JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2006, p. 854–856 Vol. 44, No. 3
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.3.854–856.2006

Use of Various Common Isolation Media To Evaluate the New VITEK 2 Colorimetric GN Card for Identification of *Burkholderia pseudomallei*

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Received 8 November 2005/Returned for modification 19 December 2005/Accepted 6 January 2006

The use of automated systems in the modern microbiology laboratory is becoming commonplace as the pressure of cost containment impacts on staff resources. With increased international travel and threats of bioterrorism, recognition and accurate identification of organisms such as *Burkholderia pseudomallei* is important. In areas where this organism is endemic, identification is not usually problematic. This study evaluates the performance of the new VITEK 2 colorimetric GN card for the identification of this organism. A total of 103 previously identified clinical isolates were tested with the new card with isolates taken from MacConkey agar, Columbia horse blood agar, Columbia sheep blood agar, and Trypticase soy agar in order to determine identification performance and to see if there was any variability in results due to the agar. Columbia horse blood agar produced the highest rates of identification (81%), followed by Trypticase soy agar (78%), Columbia sheep blood agar (75%), and MacConkey agar (63%). There was considerable variability in some of the reactions obtained. Seven isolates failed to identify from any of the agars used. This study highlights issues with the identification of this organism with the new VITEK 2 GN card. Enhancements of the algorithm parameters for the GN card are warranted and are in progress. Laboratory personnel need to be aware of the current limitations with this GN card and the software (version 4.02 or older for the VITEK 2 60/XL and version 1.02 or older for VITEK 2 Compact) and rely on clinical history, a high index of suspicion, and basic microbiology tests to confirm the identification of this organism.

Burkholderia pseudomallei is the agent of melioidosis, causing diverse clinical presentations including septicemia, organ abscesses, pneumonia, joint infections, and localized skin lesions. Endemic regions include Southeast Asia and the northern regions of Australia (1). The organism is a soil saprophyte which causes infection by inhalation, inoculation, or ingestion (1). In the acute form of the disease, fulminant septicemia may result in death within a few days of exposure. A high index of suspicion by clinical and/or laboratory staff and rapid identification of the isolate is essential in order to reduce the mortality rate for this disease.

Culture of the organism is recognized as the "gold standard" for diagnosis of *B. pseudomallei*. Variable rates of success with commercial identification systems for isolates have been reported (5, 6, 7), leading to investigation of other methods for diagnosis and identification, including serology, antigen detection, immunofluorescence, and molecular methods (1, 4, 8, 9). Laboratories in regions where the bacterium is endemic usually have little difficulty with the identification of *B. pseudomallei*. Microbiology laboratories not familiar with this organism, however, may handle it inappropriately, misidentify it, or have delayed identification, and in doing so they may expose personnel to an extremely hazardous organism (2). This organism should always be handled in a biohazard safety cabinet class II or higher.

Automation in the modern microbiology laboratory is becoming commonplace as the pressure of cost containment impacts staff resources. Health care institutions demand the rapid and improved results promised by automated methods to ensure that patient management is more cost effective. In addition, employees increasingly view the workplace as a means to an end, not a chosen career path that was once commonplace. Staff turnover contributes to a deskilled workforce increasingly reliant on technology. With international travel now common, it is more likely that laboratories will face the challenge of having to identify organisms not previously encountered. The omnipresent threat of terrorism, possibly involving biological agents, must also be considered.

The VITEK 2 (bioMérieux, Marcy-l'Etoile, France) fluorometric ID-GNB card has been previously shown not to be suitable for the identification of *B. pseudomallei* (7). The new colorimetric GN card for clinically relevant gram-negative bacilli has an expanded database, including claims for 138 taxa,

TABLE 1. Identification results for all media analyzed using software version VT2-R4.01

Medium type	Identification	% Of strains
Columbia horse blood agar	*Burkholderia pseudomallei*	81
	Burkholderia cepacia group	19
Trypticase soy agar	*Burkholderia pseudomallei*	78
	Burkholderia cepacia group	18
	Pseudomonas aeruginosa	2
	Sphingomonas paucimobilis	1
	Burkholderia putida	1
Columbia sheep blood agar	*Burkholderia pseudomallei*	75
	Burkholderia cepacia group	24
	Unidentified organism	1
MacConkey agar	*Burkholderia pseudomallei*	63
	Burkholderia cepacia group	35
	Pseudomonas aeruginosa	1
	Unidentified organism	1

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TABLE 2. Reactions for all media analyzed using software version VT2-R4.01

