



The Importance of Distinguishing Between Aerobic Vaginitis and Bacterial Vaginosis

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Learning Objectives

- Discuss the dynamics involved in the Vaginal Microbiome
- Identify key infectious states associated with vaginal dysbiosis, namely Aerobic Vaginitis and Bacterial Vaginosis
- Describe the limitations with traditional methods
- Propose a molecular testing solution for identifying and differentiating between pathogenic states of the vaginal microbiota

Why is Vaginal Microbiota Research Important?

Globally, there are large numbers of cases...

Urogenital infections, including vaginitis, afflicts over

1 billion women each year

21.2 million

women affected per year
in the U.S.¹

Most common

vaginal condition affecting women ages 15-44.¹

Aerobic vaginitis

affects 5-13% of asymptomatic women and a more
significant proportion of symptomatic women.

Increases a woman's risk

of contracting STDs, premature
delivery and low birth weight
newborns¹

Research to better characterize the vaginal microbiota is important
for the health of women, their fetuses, and newborns

1: <http://www.cdc.gov/std/bv/stats.htm> Accessed 17-March-2021

Pathogenesis of the Vaginal Microbiota

- Disruption of the healthy vaginal ecosystem contributes to the overgrowth of pathogens which cause complicated vaginal infections such as Bacterial Vaginosis (BV), Aerobic Vaginitis (AV), sexually transmitted infections (STIs), vulvovaginal candidiasis, and trichomoniasis¹.

Vaginosis/Vaginitis

- **Vaginitis:** inflammation of the vagina; discharge, itching and pain (11 million office visits per year)
 - **Bacterial vaginosis:** associated with an altered microbial flora (absence of gross appearance of inflammation)
 - **Vulvovaginal candidiasis:** caused by naturally occurring fungus of *Candida spp.*
 - **Trichomonal vaginitis:** sexually transmitted parasite *Trichomonas vaginalis*
 - **Vaginal atrophy (atrophic vaginitis):** reduced estrogen levels after menopause

Aerobic Vaginitis

- Abnormal vaginal flora, distinct from bacterial vaginosis
- Replacement of healthy *Lactobacillus* species with aerobic pathogens, mostly of intestinal origin
 - *Escherichia coli*, *Group B Streptococcus*, *Staphylococcus aureus*, *Enterococcus faecalis*
- Localized vaginal inflammatory immune response
- More extreme inflammatory changes than BV
- Increased numbers of toxic leukocytes visible in vaginal smears
- Presence of parabasal epithelial cells
- Confused with common vaginitis etiologies
 - BV, Vulvovaginal Candidiasis and Trichomoniasis
- AV has been observed in 8–11% of pregnant women and 5–24% of women reporting vaginal complaints

Modes of Infection and Potential Complications

Bacterial Vaginosis(BV)

Aerobic Vaginitis(AV)

Modes of Infection

Recent use of broad-spectrum antibiotics, Hormonal changes, IUD's, and an increased number of sexual partners

Unclear whether the primary cause is pathogenic organisms, lack of lactobacilli, immune dysregulation and pro-inflammatory cytokines, or insufficient oestrogen in the vaginal mucosa

Potential Complications

In most women there are minimal complications, and it is possible that these complications were due to Aerobic Vaginitis, which is only recently being recognized as distinct from bacterial vaginosis

Infection-related miscarriage, preterm birth, activation of HPV infection and cervical cancer, increased risk of acquiring STD's, Pelvic inflammatory disease and infertility

Symptoms for BV & AV

Bacterial Vaginosis(BV)

Aerobic Vaginitis(AV)

- Lack of specific symptoms
 - Greyish-white, watery vaginal discharge with fishy odor
 - Lack of inflammation on vaginal examination
 - Microscopy findings of decreased lactobacilli and granular flora
- Vaginal stinging and burning sensations
 - Dyspareunia
 - Sticky yellow or green discharge with a rotten odor
 - Symptoms can fluctuate over time

