

# EVALUATION OF TWO AMIES CHARCOAL TRANSPORT SWABS: IMPACT OF REDUCED CHARCOAL ON GRAM STAINING AND VIABILITY

M. Desjardins<sup>1,2,3</sup>, C. Seetaram<sup>2</sup>, K. Ramotar<sup>1,2,3</sup>

<sup>1</sup>The Ottawa Hospital, <sup>2</sup>Ottawa Hospital Research Institute, <sup>3</sup>University of Ottawa, Ottawa ON

## Abstract (revised)

**Background:** Charcoal in Amies transport swabs improves the recovery of fastidious organisms from clinical specimens but interferes with the quality of the Gram stain. The Starplex Amies Plus SP140X swab is designed with reduced charcoal to improve Gram staining while maintaining the viability of organisms. Here we compared both the regular Amies transport swab with charcoal (Amies Charcoal SP131X) with the Amies plus swab.

**Method:** 6 ATCC strains were tested for survival on Amies Charcoal SP131X and the Amies Plus SP140X swab using CLSI M40 guidelines. Strains included *N. gonorrhoeae* (NG) ATCC 49221, *H. influenzae* (HI) ATCC 10211, *S. pneumoniae* (SPN) ATCC 6305, *S. pyogenes* (SP) ATCC 19615, *P. aeruginosa* (PA) ATCC 27853, *S. aureus* (SA) ATCC 25923 and *E. coli* (EC) ATCC 25922. Swabs inoculated with NG, HI, SPN and were held 4°C and those inoculated with SP, PA, SA and EC were held at room temperature (RT). Organism survival was evaluated by determining CFU/mL at 0, 6, 24 and 48 hours by plating 100µL of the suspension to appropriate aerobic media. Dilutions with visible growth at 48 hours incubation with a range of 30-300 CFU were evaluated. Combinations were tested in triplicate and compared using a two-tailed student t-Test. For the Gram stain, both swabs were inoculated with 0.5 McFarland suspensions of each strain and held for ½ hour at RT prior to preparing and staining.

**Results:** Except for SP, there was no statistical difference in recovery of viable organisms between the two swabs. For SP (ATCC strain) 10% of the original inoculum was recovered at 48 hours with the Amies Plus SP140X compared to 74% with the Amies Charcoal SP131X (p=0.008). Interpretation of the Gram stain was significantly improved with the Amies Plus SP140X swab.

**Conclusions:** The Amies Plus 140X swab containing reduced charcoal significantly reduces the interference of charcoal particles and improves the interpretation of the Gram stain but continues to maintain recovery of fastidious organisms for up to 48 hours.

## Background

Appropriate specimen transport remains an essential component for providing accurate, reliable information for patient management. Maintaining the viability of clinically relevant pathogens is a major problem especially in the out patient setting where delay in transport may often occur. Recovery of pathogens, in particular fastidious organisms such as *N. gonorrhoeae*, *H. influenzae* and *S. pneumoniae*, can be influenced by the transport system, storage temperature and delays in specimen processing.

Different transport media formulations have been devised to improve recovery rates especially for fastidious pathogens. Although addition of charcoal can improve survival it does interfere with the quality of the Gram stain. Charcoal particles in the smear can mask or even be confused with Gram positive organisms.

Here we evaluate a new Starplex Amies swab with reduced charcoal (SP140X) for its ability to maintain viability and to improve the Gram stain by comparing it with the regular Amies charcoal swab (SP131X).

## Methods

- Evaluation of the Starplex Amies transport swabs with charcoal (SP131X) and the new Amies Plus (SP141X) was performed according to CLSI M40 guidelines: direct swabbing/roll plate method.
- One ATCC and one clinical isolate of each of the following were tested: NG (ATCC 49221), HI (ATCC 10211), SPN (ATCC 6305), SP (ATCC 19615), PA (ATCC 27853), SA (ATCC 25923), EC ATCC (25922) and PAATCC (27337).
- The inoculum was prepared from an 18-24 hour subculture of each isolate. For the Gram stain, each swab was inoculated with a 0.5 McFarland suspension of each organism and held at room temperature for 30 minutes prior to smear preparation.
- Based on previous experience, swabs inoculated with fastidious organisms (NG, HI, SPN) were stored at 4°C, with the remaining swabs stored at room temperature.
- Proportion of viable organisms compared to baseline and CFU/mL were determined based on a dilution scheme after 48 hours incubation providing a measurable count between 30-300 CFU.
- Statistical evaluation of viable growth recovered from Amies charcoal swab (SP131X) compared to Amies Plus (SP140X) was performed using a two-tailed student t-test.