Test name	Medium and identification results[a]											
	CBA			TSA			SBA			MAC		
	%P	%N	%E	%P	%N	%E	%P	%N	%E	%P	%N	%E
Ala-Phe-Pro-arylamidase	0	99	1	2	98	0	0	100	0	0	100	0
Adonitol	0	100	0	0	100	0	0	100	0	0	100	0
L-Pyrrolydonyl-arylamidase	0	98	2	0	99	1	0	99	1	0	100	0
L-Arabitol	0	99	1	0	100	0	0	100	0	0	100	0
D-Cellobiose	2	98	0	2	98	0	2	98	0	2	97	1
Beta-galactosidase	0	100	0	0	100	0	0	100	0	0	100	0
H_2S production	0	100	0	0	100	0	0	100	0	0	100	0
Beta-*N*-acetyl-glucosaminidase	84	15	1	80	15	5	83	14	3	52	35	13
Glutamyl arylamidase pNA	1	97	2	0	96	4	1	97	2	17	82	1
D-Glucose	100	0	0	100	0	0	100	0	0	100	0	0
Gamma-glutamyl-transferase	100	0	0	100	0	0	100	0	0	100	0	0
Fermentation/glucose	0	100	0	1	99	0	1	99	0	2	98	0
Beta-glucosidase	0	100	0	0	100	0	0	100	0	0	100	0
D-Maltose	27	72	1	11	87	2	24	76	0	6	92	2
D-Mannitol	94	2	4	88	4	8	96	2	2	96	4	0
D-Mannose	70	21	9	49	37	13	60	32	8	44	45	12
Beta-xylosidase	0	100	0	0	100	0	0	100	0	0	100	0
Beta-alanine arylamidase pNA	93	7	0	92	8	0	93	7	0	89	11	0
L-Proline arylamidase	57	40	3	92	7	1	38	61	1	12	80	8
Lipase	0	100	0	0	100	0	0	100	0	0	100	0
Palatinose	0	100	0	0	100	0	0	100	0	0	100	0
Tyrosine arylamidase	70	28	2	93	7	0	50	50	0	7	92	1
Urease	0	100	0	0	99	1	0	100	0	46	43	12
D-Sorbitol	92	4	4	84	7	9	93	4	3	92	3	5
Saccharose/sucrose	99	1	0	99	1	0	99	1	0	97	2	1
D-Tagatose	1	98	1	1	99	0	0	99	1	3	95	2
D-Trehalose	1	98	1	0	100	0	0	100	0	0	100	0
Citrate (sodium)	78	22	0	58	41	1	75	25	0	79	21	0
Malonate	31	45	24	6	83	11	36	43	21	71	25	4
5-Keto-D-gluconate	0	100	0	0	100	0	0	100	0	0	100	0
L-Lactate alkalinisation	100	0	0	100	0	0	100	0	0	100	0	0
Alpha-glucosidase	0	100	0	0	100	0	0	100	0	0	100	0
Succinate alkalinisation	99	1	0	98	2	0	99	1	0	100	0	0
Beta-*N*-acetyl-galosaminidase	68	29	3	48	44	7	68	31	1	64	35	1
Alpha-galactosidase	0	100	0	0	100	0	0	100	0	0	100	0
Phosphatase	39	30	31	14	62	24	44	31	25	91	2	7
Glyine arylamidase	0	99	1	0	100	0	0	99	1	0	98	2
Ornithine decarboxylase	0	100	0	0	100	0	0	100	0	0	100	0
Lysine decarboxylase	0	100	0	0	100	0	0	100	0	0	100	0
L-Histidine assimilation	10	86	4	8	92	0	14	84	2	4	94	2
Courmarate	74	26	0	71	29	0	77	23	0	64	36	0
Beta-glucoronidase	0	100	0	0	100	0	0	100	0	0	100	0
O/129 resistance (comp.vibrio)	82	18	0	71	28	1	78	21	1	66	33	1
Glu-Gly-Arg-arylamidase	0	100	0	0	100	0	0	100	0	0	100	0
L-Malate assimilation	20	78	2	13	86	1	22	77	1	6	93	1
Ellman	72	8	20	63	14	23	68	16	16	2	96	2
L-Lactate assimilation	11	86	3	8	89	3	11	87	2	3	95	2

[a] Percent first choice for *B. pseudomallei* for Columbia horse blood agar (CBA), 81%; for Trypticase soy agar (TSA), 78%; for Columbia sheep blood agar (SBA), 75%; for MacConkey agar (MAC), 63%. %P, percentage of isolates positive; %N, percentage of isolates negative; %E, percentage of isolates equivocal.

compared to 104 taxa claims in the fluorometric identification card. The card has 47 biochemical tests and produces results in approximately 10 h. Isolates can be cultured on a wide variety of media prior to testing. Recently, published studies supported the utility of this new card (3). Strains of *B. pseudomallei* previously studied (7) were tested with the new GN card to evaluate its utility with this organism.