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- **Vaginal condition distinct from BV, require different management, distinct risks**
 - **More extreme inflammatory changes than BV**
 - **Often confused with BV**

Management Types

Supportive Management

- Topical corticosteroids
- Topical oestrogen
- Antiseptic
- Probiotics/Microbiome Therapy

Antimicrobial Management

- Kanamycin, fluoroquinolones, penicillin, ampicillin, amoxicillin
- Unlike BV, AV does not respond well to metronidazole, which is commonly used for the treatment of *T. vaginalis* and BV

BV & AV Considerations

- **Aerobic Vaginitis (AV)**

- Assessment
- Microscopic Scoring

- **Bacterial Vaginosis (BV)**

- Nugent Scoring using Microscopy
- Amsel Criteria



- **Limitations to consider:**

- Subjective methodologies
- Lacks sensitivity/specificity
- Labor Intensive
- Challenges in finding trained microscopists
- Time consuming

Conventional microbiological approaches – limited utility

<https://pubmed.ncbi.nlm.nih.gov/26781259/>

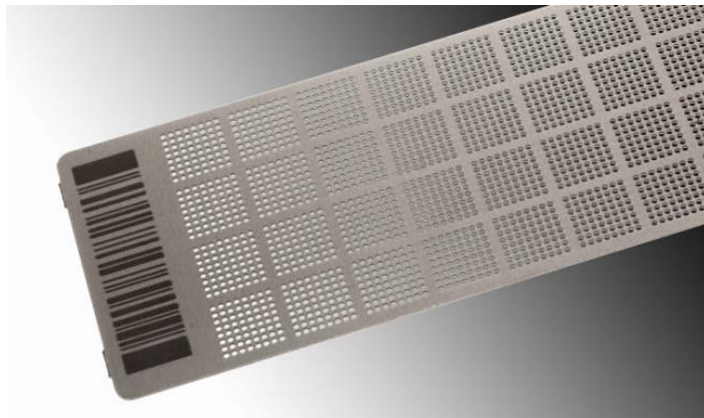
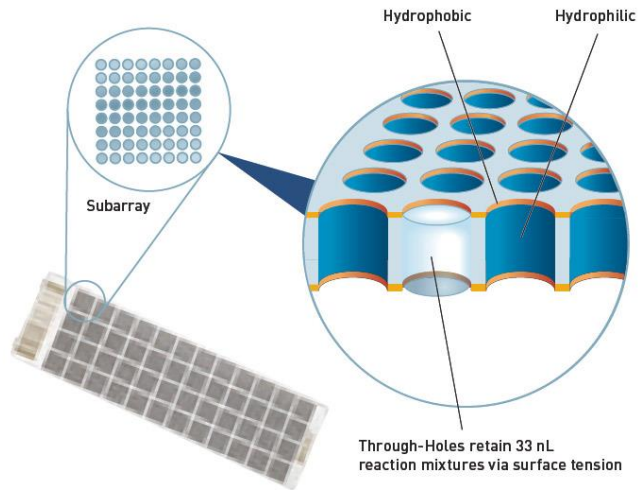
Rationale

- Quantitative/semi-quantitative PCR
 - Lack of standardization (different marker organisms, different thresholds)
 - Limited number of organisms
- Need for a multi-variate analysis of vaginitis- associated marker organisms
 - Microbiome

