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Sunday

Can CAP Proficiency Testing Samples be Adequately Processed with Laboratory Automation? A Proof of Concept Study.

Scott Oliver[1], Laura Navarria[2], Arnalda Giambra[2], Susan Sharp[1]. [1]Copan Diagnostics US, Murrieta, CA, [2]Copan Italia, Brescia, Italy

Copan Diagnostics, Inc., US
800-216-4016
scott.oliver@copanusa.net

ABSTRACT

Background: Microbiology laboratories are required to test proficiency samples to assess performance. The CAP provides multiple proficiency testing (PT) samples annually to laboratories to be tested per their routine procedures. For many bacterial challenges, PT samples consist of fiber swabs inoculated with organisms that are to be processed manually per CAP instructions. However, some laboratories are processing patient samples using laboratory automation (LA); thus, there is a need for these laboratories to have information on the appropriate automated method for processing PT samples. This study was undertaken to design a workflow which would allow CAP PT samples to be processed using LA.

Methods: The first step of the study consisted of defining a possible automated workflow that would equate to the manual procedure currently included in CAP PT instructions. For this, 15 blank CAP fiber swabs were inoculated with suspensions containing 100µl of 10^8, 10^6 and 10^4 CFU/mL of E. coli ATCC 25922 (5 swabs per concentration). One from each concentration was processed manually per CAP instructions (method 1), the remaining swabs were processed using 4 different automated methods (2A, 2B, 3A, 3B). The second step consisted of using the WASP®DT: Walk-Away Specimen Processor to process 3 retained CAP PT samples with the method selected as optimal from the first step. Plates were incubated using Full Laboratory Automation system. WASPLab™ and images of growth taken after 18, 24, 36 and 48 hours of incubation.

Results: The swab inoculated with 10^4 CFU/mL of E. coli and processed with method 3A was determined to be optimal. Method 3A compared favorably to the CAP PT direct manual inoculation procedure (showing 63 CFU and 196 CFU, respectively) and it was the most efficient method studied. Briefly, method 3A consisted of vigorously mixing the PT fiber swab in 1ml of ESwab™ Amies medium, then pressing and rotating the swab against the side to the tube before discarding. For the 3 PT samples assayed using this method, the manual and automatic processing showed the same results: PT #1 grew gram negative rods, PT #2 grew gram positive cocci, and PT #3 also grew gram positive cocci, all in similar quantities.

Conclusion: Further testing with more challenging PT samples (including fastidious organisms, anaerobic organisms, and mixed samples) is needed, but this preliminary study suggests that the automatic processing of PT samples by LA is feasible. This will allow the laboratory to assess its performance using its routine automated workflow, as well as follow CLIA PT guidance in processing PT samples in a similar manner to that used for patient specimens.

INTRODUCTION

Currently, more sophisticated plating instrumentation along with additional laboratory automation has been developed, such as the WASPLab™ (Copan Diagnostics) and the Kiestra™ TLA System (Becton Dickinson). The newer instruments not only automate the plating of liquid specimens but allow for automated and continuous incubation of cultures along with digital imaging of plates for analysis. The WASPLab™ also has the capability to 'read' cultures with the use of innovative artificial intelligence and interpretive algorithms.

All laboratory specialties are legally required to participate in proficiency testing to assess their clinical performance. The College of American Pathologists (CAP) provides multiple proficiency testing (PT) samples annually to laboratories to be analyzed per their routine procedures. Many bacterial PT challenges consist of fiber swabs inoculated with organisms that are to be processed manually per CAP instructions. However, some laboratories are processing patient samples using the previously described laboratory automation platforms and are no longer processing patient swab collections manually. This study was undertaken to design a workflow which would allow CAP PT samples to be adequately processed using laboratory automation.

MATERIALS AND METHOD

To investigate the optimal method for PT sample processing by the WASP™ and WASPLab™ system (Figure 1), two approaches were taken utilizing both; I) contrived samples with E.coli, and II) three separate retained CAP PT samples.

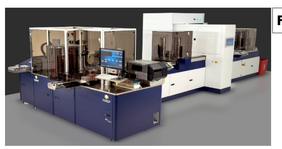


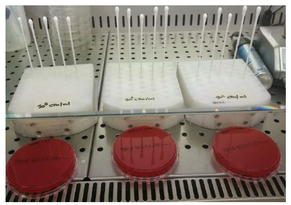
Figure 1: WASP™ and WASPLab™ system

Figure 2: ESwab™ tube

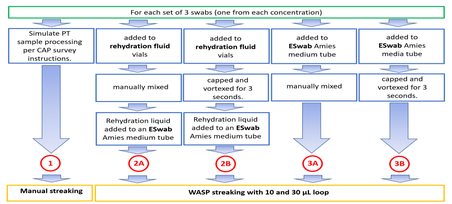
MATERIALS AND METHOD

Contrived sample preparation and processing: 3 suspensions of E. coli ATCC 25922 at different concentrations (10^8 CFU/ml, 10^6 CFU/ml and 10^4 CFU/ml) were used to inoculate 15 blank PT swabs. For each concentration, 100 µl were inoculated onto five swabs. The swabs were dried at RT for 10 minutes (Figure 3) and processed as illustrated below in Scheme I.

