# COMPARATIVE STUDY OF THE BACTERIOLOGICAL PERFORMANCE OF COMMERCIAL AMIES AGAR SWAB TRANSPORT DEVICES WITH A TRADITIONAL STUART AGAR TRANSPORT SYSTEM

Eva Blomgren, Margareta Larsson & Lennart Sjoberg

## ABSTRACT

(Revised 5/7/01)
Many bacteriology specimens are received on swabs, which are being transported over increasing distances and lengths of time. To maximize organism survival and sample quality our laboratory has chosen to use a relatively expensive swab transport device based on Stuart's original description. This is a steam-sterilized medium dispensed in glass screw-cap tubes and supplied with a charcoal coated swab (SSI-CS, Statens Serum Institut, Denmark). We conducted a comparative viability study to investigate the consequences of changing from this to a lower cost system. Three devices; Starswab II Amies without Charcoal (SA, Starplex Scientific, Ontario, Canada), Copan Amies without Charcoal (CA, Copan, Brescia, Italy), both supplied with plain swabs and Copan Amies without Charcoal supplied with a charcoal coated swab (CCS, Copan, Brescia, Italy) were evaluated against SSI for their efficacy in promoting fastidious aerobe and anaerobe survival over a 48 hour period. Swabs were inoculated with 100µL of $10^7$ CFU/ml saline organism suspensions of Neisseria gonorrhoeae (2 strains NGA, NGS), Neisseria meningitidis (2 strains NMA, NMC), Streptococcus pneumoniae (SPN), Streptococcus pyogenes (SPY), Haemophilus influenzae (HI), Peptostreptococcus anaerobius (PA) and Fusobacterium nucleatum (FN). The swabs were placed into SA, CA, CCS and SSI devices and incubated at 22°C. Triplicate swabs of each organism/device were removed at 0hr, 6h, 24h and 48h and quantitative cultures were performed on plated media to calculate zero time baseline counts and percentage organism recovery at each time point thereafter. All devices maintained the viability of test organisms at 6h. At 24h SA recovered 7/9 organisms while CA, CCS and SSI-CS 9/9. SA failed to recover NGA and PA at 24h and demonstrated lower recovery counts for HI 0.02%, NMA 0.01%, SPN 2.47% compared with CA HI 6.2%, NMA 22.9%, SPN 7.8%, CCS HI 3.1%, NMA 40.6%, SPN 22.9%, SSI-CS HI 12.6%, NMA 3.8% and SPN 7.8%. At 48h SA recovered 2/9 organisms, CA 5/9, CCS 9/9 and SSI-CS 9/9. Charcoal coated swabs provided with CCS and SSI-CS may prolong the survival of certain bacteria. CCS performed the most comparable to SSI-CS. The final poster presentation includes additional performance data collected for two transport devices SAC and CAC, both these devices contain Amies medium with charcoal and are supplied with a plain swab.

Örebro Medical Centre Hospital

Dept. of Clinical Microbiology and Immunology

Eva Blomgren, Margareta Larsson & Lennart Sjoberg
Clinical Microbiology & Immunology
Örebro Medical Centre Hospital, SE - 701 85, Örebro, Sweden
E-mail: eva.blomgren@orebroll.se, lennart.sjoberg@orebroll.se

## INTRODUCTION

Specimen collection and transport is one of the most critical parts of pre-analytical phase and the total microbiology testing process. The significance of pre-analytical steps including specimen selection, sampling, and transportation are most often underestimated and those who perform specimen sampling usually know the least. Accurate diagnosis of microbiology disease relies on the collection of an adequate specimen at the right time, from the correct site with the appropriate sampling device. With the growing trend in centralization of laboratory services resulting in significant delays and extended transport times, increasing demands are being placed on traditional microbiology collection and transport devices to maintain the viability of fastidious organisms in clinical samples. A variety of specimens arrive in the microbiology laboratory each day in swab transport systems. Evidence suggests that swab specimens are inferior to fluid specimens when wounds, exudates and drainage are collected, especially for anaerobic culture[1,2,3]. However, the ready availability and ease of swab collection results in the receipt of many such specimens. Our laboratory has always paid careful attention to the quality of all microbiology specimens we receive and for many years we have supplied a swab transport medium system manufactured by the Statens Serum Institut in Denmark (SSI)[4]. The SSI swab transport system is based upon and closely resembles the original description of a collection and transport medium system described by Stuart[5,6]. It comprises of a glass screw cap tube filled with 8 ml of clear Stuart Agar Medium to within 1 – 1.5 cm from the cap. The product is sterilized by steam, has a methylene blue indicator to indicate oxidation, a relatively short shelf life of 9 months at 2-8°C or 4 months at 20-25°C and costs approximately 62 cents for each tube of agar medium plus an additional 15.5 cents for a sterile packed charcoal swab or 32 cents for a sterile packed plain swab. We decided to evaluate a variety of commercially manufactured transport swabs to compare their performance with our current SSI products. Until now we have been satisfied with the quality and performance of the SSI products however, commercially manufactured swabs offer a number of important advantages including significant cost saving, longer shelf life, wider selection of medium and swab applicator formats and a plastic medium tube, which would improve safety by eliminating any risk of breakages and sample leakage during transport.

Our study was conducted in two phases. In phase one we compared the SSI Stuart Agar transport systems with Starplex Starswab II (Starplex Scientific, Ontario, Canada) and Copan Transystem (Copan Italia, Brescia, Italy) Amies Agar Medium with Charcoal and Amies Medium without Charcoal for their ability to maintain the viability of 9 bacteria that comprised fastidious aerobes and anaerobes. In phase two, using the same group of 9 aerobic and anaerobic bacteria, we compared the viability performance of just the SSI Stuart Agar transport system supplied with a charcoal coated swab with a newly developed Copan Amies Agar Medium transport supplied with a charcoal coated swab

