

C-170

EVALUATION OF AUTOMATION FOR STREAKING PRIMARY MEDIA INOCULATED WITH CLINICAL SPECIMENS

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INTRODUCTION

InocuLAB (Dynacon Inc, Mississauga, Canada) is a fully automated system for the processing (inoculating, streaking and barcoding) of urine specimens on media for semi-quantitative assessment. The instrument also operates in a 'streak mode' for streaking, using a conventional four-quadrant pattern, media that has been inoculated from other specimen types. InocuLAB was evaluated in streak mode for carry over of bacteria between specimens, comparable results to manual streaking and for labor saving with this automated approach.

MATERIAL AND METHODS

To assess carry over between specimens

One hundred blood agar plates were inoculated with stool specimens and alternated interspaced with uninoculated BAP media plates and then streaked automatically by InocuLAB. The BAPs were incubated at 35°C in 5% CO₂ for 48 hours.

Colony isolation comparison

1) Simulated specimens

0.1mL of an 18-hour culture of each of the above specimens was added to 5 mL of trypticase soy broth in the following combinations:

- a) *E. coli*, *P. aeruginosa* and *S. bovis*
- b) *S. pyogenes* and *S. aureus*
- c) *S. epidermidis*, *K. pneumoniae* and *S. pneumoniae*
- d) *S. aureus* and *E. coli*
- e) *S. aureus*, *E. coli* and *S. bovis*

The following ATCC strains were used:

Escherichia coli ATCC 25922,
Klebsiella pneumoniae ATCC 700603,
Pseudomonas aeruginosa ATCC 27853
Streptococcus bovis ATCC 49147,
Streptococcus pneumoniae ATCC 49619,
Staphylococcus aureus ATCC 291213,
Staphylococcus epidermidis ATCC 12228
Streptococcus pyogenes ATCC 12384

Procedure

One microliter (1µL) of the above combination of organisms was inoculated on blood, chocolate and MacConkey agar plates. Two sets of media were streaked manually and by InocuLAB in duplicate. All media were incubated at 35°C in 5% CO₂ for 48 hours. The cultures were evaluated based on growth in the 1st, 2nd, 3rd, and the 4th quadrant.

2) Clinical specimens

- a) The swabs of 9 specimens submitted for the isolation of a specific organism were vortexed in 1.0 mL of trypticase soy and 1µL was inoculated on blood, chocolate and MacConkey agar plates and streaked manually and by InocuLAB. This procedure was completed in duplicate creating 108 media plates to be compared. All media were incubated at 35°C in 5% CO₂ for 48 hours. The cultured media was evaluated based on growth in the 1st, 2nd, 3rd, and the 4th quadrant.

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- b) Twenty-eight (28) sputum specimens were inoculated on blood, chocolate and MacConkey agar plates and streaked manually and by InocuLAB. This procedure was completed in duplicate. All media were incubated at 35°C in 5% CO₂ for 18 – 24 hours. The cultured media was evaluated based on growth in the 1st, 2nd, 3rd, and the 4th quadrant.
- c) Wound specimens: 148 wound specimens were inoculated on colistin nalidixic acid (CNA), chocolate and MacConkey agar and streaked both manually and using InocuLAB. All media were incubated at 35°C in 5% CO₂ for 48 hours and evaluated for the number of isolated colonies. The manually streaked plates were processed first.
- d) Genital specimens: Eight five (85) genital were inoculated on CNA and human blood agar. All media were incubated at 35°C in 5% CO₂ for 48 hours and evaluated for the number of isolated colonies. The manually streaked plates were processed first.

Labor savings

- 1) The time to label, inoculate and streak human blood agar and CNA blood agar plates and to label, inoculate and load InocuLAB was determined.
- 2) The time to label, subculture and streak 100 18 hour Todd Hewitt broth culture and to label, inoculate and load InocuLAB was determined.

