PCRassays.com

ASSAY NAME: AMR1_QS (Antimicrobial resistance genes panel 1 for QuantStudio)

Quantity: 100 x 20µL PCR reactions

5-plex assay: mecA, mecC, vanA, vanB, and human RPP30 DNA

SKU: PNP-AMR1-D-QS-100 (QuantStudio)

(RUO). Research Use Only. Not for use in Diagnostic Procedures.

SCOPE OF THIS PRODUCT INFORMATION SHEET (PIS):

The oligonucleotide recipes are optimized for each instrument (BioRad, QuantStudio, MIC). The verification data presented in this PIS were performed using PNP-AMR1-D-QS-100 on a QuantStudio™ 7 Flex Real-Time System. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if you need to use a different qPCR instrument.

CONTENTS

The AMR1 multiplexed qPCR kit contains a mixture of primers/probes targeting the genes for mecA (PBP2a family peptidoglycan transpeptidase), mecC (PBP2a family beta-lactam-resistant peptidoglycan transpeptidase), vanA, and vanB. The primers and probes in the AMR1 assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 antimicrobial resistance genes (AMR) and a human extraction control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
mecA	FAM	BHQ-1	1,2
vanB	HEX	BHQ-1	3
RPP30-DNA control	TAMRA	BHQ-2	4,5
mecC	TEX615	BHQ-2	6
vanA	Cy5	BHQ-2	7

The probes are designed as TaqMan[8] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

Table of AMR Genes and the Drugs and Organisms where resistance is conferred:

AMR Gene	Drugs	Organisms
mecA	Methicillin, Oxacillin	*S. argenteus, S. aureus, S. capitis, S. caprae, S. cohnii, S. epidermidis, S. fleurettii, S. haemolyticus, S. hominis, S. lugdunensis, S. pseudintermedius, S. saprophyticus, S. schleiferi, S. sciuri, S. warneri, Mammaliicoccus lentus, Mammaliicoccus sciuri, S. kloosii, S. vitulinus*
mecC	Methicillin, Oxacillin	*S. aureus, S. caprae, S. edaphicus, S. esaphicus, S. saprophyticus, S. sciuri, S. stepanovicii, S. warneri, and S. xylosus*
vanA	Vancomycin, Teicoplanin	*Enterococcus faecalis, Enterococcus faecium, and Staphylococcus aureus*
vanB	Vancomycin	*Enterococcus faecalis, Enterococcus faecium*

Assay contents:

Tube 1: Primer/Probe mix (5X) for mecA, mecC, vanA, vanB, and hRPP30DNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: 5000 copies/µl positive controls of synthetic 500 bp DNA fragments of mecA, mecC, vanA, vanB, and hRPP30DNA.

Tube 3: InhibiTaq Standard qPCR enzyme Mastermix (enough for 100 rxns. with 20 µL total volume). This is a custom formulation from Fortis Life Sciences to the specifications of PCRassays.com.

ASSAY HANDLING

The AMR1 assay is shipped at ambient temperature, and should be stored at -20 °C. The tubes should be kept on ice once thawed. Do not subject the enzyme to multiple freeze-thaw cycles.

Contamination should be avoided by using appropriate personal protective equipment (PPE), powder free gloves, aerosol barrier pipette tips, and a clean hood.

EXPERIMENTAL

Perform nucleic acid extraction/purification (recommended).

Set up your reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaq qPCR enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample	2
Water	4

Notes: To improve assay sensitivity, up to 6 µL of sample can be added (water volume adjusted accordingly) for a total reaction volume of 20 µL. For positive control rxns., add 2 µL of the solution from Tube 2.
Molecular biology grade water (NOT included) should be used to prepare the PCR reactions.

A PCR protocol was used for verification on a QuantStudio™ 7 Flex Real-Time system, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 95 °C for 2 minutes
2	Incubate @ 95 °C for 3 seconds
3	Incubate @ 55 °C for 22 seconds
4	Plate Read
5	Go to Step 2, repeat 44× more

RESULT INTERPRETATION

After running the qPCR reaction, perform a regression analysis on the data to determine the quantification cycle, Cq. (Cq is preferred over Ct). Each fluorescence channel with a Cq < 38 cycles and final RFU > 200,000 on QuantStudio 5, 6, 7, 12K instruments is considered "positive" or "+" in the Table below.