## Phase One Study

| Description | Type of swab provided | Abbreviation | Manufacturer |
|---|---|---|---|
| SSI Transport Medium (Stuart Agar) | Charcoal coated Rayon swab | SSI-CS | Statens Serum Institut, Copenhagen, Denmark |
| SSI Transport Medium (Stuart Agar) | Plain Rayon swab | SSI-PS | Statens Serum Institut, Copenhagen, Denmark |
| Copan Amies Agar Medium without Charcoal | Plain Rayon swab | CA | Copan Italia, Brescia, Italy |
| Copan Amies Agar Medium with Charcoal | Plain Rayon swab | CAC | Copan Italia, Brescia, Italy |
| Starplex Starswab II Amies Medium without Charcoal | Plain Rayon swab | SA | Starplex Scientific, Ontario, Canada |
| Starplex Starswab II Amies with Charcoal | Plain Rayon swab | SAC | Starplex Scientific, Ontario, Canada |

## Phase Two Study

| Description | Type of swab provided | Abbreviation | Manufacturer |
|---|---|---|---|
| SSI Transport Medium (Stuart Agar) | Charcoal coated Rayon swab | SSI-CS | Statens Serum Institut, Copenhagen, Denmark |
| New Copan Amies Agar Medium without Charcoal | Charcoal coated Rayon swab | CCS | Copan Italia, Brescia, Italy |

## MATERIALS

GC Agar (GCAGP), a modified Thayer and Martin medium.
Tubes of 0.85% NaCl (NACLR) solution (1.8ml, 1ml and 0.9ml volumes) for making dilutions
Disposable spreaders (Servant™)
Biohit Proline 5-50µL and Proline 50-200µL accurate pipettors
GasPak Plus BBL™ Anaerobic System

Seven lyophilized ATCC cultures:
Streptococcus pyogenes ATCC 19615 (SPY)
Neisseria gonorrhoeae ATCC 43069 (NGA)
Haemophilus influenzae ATCC 10211 (HI)
Fusobacterium nucleatum ATCC 25586 (FN)
Streptococcus pneumoniae ATCC 6305 (SPN)
Neisseria meningitidis ATCC 13090 (NMA)
Peptostreptococcus anaerobius ATCC 27337 (PA)

Two reference cultures:
Neisseria meningitidis KMA 2000-590-00158 (NMC - clinical isolate)
Neisseria gonorrhoeae RS18, CCUG 10130, EF 899/80 (NGS - Swedish control strain)

## SSI SWABS



Glass tubes with screw cap filled with 8ml of Stuart Agar Medium 1-1.5 cm headspace between top of agar medium and cap. Steam sterilized. Shelf life 9 months at 2-8°C and 4 months at 20-25°C. Supplied with plain or charcoal coated Rayon swabs.

## COPAN SWABS


Plastic tubes filled with 5ml of Amies Agar Medium. Sterilized by ionizing irradiation. Shelf life 15 months from date of manufacture.

## STARPLEX SWABS


Plastic tubes filled with 5ml of Amies Agar Medium. Sterilized by ionizing irradiation. Shelf life 24 months from date of manufacture.

## METHODS

This comparative swab study was performed in two phases using the same protocol in each phase. The protocol used was similar to previously published studies[7,8,9,10,11]. Phase 1 compared SSI-CS, SSI-PS, CA, CAC, SA, and SAC systems. Phase 2 compared SSI-CS and CCS systems.

Fresh subcultures of two local bacterial reference strains of Neisseria meningitidis KMA 2000-590-00158 (clinical isolate) and Neisseria gonorrhoeae RS18, CCUG 10130, EF 899/80 (Swedish control strain) were made on GCAGP medium. New vials of lyophilized ATCC cultures of Streptococcus pyogenes ATCC 19615, Streptococcus pneumoniae ATCC 6305, Neisseria meningitidis ATCC 13090, Neisseria gonorrhoeae ATCC 43069, Haemophilus influenzae ATCC 10211, Peptostreptococcus anaerobius ATCC 27337, Fusobacterium nucleatum ATCC 25586 were reconstituted and cultured on GCAGP medium.18 - 24 hours growth from culture plates were then used to prepare suspensions of each bacterium in saline equivalent to a 0.5 McFarland Standard (approximately 1.5 x $10^8$ CFUs per ml). A 1:10 dilution of these McFarland suspensions of bacteria were made in sterile saline to create the final working volume for inoculation of swabs. For each bacterium tested, three swabs from each transport system and for each time point evaluated were inoculated with 100µL of bacterial suspension using an accurate volumetric pipettor.

Example: S. pyogenes versus SSI-CS

Time: 0 hr 3 swabs 6 hrs 3 swabs 24 hrs 3 swabs 48 hrs 3 swabs. Total number of swabs inoculated 12 for each organism evaluated.

Inoculated swabs were placed into their respective transport devices. For a zero time bacterial count 3 swabs, designated as the 0 hr swabs, were immediately removed and place into 1ml of sterile saline for further processing. All other inoculated swab systems were held at 22°C and were removed for processing at the designated time points.

At each designated time point 0 hr, 6 hrs, 24 hrs and 48 hrs, swabs were processed and cultured as follows to provide a quantitative bacterial count:

1. Swabs are removed from transport devices and place in tubes containing 1ml of sterile saline.
2. Vortex mix each swab vigorously for 30 seconds and thoroughly squeeze out any liquid from the swab tip by pressing against the sidewalls of the tube, then discard the swab.
3. Using a Biohit pipettor 100µL of the vortex suspension is removed and two 10-fold dilutions in saline are made.
4. Using a Biohit pipettor 100µL from the original vortex swab suspension and from each 10-fold dilution is removed and spread evenly over the entire surface of an appropriate culture plate. This procedure is performed in duplicate so there are two plate counts for the original vortex suspension and two for each saline dilution.
5. Plates are incubated in the appropriate atmospheric conditions for the test organism for 24 to 48 hours before examining and counting. Results for the duplicate plate cultures of each serial dilution are counted and averaged.

Three swabs from each manufacturer were analyzed on each bacterium at each time point. With duplicate plate counts being performed on each subsequent swab vortex suspension and two 10-fold dilutions, this meant that as many as 18 plate count cultures were performed for each individual test parameter. Bacterial survival rates were compared by expressing recovery as a percentage of 0 hr time plate counts.

