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Comparison of a Molecular Multiplex Panel to API® RapiD Strips for Bacterial Identification

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Abstract

Background: Conventional microbiological methods are the gold standard for bacterial identification (ID) in clinical laboratories. While accurate, these phenotypic and biochemical tests are laborious and time-consuming. Molecular methods of ID are as sensitive and specific as conventional methods and take hours instead of days for result reporting. Rapid diagnosis is increasingly important for patient therapy where laboratory results can alter treatment. In this study, we compare the performance of Target Enriched Multiplex PCR (TEM-PCR™) to gold-standard API® RapiD 20 E™ strips (BioMérieux, Marcy-l'Étoile, France) for bacterial ID.

Methods: Fifty-two well-characterized, blinded isolates were obtained from IHMA, Inc. Isolates were cultured on non-selective media, and 0.5 McFarland standards were prepared. API® RapiD 20 E™ strips were inoculated for ID of *Enterobacteriaceae*. Simultaneously, nucleic acids were extracted using a KingFisher™ Flex instrument (Thermo Fisher Scientific, Waltham, MA) and amplified with TEM-PCR™, utilizing a panel of fourteen gram-negative and gram-positive pathogen targets. Amplified products were hybridized to target probes on microarray plates and detected using a fluorescence imaging reader. Results were reported as "Detected" or "Not Detected". For true method comparison, clinical specimens submitted to Diatherix Laboratories for routine GNEG Panel testing were selected for testing on API® strips.

Results: Bacterial ID of each isolate was confirmed with IHMA upon completion of TEM-PCR™ and API® testing. Results obtained with TEM-PCR™ had 100% correlation with API® testing for targets present in the TEM-PCR™ panel. Gene targets for *Providencia rettgeri* and *Serratia marcescens* were not included in the TEM-PCR™ panel, therefore not detected, yielding an overall correlation of 96% between the two methods.

Conclusions: Molecular and microbiological techniques are both accurate means for determining bacterial ID, but molecular multiplex methods have added benefits over traditional gold standard testing. TEM-PCR™ is performed directly from the patient specimen and can detect multiple pathogens in a single reaction. Bacterial ID from API® strips takes at least two days, while molecular tests can provide results in less than eight hours, eliminating empirical antibiotic therapy and resulting in faster, more accurate patient treatment.

*Abstract has been amended to include additional data collected after submission.

Introduction

Conventional microbiological methods are the gold standard for bacterial identification (ID) in clinical laboratories. While accurate, these phenotypic and biochemical tests can be subjective, laborious, and time-consuming. Bacterial culture and purified isolates are required for phenotypic testing, which can be complicated by polymicrobial infections. Furthermore, methods that rely on growth of organisms can yield false negative results if antibiotic therapy has been initiated. API® strips are the established reference technique worldwide, used to assess the performance of other ID methods. In this study, we compare the performance of Target Enriched Multiplex PCR (TEM-PCR™) to gold-standard API® RapiD 20 E™ strips (BioMérieux, Marcy-l'Étoile, France) for bacterial ID. Molecular methods of ID are as sensitive and specific as conventional methods and take hours instead of days for result reporting. Rapid and accurate diagnosis is increasingly important for patient therapy where laboratory results can alter treatment.

Materials & Methods

Samples

- Fifty-two blinded, gram-negative bacterial isolates were received from International Health Management Associates, Inc. (IHMA, Schaumburg, Illinois). Isolates were cultured on Tryptic Soy Agar plates (Teknova, Hollister, CA), and incubated for 16-18 hours at 37°C. Post-incubation, 0.5 McFarland suspensions were immediately prepared for each isolate. Bacterial ID of each isolate was confirmed with IHMA upon completion of API® strip and TEM-PCR™ testing.
- Eleven routine clinical samples submitted for testing on the GNEG Panel, with subsequent positive bacterial detection, were selected for culture and testing on API® strips as per manufacturer protocol.

