Sequence-specific validation of LAMP amplicons in real-time optomagnetic detection of Dengue serotype 2 synthetic DNA

We report on an optomagnetic technique optimised for real-time molecular detection of Dengue fever virus under ideal as well as non-ideal laboratory conditions using two different detection approaches. The first approach is based on the detection of the hydrodynamic volume of streptavidin coated magnetic nanoparticles attached to biotinylated LAMP amplicons. We demonstrate detection of sub-femtomolar Dengue DNA target concentrations in the ideal contamination-free lab environment within 20 min. The second detection approach is based on sequence-specific binding of functionalised magnetic nanoparticles to loops of LAMP amplicons. Melting studies reveal that true positive and spurious amplicons have different melting points and this allows us to discriminate between them. This is found to be in a good agreement with subsequent studies on real-time sequence-specific discrimination of LAMP amplicons. The specific binding causes clustering of magnetic nanoparticles via binding to multiple sites (loops) emerging in the elongation phase of LAMP. Formation of nanoclusters is monitored via the depletion of the optomagnetic signal due to free nanoparticles. After sequence-specific validation, we claim detection of down to 100 fM of Dengue target after 20 min of LAMP with a contamination background.

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