## RESULTS

Phase 1

Only the SSI-CS swab device was capable of maintaining the viability of all 9 test organisms at all time points. At 48 hrs SSI-PS maintained 8/9 organisms while CAC and SAC supported 6/9, CA 5/9 and SA 2/9. At 24 hrs SSI-PS, CA, CAC and SAC maintained 9/9 organisms while SA supported 7/9. Neisseria gonorrhoeae ATCC 43069 strain and Peptostrep. anaerobius both failed to survive in SA at 24 hours. The percentage recovery varied significantly between different swab devices with different bacteria. Analysis of CFU counts at the 24 hours time point for those devices without charcoal in the medium or on the swab generally showed a lower percentage recovery for bacteria held in Starplex SA compared with SSI-PS and CA. For example, recovery rate for SPN were 29.67% (SSI-PS), 7.87% (CA), 2.47% (SA), for HI 40.05% (SSI-PS), 6.21% (CA), 0.02% (SA), for NMA 0.04% (SSI-PS), 22.99% (CA), 0.01% (SA) for FN 0.47% (SSI-PS), 1.53% (CA), and 0.38% (SA). Analysis of CFU counts at 24 hours with devices containing charcoal in the medium or on the swab, demonstrated that certain bacteria faired better in CAC notably HI, NMA and NMC. Other organisms in charcoal containing transport devices followed a similar trend as noted with non-charcoal containing devices with SAC averaging lower percentage recoveries at 24 hours except with FN. SSI-CS was singled out for further comparative study in Phase 2, with a new device CCS, because of it's capacity to maintain acceptable recovery of organisms at all time points.

Phase 2

Both CCS and SSI-CS were able to maintain the viability of all 9 test organisms for 48 hrs. Survival of the two anaerobic bacteria was significantly reduced compared to aerobes in both these transport systems. Colony counts for PA at the 24 hrs and 48 hrs time points were very low but growth was still detectable: 24 hrs <0.01% (SSI-CS) versus 0.66% (CCS) and at 48 hrs <0.01% (SSI-CS) versus 0.02% (CCS). Both strains of gonococcus and meninigococcus survived well in these transport systems as did HI, SPN and SPY. At the 24 hr time point, with 6/9 bacteria, we recorded a higher percentage recovery from the CCS device most notably with Neisseria species.

## DISCUSSION

This study was designed to compare the ability of various swab transport systems to maintain the viability of a range of clinically important aerobes and anaerobes. Arguably this study may not reflect the conditions of clinical samples, which vary in viscosity and contain cellular and chemical constituents that may act as nutrients or toxins and may also contain mixtures of bacteria. These factors all have the potential to affect organism viability. In this case our study, using pure cultures in saline suspensions, devoid of nutrient, with no positive or negative contribution from patient sample, may provide a means of predicting the likely outcome when the product is used in a clinical setting. The test protocol we used has been described in previously published studies. The protocol

provided easily countable colonies enabling us to quantify and accurately measure the reduction in bacterial viability and was sufficiently sensitive to show subtle differences in performance of different swab transport devices.

For many years we have been using a swab transport system manufactured by the Statens Serum Institut (SSI), which is manufactured using traditional methods of culture media production. The product is filled in glass screw capped tubes, steam sterilized, supplied with a charcoal swab, and is very similar to the format originally described by Stuart in 1946 and 1954. We believe that this product represents a high quality standard in the field of swab transport. We have been interested in testing commercially produced swabs as they offer some interesting advantages and potential savings however, we were curious to learn whether commercial mass-produced transport systems in plastic tubes, sterilized using ionizing irradiation, would perform similarly to the SSI products. The first phase of our study demonstrated that, generally, transport systems incorporating charcoal outperformed equivalent systems without charcoal and that SSI in combination with a charcoal coated swab performed the best. Some non-charcoal systems notably, Starplex Starswab™ II Amies Agar (SA), recorded lower percentage recoveries at most time points. The superior performance of charcoal containing systems might be specifically connected to charcoal coating of the swabs, which may work differently than charcoal suspended inside transport medium. Stuart advocated the use of charcoal coated swabs over incorporating charcoal in the medium[5]. Charcoal is known to have beneficial affects of absorbing toxic substances and fatty acids that can be found in varying amounts in agar and swab fibers. Charcoal may also absorb free radicals and superoxides that accumulate during storage of agar medium. We suspect that charcoal may have some other positive contribution to organism recovery that is no yet fully understood. Charcoal is used extensively in the electronics industry for minimizing and controlling the build up of static electricity in sensitive equipment. We can hypothesize that charcoal may have the affect of neutralizing the electrostatic charges on swab fibers, which in turn, could minimize the electrostatic attachment of bacteria to these fibers. If this neutralizing theory could be proven then it might account for a greater release of bacteria from the swab and improved percentage recovery. During the course of our study we discovered that Copan were also researching a new prototype charcoal coated swab for use in conjunction with their Amies Agar Medium. We tested this new Copan product (CCS) in our second phase study and found that the product performed very similarly to the SSI-CS.

In 1967 Amies described a modified formula for the preparation of Stuart's transport medium in which charcoal was incorporated into the medium[12]. The reasons given for this change were that preparation of charcoal coated swabs was time consuming and the unsightly appearance of the swab brought it into disfavor, albeit with patients or clinicians. In the last decades there has been an increasing trend in the use of clear agar gel medium without charcoal used in conjunction with a plain swab and in the U.S. there has been widespread use of liquid transport medium. Anecdotal reports suggest that people prefer these charcoal free transports as they offer greater convenience, ease of use and are less problematic when making and reading gram stain smears. Examining smears from charcoal medium or charcoal swabs requires a more skillful and careful inspection as large charcoal particles may obscure bacteria or white cells.

## SUMMARY/CONCLUSION

1. Swab transport systems vary in their ability to provide recoverable numbers of fastidious aerobes and anaerobes
2. In our study charcoal containing swab transport systems generally supported the viability of organisms better than non-charcoal containing systems.
3. In the Phase 1 study only SSI-CS was able to maintain 9/9 organisms tested at 48 hrs compared with SSI-PS 8/9, CAC 6/9, SAC 6/9, CA 5/9 and SA 2/9. At 24 hrs all swab system were able to maintain 9/9 organisms with the exception of Starplex Starswab™ II (SA), which supported 7/9 bacteria.
4. The SSI-CS overall performed the best and in our Phase 2 study SSI-CS performed comparable to a new commercial transport swab format from Copan incorporating a charcoal coated swab (CCS).
5. Further research needs to be done on the beneficial affects of charcoal coated swabs used in combination with clear agar gel transport medium as originally described by Stuart. This swab and medium combination might provide the optimum balance of reduced agar medium environment for maintaining organism viability coupled with improved release effect of bacteria from the swab fibers.
6. By avoiding the use of charcoal in some form or another inside swab transport systems we may risk losing some significant percentage recovery of certain bacteria.