API® RapiD Strip Testing

- API® RapiD 20 E™ strips were inoculated with 50 µL of 0.5 McFarland bacterial suspensions to reconstitute the dehydrated substrates within the microtubes of the test strip. Strips were incubated for 4.5 hours at 37°C, according to manufacturer's recommendations.
- During incubation, metabolism of the substrates produces color changes that are either spontaneous or revealed by the addition of catalysts added post-incubation: VP 1 and VP 2, JAMES, and Oxidase reagents for the processing of the Voges-Proskaeur, Indole, and Oxidase tests, respectively.
- After final processing, each biochemical test result was immediately read and recorded on result sheets according to the Reading Table provided by the vendor. Identification of each isolate was obtained by referring to the apiweb™ identification software (*https://apiweb.biomerieux.com*).

Materials and Methods *(continued)*

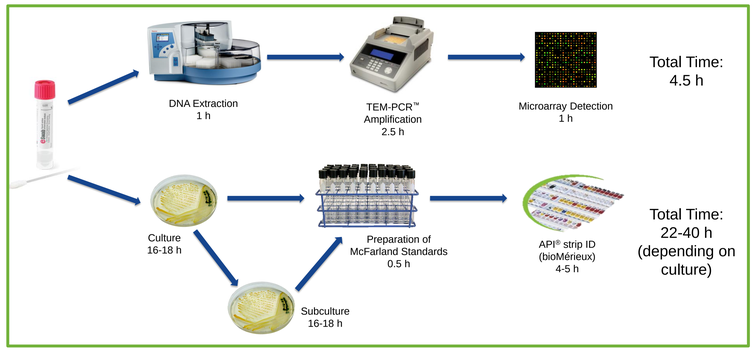

Figure 1. Method comparison between API® RapiD strip and TEM-PCR™ procedures.

TEM-PCR™ Testing

DNA Extraction

- Nucleic acids were extracted from McFarland suspensions and routine clinical samples using an in-house developed method, utilizing reagents from Omega Bio-Tek, Inc. (Norcross, GA) and MO BIO Laboratories, Inc. (Carlsbad, CA) on a KingFisher™ Flex semi-automated, magnetic, bead-based platform. With this method, nucleic acid extraction is accomplished for up to 96 samples per instrument in less than one hour.

Target Enriched Multiplex Polymerase Chain Reaction (TEM-PCR™)

- TEM-PCR™ is a highly multiplexed, nested, end-point PCR technique, in which the primer mix contains two pairs of gene-specific primers for each target. Inside nested primers have a unique tag sequence complementary to proprietary superprimers, also included in the primer mix. TEM-PCR™ cycling includes initial enrichment and tagging of each target, followed by traditional PCR amplification (Figure 2).

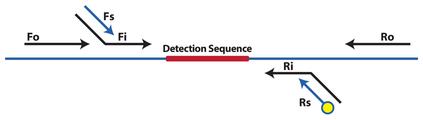

Figure 2. TEM-PCR™ scheme. Low concentrations of nested gene-specific primers (Fo – forward out; Fi – forward in; Ri – reverse in; and Ro – reverse out) are designed to enrich the genetic targets during the initial PCR cycles. Later in the procedure, a pair of universal SuperPrimers (Fs and Rs) are used to amplify all targets. The reverse SuperPrimer is labeled with biotin. Target-specific biotinylated PCR products are detected with a complimentary detection probe which is covalently coupled to a glass microarray.

- Isolated DNA from each isolate was amplified with the Diatherix GNEG TEM-PCR™ Panel (Figure 3). The GNEG reagent panel contains sixty-seven primers for the detection of the fourteen pathogenic organisms and two genes responsible for vancomycin resistance.

Acinetobacter baumannii	*Enterococcus faecium*	*Pseudomonas aeruginosa*
Citrobacter freundii	*Escherichia coli*	*Proteus mirabilis*
Enterobacter aerogenes	*Klebsiella oxytoca*	*Proteus vulgaris*
Enterobacter cloacea	*Klebsiella pneumoniae*	*Staphylococcus saprophyticus*
Enterococcus faecalis	*Morganella morganii*	Vancomycin resistance (*vanA*/*vanB*)

Figure 3. Diatherix TEM-PCR™ GNEG Panel.

