

ASSAY NAME: BVPN (Bacterial Vaginosis Panel)
Quantity: 100 x 20µL PCR reactions
5-plex assay: *Atopobium vaginae*, BVAB-2, *Gardnerella vaginalis*, *Megasphaera type1*, and human RPP30 DNA
SKU #'s:
PNP-BVPN-D-QS-100 (QuantStudio)
(RUO). Research Use Only. Not for use in Diagnostic Procedures.
SCOPE OF THIS DOCUMENT:

The oligonucleotide recipes are optimized for QuantStudio. The verification data presented in this product information sheet were performed using PNP-BVPN-D-QS-100 on a QuantStudio™ 7 Flex Real-Time System. The performance on other QuantStudio instruments should be similar (presuming they are equipped with the proper wavelength filters). Contact PCRassays.com if you are planning to use a different instrument.

CONTENTS

The primers and probes in the BCPN assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 pathogens and a human control. The BVAB-2 assay detects both *Amygdalobacter indicium* and *Amygdalobacter nucleatus*.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
BVAB-2	FAM	BHQ-1	1,2
<i>A. vaginae</i>	HEX	BHQ-1	3
RPP30-DNA control	TAMRA	BHQ-1	4
<i>G. vaginalis</i>	TEX615	BHQ-2	5, 6
<i>Megasphaera type1</i>	Cy5	BHQ-2	7

The probes are designed as TaqMan⁸ cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

ASSAY HANDLING AND CONTAMINATION

The BVPN assay is shipped at ambient temperature, and should be stored at -20 °C. The assay 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.

Note: molecular biology grade water should be used to prepare the PCR reactions, which is NOT included in this assay.

ASSAY CONTENTS:

Tube 1: 5X Primer/Probe mix for *Atopobium vaginae*, BVAB-2, *Gardnerella vaginalis*, *Megasphaera type1*, and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: 5000 copies/µl of synthetic 500 bp DNA fragments for *A. vaginae*, BVAB-2, *G. vaginalis*, *Megasphaera type1*, and hRPP30DNA.

Tube 3: InhibiTaq 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.


EXPERIMENTAL

Perform nucleic acid extraction/purification (recommended). Set up your PCR reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaq enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample or positive control	2
Molecular biology grade 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.

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

Step	Thermocycling Protocol:
1	Incubate @ 85 °C for 1 second
2	Incubate @ 94 °C for 2 minutes
3	Incubate @ 94 °C for 3 seconds
4	Incubate @ 55 °C for 22 seconds
5	Plate Read
6	Incubate @ 85 °C for 1 second
7	Incubate @ 94 °C for 3 seconds
8	Incubate @ 55 °C for 22 seconds
9	Plate Read
10	Go to Step 6, repeat 43× more

RESULT INTERPRETATION

After running the qPCR reaction, use the instrument software to determine the quantification cycle, C_q (or use C_T if your instrument does not have the capability to compute a C_q). Fluorescence channels with a C_q < 38 cycles, and final RFU > Threshold is considered “positive” or “+” in the Table below. The “Threshold” value for calling a PCR positive is dependent on the instrument model, well size, and sample volume; thus the user must determine the threshold that is appropriate for their method. For our QuantStudio 7 Flex with 96 well plate with 100 µL wells and 20 µL reaction volume, the average RFU was approximately 1,000,000 and we used a threshold of 200,000 for calling positives or “+” in the Table below.

<i>BVAB-2</i> FAM TM	<i>A. vaginae</i> HEX TM	<i>G. vaginalis</i> TEX615 TM	<i>Megasphaera type1</i> Cy5 TM	hRPP30 TAMRA TM	Recommended Interpretation
-	-	-	-	-	The PCR reaction failed. Please repeat the experiment
-	-	-	-	+	The sample does not contain bacterial DNA of interest. The sample contains human RPP30 DNA.
+	-	-	-	-	The sample contains <i>BVAB-2</i> DNA. The sample may not contain human RPP30 DNA.
+	-	-	-	+	The sample contains <i>BVAB-2</i> DNA and human RPP30 DNA.
-	+	-	-	-	The sample contains <i>A. vaginae</i> DNA. The sample may not contain human RPP30 DNA.
-	+	-	-	+	The sample contains <i>A. vaginae</i> DNA and human RPP30 DNA.
-	-	+	-	-	The sample contains <i>G. vaginalis</i> DNA. The sample may not contain human RPP30 DNA.
-	-	+	-	+	The sample contains <i>G. vaginalis</i> DNA and human RPP30 DNA.
-	-	-	+	-	The sample contains <i>Megasphaera type1</i> DNA. The sample may not contain human RPP30 DNA.
-	-	-	+	+	The sample contains <i>Megasphaera type1</i> DNA and human RPP30 DNA.
+	+	+	+	-	The sample contains <i>BVAB-2</i> , <i>A. vaginae</i> , <i>G. vaginalis</i> , <i>Megasphaera type1</i> DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains <i>BVAB-2</i> , <i>A. vaginae</i> , <i>G. vaginalis</i> , <i>Megasphaera type1</i> DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The BVPN assay verification was carried out as a 5-plex assay, which simultaneously detects DNA from *A. vaginae*, BVAB-2, *G. vaginalis*, *Megasphaera type1*, and human RPP30 DNA, which serves as a positive extraction-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 synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genomes, human RPP30 DNA gene, and human genomic DNA. The results of these experiments are shown in **Figure 1** and indicate that the 5-plex specifically detects the different bacterial species in the human genomic DNA matrix.