Potential Utility

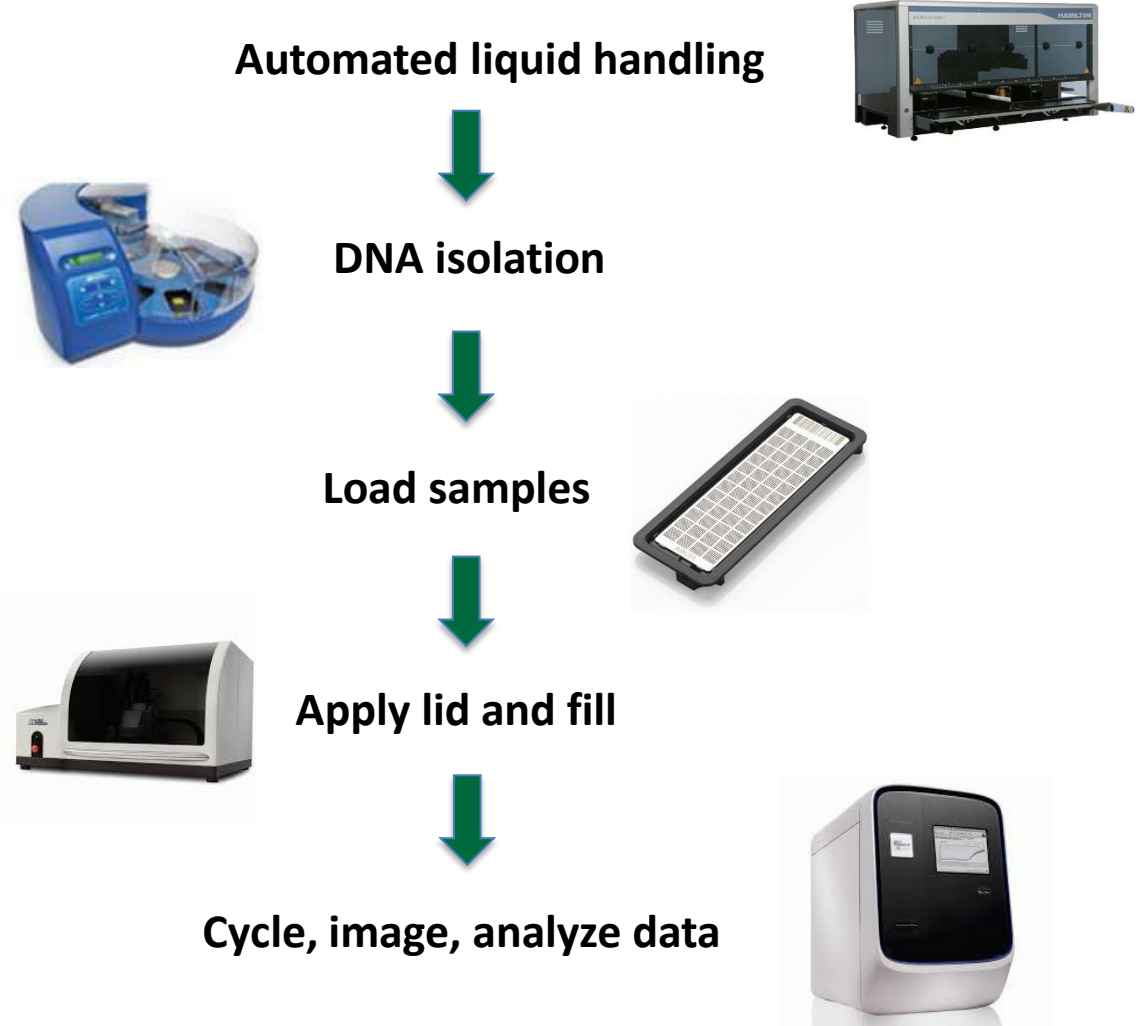
- Custom panel/s based on potential utility
- Consolidation of targets
- Flexibility
- Sensitive, specific, rapid
- Detection of BV, non-BV targets
- Variability of specimen collection, multiplicity of targets

OpenArray™ from Thermo Fisher Scientific



- **OpenArray Realtime PCR plates**
 - 48 subarrays; 64 through-holes/subarray
 - 3,072 individual real-time PCR assays in parallel on a single OpenArray
 - 4 arrays can be run at the same time (12,288 assays)
 - 33 nL reaction volume
 - Hydrophilic interior & external hydrophobic coatings
- **48 specimen per OpenArray**
 - 192 specimens per run (6 hours)
 - 26 organisms per specimen
- **Simple workflow**