TEM-PCR™ Hybridization and Detection

- Amplified TEM-PCR™ products were hybridized to target detection probes on custom microarray plates (Microarrays, Inc., Huntsville, AL). Streptavidin-phycoerythrin conjugate (Moss, Inc., Pasadena, Maryland) was added to the hybridization reaction, followed by a series of wash steps.
- Fluorescence signals were analyzed on a SensoSpot Fluorescence Low Density Microarray Analyzer (Sensovation, Radolfzell, Germany).
- Results for each target were reported as "Detected" or "Not Detected" based on signal intensity above background.

Results

Table 1. Comparison of bacterial ID results between gold-standard API® RapiD 20 E™ strips and TEM-PCR™ using blinded isolates. API® test results indicated additional testing recommended for 34.6% of isolates (shown in red). TEM-PCR™ failed to detect two isolates.

Sample Number	Organism ID	Identification by API® RapiD 20 E™	Detected by TEM-PCR™
1	*Klebsiella pneumoniae*	Good	✓
2	*Klebsiella pneumoniae*	Very Good	✓
3	*Klebsiella pneumoniae*	Very Good	✓
4	*Pseudomonas aeruginosa**	Very Good	✓
5	*Pseudomonas aeruginosa**	Very Good	✓
6	*Pseudomonas aeruginosa**	Very Good	✓
7	*Escherichia coli*	Very Good	✓
8	*Klebsiella pneumoniae*	Low Discrimination	✓
9	*Proteus mirabilis*	Very Good	✓
10	*Pseudomonas aeruginosa**	Doubtful Profile	✓
11	*Klebsiella pneumoniae*	Very Good	✓
12	*Klebsiella pneumoniae*	Very Good	✓
13	*Pseudomonas aeruginosa**	Very Good	✓
14	*Pseudomonas aeruginosa**	Very Good	✓
15	*Escherichia coli*	Good	✓
16	*Klebsiella pneumoniae*	Good	✓
17	*Pseudomonas aeruginosa**	Very Good	✓
18	*Klebsiella pneumoniae*	Good	✓
19	*Escherichia coli*	Very Good	✓
20	*Escherichia coli*	Good	✓
21	*Pseudomonas aeruginosa**	Very Good	✓
22	*Klebsiella pneumoniae*	Low Discrimination	✓
23	*Providencia rettgeri*	Excellent	
24	*Klebsiella pneumoniae*	Low Discrimination	✓
25	*Acinetobacter baumannii**	Very Good	✓
27	*Klebsiella pneumoniae*	Very Good	✓
28	*Klebsiella pneumoniae*	Very Good	✓
29	*Pseudomonas aeruginosa**	Doubtful Profile	✓
30	*Klebsiella pneumoniae*	Very Good	✓
31	*Pseudomonas aeruginosa**	Doubtful Profile	✓
32	*Klebsiella pneumoniae*	Good	✓
33	*Pseudomonas aeruginosa**	Doubtful Profile	✓
34	*Escherichia coli*	Good	✓
35	*Escherichia coli*	Acceptable	✓
36	*Escherichia coli*	Very Good	✓
37	*Klebsiella pneumoniae*	Low Discrimination	✓
38	*Klebsiella pneumoniae*	Low Discrimination	✓
39	*Klebsiella pneumoniae*	Low Discrimination	✓
40	*Escherichia coli*	Doubtful Profile	✓
41	*Serratia marcescens*	Good	
42	*Escherichia coli*	Good	✓
43	*Klebsiella pneumoniae*	Low Discrimination	✓
44	*Klebsiella pneumoniae*	Low Discrimination	✓
45	*Klebsiella pneumoniae*	Low Discrimination	✓
46	*Klebsiella pneumoniae*	Low Discrimination	✓
47	*Pseudomonas aeruginosa**	Doubtful Profile	✓
48	*Proteus mirabilis*	Unacceptable Profile	✓
49	*Klebsiella pneumoniae*	Low Discrimination	✓
50	*Klebsiella pneumoniae*	Good	✓
51	*Klebsiella pneumoniae*	Very Good	✓

