaginosa	*Rhodotorula glutinis* *Rhodotorula mucilaginosa*

Table 15. Species That May Be Non-Reactive on the YST Card.

Candida sake
Candida zeylanoides
Malassezia furfur
Malassezia pachydermatis
Zygosaccharomyces bailii

Table 16. Test Substrates on YST Card.

Well	Test	Mnemonic	Amount/Well
3	L-Lysine-ARYLAMIDASE	LysA	0.0228 mg
4	L-MALATE assimilation	lMLTa	0.15 mg
5	Leucine-ARYLAMIDASE	LeuA	0.0234 mg
7	ARGININE GP	ARG	0.15 mg
10	ERYTHRITOL assimilation	ERYa	0.3 mg
12	GLYCEROL assimilation	GLYLa	0.16 µL
13	Tyrosine ARYLAMIDASE	TyrA	0.0276 mg
14	BETA-N-ACETYL-GLUCOSAMINIDASE	BNAG	0.0408 mg
15	ARBUTIN assimilation	ARBa	0.3 mg
18	AMYGDALIN assimilation	AMYa	0.3 mg
19	D-GALACTOSE assimilation	dGALa	0.3 mg
20	GENTIOBIOSE assimilation	GENa	0.3 mg
21	D-GLUCOSE assimilation	dGLUa	0.3 mg
23	LACTOSE assimilation	LACa	0.96 mg
24	METHYL-A-D-GLUCOPYRANOSIDE assimilation	MAdGa	0.3 mg
26	D-CELLOBIOSE assimilation	dCELa	0.3 mg
27	GAMMA-GLUTAMYL-TRANSFERASE	GGT	0.0228 mg
28	D-MALTOSE assimilation	dMALa	0.3 mg
29	D-RAFFINOSE assimilation	dRAFa	0.3 mg
30	PNP-N-acetyl-BD-galactosaminidase 1	NAGA1	0.0306 mg
32	D-MANNOSE assimilation	dMNEa	0.3 mg
33	D-MELIBIOSE assimilation	dMELa	0.3 mg
34	D-MELEZITOSE assimilation	dMLZa	0.3 mg
38	L-SORBOSE assimilation	lSBEa	0.3 mg
39	L-RHAMNOSE assimilation	lRHAa	0.3 mg
40	XYLITOL assimilation	XLTa	0.3 mg
42	D-SORBITOL assimilation	dSORa	0.1875 mg
44	SACCHAROSE/SUCROSE assimilation	SACa	0.3 mg
45	UREASE	URE	0.15 mg
46	ALPHA-GLUCOSIDASE	AGLU	0.036 mg
47	D-TURANOSE assimilation	dTURa	0.3 mg
48	D-TREHALOSE assimilation	dTREa	0.3 mg
49	NITRATE assimilation	NO3a	0.03 mg
51	L-ARABINOSE assimilation	lARAa	0.3 mg
52	D-GALACTURONATE assimilation	dGATa	0.15 mg
53	ESCULIN hydrolysis	ESC	0.225 mg
54	L-GLUTAMATE assimilation	lGLTa	0.15 mg
55	D-XYLOSE assimilation	dXYLa	0.3 mg
56	DL-LACTATE assimilation	LATa	0.15 mg
58	ACETATE assimilation	ACEa	0.15 mg
59	CITRATE (SODIUM) assimilation	CITa	0.15 mg
60	GLUCURONATE ASSIMILATION	GRTas	0.15 mg
61	L-PROLINE assimilation	lPROa	0.15 mg
62	2-KETO-D-GLUCONATE assimilation	2KGa	0.15 mg
63	N-ACETYL-GLUCOSAMINE assimilation	NAGa	0.15 mg
64	D-GLUCONATE assimilation	dGNTa	0.15 mg

BCL Card

The BCL card is used for the automated identification of 38 taxa of the most significant aerobic endospore-forming species of the family *Bacillaceae*. The list of claimed species is shown in Table 17. Of the 38 taxa, four are grouped taxa in slashline designations. When representative species of these slashlines are included, the total number of taxa claimed by BCL is 42. As with low discrimination identifications, grouped taxa can be separated into their component taxa by supplemental testing and/or observation. A list of the slashline taxa is shown in Table 18. There are informational notes that appear with certain taxa and these are shown in Table 19. When no reactivity is encountered, one is referred to a list of species that may be associated with a non-reactive biopattern (see Table 20).

