Performance of the Sensititre® Gram-Negative Identification Plate (GNID) compared to the Vitek I AutoMicrobic System GNI+ card and the Microscan WalkAway System Dried Neg ID Type 2 panel

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ABSTRACT

A two-site trial was conducted in the U.S.A, to predict the performance of the Sensititre GNID (Gram-Negative Identification plate, Trek Diagnostic Systems Ltd, UK) in correctly identifying *Enterobacteriaceae* and non-*Enterobacteriaceae.* The GNID is an upgrade of the current AP80 AutoIdentification plate, which will be released concurrently with upgraded Sensititre software.

Performance of GNID was compared to two commonly used automated bacterial identification systems for gram-negative bacteria: the Vitek I AutoMicrobic System GNI+ card (bioMerieux Vitek Systems Inc., U.S.A) and the Microscan WalkAway System Dried Neg ID Type 2 panel (Dade Behring, U.S.A). A total of 251 clinical isolates consisting 198 *Enterobacteriaceae* (143 tested on Vitek, 55 on Microscan) and 53 non-*Enterobacteriaceae* (37 tested on Vitek, 16 on Microscan) were tested on GNID to determine overall agreement to the species and genus levels. Where identification results from GNID and another automated system were not in agreement, a manual identification kit (API, bioMerieux, France or IDS, Remel, U.S.A) was used to arbitrate. Where an arbitration result was not conclusive, the isolate was sent to a reference laboratory (Public Health Laboratory Service, Colindale, U.K.) for confirmatory testing.

GNID correctly identified 96% of *Enterobacteriaceae* and 98% of non-*Enterobacteriaceae* to species. The Vitek GNI+ correctly identified 99% of Enterobacteriaceae to species and 95% of non-*Enterobacteriaceae.* Microscan Dried Neg ID Type 2 panel correctly identified 96% of *Enterobacteriaceae* and 94% of non-*Enterobacteriaceae.* Overall performance of GNID was 96% of isolates correctly identified to species and 99% to genus. This compared to Vitek which achieved 98% correct to species and 99% to genus and Microscan which achieved 96% to species and 99% to genus. The performance of GNID compared satisfactorily to the performance of the other two automated systems in correctly identifying clinically significant gram-negative bacteria. The performance of GNID in identifying non-*Enterobacteriaceae* exceeded that of both the Vitek GNI+ and the Microscan Dried Neg ID Type 2 panel.

INTRODUCTION

The Sensititre AP80 AutoIdentification plate (Trek Diagnostic Systems Ltd, U.K.) is an *in-vitro* diagnostic product for the automated identification of common, clinically significant *Enterobacteriaceae* and non-*Enterobacteriaceae.* The plate is part of the Sensititre automated diagnostic system which includes both bacterial identification and antimicrobic susceptibility testing.

The current AP80 plate consists of 32 biochemical tests pre-dosed and dried into a 96-well microtitre plate. The configuration of tests is repeated three times allowing three isolates to be tested on each plate. The tests consist of 22 standard biochemical tests (formulated to allow fluorometric reading) in addition to 10 novel fluorogenic substrates.

Trek Diagnostic Systems Ltd have developed an improved database for use with the new GNID (Gram-Negative Identification plate) which will replace the current AP80 plate. In-house studies have shown the performance of the new database to be 93 to 95% agreement with reference methods for clinically significant, gram-negative bacteria. Results from a study at Limerick Regional Hospital, Eire, using the new GNID plate for *Enterobacteriaceae* and non-*Enterobacteriaceae* found 95% of all isolates were identified correctly to genus and species [1]. This compared favourably to the identification results generated by API 20E/20NE and IDS RapID ONE/RapID NF manual identification products [1].

In addition to the database improvement, a new bi-functional esculin-nitrate test has also been added to the plate to allow offline testing for nitrate reduction to aid the differentiation of *Pseudomonas aeruginosa* from *Pseudomonas fluorescens* isolates.

OBJECTIVE

The objective of this study was to further determine the overall performance of the GNID plate. A two-site clinical trial was conducted in the U.S.A, comparing GNID results with those from one of two other commonly used automated bacterial identification systems.

