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Original Article

Towards automated detection, semi-quantification and identification of microbial growth in clinical bacteriology: A proof of concept

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ABSTRACT

Background: Automation in microbiology laboratories impacts management, workflow, productivity and quality. Further improvements will be driven by the development of intelligent image analysis allowing automated detection of microbial growth, release of sterile samples, identification and quantification of bacterial colonies and reading of AST disk diffusion assays. We investigated the potential benefit of intelligent imaging analysis by developing algorithms allowing automated detection, semi-quantification and identification of bacterial colonies.

Methods: Defined monomicrobial and clinical urine samples were inoculated by the BD Kiestra™ InoqulA™ BT module. Image acquisition of plates was performed with the BD Kiestra™ ImagA BT digital imaging module using the BD Kiestra™ Optis™ imaging software. The algorithms were developed and trained using defined data sets and their performance evaluated on both defined and clinical samples.

Results: The detection algorithms exhibited 97.1% sensitivity and 93.6% specificity for microbial growth detection. Moreover, quantification accuracy of 80.2% and of 98.6% when accepting a 1 log tolerance was obtained with both defined monomicrobial and clinical urine samples, despite the presence of multiple species in the clinical samples. Automated identification accuracy of microbial colonies growing on chromogenic agar from defined isolates or clinical urine samples ranged from 98.3% to 99.7%, depending on the bacterial species tested.

Conclusion: The development of intelligent algorithm represents a major innovation that has the potential to significantly increase laboratory quality and productivity while reducing turn-around-times. Further development and validation with larger numbers of defined and clinical samples should be performed before transferring intelligent imaging analysis into diagnostic laboratories.

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At a glance commentary

Scientific background on the subject

The introduction of laboratory automation has revolutionized conventional clinical bacteriology from samples inoculation to plates incubation and reading. With this new technology, the reading of plates is performed on digital images by technicians that can select microbial colonies for subsequent follow-up work such as identification and antibiotic susceptibility testing.

What this study adds to the field

The study shows as a proof of concept that artificial intelligence may represent a driving innovation in diagnostic bacteriology. Intelligent algorithms for plates analysis linked to expert systems may provide a fully automated approach for microbial growth reading and interpretation that could eventually replace and/or support human-based decisions.

For many years, diagnostic microbiology was not considered as being adapted for laboratory automation due to the variability of the specimen types, the complexity of the various analytical processes and a relatively low level of analytical samples volume compared to other diagnostic units such as chemistry and molecular biology. However, the gradual increase in samples number, limited budget, personal shortage and quality issues as well as laboratories consolidation and liquid-based transport devices have triggered the development and the introduction by different manufacturers of laboratory automation solutions into diagnostic bacteriology laboratories [1–4]. Several peer-reviewed publications have demonstrated that laboratory automation have the potential to greatly improve the diagnostic processes in bacteriology by increasing the productivity, the quality and the throughput but also by decreasing the time-to results and laboratory cost [1,5–13]. Even though the indirect impact of lab automation on patient management remained to be demonstrated in objective, comparative and prospective clinical studies performed by independent laboratories, the shortening of time-to results observed after implementation of laboratory automation strongly suggests that automation will positively improve the clinical management of patients suffering from infectious diseases.

The partial automation available in bacteriology covers four main laboratory processes: inoculation, plate management, incubation and digital imaging [1]. However, a significant part of diagnostic microbiology such as samples preprocessing, microscopy, reading and follow-up work such as identification (ID) and antibiotic susceptibility testing (AST) of isolated colonies remain to be automatized to reach a true total lab automation. Several manufacturers are working on additional hardware solutions to further increase the level of automation in bacteriology such as (1) sample input track, (2) automated colony picking modules including automated deposition of the samples on MALDI-TOF plates as well as automated preparation of bacterial suspension for automated or disk diffusion AST, (3) automated disk dispensing modules and (4) broth incubators [1].

Even though these new technologies will further improve laboratory automation with increased productivity, the next revolution with a major impact on diagnostic microbiology will likely arise from the development of intelligent algorithms and applications linked to expert systems that may in the future monitor several laboratory processes from inoculation to ID/AST without human intervention. To reach such a level of intelligent automation, several algorithmic and application tools need to be developed and validated before being used by intelligent expert systems for the monitoring of laboratory processes. Thus, further improvements will be driven by the development of intelligent image analysis algorithms allowing earlier detection of microbial growth, automated detection and auto-release of sterile samples, automated identification and quantification of bacterial colonies as well as automated reading of AST disk diffusion assays.

We thus investigated the potential benefit of intelligent imaging analysis by developing several algorithms and applications allowing automated detection, identification and semi-quantification of bacterial colonies from both defined and clinical urine samples.

Material and methods

Strains, media, and bacterial suspensions

Most bacterial and yeast strains used in this study (Tables A.1–A.3) were selected according to the most prevalent strains isolated in clinical urine samples in 2014 at the University Hospital of Lausanne (CHUV), Switzerland. The strains were grown on Columbia agar with 5% sheep blood (Columbia III agar; BD, Franklin Lakes, NJ, USA) at 37 °C in normal atmosphere or in 5% CO_2 atmosphere incubators. Colonies of each bacterial species were utilized to prepare a bacterial suspension in saline solution adjusted to a 0.5 McFarland turbidity measured with a DensiCheck densitometer instrument (bioMérieux, Marcy l'Etoile, France). The exact bacterial concentration corresponding to a 0.5 McFarland were assessed for each bacterial and yeast species and for each experimental run by measuring the colony forming units (CFU) on Columbia agar with 5% sheep blood (Table A.1). Different concentrations of monomicrobial suspensions were prepared by doing serial 10-fold dilutions in saline solutions, ranging from 1 to 10^{-5}.

Clinical urine sample collection and processing

A total of 218 clinical urine samples were collected with UriSwab™ tubes (Copan, Brescia, Italy) during a 2-months period from outpatients and hospitalized patients at the CHUV, without selection criteria. All clinical urine samples were deidentified prior to testing. Selected urinary samples were immediately processed or stored for maximum 8 h at 4 °C until inoculation. UriSwab™ contains preservative substances (boric acid and sodium formate) that both preserve

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