Smart handy plate: can it help in microbiology practical sessions? (S-03)

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Introduction

A pure bacterial culture is the foundation for the diagnosis of bacterial infection, determining antibiotic susceptibility, and/or studying bacteria itself. Furthermore, microbiologists still use it because of its cost and time-effectiveness. Bacterial culture involves many techniques that are mostly critical in the assessment of the culture. Plate counting and morphological assessment of the colonies are two critical steps for the determination and enumeration of bacteria, respectively. Both steps can be performed manually or automatically using computerized systems. The manual standard of care (SOC) for colony counting is time-consuming and prone to error. It also requires an expert in the field for highly accurate and precise results. Meanwhile, the automated plate assessment system (APAS) has high accuracy and precision, is less time-consuming and not affected by expertise, and has nearly 95.13% accuracy of manual enumeration [1], but it is highly expensive, non-portable, and not affordable for small labs, or multiple instruments may be required for large facilities and research centers. In this study, we are introducing the smart handy plate (SHP) with computer software to test whether it can be used for small-scale assessment of bacterial culture by identifying the organism using its morphology and counting bacterial colonies or not. This tool may be of help to non-experts, especially students, in their practical sessions.

Materials and methods

a) Plate design

For the SHP design, we used low-cost materials like a modified petri dish with black color and longer diameter than ordinary Petri dishes to serve as a holder for other prepared agar plates, a microcontroller (Arduino), light-emitting diodes (LEDs), Raspberry Pi high quality camera with 12.3 megapixels IMX477 sensor, a universal serial bus (USB) port, and a USB cable with two male ends.

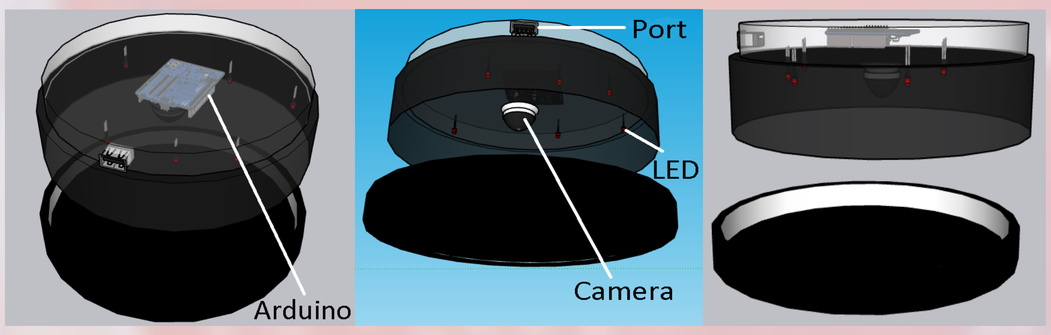


Figure (1) SHP "black version" 3D design using SketchUp application

b) Software development

For image processing, we developed computer software that can either be loaded on the microcontroller board or installed on the computer. We called this software SHP-Station™.

c) Morphology Identification

We used *Staphylococcus aureus (S.aureus)* and *Staphylococcus epidermidis (S.epidermidis)* to test the software and observe whether it can differentiate between them or not although there are minor differences between them on selective media. We cultured *S. aureus* and *S. epidermidis* using selective media as mannitol salt agar (MSA) and blood agar and tested their identification by the software based on a library with stored high-quality standard images from the internet.[6]

d) Colony enumeration

For colony enumeration, we used the pour plate method to prepare two agar plates, one of which contains *S.aureus* colonies and the other contains *S.epidermidis* colonies, to count them manually and automatically using the software.

e) Infection control and hygiene

The aseptic technique for all steps was followed. In addition, we wore personal protective equipment (PPE) like masks, gloves, and gowns for SHP cleaning. Moreover, we used isopropyl alcohol (IPA) before and after SHP use. We found that IPA is the most suitable for cleaning, especially for electronic devices like the microcontroller, LEDs, and camera.