## Results

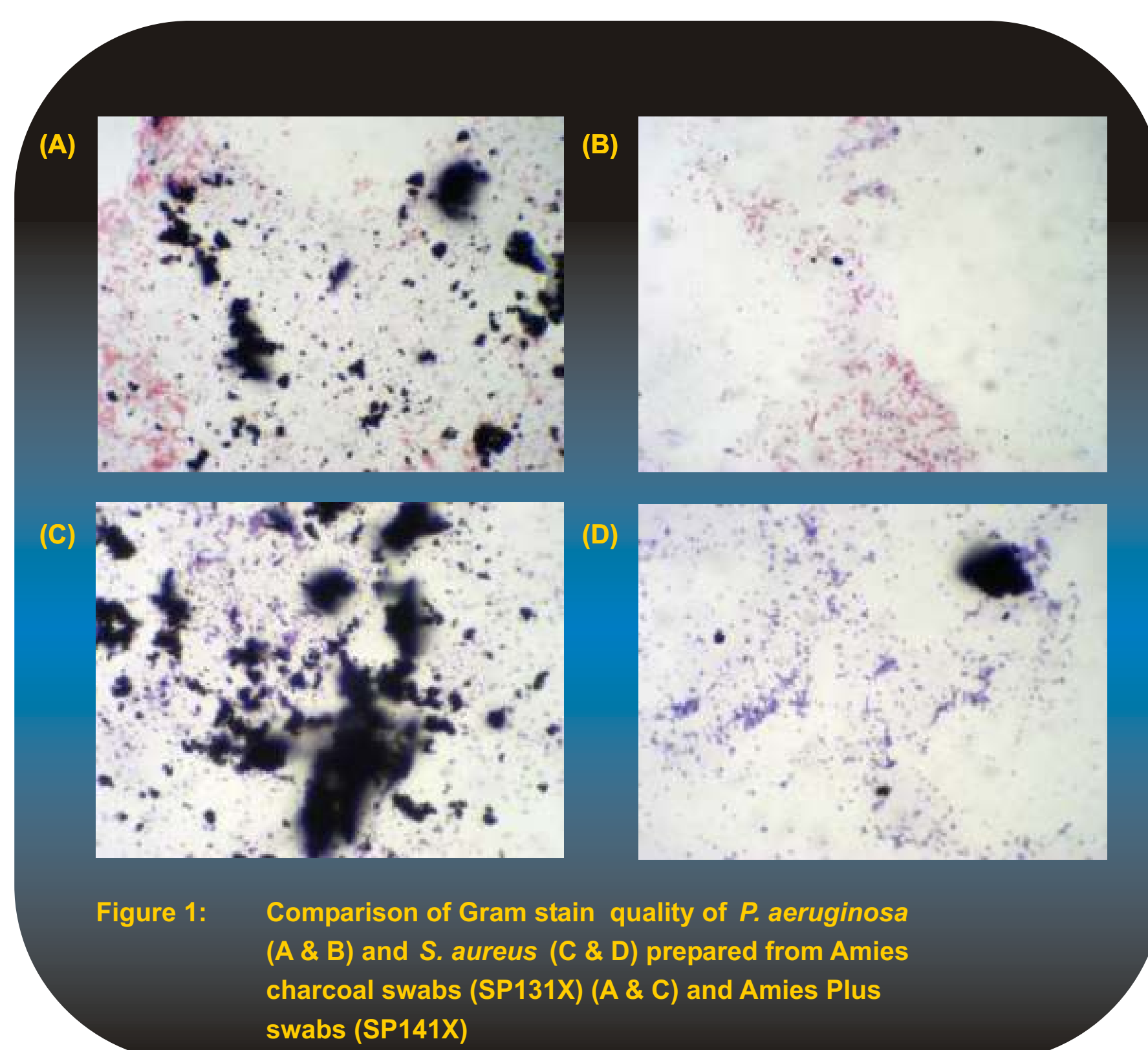


Figure 1: Comparison of Gram stain quality of *P. aeruginosa* (A & B) and *S. aureus* (C & D) prepared from Amies charcoal swabs (SP131X) (A & C) and Amies Plus swabs (SP141X) (B & D).