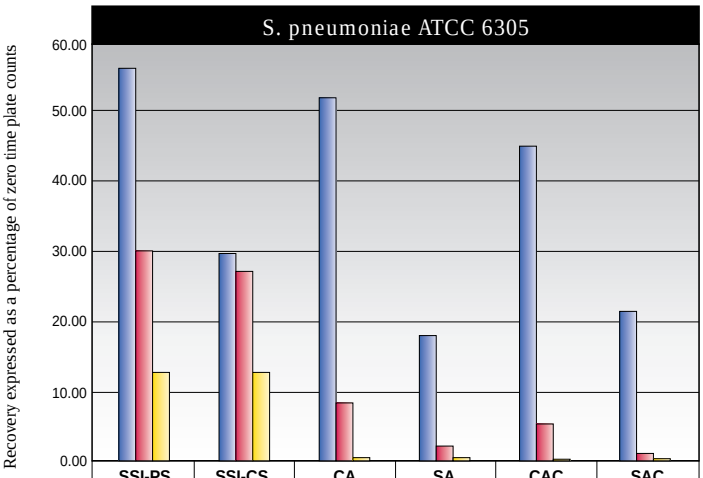
## REFERENCES

1. Holden, J. 1992. Collection and transport of clinical specimens for anaerobic culture, p. 2.2.1-2.2.6. In H. D. Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
2. Summanen P, Baron E.J., Citron D.M., Strong C.A., Wexler H.M., and Finegold S.M. 1993. In Wadsworth Anaerobic Bacteriology Manual. Fifth Edition. Star Publishing Company, Belmont, CA, 1993.
3. Miller J. M. 1999. A Guide to Specimen Management in Clinical Microbiology (2nd Edition). American Society for Microbiology, Washington, D.C.
4. Statens Serum Institut, 5 Artillerivej, DK-2300 Copenhagen, Denmark. www.ssi.dk.
5. Stuart, R.D. 1946. The diagnosis and control of gonorrhoeae by bacteriological cultures. Glasgow Medical Journal. 27: 131-142.
6. Stuart, R. D., Toshach, S. R., and Patsula, T. M. 1954. The problem of transport of specimens for culture of gonococci. Canadian Journal of Public Health. 45: 73-83.
7. Perry, J. L. 1997. Assessment of Swab Transport Systems for Aerobic and Anaerobic Organism Recovery. Journal of Clinical Microbiology. Vol 35, No. 5: 1269-1271.
8. Van Horn K., Warren K., and Tóth C. 1999. Comparison of Four Swab Systems for Recovery of Anaerobic Bacteria. C32. In Abstracts of the 99th General Meeting of the American Society for Microbiology, Chicago 1999. American Society for Microbiology, Washington D.C.
9. Allen S. D., Siders J. A., Emery et. al. 1999. Evaluation of the New BBL™ CultureSwab Plus™ Transport Device (Becton Dickinson) for Recovery of Anaerobes. C16. In Abstracts of the 99th General Meeting of the American Society for Microbiology, Chicago 1999. American Society for Microbiology, Washington D.C.
10. Campos J. M., Ruthman L., and M. Tshimanga. 2000. Survival of Fastidious Bacteria on Specimen Collection Swabs Stored at Room Temperature. C-150. In Abstracts of the 100th General Meeting of the American Society for Microbiology, Los Angeles 2000. American Society for Microbiology, Washington D.C.
11. Hindiyeh M., Acevedo V., and Carroll K. C. 2001. Comparison of Three Transport Systems (Starplex StarSwab II, the New Copan Vi-Pak Amies Agar Gel Collection and Transport Swabs, and BBL Port-a-cul) for Maintenance of Anaerobic and Fastidious Aerobic Organisms. Journal of Clinical Microbiology. Vol. 39. No. 1: 377-380
12. Amies C.R. 1967. A modified formula for the preparation of Stuart's medium. Canadian Journal of Public Health, July 1967. Vol. 58, 296-300.