Previous studies of automated bacterial identification systems found non-fermenters are often less reliably identified than other groups of gram-negative bacteria due to their slower metabolism [2-4]. However, non-*Enterobacteriaceae* are important opportunistic pathogens implicated in nosocomial infections [5-7] and infections of immuno-compromised individuals such as burns victims, cancer patients and patients with cystic fibrosis [8-9]. With the increase in multi-resistant strains of clinically significant species such as *Pseudomonas aeruginosa, Acinetobacter baumanii* and *Stentotrophomonas maltophilia* [5-7] it is essential that clinical laboratories are able to accurately identify these species.

This study describes a two-site trial conducted in the U.S.A to predict the likely performance of GNID in the field.

MATERIALS AND METHODS

Organisms

A total of 251 clinical isolates were obtained for testing from major diagnostic laboratories in the U.S.A. These included Cleveland Clinic, Cleveland, OH (121 isolates), Lahey Clinic, Burlington, MA (71 isolates), US Lab, Boston, MA (39 isolates), St Vincents Medical Centre, New York City, NY (9 isolates) and Worcester Medical Centre, Worcester, MA (10 isolates). All isolates were tested either at Trek diagnostic Systems Inc. (U.S.A) Research and Development facilities (Westlake, OH) or at Lahey Clinic Department of Laboratory Medicine (Burlington, MA). All isolates had previously been identified using either the Vitek AutoMicrobic GNI+ card (bioMerieux Vitek Systems Inc., Hazelwood, MO) or the Microscan WalkAway Dried Neg ID Type 2 panel (Dade Behring, West Sacramento, CA). Isolates were supplied on tryptone soya agar (TSA) with or without 5% sheep blood or MacConkey agar plates and were subcultured overnight at 34-36°C on TSA with 5% sheep blood (BBL, USA).

GNID plate inoculation

GNID plates were inoculated according to the manufacturer's recommendations, using a Sensititre AutoInoculator™ (Trek Diagnostic Systems Ltd, U.K.). Plate details were entered into SAMS (Sensititre Automated Microbiology System) software and plates incubated in a Sensititre ARIS® (Automated Reading and Incubation System) at 34-36°C for 18 hours before reading. A purity plate from each inoculum was set up at the time of testing.

Comparative Methods

The identification generated by the improved software database for GNID was compared to the identification generated by either the Vitek I or Microscan system used by the testing laboratory. When identification results from the GNID were not in agreement with the automated system used in the testing laboratory, the isolate was re-tested on a manual identification system. *Enterobacteriaceae* were tested on either API 20E (bioMerieux, France) or IDS RapID ONE (Remel Inc, USA), non-*Enterobacteriaceae* on API 20NE (bioMerieux, France) or IDS RapID NF (Remel Inc, U.S.A). All bacterial identification systems were set up and read according to the appropriate manufacturer's recommendations.

An identification was assumed to be correct when two or more systems were in agreement (at the 'Acceptable' or equivalent level or above). When a different result was generated from each of the three systems tested, the isolate was sent to a reference laboratory for arbitration (Central Public Health Laboratories, London, U.K.).

Quality Control

Six quality control strains (*Edwardsiella tarda* ATCC 15947, *Klebsiella oxytoca* ATCC 87724, *Morganella morganii* ATCC 25820, *Proteus vulgaris* ATCC 6896, *Pseudomonas aeruginosa* ATCC 10145 and *Shigella sonnei* ATCC 25931) were tested daily, as recommended by the manufacturer. Quality control organisms were supplied as freeze-dried gelatin disks, stored at 2-8°C. To prepare organisms for testing, a single disk was aseptically placed on a blood agar plate and held at 34-36°C for ten minutes to allow the disk to melt. The culture was then streaked across the plate and incubated overnight at 34-36°C. Each organism was sub-cultured twice before testing.

Data analysis

Data collected in the field using GNID plates were run through the improved GNID database. Identification results generated by the GNID database were compared to the other automated systems identification results to determine the performance of the GNID plate.

