S.M.A.R.T. Novel, Real-Time, Simple RNA-based Molecular Diagnostic at Point-of-Care for Influenza and Other Diseases (Case 1964)

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Technology description

Brief Description:

A major diagnostic problem in clinical medicine is the lack of a rapid, sensitive, reliable point-of-care device for detecting RNA-based pathogens such as H5 influenza, among others. Faster, more sensitive and efficient diagnosis via RNA would lead to expedited treatment resulting in potentially better patient outcomes and improved public health safety. A current methodology, the polymerase chain reaction (PCR) assay and variations of require precise equipment and conditions that are not conducive to a point-of-care solution. Another approach, Nucleic Acid Sequence-Based Amplification (NASBA) may currently be the best choice for a point-of-care device as it involves using an isothermal amplification step lasting only 90 minutes. However, NASBA has not performed as expected in the laboratory potentially due to secondary structure in the RNA that prevents efficient primer binding and enzyme progression or may even stop the amplification reaction.

Here, we offer the ‘Simple Method to Amplify RNA Targets’ or S.M.A.R.T., a compelling alternative to NASBA to provide a solution to secondary structure issues that can occur in amplification processes. The invention, S.M.A.R.T. is a new instant assay method to detect, in real-time, short RNA sequences. This innovation extends beyond NASBA particularly where NASBA is ineffective due to molecular constraints. The S.M.A.R.T. technique also provides advantages over PCR in that this novel assay can be translated into a portable, point-of-care device/platform in a variety of formats such as, but not limited to, microfluidic or droplet. Furthermore, additional and optional intellectual property is available that involves a microfluidic chip or droplet technology [incorporating the present method] to allow for a cost-effective option requiring the use of minimal reagents and facilitating a robust point-of-care device. Both the capture and amplification steps are incorporated in these device options.

The innovative S.M.A.R.T. method exponentially amplifies strands of target RNA, complementary to at least a portion of a target nucleotide sequence (i.e., RNA, DNA, and other nucleotides), if the target nucleotide sequence is present in a sample. The sample may be an aliquot, suspension, or fraction that contains the target nucleotide under investigation. S.M.A.R.T. employs rapid, isothermal, cyclic amplification and optimally engineers a short probe with minimal secondary structure for improved primer binding.

A variety of detection and/or quantification methods can be implemented with the S.M.A.R.T. protocol such as fluorescence or gel electrophoresis via molecular beacons. S.M.A.R.T. can detect multiple target sequences, i.e., different genotypes associated with the same clinically relevant phenotype, with the same molecular beacon combined with other optimized primer conditions; this is believed to provide more favorable amplification thermodynamics than typical multiplex NASBA reactions, which are less efficient due to the number of primers present. In addition, the use of a single beacon lowers testing costs and simplifies detection. S.M.A.R.T. has been optimized for H5 influenza, but can be used to diagnose many other disease states.

Application area

The primary market is clinical molecular diagnostic assays with applications in detection and diagnosis of human and/or animal diseases such as H5 influenza, among others, and in clinical testing laboratories and/or point-of-care scenarios – at the bedside, home or in the remote field. Market segments include human and veterinary. Another market is in scientific R&D research tools for use in experimentation to furthering various biomedical fields, e.g., infectious diseases and/or oncology.

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