## CHARTED RESULTS

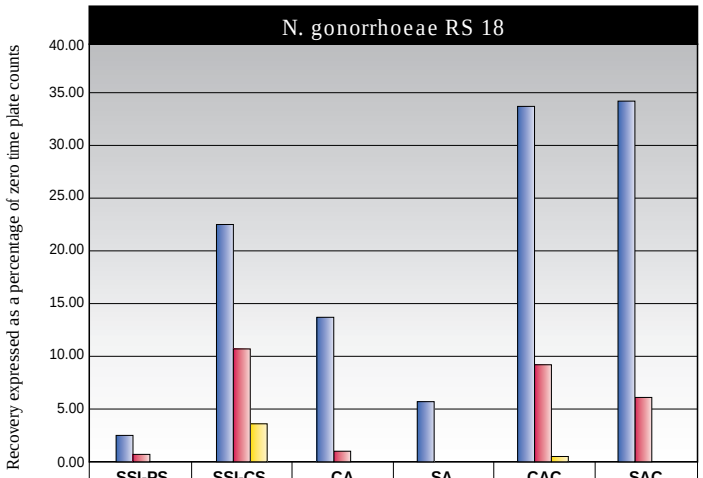
# PHASE ONE

### S. pneumoniae ATCC 6305



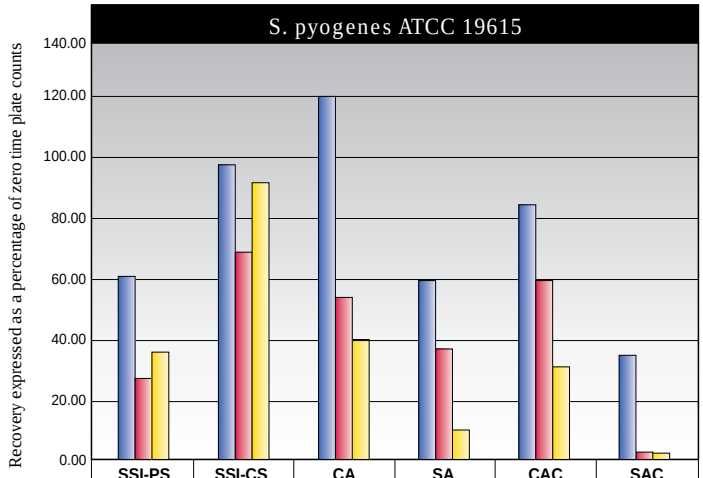

| | SSI-PS | SSI-CS | CA | SA | CAC | SAC |
|---|---|---|---|---|---|---|
| 6 hr | 56.61 | 29.53 | 51.10 | 17.85 | 44.91 | 21.15 |
| 24 hr | 29.67 | 25.59 | 7.87 | 2.47 | 5.50 | 1.33 |
| 48 hr | 14.12 | 14.07 | 0.55 | 0.31 | 0.35 | 0.15 |

### N. gonorrhoeae RS 18



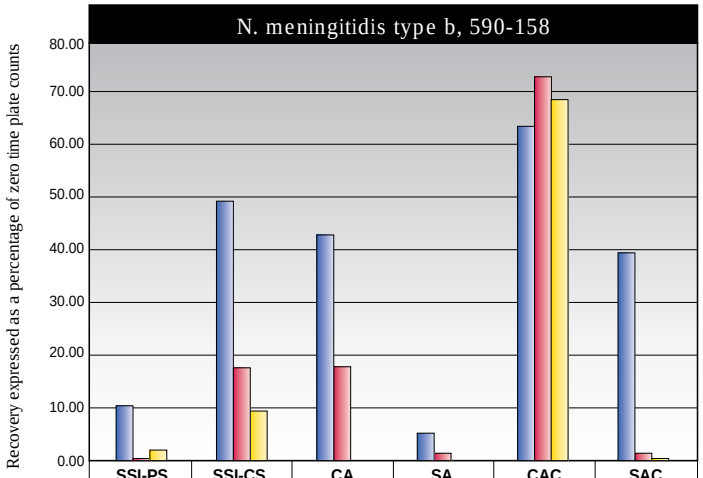

| | SSI-PS | SSI-CS | CA | SA | CAC | SAC |
|---|---|---|---|---|---|---|
| 6 hr | 2.50 | 22.74 | 13.34 | 6.00 | 33.50 | 34.00 |
| 24 hr | 0.71 | 10.79 | 0.89 | 0.01 | 9.11 | 6.39 |
| 48 hr | 0.02 | 3.04 | **0.00** | **0.00** | 0.56 | **0.00** |

### S. pyogenes ATCC 19615



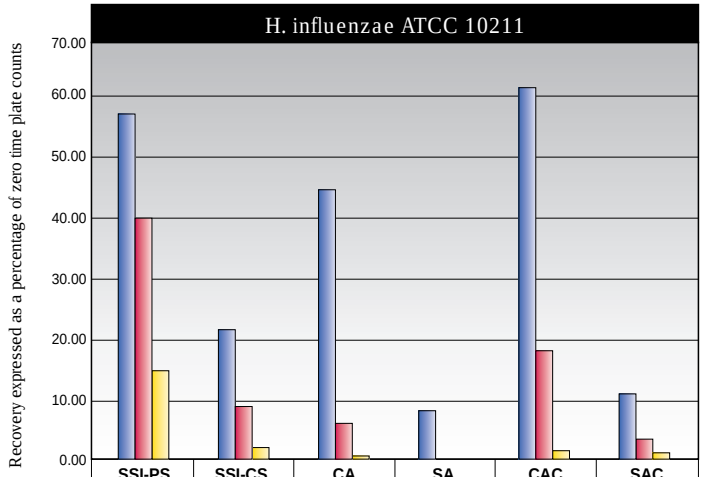

| | SSI-PS | SSI-CS | CA | SA | CAC | SAC |
|---|---|---|---|---|---|---|
| 6 hr | 60.66 | 98.09 | 120.62 | 59.17 | 86.34 | 33.68 |
| 24 hr | 29.49 | 69.57 | 51.56 | 35.14 | 59.77 | 2.78 |
| 48 hr | 36.02 | 93.19 | 40.55 | 10.80 | 31.38 | 2.15 |

### N. meningitidis type b, 590-158



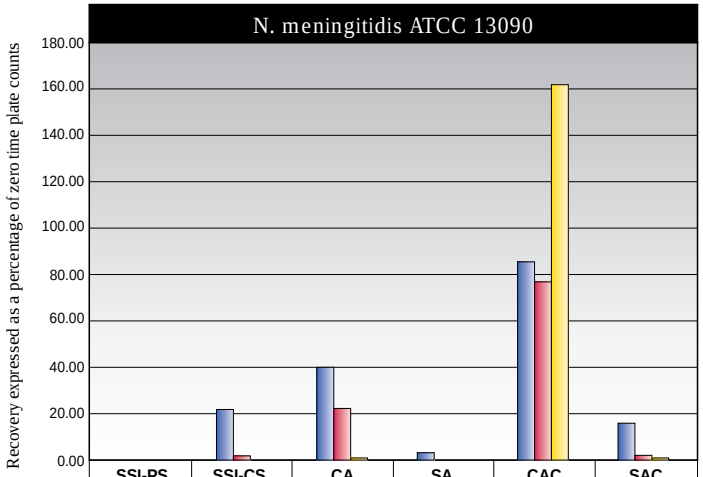

| | SSI-PS | SSI-CS | CA | SA | CAC | SAC |
|---|---|---|---|---|---|---|
| 6 hr | 10.30 | 49.28 | 43.93 | 5.82 | 64.02 | 39.28 |
| 24 hr | 0.70 | 16.48 | 16.94 | 1.34 | 73.35 | 1.85 |
| 48 hr | 2.36 | 9.40 | 0.10 | **0.00** | 68.10 | 0.80 |

