Simultaneous Identification of Select Microbial Pathogens and Determination of Antimicrobial Resistance by a Novel Assay

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ABSTRACT

Background: In a recent IDSA public policy released last year, concern was raised regarding the "overuse of our small inventory of effective antimicrobials," which the authors ascribe to the empiric use of broad-spectrum antibiotics instead of "therapy directed by the rapid identification of the infectious agent."[1] The statement places emphasis on the need for the further development of rapid tests for use in diagnosing infectious agents. Here we report the application of a newly developed test which allows for the simultaneous identification of a microbe and determination of its susceptibility to select antimicrobials.

Materials: Group B streptococcus (GBS), *Enterococcus faecalis* and *Enterococcus faecium* were purchased from ATCC. Serial dilutions were prepared, either in the presence or absence of clindamycin (for GBS) or vancomycin (for Enterococcus). Samples were either processed immediately or after an 18-hour incubation at 37ºC in enrichment broth. Microtiter wells had been previously coated with antibodies directed against the specific pathogen and then blocked. Aliquots of the specific pathogen/antibiotic dilution were added to the wells for 30 minutes. After washing with saline, horse-radish peroxidase labeled antibody was added. After washing, bound antibody was detected at 450 nm. Competition studies were performed in which the specified pathogen, either GBS, *E. faecalis* or *E. faecium*, was serially diluted while the level of a second pathogen was held constant at 10^7 bacteria/ml. Competing bacteria studied included *Escherichia coli*, methicillin-resistant *Staphylococcus aureus*, GBS (for Enterococcus studies) or *E. faecalis* (for GBS studies).

Results: Immediate processing of selected pathogens allowed detection at 10^5 bacteria/well. After an 18-hour incubation, the sensitivity increased to 10^{-1} bacteria/well. In addition, antimicrobial susceptibility of each organism was determined after an 18-hour incubation period.

Conclusion: This test allows for the rapid identification of GBS, *E. faecalis* and *E. faecium*, in 30 minutes for an inoculum greater than 10^5 bacteria/ml. For lighter bacterial burden, more time is required to detect these more dilute specimens. This added incubation step, however, allows for the simultaneous determination of antimicrobial susceptibility.

METHODS

- Dilution Curve was prepared:
  - Bacteria (GBS, *E. faecalis*, or *E. faecium*) were diluted serially, starting with 0.5 McFarland
  - OD_{600} was determined per dilution, and plates were inoculated for 24-48 hour growth
  - CFU/ml was determined at each dilution, and correlated with OD_{600}
- Microtiter wells were prepared by coating wells with antibody directed against GBS/Enterococcus, and then blocked
- Bacterial inocula were diluted out serially, and then aliquoted into wells for the 30 min test, OR
- Bacterial inocula were prepared in the presence/absence of select antibiotic, and then aliquots were placed into microtiter wells after a 18-hour incubation.
- After washing, HRP-conjugated antibody against specific bacteria was added, and OD450 was read

RESULTS

Fig 1 A

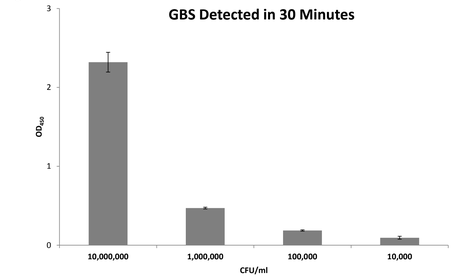


Fig 2 A

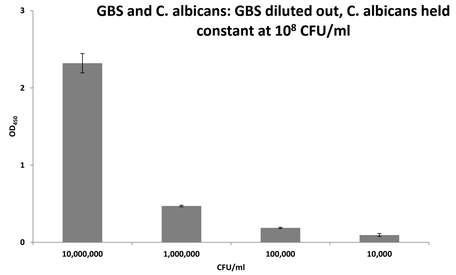


Fig 1 B

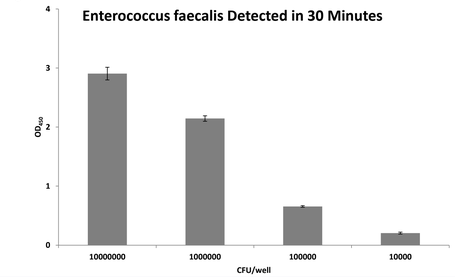
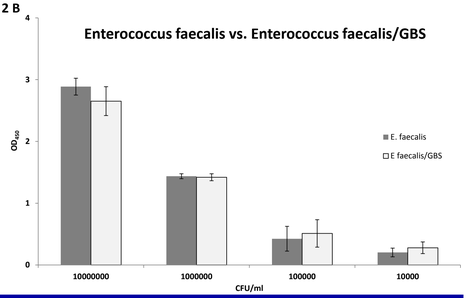


Fig 2 B



GBS	Without Clindamycin Present					With Clindamycin Present				
	10,000	1,000	100	10	1	10,000	1,000	100	10	1
Clindamycin Resistant	+	+	+	+	-	+	+	+	+	-
Clindamycin Sensitive	+	+	+	+	-	-	-	-	-	-

-Inocula are CFU/ml. Results deemed positive if OD_{450} > 1.0, negative if <0.5 after 18-hour incubation

Enterococcus	Without Antibiotic Present					With Antibiotic Present				
	100	10	1	-1	-10	100	10	1	-1	-10
Vancomycin Resistant	+	+	+	+	-	+	+	+	+	-
Vancomycin Sensitive	+	+	+	+	-	-	-	-	-	-

-Inocula are CFU/ml. Results deemed positive if OD_{450} > 1.0, negative if <0.5 after 18-hour incubation
-Vancomycin Resistant Enterococcus = E. faecium
-Vancomycin Sensitive Enterococcus = E. faecalis

CONCLUSIONS

- This assay allows for the rapid detection of a heavy inoculum of GBS or Enterococcus in 30 minutes
- Detection of lighter inocula is possible following an 18-hour incubation
- Antibiotic susceptibility may be determined within this 18-hour incubation time-period
- By substituting the capture and detection antibodies for antibodies against other pathogens, such as Neisseria gonorrhea, the assay can easily be extended to detect other pathogens of interest
- This test may be capable of detecting de novo resistance, as it detects growth of bacteria in the presence of selected antibiotics
- Application of this test in a clinical setting may facilitate more directed approach to treating clinical infections

ACKNOWLEDGEMENTS

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- The assay described in this work is licensed by Nanologix under the N-Assay trademark

REFERENCES

Caliendo AM, Gilbert DN, Ginocchio CC, Hanson KE, May L, Quinn TC, et al.; Infectious Diseases Society of America (IDSA). Better tests, better care: Improved diagnostics for infectious diseases. *Clin Infect Dis*. 2013 Dec;57 Suppl 3:S139-70