

## EVALUATION OF AUTOMATION FOR STREAKING PRIMARY MEDIA INOCULATED WITH CLINICAL SPECIMENS

Y. A. Lue, R. Salas, S. Rana and S. Shah

Quest Diagnostics Incorporated  
Teterboro, New Jersey

## 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% CO<sub>2</sub> 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 tryptic soy broth in the following combinations:

- E. coli*, *P. aeruginosa* and *S. bovis*
- S. pyogenes* and *S. aureus*
- S. epidermidis*, *K. pneumoniae* and *S. pneumoniae*
- S. aureus* and *E. coli*
- 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 combinations were 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% CO<sub>2</sub> for 48 hours. The cultures were evaluated based on growth in the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and the 4<sup>th</sup> 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 tryptic 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% CO<sub>2</sub> for 48 hours. The cultured media was evaluated based on growth in the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and the 4<sup>th</sup> quadrant.

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% CO<sub>2</sub> for 18 – 24 hours. The cultured media was evaluated based on growth in the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and the 4<sup>th</sup> 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% CO<sub>2</sub> 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% CO<sub>2</sub> for 48 hours and evaluated for the number of isolated colonies. The manually streaked plates were processed first.

## Labor savings

- The time to label, inoculate and streak human blood agar and CNA blood agar plates and to label, inoculate and load InocuLAB was determined.
- 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% CO<sub>2</sub>.

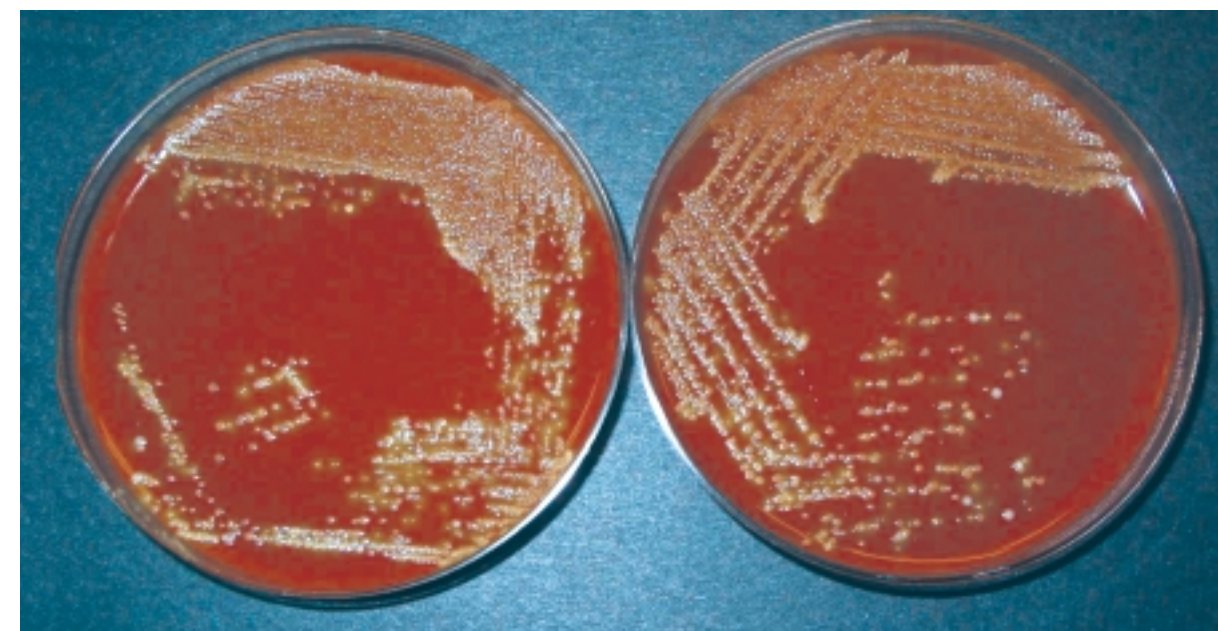
## Colony isolation comparison

## 1) Simulated specimens.

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

- E. coli*, *P. aeruginosa* and *S. bovis*
- S. pyogenes* and *S. aureus*
- S. epidermidis*, *K. pneumoniae* and *S. pneumoniae*
- S. aureus* and *E. coli*
- S. aureus*, *E. coli* and *S. bovis*

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	K. pneumoniae 3rd S. epidermidis 3rd S. pneumoniae 2nd	K. pneumoniae 3rd S. epidermidis 3rd	K. pneumoniae 3rd	K. pneumoniae 4th S. epidermidis 4th S. pneumoniae 3rd	K. pneumoniae 4th	K. pneumoniae 4th
D	3th	3rd	3rd	4th	4th	4th
E	3rd	3rd	4th	4th	4th	4th



## 2) Clinical specimens

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

## b) 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.

Address for correspondence:  
Yvonne A Lue  
Quest Diagnostics Incorporated  
One Malcolm Avenue  
Teterboro NJ 07608  
Tel: 201.393.6123  
Email: Yvonne.A.Lue@QuestDiagnostics.com