### H. influenzae ATCC 10211




| | SSI-PS | SSI-CS | CA | SA | CAC | SAC |
|---|---|---|---|---|---|---|
| 6 hr | 56.38 | 22.54 | 45.30 | 8.72 | 61.79 | 11.72 |
| 24 hr | 40.05 | 8.78 | 6.21 | 0.02 | 18.13 | 3.53 |
| 48 hr | 15.38 | 2.26 | 0.30 | **0.00** | 1.85 | 1.26 |

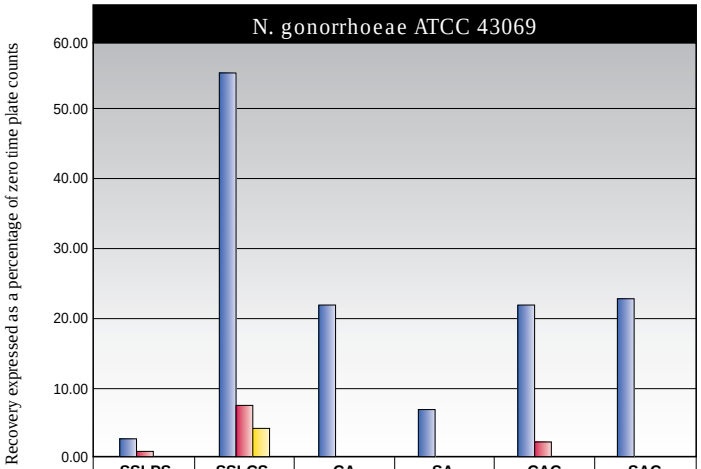
### N. meningitidis ATCC 13090



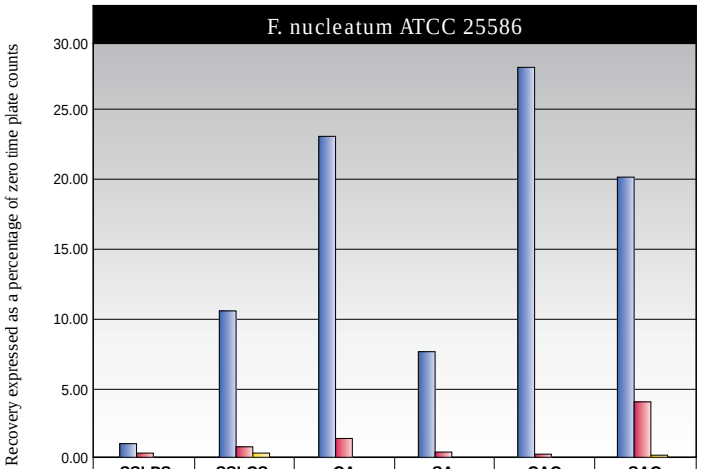

| | SSI-PS | SSI-CS | CA | SA | CAC | SAC |
|---|---|---|---|---|---|---|
| 6 hr | 0.29 | 21.82 | 39.60 | 2.05 | 87.89 | 15.18 |
| 24 hr | 0.04 | 2.68 | 22.99 | 0.01 | 75.96 | 2.37 |
| 48 hr | **0.00** | 0.01 | 0.64 | **0.00** | 161.87 | 0.88 |

## CHARTED RESULTS

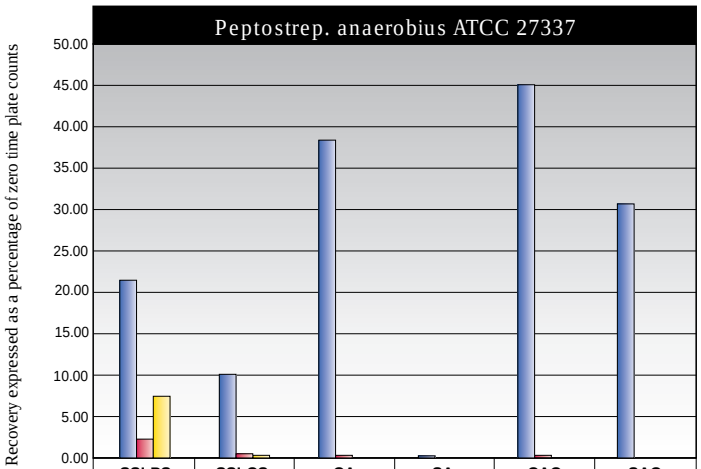
### PHASE ONE




| | SSI-PS | SSI-CS | CA | SA | CAC | SAC |
|---|---|---|---|---|---|---|
| 6 hr | 2.77 | 54.99 | 22.64 | 6.61 | 22.30 | 23.75 |
| 24 hr | 0.17 | 7.25 | 0.05 | **0.00** | 2.43 | 0.01 |
| 48 hr | 0.01 | 4.36 | **0.00** | **0.00** | **0.00** | **0.00** |

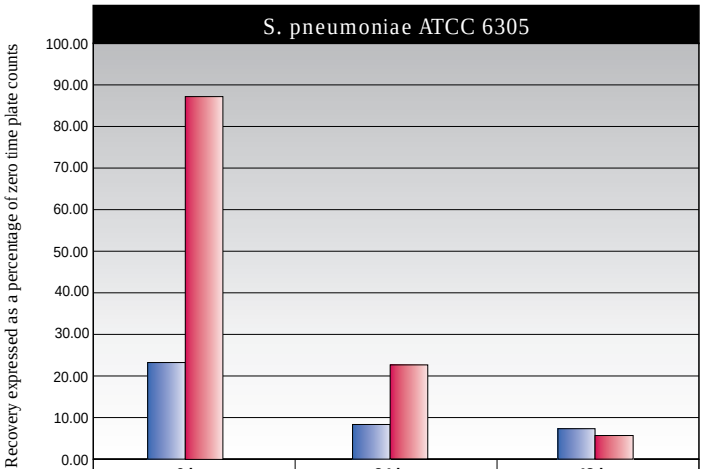



| | SSI-PS | SSI-CS | CA | SA | CAC | SAC |
|---|---|---|---|---|---|---|
| 6 hr | 1.22 | 11.08 | 22.97 | 7.81 | 27.92 | 20.14 |
| 24 hr | 0.47 | 1.26 | 1.53 | 0.38 | 0.28 | 3.97 |
| 48 hr | 0.04 | 0.34 | **0.00** | **0.00** | **0.00** | 0.15 |

