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Technical Note

Detection of *Staphylococcus aureus* using Spex 2-Color qPCR Kit

Introduction

S. aureus is a ubiquitous gram-positive bacterium that is known to infect plant tissues, as well as the soil. *S. aureus* infections are known to cause a variety of illnesses in humans, such as endocarditis, deep-seated abscesses, osteomyelitis, bacteraemia, pneumonia, and meningitis (DOI: 10.1111/j.1348-0421.1995.tb03280.x). Agricultural and nutraceutical products can become contaminated by *S. aureus* in resulting in human foodborne illnesses. Products such as cannabis, vapes and tobacco can cause *S. aureus* exposure by direct introduction of contaminated vapors into the respiratory tract.

Increasingly, ordinary treatments, such as with antibiotics like penicillin and its relatives, are becoming less effective in treating infections caused by resistant *S. aureus* bacterium. *S. aureus* (especially antibiotic resistant strains) is one of the pathogens responsible for the highest number of deaths and serious illness around the world.

There are many different *S. aureus* subtypes, such as methicillin resistant (MRSA), methicillin sensitive (MRSA and MSSA), vancomycin intermediate (VISA), and vancomycin resistant *S. aureus* (VRSA). Some strains of *S. aureus* are also know to excrete toxic substances that can cause toxic shock syndrome and food poisoning. Although MRSA is widely recognized and represents the leading cause of *S. aureus* associated illness, any *S. aureus* infection can be dangerous.

Therefore, the detection of *S. aureus* is imperative for safe agricultural products and the safe use of cannabis. Traditionally, *S. aureus* is detected via a test for the coagulase or a thermally stable nuclease enzyme in a clinical setting (DOI: 10.1186/1476-0711-9-23). However, the coagulase or nuclease activity is not unique to *S. aureus* and does not guarantee a reliable and consistent identification of *S. aureus*.

In contrast, the PCR based amplification and detection of the *nuc* gene, which encodes the thermostable nuclease, is widely considered the gold-standard for *S. aureus* detection (DOI: 10.1186/1746-0711-9-23). The *nuc* gene has been used to successfully detect over 80 clinical isolates of *S. aureus*, with no amplification of DNA from over 60 isolates of other *Staphylococcus* species and non-*Staphylococcus* strains of bacteria.

The Spex 2-Color *Staphylococcus aureus* quantitative PCR (qPCR) kit is designed to detect the presence of *Staphylococcus aureus (S. aureus)* in the DNA of plant material including cannabis flower, stem or leaf tissue. The kit is designed to detect *S. aureus* via amplification of the *nuc* gene.

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Materials

The kit comes equipped with:

- A master mix including
  - Recombinant Taq DNA polymerase
  - An aptamer-based hot start technology to inhibit non-specific amplification
  - Proprietary cocktail of PCR enhancers that promote a specific and efficient amplification of *S. aureus* DNA.
- *Staphylococcus aureus* positive control: a non-coding fragment of the *S. aureus nuc* gene was PCR amplified and cloned into a vector. The vector is linearized and used as a positive control for the kit. This positive control is detected via the FAM channel.
- Internal control template DNA – a template optimized for PCR was TA cloned and linearized for use as an internal control for PCR. This control is detected via the HEX or VIC cannel.
- ROX – an inert dye used for well-to-well normalization in qPCR
- Yellow dye (20X) – an inert yellow dye
- Water (DNase-, RNase-, Protease-free)

Method

1. Thaw all components on ice and mix tubes by inversion to ensure homogeneity
2. Before first use, ROX is added to the **2X *Staphylococcus aureus* Detection Master Mix** to ensure qPCR machine compatibility
3. Afterwards, 10 µL of the **2X *Staphylococcus aureus* Detection Master Mix** is added to designated wells of a qPCR plate
4. In a separate 1.5 mL tube, the following components are mixed:

Component	Volume (10 µL)
20X Yellow Dye	1 µL
DNA template*	1–6 µL
Internal Control Template	2 µL
Water	up to 10 µL

