

# Development of a Semi-Quantitative Women's Health Bacterial Panel on the Luminex® NxTAG® Platform

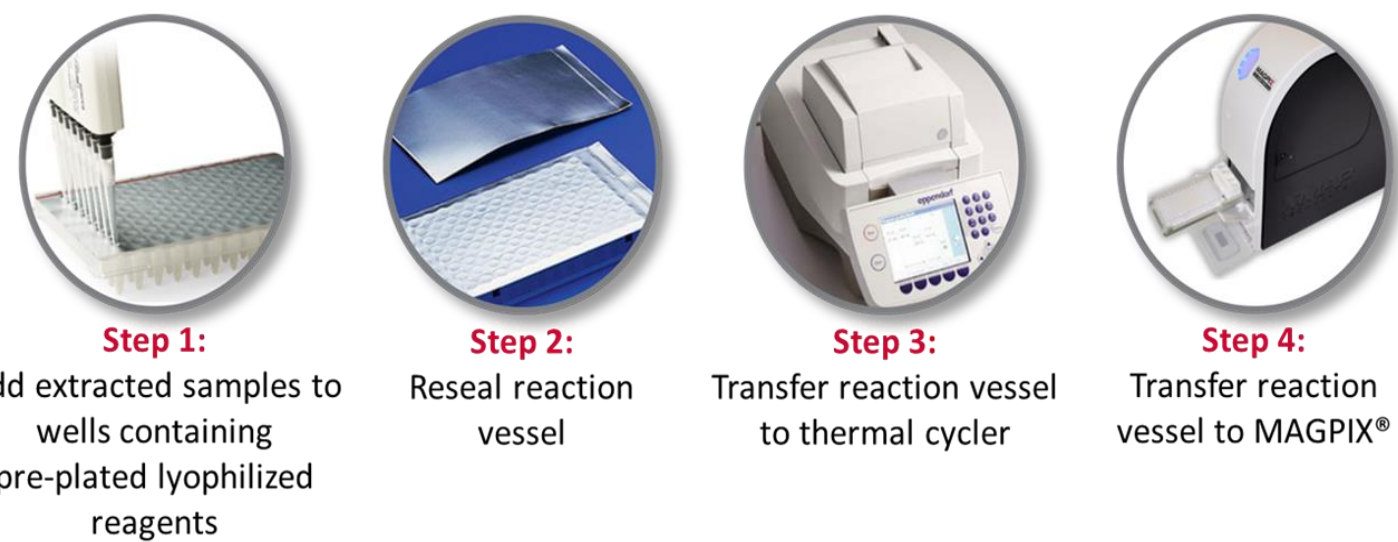
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## Background and Objective

The women's health bacterial panel (WHBP) prototype in development highlights the semi-quantitative capabilities of the Luminex® NxTAG® platform. The WHBP prototype offers simplified and high throughput testing of 1-96 samples workflow of NxTAG (Figure 1) while simultaneously detecting and providing a semi-quantitative result for *Atopobium vaginae*, bacterial vaginosis associated bacteria 2 (BVAB2), and *Megasphaera* Type 1 (*Mega 1*).

The objective of this study was to evaluate the performance of the NxTAG WHBP panel on remnant gynecological (GYN) samples. High positive, low positive, and negative calls were determined for all analytes in the panel and real-time PCR (qPCR) was used as the comparator.



**Figure 1.** Overall Assay Workflow of NxTAG WHBP Prototype

## Results

**Table 1: Summary of NxTAG WHBP Results Confirmed by qPCR**

| <i>A. vaginae</i> Result | # of Calls | # qPCR Confirmed | % Confirmed |
|--------------------------|------------|------------------|-------------|
| High Positive            | 146        | 144              | 98.6        |
| Low Positive             | 12         | 12               | 100         |
| Negative                 | 65         | 61               | 93.8        |
| Total                    | 223        | 217              | 97.3        |
| BVAB2 Result             | # of Calls | # qPCR Confirmed | % Confirmed |
| High Positive            | 110        | 106              | 96.4        |
| Low Positive             | 15         | 12               | 80          |
| Negative                 | 98         | 92               | 93.9        |
| Total                    | 223        | 210              | 94.2        |
| <i>Mega 1</i> Result     | # of Calls | # qPCR Confirmed | % Confirmed |
| High Positive            | 120        | 120              | 100         |
| Low Positive             | 43         | 30               | 69.8        |
| Negative                 | 60         | 49               | 81.7        |
| Total                    | 223        | 199              | 89.2        |

***A. vaginae*:** High positive > 3.2x10<sup>5</sup> copies/ml; low positive = 3x10<sup>2</sup> – 3.2x10<sup>5</sup> copies/ml. **BVAB2:** High positive > 1.2 x10<sup>5</sup> copies/ml; low positive = 3.2x10<sup>2</sup> – 1.2x10<sup>5</sup> copies/ml. ***Mega 1*:** High positive > 1.2x10<sup>5</sup> copies/ml; low positive = 5x10<sup>2</sup> – 1.2x10<sup>5</sup> copies/ml

**Table 2: Results of NxTAG WHBP Compared to Sample Nugent Score**

| Nugent Score | # of Samples with 2 or More High Positive Calls | # of Samples with 1 or Fewer High Positive Calls |
|--------------|---|--|
| 7-9          | 39  | 1  |
| 4-6          | 27  | 3  |

## Materials and Methods

A total of 223 remnant GYN samples were used for the study. Nucleic acids were extracted from 200 µl of raw sample collected in either PreservCyt, Universal Transport Media, or Liquid Amies using the NucliSENS® easyMAG® extractor and eluted in to 110 µl of elution buffer. An endogenous human control is used to eliminate the need for an external extraction control. Extracted nucleic acid was stored at -80°C until testing. 35 µl of extracted nucleic acid were added directly to pre-plated WHBP specific lyophilized bead reagents (LBRs) and NxTAG universal LBRs. Multiplexed PCR and bead hybridizations were performed in closed PCR tubes under a single cycling program. The sealed plates were placed directly on the MAGPIX® instrument for data acquisition. Thresholds applied to make calls are preliminary. Real-time PCR was used to confirm all calls for the three bacterial analytes detected by the NxTAG WHBP prototype.

## Conclusion

- The NxTAG WHBP prototype is able to semi-quantitatively detect all three analytes in remnant GYN samples. 97.3%, 94.2%, and 89.2% of all NxTAG WHBP calls were confirmed by qPCR for *A. vaginae*, BVAB2, and *Mega 1*, respectively. The NxTAG WHBP gives a semi-quantitative result that compares well with the absolute quantification of qPCR.
- The NxTAG WHBP prototype results also correlate well with the Nugent Score (NS). 97.5% of samples with a NS = 7-9 and 90% of samples with NS= 4-6 were high positive for at least two analytes by the WHBP prototype.
- The NxTAG WHBP prototype in development offers a high throughput and scalable (1-96 samples) platform for the semi-quantitative testing of three bacterial pathogens. The NxTAG platform also has the benefit to add additional analytes that require either a semi-quantitative or qualitative result.