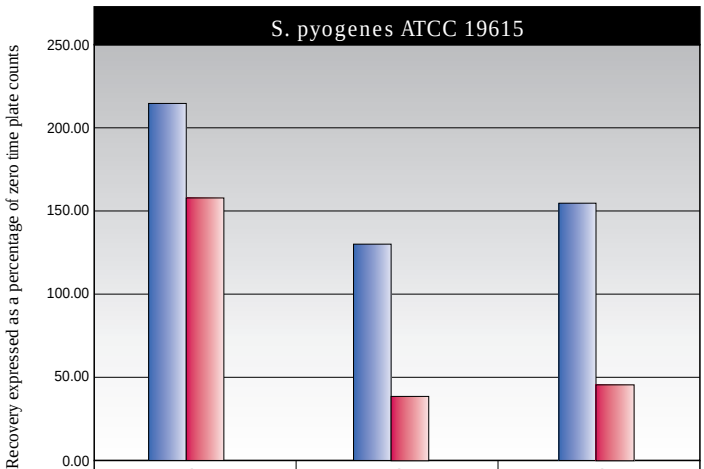



| | SSI-PS | SSI-CS | CA | SA | CAC | SAC |
|---|---|---|---|---|---|---|
| 6 hr | 21.48 | 10.02 | 33.85 | 0.37 | 44.87 | 31.05 |
| 24 hr | 2.11 | 0.61 | 0.41 | **0.00** | 0.27 | 0.03 |
| 48 hr | 7.72 | 0.36 | **0.00** | **0.00** | **0.00** | **0.00** |

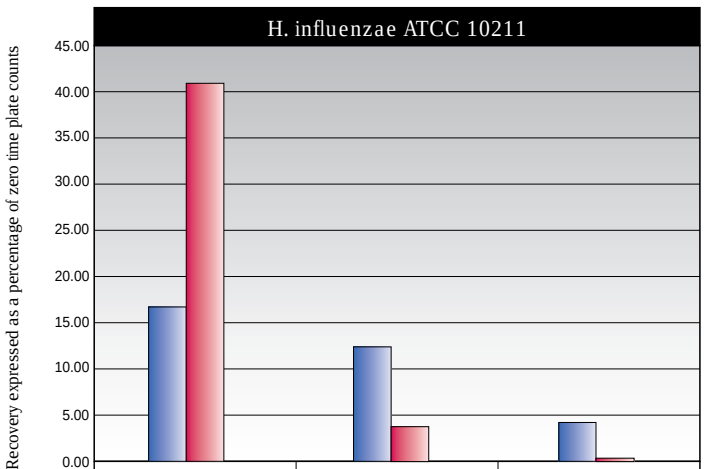
### PHASE TWO




| | 6 hr | 24 hr | 48 hr |
|---|---|---|---|
| SSI-CS | 23.05 | 7.85 | 6.76 |
| CCS | 86.09 | 22.96 | 6.05 |



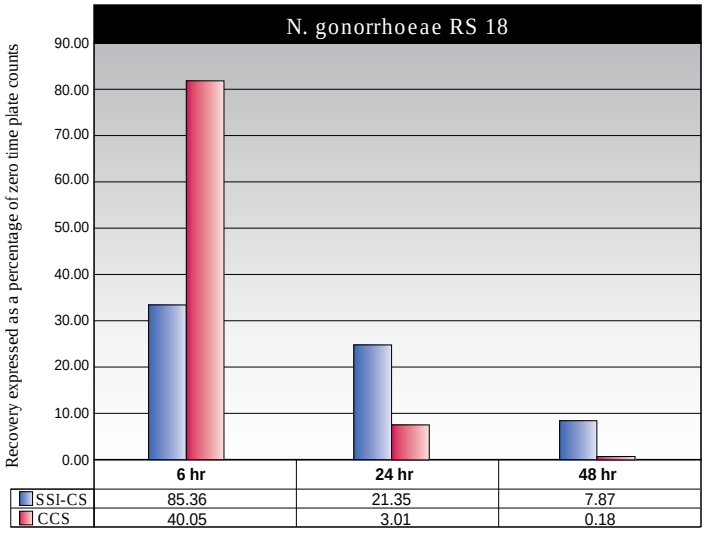

| | 6 hr | 24 hr | 48 hr |
|---|---|---|---|
| SSI-CS | 216.49 | 128.04 | 154.99 |
| CCS | 161.45 | 38.43 | 44.82 |



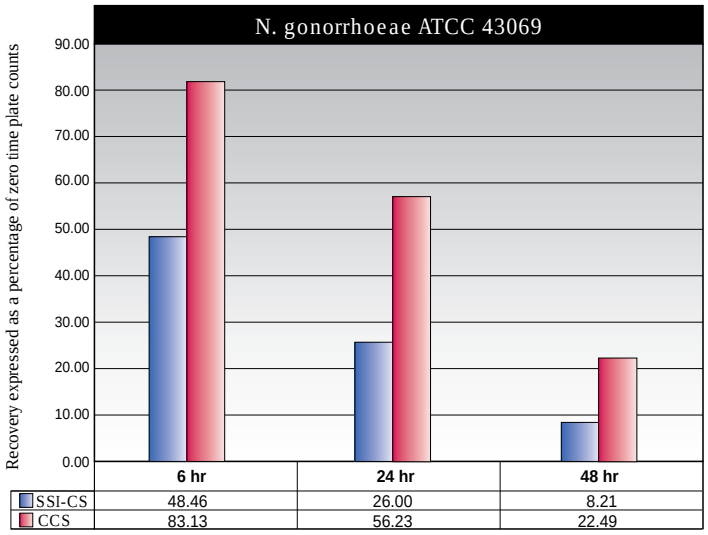

| | 6 hr | 24 hr | 48 hr |
|---|---|---|---|
| SSI-CS | 16.80 | 12.60 | 4.01 |
| CCS | 40.77 | 3.18 | 0.42 |