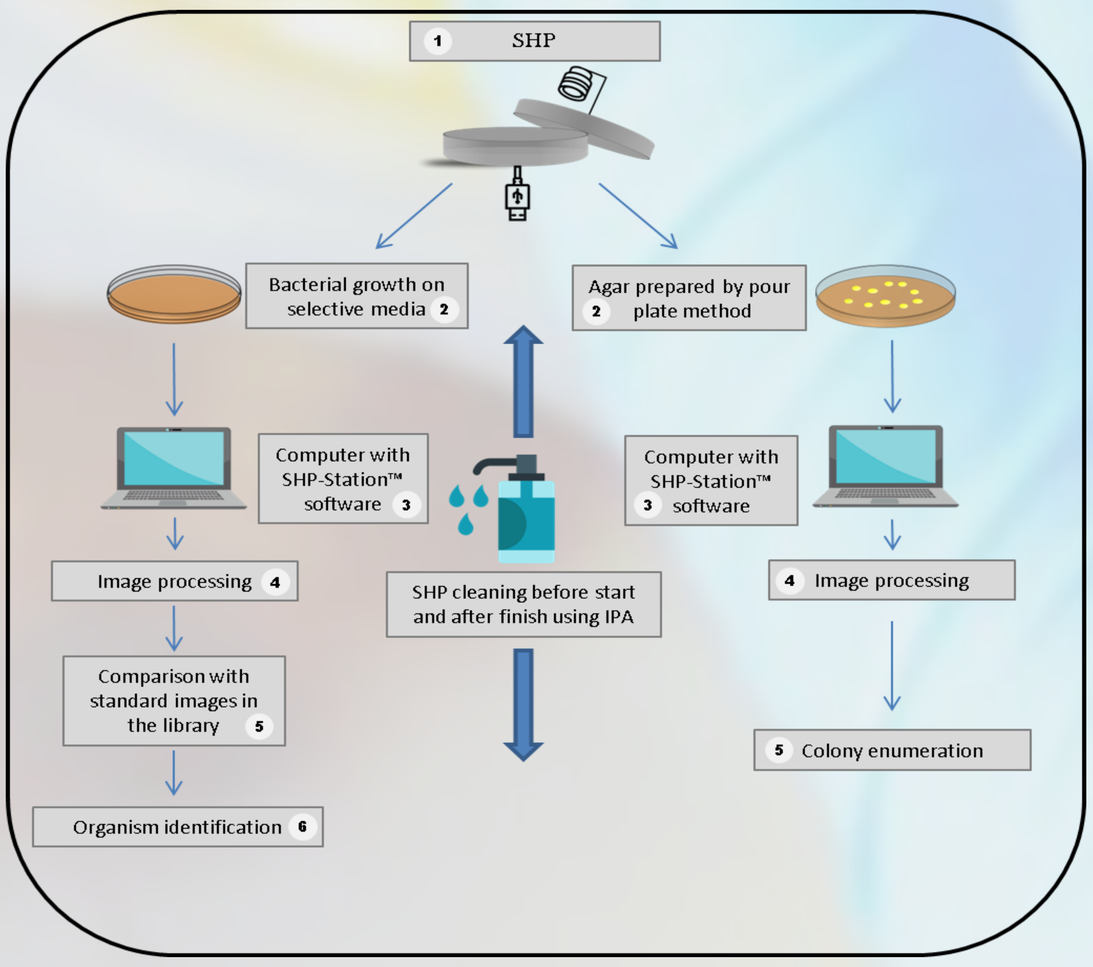


Figure (2) Schematic diagram showing the steps of using SHP

Results

Table (1) Morphology identification

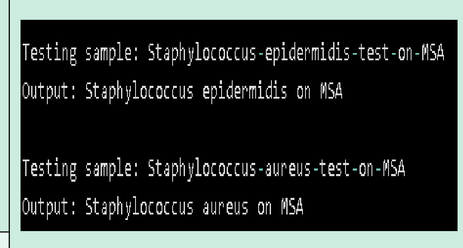
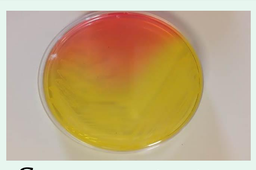
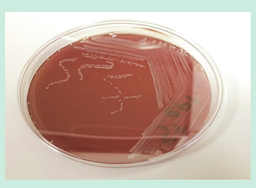
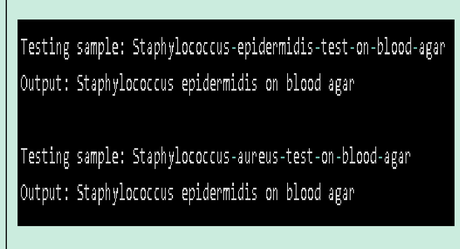
Standard "std"	Test	Results / Comments
 *S.epidermidis*-std on MSA	 *S.epidermidis*-test on MSA	 - Successful identification of the test - The average color of each picture was used by the software
 *S.aureus*-std on MSA	 *S.aureus*-test on MSA	
 *S.epidermidis*-std on blood agar	 *S.epidermidis*-test on blood agar	 - Unsuccessful identification of the test - Identification using average color is not viable
 *S.aureus*-std on blood agar	 *S.aureus*-test on blood agar	

Table (2) colony enumeration

Agar	Manual (# Colonies)	Software (# Colonies)	Accuracy (%)
S.aureus agar	146	135	92.5
S.epidermidis agar	68	67	98.5

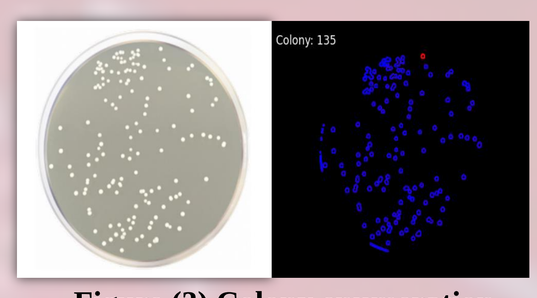


Figure (3) Colony enumeration for *S.aureus* agar plate

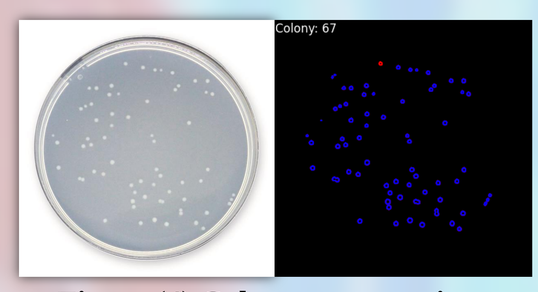


Figure (4) Colony enumeration for *S.epidermidis* agar plate

Conclusion

To sum up, SHP showed promising results in round colony enumeration, such as *S. aureus* and *S.epidermidis* colonies, and morphology identification for *S.aureus* and *S.epidermidis* on the MSA plate. On the other hand, it couldn't differentiate between *S.aureus* and *S.epidermidis* on blood agar due to close similarities in colors, which needs more advanced approaches. Another point to be considered in morphology identification is that selective media must be used to ensure morphology differences between bacterial species and aid in accurate identification. SHP is a simple, low-cost, portable, and promising tool compared to other systems in the world of digitalization in clinical microbiology.

Recommendations

In the future, we recommend carrying out further studies on SHP to add more features, like antibiotic susceptibility testing (AST), and comparing the results with those of the Clinical and Laboratory Standards Institute (CLSI) to help in the proper choice of antibiotics, thus improving clinical decisions. Moreover, we recommend using vaporized hydrogen peroxide (VHP) in the sterilization process of SHP to ensure more effective infection control. Lastly, for morphology identification, we recommend using more advanced approaches that use artificial intelligence (AI) like computer vision instead of image processing for more accurate results in color and shape identification.

References

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