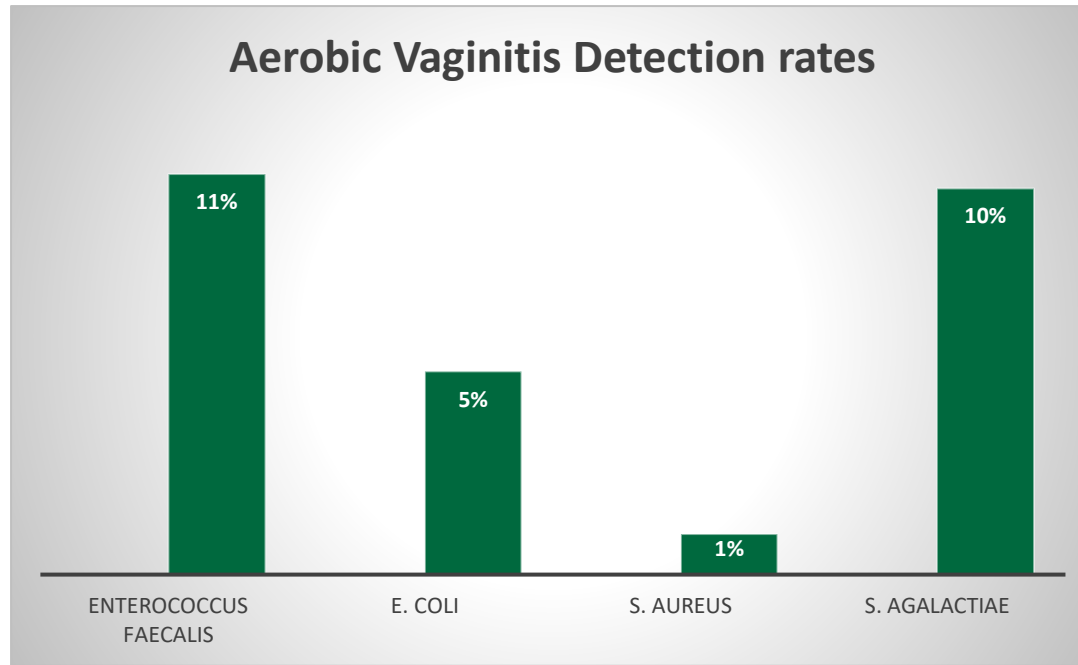
Assay Workflow



Assay Design/Verification

- Analytical LOD
- Analytical thresholds
- Relative quantitative data for each organism by comparing to total bacterial load
 - Internal Control
- Samples from our routine testing population
- Compared this data in symptomatic/asymptomatic subjects
- Reference lab results for comparison

Aerobic Vaginitis in the Clinic



- >18,000 samples tested
- 60% of AV orders presented with Vaginitis/Vaginosis symptoms/indications
- ~23% specimens positive for Aerobic Vaginitis
- **~20% of normal Bacterial Vaginosis samples were positive for Aerobic Vaginitis**

Conclusion

- High-throughput
- Sensitive/specific
- Investigation of vaginal infections by evaluating a large panel of pathogenic and commensal organisms
 - Syndromic testing
- Faster TAT, without subjectivity associated with culture
- Operational efficiency
- Integrated, pre-microbiome

Summary

- Alteration of the vaginal biota with different bacterial, clinical, and immunological characteristics with respect to the classic form of vaginitis
- Depletion of healthy *Lactobacillus* species with aerobic pathogens, mostly of intestinal origin, such as *Escherichia coli*, Group B Streptococcus (GBS), *Staphylococcus aureus*
- Localized vaginal inflammatory immune response
- Distinct signs and symptoms that can be confused with common vaginitis etiologies such as BV, *Vulvovaginal Candidiasis*, and *Trichomoniasis*
- Variety of symptoms that are not consistent
- Common side effects after treatment for BV with metronidazole
- Significant fraction of this population may have been misdiagnosed and suffer from AV, which requires antibiotic treatment specific for the aerobic bacteria
- Pregnancy-associated complications such as chorioamnionitis, preterm birth, and premature rupture of membranes

References & Acknowledgements

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