The BCL identification card is based on established biochemical methods and newly developed substrates (Atlas 1993, Claus and Berkeley 1986, Gordon et al. 1973, Logan and Berkeley 1981, Logan and Berkeley 1984, Logan et al. 1985, Logan et al. 2002, Logan and Turnbull 2003). There are 46 biochemical tests measuring carbon source utilization, enzymatic activities, inhibition, and resistance. Final identification results are available in approximately 14 hours. The list of test substrates is shown in Table 21.

The database performance of the VITEK 2 BCL was evaluated using 1436 isolates of both commonly and rarely observed species of Gram-positive aerobic spore-forming bacilli. The reference identification was determined with the api 50CHB identification kit and other conventional test methods. Overall, the VITEK 2 BCL correctly identified 96.1% of the isolates, including 9.3% low discrimination with the correct species listed. Misidentifications occurred at 2.6% and no identifications occurred at 1.3%.

Table 17. Organisms Identified by the BCL Card.

Aneurinibacillus aneurinilyticus	*Brevibacillus choshinensis*
Bacillus anthracis	*Brevibacillus invocatus*
Bacillus badius	*Brevibacillus laterosporus*
Bacillus cereus/Bacillus thuringiensis	*Brevibacillus parabrevis*
Bacillus circulans	*Geobacillus stearothermophilus*
Bacillus coagulans	*Geobacillus thermoglucosidasius/ Geob. thermodenitrificans*
Bacillus firmus	
Bacillus lentus	*Paenibacillus alvei*
Bacillus licheniformis	*Paenibacillus amylolyticus*
Bacillus megaterium	*Paenibacillus durus*
Bacillus mycoides	*Paenibacillus glucanolyticus*
Bacillus pumilus	*Paenibacillus macerans*
Bacillus smithii	*Paenibacillus pabuli*
Bacillus sphaericus/Bacillus fusiformis	*Paenibacillus peoriae*
Bacillus sporothermodurans	*Paenibacillus polymyxa*
Bacillus subtilis/Bacillus amyloliquefaciens	*Paenibacillus thiaminolyticus*
Brevibacillus agri	*Paenibacillus validus*
Brevibacillus borstelensis	*Virgibacillus pantothenticus*
Brevibacillus brevis	*Virgibacillus proomii*
Brevibacillus centrosporus	

Table 18. BCL Slashline Taxa.

Slashline	Species Represented
Bacillus cereus/Bacillus thuringiensis	*Bacillus cereus* *Bacillus thuringiensis*
Bacillus sphaericus/Bacillus fusiformis	*Bacillus fusiformis* *Bacillus sphaericus*
Bacillus subtilis/Bacillus amyloliquefaciens	*Bacillis amyloliquefaciens* *Bacillus subtilis*
Geobacillus thermoglucosidasius/Geob. thermodenitrificans	*Geobacillus thermodenitrificans* *Geobacillus thermoglucosidasius*

Table 19. Notes Associated with Certain BCL Taxa.

Note	Taxon
Possibility of *Aneurinibacillus migulanus*	*Aneurinbacillus aneurinilyticus*
Important! Presumptive identification	*Bacillus anthracis*

Table 20. Species That May Be Non-Reactive on the BCL Card.

Geobacillus thermodenitrificans
Geobacillus thermoglucosidasius

Table 21. Test Substrates on BCL Card.

