



Rigor and reproducibility of *Clostridioides difficile* susceptibility testing

Poster# 401

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BACKGROUND

- The Clinical Laboratory Standards Institute (CLSI) recommends minimum inhibitory concentration (MIC) testing for *C. difficile* through agar dilution (AD) assay, which carries logistical and time burdens compared to broth microdilution (BMD) methods.
- Rigor and reproducibility (R&R) of these assays can lead to difficulty in comparing results between studies.
- Aim to assess the intra-and inter-laboratory reproducibility of MIC testing for *C. difficile* within our lab and colleagues.

MATERIALS & METHODS

Sample / Microbiology:

- Discard stool samples transported to our centralized lab
- Stool plated on selective cefoxitin-cycloserine-fructose agar (CCFA) plates and anaerobically incubated for 48 – 72 hours

Comparison of MIC Assays:

- Vancomycin MIC testing conducted via AD and BMD in accordance with CLSI guidance
- Reduced vancomycin susceptibility was defined by MIC > 2 mg/L

Intra-laboratory Reproducibility:

- Proficiency testing with 18 isolates was implemented across 2 multi-disciplinary labs
- Essential agreement (EA) and major and minor error rates were calculated

Cost Efficiency & Reproducibility Analysis

- Media reagents cost analysis and comparative AD MIC results

Automation Implementation:

- Integra Assist Plus® versus technician assay oxygen exposure time
- Integra Assist Plus® - automated pipetting machine that has some of the capabilities of a Liquid Handler at a lower price
- A program was designed to reduce the possibility of human error and decrease the time needed to aliquot samples

RESULTS

Comparison of MIC Assays

- AD vs BMD methods with 30 isolates resulted in EA of 0% (Table 1)

Table 1.

Assay (n)	MIC (mg/L)			% Resistant	Major Error (%)	Very Major Error (%)	Essential Agreement (%)
	Range	MIC ₅₀	MIC ₉₀				
AD (30)	0.05 - >16	1	1	6.67%	0.0%	6.67%	0.0%
BMD (30)	0.0625 - 0.05	0.125	0.25	0.0%			

Major error: resistant results by the new method and susceptible results by the gold standard method; **Very major error:** susceptible result by the new method and a resistant result by the gold standard method; **Essential agreement:** MICs within ± 1 dilution

Intra-laboratory Reproducibility

- Intra-lab comparison of AD MICs yielded EA of 88.9% (16/18)
- Optimization between labs identified different drug manufacturer of vancomycin being used as well as one bottle being much older although not expired – EA of 100% after both labs used vancomycin from Sigma-Aldrich
- R&R was validated in a larger cohort between the two labs where EA of 93.9% (109/116) was found (Table 2)

Table 2.

Lab (n)	MIC (mg/L)						Essential Agreement (%)	
	Range		MIC ₅₀		MIC ₉₀			
Lab 1 (18)	2 - 8	1 - 4	4	2	8	2	88.9%	100%
Lab 2 (18)	1 - 4		2		4			

*Lab 1 results from before (left) and after (right) rigor plan of action

Cost Efficiency and Reproducibility Analysis in Media Reagents

- 30 isolates were analyzed via AD methods using different brands of media
- Overall, there were no observed differences observed across the different media brands utilized in for MIC obtainment by AD methodology (Table 3)

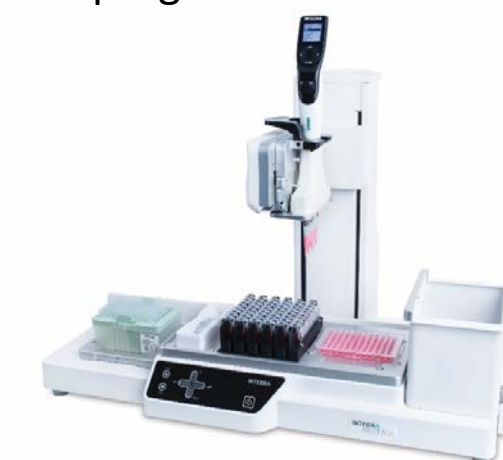
Table 3.

Brand	BD®	Remel®	Criterion®
Cost per bottle (500g)	\$346.80	\$280.80	\$105.98
Cost per preparation	\$29.82	\$24.15	\$9.11
Number of plates	25	25	25
Cost of testing 1 isolate at 1 concentration	\$0.019	\$0.015	\$0.006

RESULTS CONT.

Automation Implementation

- A total of 10'4" minutes were saved using automation (12'44" mean) versus a technician (24'40" mean)
- Supplemental handout / video available for automation program observance



CONCLUSION

- Here we present the process undertaken to ensure the rigor and reproducibility of *C. difficile* susceptibility testing.
- We developed a procedure with accurate results from two labs, minimized cost, and lowered time required.
- Future research will include validation with a larger sample and more academic partners.

REFERENCES & APPENDIX

-Integra Biosciences Assist Plus Pipetting Robot
-BD BBL™ Dehydrated Culture Media: Brucella Agar BD 211086 – Fisher Scientific
-Remel™ Brucella Agar R452652 – Fisher Scientific
-CRITERION™ Brucella Agar, Dehydrated Culture Media - Hardy Diagnostics
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