DNA hybridization assays and quantitative polymerase chain reaction using a smartphone as a chemiluminescence imager.

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ABSTRACT

Polymerase chain reaction (PCR) is a fundamental technique in nucleic acid analytical chemistry. PCR entails *in vitro* exponential amplification of DNA/RNA sequences enabling ultra-low detection levels in complicated sample matrices. Quantitative competitive PCR has found widespread applications in the health, food and environmental sectors. The determination of the amplification products has been based on electrophoresis, fluorometry, chemiluminometry, mass spectrometry and flow-cytometry. All the above methods require costly equipment and highly qualified personnel. In this work, we introduce the smartphone as a low-cost chemiluminescence imager for the development of *(a)* DNA hybridization assays and *(b)* quantitative competitive PCR assays (QCPCR). For the hybridization assay, a specific oligonucleotide probe was immobilized in microtiter wells and allowed to hybridized with biotinylated denatured dsDNA target. The hybrids were determined by adding an avidin-peroxidase conjugate in combination with a chemiluminogenic substrate. The emitted light was detected by a smartphone and the results were compared with a conventional digital camera and a microtiter-plate luminometer (with a photomultiplier). The limits of detection of the DNA target based on the smartphone, digital camera and luminometer were 1.6, 2.4 and 1 pmol/L, respectively. For QCPCR, a suitable dsDNA internal standard (competitor), with the same size and same primer binding sites as the target sequence, was synthesized and 5000 molecules were added to each amplification reaction. The amplification products from the target and competitor were determined by the hybridization assay and the ratio of the smartphone-obtained signals were related to the number of target DNA copies in the sample prior to PCR. Smartphone-based QCPCR showed an analytical range from 137 to $9x10^5$ copies of target DNA. The CVs for the QPCR ranged from 7-17%.

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