## CHARTED RESULTS

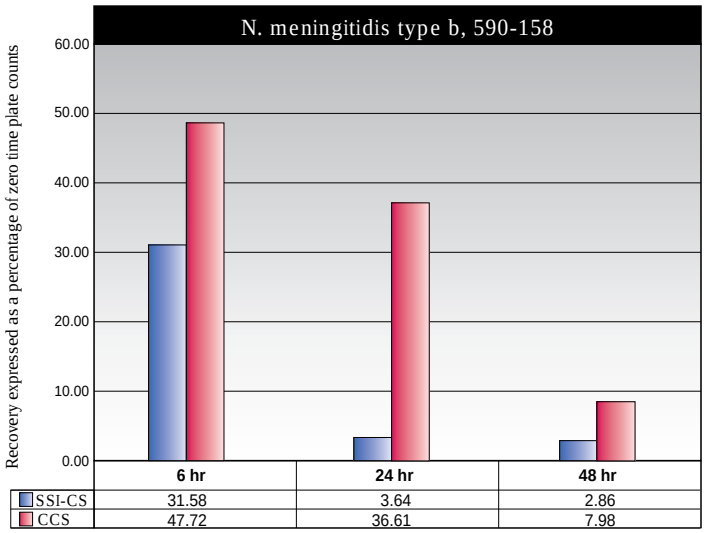
# PHASE TWO



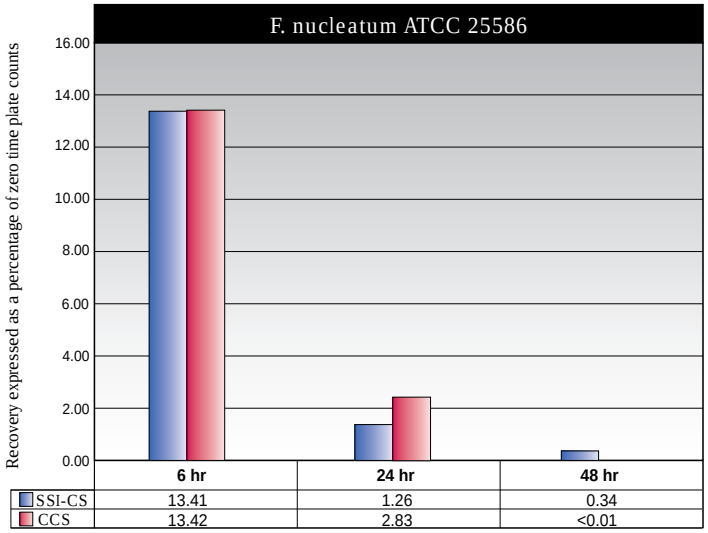

| | 6 hr | 24 hr | 48 hr |
|---|---|---|---|
| SSI-CS | 85.36 | 21.35 | 7.87 |
| CCS | 40.05 | 3.01 | 0.18 |



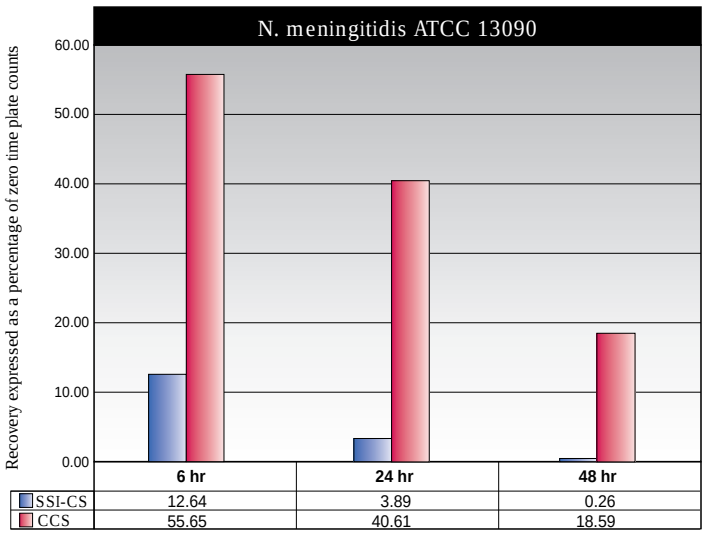

| | 6 hr | 24 hr | 48 hr |
|---|---|---|---|
| SSI-CS | 48.46 | 26.00 | 8.21 |
| CCS | 83.13 | 56.23 | 22.49 |



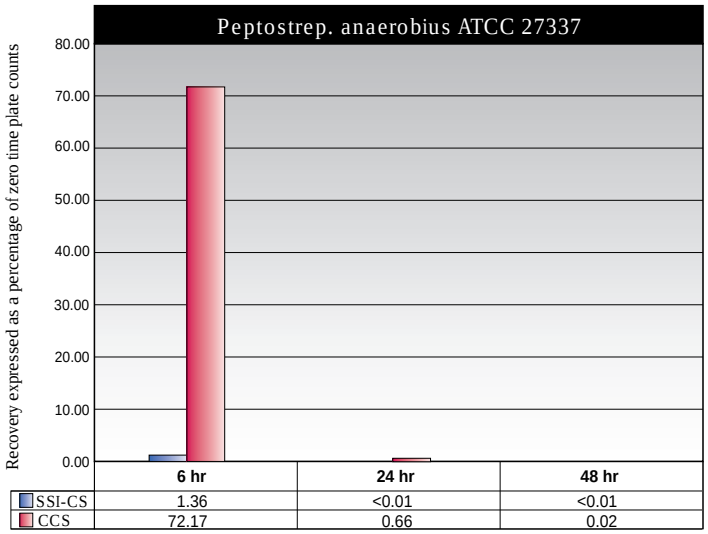

| | 6 hr | 24 hr | 48 hr |
|---|---|---|---|
| SSI-CS | 31.58 | 3.64 | 2.86 |
| CCS | 47.72 | 36.61 | 7.98 |




| | 6 hr | 24 hr | 48 hr |
|---|---|---|---|
| SSI-CS | 13.41 | 1.26 | 0.34 |
| CCS | 13.42 | 2.83 | <0.01 |




| | 6 hr | 24 hr | 48 hr |
|---|---|---|---|
| SSI-CS | 12.64 | 3.89 | 0.26 |
| CCS | 55.65 | 40.61 | 18.59 |




| | 6 hr | 24 hr | 48 hr |
|---|---|---|---|
| SSI-CS | 1.36 | <0.01 | <0.01 |
| CCS | 72.17 | 0.66 | 0.02 |