Single-probe Multiplex PCR for Identifying Pathogenic Variants (PrimeSafe II)

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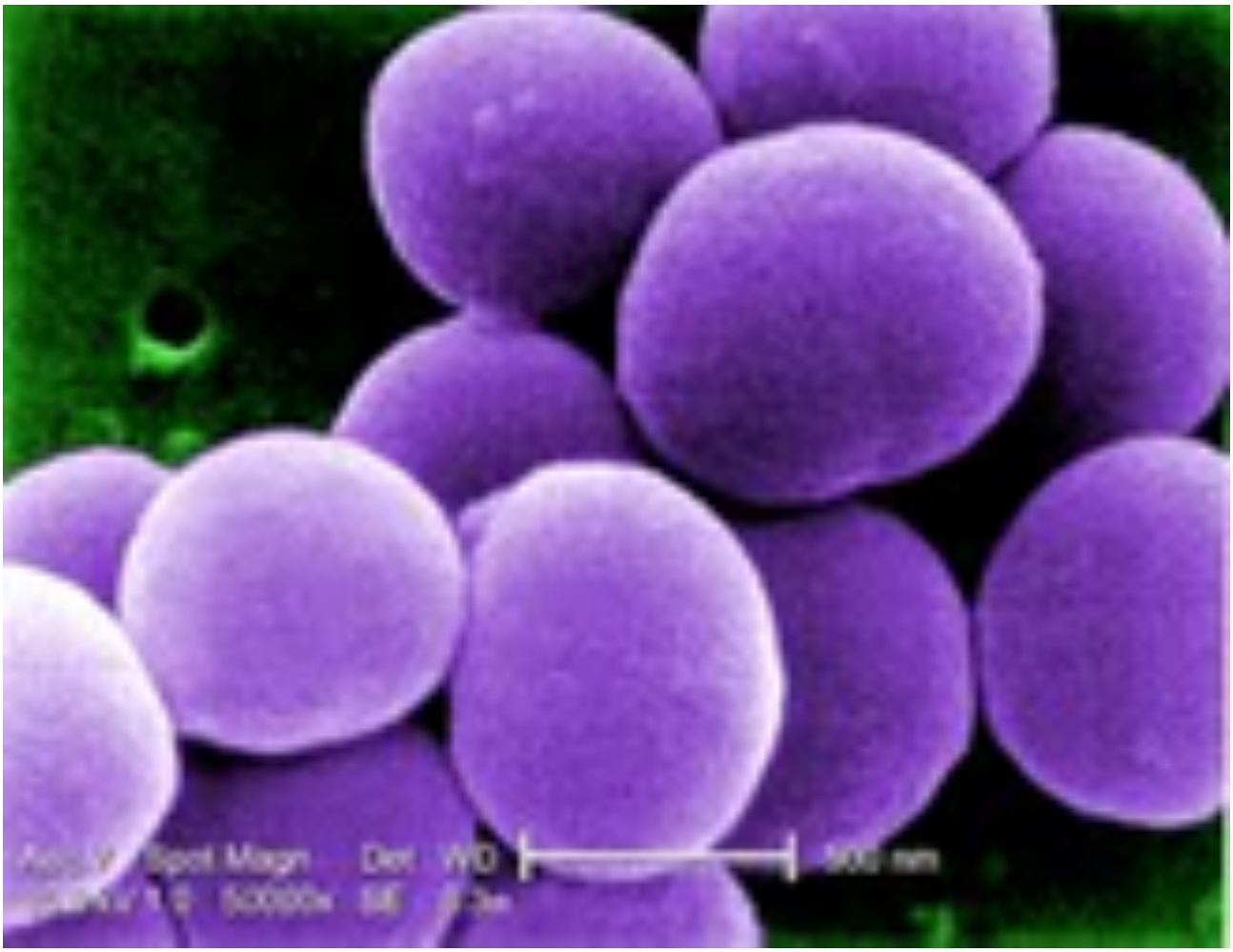
Technology description

The inventions available for licensing provide rapid methods to differentiate between various species and strains of microbial pathogens in complex samples using single-tube multiplex PCR assays that each contain a single-labeled fluorescence detection probe. The presence of a sequence variant in a sample is identified through the generation of a specific signature fluorescence signal ratio using a multiple temperature end-point signal probe detection strategy.



Staphylococcus aureus (S. aureus)is a Gram-positive commensal bacterium and opportunistic pathogen. Toxins produced by the bacteria can cause a wide range of maladies in animals and humans that vary considerably in their severity from food poisoning, simple skin infections, and even severe

life-threatening infections of the blood, bones and other organs. In the USA, ~20% of the adult population carries*S. aureus* in their nares persistently whereas ~30% of the population is intermittently colonized by *S. aureus*. The bacterium can be spread by skin-to-skin contact or through the use of shared items and surfaces so infections from *S. aureus* are common in patients at surgical sites or from implanted medical devices.

There are two broad categories of *S. aureus* based on an individual clone's susceptibility to a class of *B. lactam* antibiotics which includes methicillin – methicillin susceptible *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA). Despite the number of MRSA strains increasing in hospital and community settings over the last two decades, there are still very few antibiotics (e.g. vancomycin) known to be effective in treating these drug-resistant infections which carry an average treatment cost of more than $23,000 per incidence.

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Due to single base differences in amplicons located adjacent to the probe's hybridization site, it has been discovered that these slight sequence variations will cause the signal probe to have different annealing and melting temperatures on each of the polymorphic target sequences. As such, detection of sequence variants can be identified by using the temperature/temperature signal ration when measured at two different temperatures along a melting or binding curve. Since the hybridization site for the fluorescent probe on all the polymorphic nucleic acid targets is identical, it is believed that microbial variants are distinguished based on differences in secondary structure of the target nucleic acid sequences.

While the methods can be used to identify the presence of polymorphic nucleic acid sequence variants in multiple species, the assays have been enabled for detection of SCCmec variants in *S. aureus* containing samples using primers that target the SCCmec region of MRSA spanning the mecA-orfX boundary region (e.g. SCCmec type I; SCCmec type II; SCCmec type III; SCCmec type IV; SCCmec type V; and SCCmec type VII). The assay kits can be used to detect or rule out the presence of different *Staphylococcus* species in samples including MSSA, MSSE, MRSA, MRSE, VRSA, and VRSE.

Summary

· The single-tube assays can detect separate species of *Staphylococcus* in a quick and easy manner so the patient is treated with the correct antibiotic therapy

· The assay methods utilize asymmetric PCR amplification or LATE-PCR strategies

· A sample is exposed to multiple temperatures that allows for nucleic acid amplification in parallel to the generation of annealing and melt curves for the labeled fluorescent signaling probe to the target amplicon

· Sensitive readouts for the presence or absence of a virulent pathogen is enabled by observing the unique temperature/temperature fluorescence ratio for the probe’ s binding interactions with the amplified DNA

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