MATERIALS AND METHODS

Organisms. A total of 103 isolates of *B. pseudomallei* were subcultured onto Columbia horse blood agar from storage at −70°C. These isolates were all of clinical origin and had been previously identified using API 20NE, API 20E, and VITEK Legacy (7). Isolates were further subcultured onto MacConkey agar (all 103 strains), Columbia horse blood agar (100 of 103 strains), Columbia sheep blood agar (100 of 103 strains), and Trypticase soy agar (99 of 103 strains) and incubated at 35°C in air overnight prior to testing. Not all isolates were available to test from all media due to restrictions of card availability. This also prevented evaluation of the performance of the new cards with closely related organisms. All media were manufactured by bioMérieux in Australia.

VITEK 2 identification. The VITEK 2 GN cards were set up according to manufacturer's instructions using reagents and equipment supplied by bioMérieux. Briefly, individual colonies from a 24-h subculture on the desired agar were selected and transferred to a polystyrene test tube containing 3.0 ml of sterile saline (aqueous, 0.45% to 0.05% NaCl, pH 4.5 to 7.0). Tubes were mixed to produce a homogenous organism suspension, and the density was adjusted to be equivalent to a McFarland number of 0.50 to 0.63 using a calibrated VITEK

2 Densichek. All inoculated cards were placed in the instrument within 30 min of inoculation. Data were analyzed using software version VT2-R4.01.

RESULTS

Acceptable identification was defined as *B. pseudomallei* as either first- or second-choice identification. The possibility of this organism from culture in the laboratory should immediately signal the requirement for increased safety precautions by all staff and notification of a clinical microbiologist. The VITEK 2 GN card showed variable rates of acceptable identification. An unexpected finding was that the rates of identification varied depending on the culture medium isolates were taken from. For the same set of organisms, regardless of culture medium used, improvement was seen in comparison to the VITEK 2 ID-GNB fluorometric card, which had only 19% acceptable identification (7). The most common incorrect identification was the *Burkholderia cepacia* group. Seven isolates were incorrectly identified from all media. The percentage identifications for each medium type are shown in Table 1.

DISCUSSION

The new VITEK 2 colorimetric GN card for the identification of clinically relevant gram-negative bacilli showed less than ideal identification rates for *B. pseudomallei*. An improved level of identification of *B. pseudomallei* in comparison to its fluorometric predecessor was noted (7). An interesting aspect of the results was the variable level of acceptable identification when the same isolate was taken from different culture media. A recent study suggested that medium composition was not an important consideration when taking isolates for inoculation into the VITEK 2 system (3). The VITEK 2 Systems product information for the GN card (version 069041-1EN1) lists Trypticase soy agar, Columbia sheep blood agar, and MacConkey agar as the medium types that will give optimal performance (bioMérieux). However, the medium that produced the highest level of acceptable identification in this study was Columbia horse blood agar (81%). Depending on laboratory protocol, this may not be the first choice of medium for the isolation of this organism.

Considerable variability in the reactions obtained from the different media was noticed. While some reactions are very consistent across all media types (e.g., ornithine decarboxylase, lysine decarboxylase, saccharose/sucrose, and D-sorbitol), others are extremely variable (e.g., ellman, urease, and phosphatase). It is therefore not unexpected that variable rates of identification were noted. The complete list of reactions obtained is shown in Table 2.

The increasing use of automation in the laboratory and associated interface to the laboratory or hospital information system means that there is extra reliance on the generation of accurate results by the microbiology laboratory. If, as in this case, the system produces a result listed as very good (93 to 95% probability) or excellent (96 to 99% probability) identification of *B. cepacia* group for an isolate from a respiratory specimen, the laboratory scientist not familiar with the morphology of the organism and perhaps the characteristic susceptibility profile is likely to issue the result, possibly with disastrous consequences for the patient.

TABLE 3. Identification results for all media analyzed using software version VT2-R4.03

Media type	Identification	% Of strains
Columbia horse blood agar	*Burkholderia pseudomallei*	99
	Burkholderia cepacia group	1
Columbia sheep blood agar	*Burkholderia pseudomallei*	99
	Burkholderia cepacia group	1
Trypticase soy agar	*Burkholderia pseudomallei*	99
	Burkholderia cepacia group	1
MacConkey no. 2 agar	*Burkholderia pseudomallei*	97
	Burkholderia cepacia group	2
	Pseudomonas aeruginosa	1

It is imperative that organisms such as *B. pseudomallei*, which are potential bioterrorism agents, are accurately identified by commercial systems. bioMérieux has recognized this as an important issue and is addressing this by altering the algorithm parameters used in the GN identification knowledge base. This improvement will be included in the software release (version 4.03 for VITEK 2 60/XL and version 2.01 for VITEK 2 Compact). Reanalysis of data with the new software has shown a dramatic improvement in identification rates from all media types used. These data are shown in Table 3.

It will be important following the release of this new version of software that it be evaluated in the field, not only with these isolates but also with additional wild strains of this organism and also with closely related organisms to ensure that there has not been a reduction in specificity. If these results are reproducible, the VITEK 2 with the new software version could be recommended for the reliable identification of *B. pseudomallei*.

ACKNOWLEDGMENT

The support of bioMérieux is gratefully acknowledged.

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