** For the positive control, replace the DNA template with 2 µL of the Staphylococcus aureus positive control template. For negative control, do not add any DNA.*

5. Briefly centrifuge components and pipette to wells containing the **2X *Staphylococcus aureus* Detection Master Mix** and qPCR is performed using the following conditions:

Step	Temperature (°C)	Time	Number of Cycles
Denature	95	1 minute	1
qPCR Detection	95	10 seconds	35
	60	30 seconds	

Results and Discussion

Results from the qPCR should be available within 45–60 minutes depending on the ramp rates of the qPCR instrument. The figure below demonstrates results that can be expected when using the 2-color S. aureus qPCR kit.

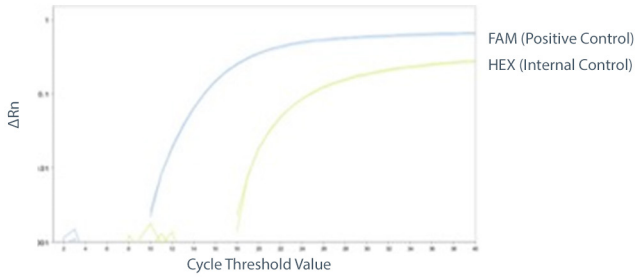

Figure 1. Expected results when using the 2-Color *S. aureus* qPCR Kit. The positive and internal control template activates the FAM and HEX channels, respectively.

The limit of detection was determined to be less than 50 copies of S. aureus genomic DNA. Importantly, the primers and probes in the master mix generate a linear increase in cycle threshold values throughout the entire dilution range (Figure 2).

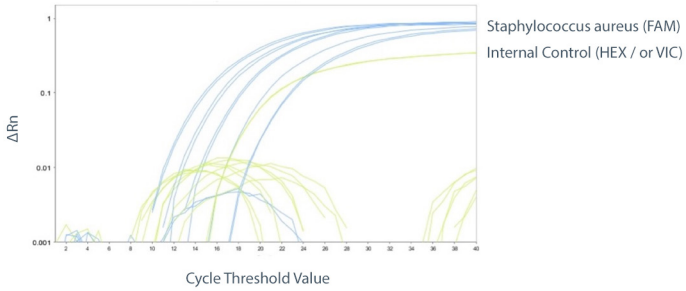

Figure 2. Serial dilution of *Staphylococcus aureus* DNA to determine limit of detection. The genomic DNA from *Staphylococcus aureus* was serially diluted and 2 µL of the diluted DNA was used for qPCR using the Spex 2-Color *S. aureus* detection master mix (FAM channel) in duplicate. Internal control DNA was detected via the HEX or VIC channel.

Analysis of the cycle threshold values versus negative Log_{10} of DNA copy number was also performed. An R-squared value of 0.96 was observed (Figure 3), indicating that primers and probes are optimized and generate a linear increase in cycle threshold values with increase in *S. aureus* genomic DNA concentration.

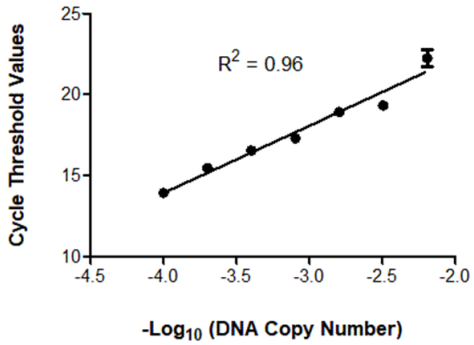

Figure 3. Dilution curve of S. aureus DNA. The positive control DNA from *Staphylococcus aureus* was serially diluted and 2 µL of the diluted DNA was used for qPCR using the 2-color *S. aureus* detection master mix (FAM channel) in duplicate. Then, the negative log of DNA copy number was graphed against the average cycle threshold value. A linear relationship between DNA copy number and cycle threshold values were observed (R-squared value: 0.96).

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