Table 1
Performance of GNID, Vitek GNI+ and Microscan Dried Neg ID Type 2 panel in identifying Gram-negative bacteria

Organism	Percent Correct GNID (n)	Percent Correct Vitek GNI+ (n)	Percent Correct Microscan Dried Neg ID Type2 (n)
Citrobacter diversus	83 (6)[1]	83 (6)[1]	-
Citrobacter freundii	100 (13)	100 (10)	100 (3)
Enterobacter aerogenes	91 (11)[1]	100 (7)	100 (4)
Enterobacter cloacae	75 (20)[1]	100 (10)	80 (10)[1]
Enterobacter gergoviae	75 (4)[1]	-	100 (4)
Escherichia coli	100 (30)	100 (30)	-
Klebsiella oxytoca	100 (6)	100 (3)	100 (3)
Klebsiella pneumoniae	100 (44)	100 (24)	100 (20)
Morganella morganii	100 (7)	100 (7)	-
Panteoa agglomerans	100 (1)	100 (1)	-
Proteus mirabilis	100 (34)	100 (24)	100 (10)
Proteus vulgaris	100 (2)	100 (1)	100 (1)
Providencia stuartii	100 (9)	100 (9)	-
Salmonella species	100 (1)	100 (1)	-
Serratia marcescens	100 (10)	100 (10)	-
Total *Enterobacteriaceae*	**96 (198)**	**99 (143)**	**96 (55)**
Acinetobacter baumanii	100 (4)	100 (4)	-
Alcaligenes xylosoxidans	0 (1)	-	0 (1)[1]
Pseudomonas aeruginosa	100 (41)	94 (31)	100 (10)
Stenotrophomonas maltophilia	100 (7)	100 (2)	100 (5)
Total non-*Enterobacteriaceae*	**98 (53)**	**95 (37)**	**94 (16)**
Total Gram-Negative (species)	**96 (251)**	**98 (180)**	**96 (71)**
Total Gram-Negative (genus)	**99 (251)**	**99 (180)**	**99 (71)**

[1] 100% correct to genus
[2] 95% correct to genus
[3] 90% correct to genus

- Not tested on that identification system

Table 2 Discrepancies between systems

Final ID Designation	Vitek Result	Microscan Result	GNID Result	API Result	IDS Result	Reference Lab Result
Alcaligenes xylosoxidans	-	*Alcaligenes species*[a]	*Acinetobacter lwoffii*[a]	*Alcaligenes xylosoxidans*	-	*Alcaligenes xylosoxidans*
Pseudomonas aeruginosa	*Alcaligenes xylosoxidans*[a]	-	*Pseudomonas aeruginosa*	*No ID*[a]	-	*Pseudomonas aeruginosa*
Pseudomonas aeruginosa	*Alcaligenes xylosoxidans*[a]	-	*Pseudomonas aeruginosa*	*Ralstonia picketti*[a]	-	*Pseudomonas aeruginosa*
Citrobacter diversus	*Citrobacter freundii*[a]	-	Low Selectivity *Citrobacter diversus*[a]	*Citrobacter diversus*	-	*Citrobacter diversus*
Enterobacter gergoviae	-	*Enterobacter gergoviae*	*No ID*[a]	Genus *Enterobacter gergoviae*[a]	*Enterobacter sakazaki*[a]	*Enterobacter gergoviae*
Enterobacter aerogenes	*Enterobacter aerogenes*	-	Low Selectivity *Enterobacter aerogenes*[a]	-	*Enterbacter aerogenes*	-
Klebsiella pneumoniae	*Enterobacter cloacae*[a]	-	*Klebsiella pneumoniae*	-	*Klebsiella pneumoniae*	-
Enterobacter cloacae	*Enterobacter cloacae*	-	Excellent Group *Enterobacter species*[a]	*Enterobacter cloacae*	-	-
Enterobacter cloacae	*Enterobacter cloacae*	-	Excellent Group *Enterobacter species*[a]	*Enterobacter cloacae*	-	-
Enterobacter cloacae	*Enterobacter cloacae*	-	Excellent Group *Enterobacter species*[a]	*Enterobacter cloacae*	-	-
Enterobacter cloacae	*Enterobacter cloacae*	-	Excellent Group *Enterobacter species*[a]	*Enterobacter cloacae*	-	-
Enterobacter cloacae	*Enterobacter cloacae*	-	*Enterobacter amnigenus*[a]	*Enterobacter cloacae*	-	-
Enterobacter cloacae	-	*Kluyvera species*[a]	*Enterobacter cloacae*	*Enterobacter aerogenes*[a]	*Enterobacter asburiae*[a]	*Enterobacter cloacae*
Enterobacter cloacae	-	*Kluyvera species*[a]	*Enterobacter cloacae*	*Enterobacter aerogenes*[a]	*Enterobacter asburiae*[a]	*Enterobacter cloacae*

[a] Mis-identification (i.e. not correct to species at the 'Acceptable' level or equivalent)
- Not tested

RESULTS

Performance of GNID in correctly identifying members of the *Enterobacteriaceae* was 96% of isolates tested (n = 198) correct to species and 99% to genus. This compares to 99% of isolates tested (n = 143) correct to species/genus for Vitek GNI+ and 96% (n = 55) to species and 98% to genus for Microscan Dried Neg ID Type 2 panel.