Table I:

Proportion of viable organisms after incubation compared to baseline for ATCC strains

Organisms	% Viability Compared to Baseline					
	Amies Charcoal (SP131X)			Amies Plus (SP140X)		
	Time of Incubation (hrs)					
	6	24	48	6	24	48
<i>S. pyogenes</i>	53	34	15	86	55	52
<i>S. pneumoniae</i>	80	93	74	64	64	10
<i>P. aeruginosa</i>	82	TNTC	TNTC	143	TNTC	TNTC
<i>S. aureus</i>	104	67	40	72	85	88
<i>E. coli</i>	110	TNTC	TNTC	211	TNTC	TNTC
<i>N. gonorrhoeae</i>	61	2	1	13	34	1
<i>H. influenzae</i>	90	59	12	56	33	53
<i>P. anaerobius</i>	14	35	0	36	3	0

Table II:

Proportion of viable organisms after incubation compared to baseline for clinical strains

Organisms	% Viability Compared to Baseline					
	Amies Charcoal (SP131X)			Amies Plus (SP140X)		
	Time of Incubation (hrs)					
	6	24	48	6	24	48
<i>S. pyogenes</i>	195	31	13	53	34	31
<i>S. pneumoniae</i>	71	74	13	140	103	34
<i>P. aeruginosa</i>	40	TNTC	TNTC	61	TNTC	TNTC
<i>S. aureus</i>	38	21	12	135	264	348
<i>E. coli</i>	259	TNTC	TNTC	TNTC	TNTC	TNTC
<i>N. gonorrhoeae</i>	20	3	0	67	5	0
<i>H. influenzae</i>	52	17	17	54	15	5
<i>P. anaerobius</i>	151	24	0	90	8	0