*API® RapiD 20 E™ strips are not recommended for testing of non-*Enterobacteriaceae*

Table 2. Blinded isolate testing with API® strips correctly identified 100% of samples; however, 18/52 isolates produced presumptive or uncertain results. TEM-PCR™ testing failed to identify two isolates which were not targeted by the GNEG Panel.

	API® RapiD 20 E™	TEM-PCR™
No. of bacterial isolates tested	52	52
% isolates correctly detected	100%	96%
% False Positives	0%	0%
% False Negatives	0%	4%

Sample Number	Identification by API® RapiD 20 E™	Detected by TEM-PCR™
15024	*Providencia stuartii* (Low Discrimination)	*Enterobacter cloacea*
08283	*Escherichia coli* or *Salmonella spp.* or *Kluyvera spp.* (Doubtful Profile)	*Escherichia coli*, *Klebsiella pneumoniae* & *Proteus mirabilis*
99682	ID Not Valid	*Escherichia coli*
25225	Unacceptable Source	*Enterobacter cloacea*
41868	*Proteus mirabilis* (Good)	*Enterococcus faecium* & *Proteus mirabilis*
90570	*Enterobacter aerogenes* (Very Good)	*Enterobacter aerogenes*
08285(A)	*Citrobacter freundii* (Good)	*Citrobacter freundii*, *Enterococcus faecalis*, *Klebsiella spp.*, & *Staphylococcus saprophyticus*
08285(B)	*Klebsiella pneumoniae* or *Enterobacter aerogenes* (Low Discrimination)	*Klebsiella oxytoca*
62850	*Klebsiella oxytoca* (Good)	*Klebsiella oxytoca*
90569	*Proteus mirabilis* or *Morganella morganii* or *Yersinia enterocolitica* (Low Discrimination)	*Pseudomonas aeruginosa*

Table 3. Clinical comparison. Eleven clinical samples submitted to Diatherix Laboratories for routine GNEG reagent testing were selected for culture and testing on API® strips. Two samples failed to yield any bacterial growth, thus could not be tested. One sample, 08285, had two colony types and was subcultured. Each colony type was tested using individual API® strips.

Conclusions

- This study has shown that gold-standard microbiological and molecular techniques are both accurate means for determining bacterial ID, but molecular multiplex methods have additional benefits over traditional gold standard testing.
- Bacterial ID from API® strips takes at least two days, requiring pure culture and selection of isolates prior to testing. Test methodology and results interpretation can be subjective, depending on the skill level of the technician, leading to incorrect or missed diagnoses. Additionally, complementary tests not included in the API® system are often recommended for samples with low discrimination or uncertain IDs.
- Molecular tests such as TEM-PCR™ can provide results directly from the patient specimen in about five hours, eliminating the need for culture, which results in faster and more accurate patient treatment.
- TEM-PCR™ can accurately detect polymicrobial infections with high sensitivity and specificity. In comparison, several API® strips would have be used following additional days of subculture and incubation to obtain the same information.
- API® strips failed to identify TEM-PCR™ detected organisms in 80% of the clinical samples tested in this study. Comparison data indicated a 33% correlation between methods when only one organism was identified with TEM-PCR™ (2/6 samples). Four samples showed no correlation of results, due to invalid or low discrimination results with API® strips. When polymicrobial infections were indicated with TEM-PCR™, at least one organism was simultaneously detected with 100% of the API® strips tested on these samples. However, testing with API® RapiD 20 E™ strips alone would have missed several potential pathogens, which could lead to poor outcomes in patient therapy.

Acknowledgements

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