RESULTS

To assess carry over between specimens

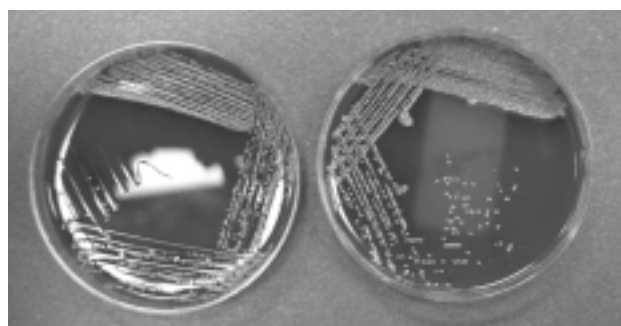
There was no growth for all 100 uninoculated BAPs after incubation for 48 hours at 35°C in 5% CO₂.

Colony isolation comparison

1) Simulated specimens.

0.1mL of an 18-hour culture of each of the above specimens was added to 5 mL of trypticase soy broth in the following combinations:

- a) *E. coli*, *P. aeruginosa* and *S. bovis*
- b) *S. pyogenes* and *S. aureus*
- c) *S. epidermidis*, *K. pneumonia* and *S. pneumoniae*
- d) *S. aureus* and *E. coli*
- e) *S. aureus*, *E. coli* and *S. bovis*



Agar plate on the left was manually streaked, plate on right streaked by InocuLAB

Organisms	Manually Last quadrant isolated growth			InocuLAB Last quadrant isolated growth		
	Blood	Chocolate	MacConkey	Blood	Chocolate	MacConkey
A	3th	3th	3th	4th	4th	4th
B	3rd	3rd	3rd	4th	4th	4th
C	<i>K. pneumoniae</i> 3rd <i>S. epidermidis</i> 3rd <i>S. pneumoniae</i> 2nd	<i>K. pneumoniae</i> 3rd <i>S. epidermidis</i> 3rd	<i>K. pneumoniae</i> 3rd	<i>K. pneumoniae</i> 4th <i>S. epidermidis</i> 4th <i>S. pneumoniae</i> 3rd	<i>K. pneumoniae</i> 4th	<i>K. pneumoniae</i> 4th
D	3th	3rd	3rd	4th	4th	4th
E	3rd	3rd	4th	4th	4th	4th

2) Clinical specimens

a) Vortex specimen swabs	Last quadrant with isolated growth	
	3rd	4th
No. of specimen streaked Manually	7	2
No. of specimen streaked by InocuLAB		9

b) Sputum specimens	Last quadrant with isolated growth	
	3rd	4th
No. of specimen streaked Manually	15	13
No. of specimen streaked by InocuLAB	8	20

Comments: For all 28 specimens the media streaked by InocuLAB had more isolated colonies in the 3 or 4th quadrant. These colonies were larger and easier to identify morphologically.

c) Wound specimens

For the 148 specimens no appreciable difference was observed between the two streaking procedures

Comments: Possible reason for no appreciable difference between these methods could be because of the concentration of organisms on the swabs since the manually streaked media was inoculated first.

d) Genital specimens

For 85 specimens streaked by InocuLAB

No. with significantly more isolated colonies of:

Group B Streptococci	21 (24.7%)
Yeast	18 (21.0%)
Normal flora	26 (30.5%)

Labor savings

For 88 genital specimens

Label, inoculate and streak	60 minutes
Label, inoculate and load InocuLAB	37 minutes

For 100 vaginal specimens in Todd Hewitt broth

Label, inoculate and streak	70 minutes
Label, inoculate and load InocuLAB	55 minutes

SUMMARY

- There is no carry over between specimens
 - For all specimens types except wounds there was significant increase in isolated colonies to the 4th quadrant for the media streaked using InocuLAB.
 - The isolated colonies for the specimens streaked by InocuLAB were larger and easier to identify morphologically.
 - Technician time was reduced by 29% using InocuLAB to streak primary media.
 - Technologists evaluating the primary media considered the media streaked by InocuLAB was easier to evaluate because of the isolation of the colonies.
 - The use of the InocuLAB could reduce possible repetitive motion injuries in large specimen volume laboratories.
 - There was a cost saving in disposable loops because InocuLAB uses inexpensive calibrated nichrome loops which are replaced infrequently.
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