Well	Test	Mnemonic	Amount/Well
1	BETA-XYLOSIDASE	BXYL	0.0324 mg
3	L-Lysine-ARYLAMIDASE	LysA	0.0228 mg
4	L-Aspartate ARYLAMIDASE	AspA	0.024 mg
5	Leucine-ARYLAMIDASE	LeuA	0.0234 mg
7	Phenylalanine ARYLAMIDASE	PheA	0.0264 mg
8	L-Proline ARYLAMIDASE	ProA	0.0234 mg
9	BETA-GALACTOSIDASE	BGAL	0.036 mg
10	L-Pyrrolydonyl-ARYLAMIDASE	PyrA	0.018 mg
11	ALPHA-GALACTOSIDASE	AGAL	0.036 mg
12	Alanine ARYLAMIDASE	AlaA	0.0222 mg
13	Tyrosine ARYLAMIDASE	TyrA	0.0282 mg
14	BETA-N-ACETYL-GLUCOSAMINIDASE	BNAG	0.0408 mg
15	Ala-Phe-Pro ARYLAMIDASE	APPA	0.0384 mg
18	CYCLODEXTRIN	CDEX	0.3 mg
19	D-GALACTOSE	dGAL	0.3 mg
21	GLYCOGEN	GLYG	0.1875 mg
22	myo-INOSITOL	INO	0.3 mg
24	METHYL-A-D-GLUCOPYRANOSIDE acidification	MdG	0.3 mg
25	ELLMAN	ELLM	0.03 mg
26	METHYL-D-XYLOSIDE	MdX	0.3 mg
27	ALPHA-MANNOSIDASE	AMAN	0.036 mg
29	MALTOTRIOSE	MTE	0.3 mg
30	Glycine ARYLAMIDASE	GlyA	0.012 mg
31	D-MANNITOL	dMAN	0.3 mg
32	D-MANNOSE	dMNE	0.3 mg
34	D-MELEZITOSE	dMLZ	0.3 mg
36	N-ACETYL-D-GLUCOSAMINE	NAG	0.3 mg
37	PALATINOSE	PLE	0.3 mg
39	L-RHAMNOSE	IRHA	0.3 mg
41	BETA-GLUCOSIDASE	BGLU	0.036 mg
43	BETA-MANNOSIDASE	BMAN	0.036 mg
44	PHOSPHORYL CHOLINE	PHC	0.0366 mg
45	PYRUVATE	PVATE	0.15 mg
46	ALPHA-GLUCOSIDASE	AGLU	0.036 mg
47	D-TAGATOSE	dTAG	0.3 mg
48	D-TREHALOSE	dTRE	0.3 mg
50	INULIN	INU	0.12 mg
53	D-GLUCOSE	dGLU	0.3 mg
54	D-RIBOSE	dRIB	0.3 mg
56	PUTRESCINE assimilation	PSCNa	0.201 mg
58	GROWTH IN 6.5% NaCl	NaCl 6.5%	1.95 mg
59	KANAMYCIN RESISTANCE	KAN	0.006 mg
60	OLEANDOMYCIN RESISTANCE	OLD	0.003 mg
61	ESCULIN hydrolysis	ESC	0.0225 mg
62	TETRAZOLIUM RED	TTZ	0.0189 mg
63	POLYMIXIN_B RESISTANCE	POLYB_R	0.00093 mg

VALIDATION PROCEDURE

Quality control organisms and their expected results are listed for each product and are tested according to the procedures outlined in the on-line product information. Frequency of testing is dependent on local regulations.

POTENTIAL NEW APPLICATIONS

A new application to be released soon is the NH card, used to identify species of *Neisseria*, *Haemophilus*, and other fastidious genera including *Campylobacter*.

CONCLUSION

The VITEK 2 is an automated microbial identification system that provides highly accurate and reproducible results as shown in multiple independent studies. With its colorimetric reagent cards, and associated hardware and software advances, the VITEK 2 offers a state-of-the-art technology platform for phenotypic identification methods.

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