mecA FAM™	vanB HEX™	hRPP30 TAMRA™	mecC TEX615™	vanA Cy5™	Recommended Interpretation
–	–	–	–	–	The PCR reaction failed. Please repeat the experiment.
–	–	+	–	–	The sample contains human RPP30 DNA. The sample doesn't contain AMR DNA.
+	–	–	–	–	The sample contains mecA DNA. The sample may not contain human RPP30 DNA.
+	–	+	–	–	The sample contains mecA DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains vanB DNA. The sample may not contain human RPP30 DNA.
–	+	+	–	–	The sample contains vanB DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains mecC DNA. The sample may not contain human RPP30 DNA.
–	–	+	+	–	The sample contains mecC DNA and human RPP30 DNA.
–	–	–	–	+	The sample contains vanA DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains vanA DNA and human RPP30 DNA.
+	+	–	+	+	The sample contains mecA, vanB, mecC, and vanA DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains mecA, vanB, mecC, vanA, and human RPP30 DNA.

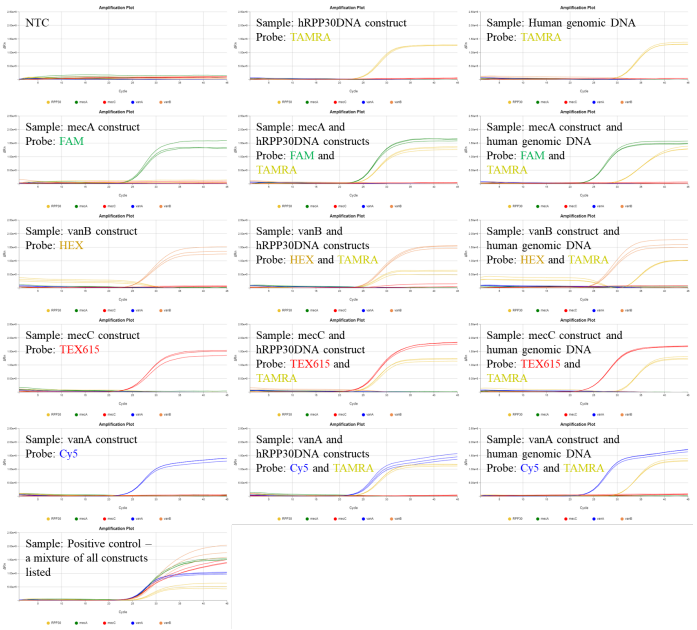

Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

VERIFICATION EXPERIMENTS

The AMR1 assay verification was carried out as a 5-plex assay that simultaneously detects DNA from mecA, mecC, vanA, vanB, and human RPP30 DNA, which serves as a positive control assay.

Experiments were performed in triplicate using the experimental procedure given above, but with different samples added to each reaction. The samples used for the verification experiments contained 1×10^4 copies/reaction of synthetic 500 bp DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genomes, human RPP30 DNA gene, and human genomic DNA. **Figure 1** shows the results of these experiments, which indicate that the 5-plex specifically detects the different AMR genes.

The limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). For dilution series only one target construct was added. The results show a limit of detection (LOD) <10 copies/reaction.

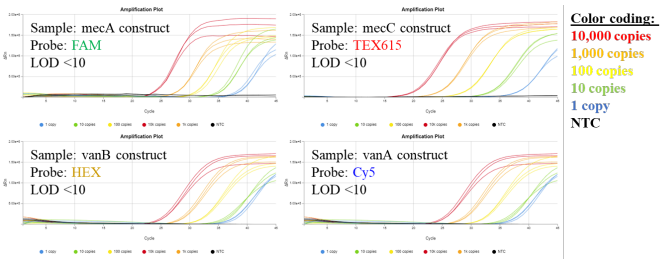

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the 5-plex primers and probes specifically detect and differentiate the four antimicrobial resistance genes and are also compatible with RPP30_DNA positive control primers. Human genomic DNA doesn't interfere with the detection of the pathogens.

NOTES

[1] FAM™ (Carboxyfluorescein), a trademark of Life Technologies Corporation.

[2] BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

[3] HEX™ (Hexachloro-fluorescein), a trademark of Thermo Fisher Scientific.

[4] TAMRA (Carboxyltetramethylrhodamine) is a trademark of Applera Cor.

[5] BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

[6] TEX615™ is a trademark of Thermo Fisher Scientific.

[7] Cy5™, a trademark of GE Healthcare.

[8] TaqMan™ is a trademark of Roche Diagnostics, Inc.

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