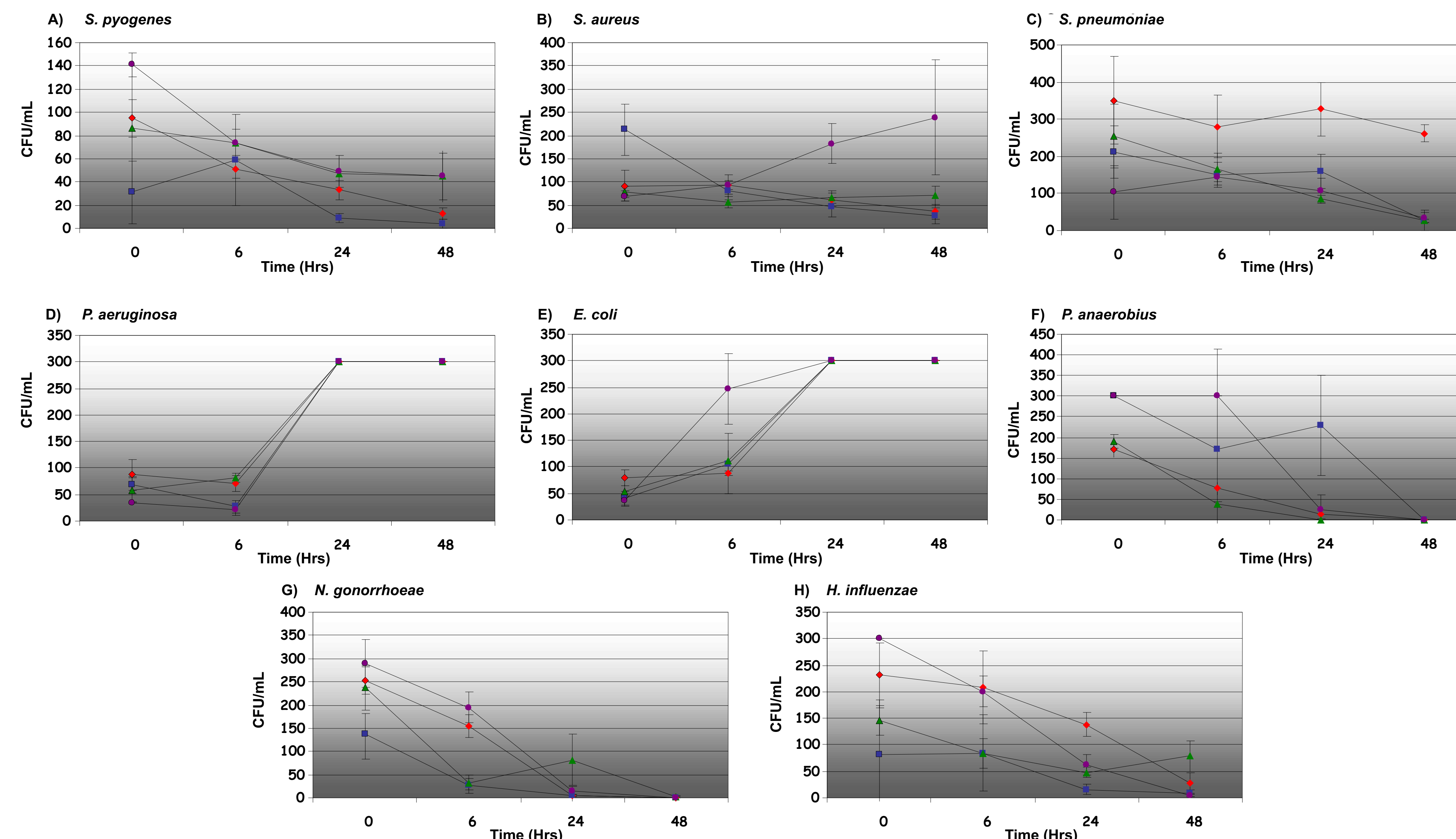


Figure 2: Evaluation of reduced charcoal content on organism viability. Inoculated swabs were incubated for 6, 24 and 48 hours at 4°C for NG, SPN, HI, or room temperature for SP, SA, PA, EC. Clinical isolates (blue and purple lines) were cultured from Amies charcoal swabs (SP131X) or Amies Plus (SP140X) and ATCC isolates (red and green lines) were cultured from Amies charcoal (SP131X) or Amies Plus (SP140X).

## Discussion

- Except for *S. pneumoniae*, there was no statistical difference between the regular Amies charcoal swab (SP131X) and the modified Amies Plus (SP140X) swab in their ability to maintain the viability of clinical and ATCC isolates.
- For both the clinical and ATCC *S. pneumoniae* strains, there was a significant reduction in viable colony count after 48 hours.
- The reduction in viable count was associated with the Amies Plus swab (SP140X) (p=0.009) for the ATCC strain compared to the Amies charcoal swab (SP131X) for the clinical isolate (p=0.02).
- For the ATCC strain the proportion of viable counts at 48 hours was 74% for the Amies charcoal swab (SP131X) and 10% for the Amies Plus swab (SP140X) compared to 13% and 34% respectively for the clinical strain.
- The reduction in charcoal significantly improved the Gram stain for all organism tested. There was a significant reduction in particles with improved visualization of organisms and background staining.

## Conclusion

Using the CLSI guidelines for evaluation of swab performance, we found that the recovery of most organisms was not negatively affected by the reduction in charcoal. The discrepancy in viable counts observed for SPN between the clinical and ATCC strains recovered from both swabs is likely a reflection of the fastidious nature of the organism or variability in colony counts and not the reduced charcoal in the Amies Plus swabs. The reduction in charcoal significantly improved the Gram stain appearance. The reduction in particles and improved staining characteristics provides an advantage especially with paucibacillary specimens without compromising the recovery of fastidious organisms.

## Acknowledgment

We wish to thank Starplex Scientific Inc. for financially supporting this poster with an unrestricted educational grant as well as providing the transport swabs.