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Rapid, Direct, Real-Time Method for Bacteria Detection on Surfaces

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Abstract : Preventing the spread of infectious diseases throughout the worldwide is one of the most important tasks of modern health care. Infectious diseases not only account for one fifth of the deaths in the world, but also cause many pathological complications for the human health. Touch surfaces pose an important vector for the spread of infections by varying microorganisms, including antimicrobial resistant organisms. Further, antimicrobial resistance is reply of bacteria to the overused or inappropriate used of antibiotics everywhere. The biggest challenges in bacterial detection by existing methods are non-direct determination, long time of analysis, the sample preparation, use of chemicals and expensive equipment, and availability of qualified specialists. Therefore, a high-performance, rapid, real-time detection is demanded in rapid practical bacterial detection and to control the epidemiological hazard. Among the known methods for determining bacteria on the surfaces, Hyperspectral methods can be used as direct and rapid methods for microorganism detection on different kind of surfaces based on fluorescence without sampling, sample preparation and chemicals. The aim of this study was to assess the relevance of such systems to remote sensing of surfaces for microorganisms detection to prevent a global spread of infectious diseases. Bacillus subtilis and Escherichia coli with different concentrations (from 0 to 10x8 cell/100µL) were detected with hyperspectral camera using different filters as visible visualization of bacteria and background spots on the steel plate. A method of internal standards was applied for monitoring the correctness of the analysis results. Distances from sample to hyperspectral camera and light source are 25 cm and 40 cm, respectively. Each sample is optically imaged from the surface by hyperspectral imaging system, utilizing a JAI CM-140GE-UV camera. Light source is BeamZ FLATPAR DMX Tri-light, 3W tri-colour LEDs (red, blue and green). Light colors are changed through DMX USB Pro interface. The developed system was calibrated following a standard procedure of setting exposure and focused for light with λ=525 nm. The filter is ThorLabs KuriousTM hyperspectral filter controller with wavelengths from 420 to 720 nm. All data collection, pro-processing and multivariate analysis was performed using LabVIEW and Python software. The studied human eye visible and invisible bacterial stains clustered apart from a reference steel material by clustering analysis using different light sources and filter wavelengths. The calculation of random and systematic errors of the analysis results proved the applicability of the method in real conditions. Validation experiments have been carried out with photometry and ATP swab-test. The lower detection limit of developed method is several orders of magnitude lower than for both validation methods. All parameters of the experiments were the same, except for the light. Hyperspectral imaging method allows to separate not only bacteria and surfaces, but also different types of bacteria, such as Gram-negative Escherichia coli and Gram-positive Bacillus subtilis. Developed method allows skipping the sample preparation and the use of chemicals, unlike all other microbiological methods. The time of analysis with novel hyperspectral system is a few seconds, which is innovative in the field of microbiological tests.

Keywords : Escherichia coli, Bacillus subtilis, hyperspectral imaging, microorganisms detection
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