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

Conclusion: The data in **Figure 1** indicate that the 5-plex primers and probes specifically detect and differentiate the bacterial types and are also compatible with RPP30_DNA positive control primers. Human genomic DNA matrix doesn't affect detection of pathogen DNA.

NOTES

- ¹ FAMTM (Carboxyfluorescein), a trademark of Life Technologies, Inc
- ² BHQ-1TM (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.
- ³ HEXTM (Hexachloro-fluorescein), a trademark of Applera Corp.
- ⁴ TAMRA (Carboxyltetramethylrhodamine), a trademark of Applera Corp.
- ⁵ TEX615TM is a trademark of Thermo Fisher Scientific.
- ⁶ BHQ-2TM (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.
- ⁷ Cy5TM, a trademark of GE Healthcare.
- ⁸ "TaqMan" is a trademark of Roche Molecular Systems, Inc.

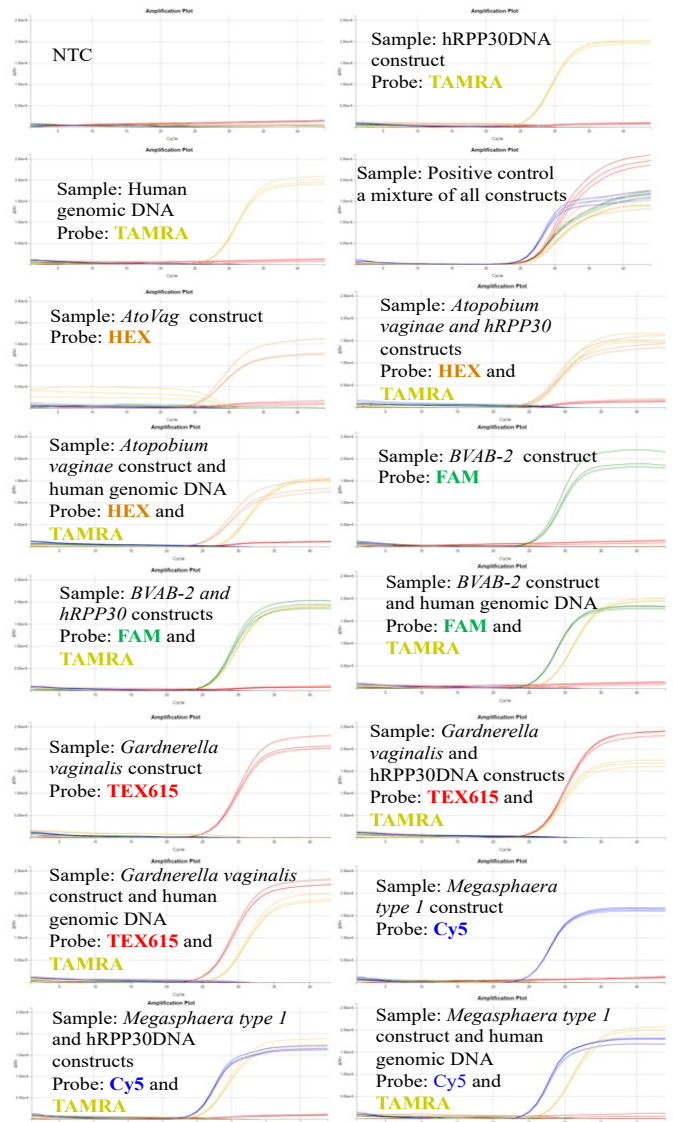


Figure 1: Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

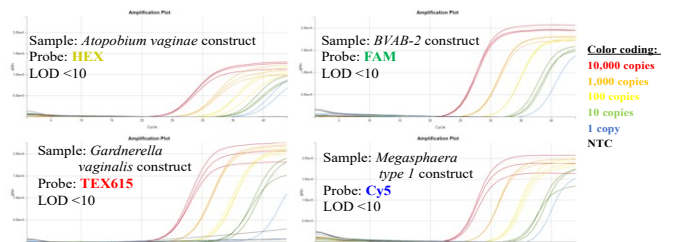


Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

CONTACT US

For assistance, please contact DNA Software using the link: <https://www.pcrassays.com/contact/>

Address: Michigan Life Science and Innovation Center,
46701 Commerce Center Dr, Plymouth, MI 48170

Phone: (734) 222-9080