Figure 3: Contrived sample inoculation



Scheme 1: Contrived sample processing

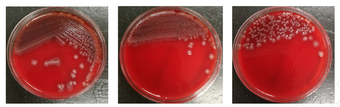


RESULTS (Contrived samples with E.coli)

Table 1: CFU/plate obtained using the 30 µl loop inoculum

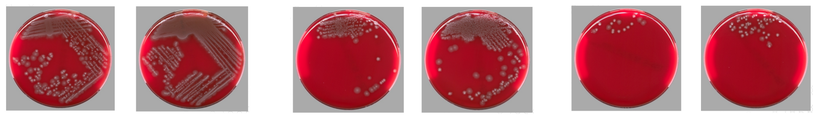
METHOD	E. coli Colony Count (30µl loop)
1 (Manual)	196 CFU/plate
2 A	36 CFU/plate
2 B	31 CFU/plate
3 A	63 CFU/plate
3 B	98 CFU/plate

1. Manual streaking per CAP recommendations



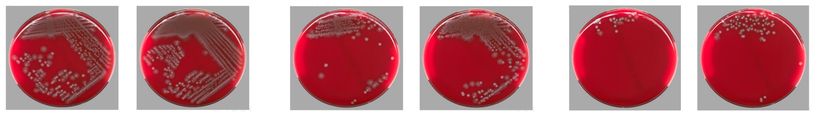
10^8 CFU/ml 10^6 CFU/ml 10^4 CFU/ml

3A. Swab directly into ESwab™ tube (Figure 2) and manually mixed



10^8 CFU/ml - 10µl volume 10^8 CFU/ml - 30µl volume 10^6 CFU/ml - 10µl volume 10^6 CFU/ml - 30µl volume 10^4 CFU/ml - 10µl volume 10^4 CFU/ml - 30µl volume

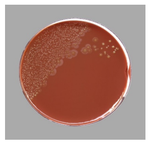
3B. Swab directly into ESwab™ tube and vortexed



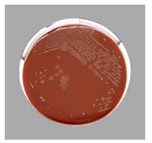
10^8 CFU/ml - 10µl volume 10^8 CFU/ml - 30µl volume 10^6 CFU/ml - 10µl volume 10^6 CFU/ml - 30µl volume 10^4 CFU/ml - 10µl volume 10^4 CFU/ml - 30µl volume

RESULTS (CAP PT Samples)

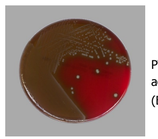
Contrived samples processed with the 3B method most closely matched the results of manual processing. However, as the vortex step may be inconvenient for some laboratories, method 3A could also be a reasonable method for processing PT samples. Method 3A using a 30 µl loop was selected for processing of the PT samples in the WASP™ and WASPLab™ system and is shown below.



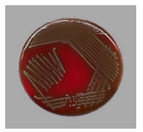
PT I: Manual streaking on chocolate agar/48 hours/CO_2 (Eikenella corrodens)



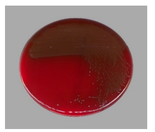
PT I: WASP™ streaking on chocolate agar/48 hours/CO_2 (Eikenella corrodens)



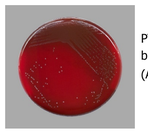
PT II: Manual streaking on blood agar/24 hours/CO_2 (Enterococcus casseliflavus)



PT II: WASP™ streaking on blood agar/24 hours/CO_2 (Enterococcus casseliflavus)



PT III: Manual streaking on blood agar/24 hours/CO_2 (Aerococcus urinae)



PT III: WASP™ streaking on blood agar/24 hours/CO (Aerococcus urinae)

DISCUSSION

As required by CLIA, and implemented by CAP and other proficiency testing organizations, PT samples must be tested with the laboratory's regular workload, using routine methods, and testing the PT samples the same number of times it routinely tests patient specimens. Laboratories that utilize microbiology culture processing and other automated features should be able to process and read PT samples as described by CLIA.

This preliminary study looked at various concentrations of organisms and procedures to allow automated sample processing that would be comparable to manual PT standard methods. Based on our initial studies, the described method 3A worked well for automated processing of PT samples. Additional studies utilizing fastidious organisms, anaerobic organisms, and mixed cultures should be evaluated but this preliminary study suggests that the processing and reading of PT samples by automation is feasible.