Performance of GNID in correctly identifying non-*Enterobacteriaceae* was 98% to species (n = 53). This compares to 95% (n = 37) for Vitek GNI+ and 94% (n = 16) for Microscan Dried Neg ID Type 2 panel.

Overall total performance of GNID was 96% of isolates (n = 251) correctly identified to species level and 99% to genus. This compared to Vitek GNI+ which achieved 98% correct (n = 180) to species and 99% to genus and Microscan Dried Neg ID Type 2 panel which achieved 96% to species (n = 71) and 99% to genus (Table 1).

Details of isolates mis-identified by GNID, Vitek or Microscan are given in Table 2.

DISCUSSION

The performance of the new GNID plate in identifying clinically significant gram-negative bacteria was comparable to the performance of two other commercially available automated bacterial identification systems tested in this study. From the data presented, the GNID performed as well as the other systems already widely used in the field, and exceeded their performance in correctly identifying commonly encountered non-*Enterobacteriaeae.*

Overall performance of 96% to species level compares well to the overall performance of the Vitek GNI+ (98%) and Microscan Dried Neg ID Type 2 panel (96%). These data also reinforce the 95% agreement to species level obtained in a clinical trial conducted at Limerick Regional Hospital, Eire, compared to API 20E/20NE (74% agreement) and IDS RapID ONE/RapID NF (88% agreement) manual identification products [1].

Performance of the GNID in correctly identifying non-*Enterobacteriaceae* exceeded its performance on *Enterobacteriaceae*, achieving 98% to species level, compared to 95% on Vitek GNI+ and 94% on Microscan Dried Neg ID Type 2 panel. This compares to the 92% for Vitek GNI+ and 96% for Microscan reported by Sung *et al*. [10] for non-*Enterobacteriaceae*.

It is extremely important that members of non-*Enterobacteriaceae* can be accurately identified due to their significance as opportunistic pathogens [5-9] and their increasing levels of resistance to antimicrobics [5-7]. GNID correctly identified all isolates of *Acinetobacter baumanii, Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* tested. The one non-*Enterobacteriaceae* mis-identified by GNID was an *Alcaligenes xylosoxidans* isolate which identified as *Acinetobacter lwoffii* due to its slow growth and consequent biochemical inactivity. This isolate was not identified to species level by Microscan Dried Neg ID Type 2 panel (Table 2). Many comparative studies of automated bacterial identification systems have found identification of non-fermentative organisms to be less reliable than that of *Enterobacteriaceae* due to their slow metabolism [2-4, 11].

Overall, the performance of the GNID in identifying *Enterobacteriaceae* was good, comparing satisfactorily to the other systems. GNID achieved 96% compared to 99% for Vitek GNI+ and 96% for Microscan Dried Neg ID Type 2.

Eight *Enterobacteriaceae* out of 198 tested were mis-identified by GNID. One *Citrobacter diversus* and one *Enterobacter aerogenes* identified as such but only at the level of 'Low Selectivity' and one *Enterobacter gergoviae* yielded No Identification Possible. Of the five remaining isolates, four *Enterobacter cloacae* isolates identified as Excellent group *Enterobacter* species and one as *Enterobacter amnigenus* (Table 2).

The GNID plate and the improved Sensititre software should offer the clinical microbiologist a flexible, efficient means of identifying *Enterobacteriaceae* and non-*Enterobacteriaceae*. The GNID plate can test up to three isolates, with unique fluorescent test substrates providing high resolution results with excellent differentiating abilities. Windows-based interactive software provides the user with greater flexibility, by giving them the ability to enter common additional tests results such as indole, oxidase, nitrate reduction, pigment, motility and growth on MacConkey agar at any stage during isolate processing.

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