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A Novel, Rapid Diagnostic Molecular Method for Sars-Cov-2 Detection by Nanoprobes

Pablo Cea-Callejo [1,2], Sonia Arca-Lafuente [2], Laura Benítez Rico[1], Ricardo Madrid [2]
[1]Departamento de Genética, Fisiología y Microbiología, Facultad de Biología, Universidad Complutense de Madrid
Av. Séneca, 2, 28040 Madrid, Spain
pcea@ucm.es, lbenitez@ucm.es
[2]BioAssays SL
c/Faraday, 7, Parque Científico de Madrid, Campus de Cantoblanco, 28049 Madrid, Spain
sarca@arquimea.com, rmadrid@arquimea.com

Extended Abstract

Early diagnosis of active viral infections is crucial to prevent silent transmission among the population, as we have seen during the present COVID-19 pandemic. Single stranded RNA viruses, including SARS-CoV-2, are currently detected using molecular methods, such as the gold standard qRT-PCR. These methods require expensive equipment and specialized training, which limits its use as point-of-care (POC) diagnostic systems. We have developed a novel, fast and simple diagnostic method, based in the combination of Isothermal Loop Amplification (LAMP) [1], [2] and oligonucleotide-functionalized gold nanoparticles (AuNPs). If viral RNA is amplified and recognized by de DNA probe, a change in gold nanoparticles aggregation state initiates, leading to a wavelength change in the region of the visible spectrum, evident by naked eye.

The developed system has been designed by BioAssays SL to detect SARS-CoV-2. After successful results detecting synthetic targets, we have pre-validated our novel diagnostic system with a 364 sample panel from SARS-CoV-2 positive patients (83 positive samples and 281 negative samples). We analyzed two different primer sets, targeting Gen-E and Gen-N respectively, and we determined that the Gen-N primer set reliably detected SARS-CoV-2 RNA with an RT-qPCR cycle threshold (CT) number of up to 30, with a 98% sensitivity and 100% specificity compared to commercial qRT-PCR diagnostic kits. These results showed that RT-LAMP is sensitive enough to detect viral RNA within 30 min and a detection limit of 150 copies per mL when using these primers.

To allow a colorimetric visualization of the results, we coupled LAMP amplification to AuNPs detection [3], [4]. DNA-AuNPs (nanoprobes) were able to detect LAMP results in a saline buffer in 15 minutes, leading to aggregation in the absence of RT-LAMP amplification. Then, nanoprobes remained stable in saline buffer when they specifically recognized SARS-CoV-2 RT-LAMP product, and the red colour of the solution was indicative of a positive result. All positive samples amplified by RT-LAMP were successfully detected by nanoprobes.

In conclusion, our LAMP detection system coupled to nanoprobe visualization is sensitive enough to detect viral RNA within less than 60 min by the naked eye, with 98% sensitivity and 100% specificity, overcoming sensitivity limitations of previously described nanoprobe systems [5]. Due to the potential threat of re-emerging we have also designed a LAMP-nanoprobe detection system for Hepatitis C virus and we are currently starting pre-validation phases too. Upon *in vivo* validation, a marketable diagnostic kit will be developed that can be implemented in the health system as a routine method for assay. The estimated cost for the diagnostic kit is 5€ per assay. The simplicity of this technology allows its ready transfer and optimization for to the detection of other viral diseases by RNA or ssDNA viruses such as measles virus, Zika, Dengue, HIV-1, or even animal viruses, such as Avian Metapneumovirus (aMPV).

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