Bacterial Vaginosis:
Pathogenesis, Presentation, and Diagnosis

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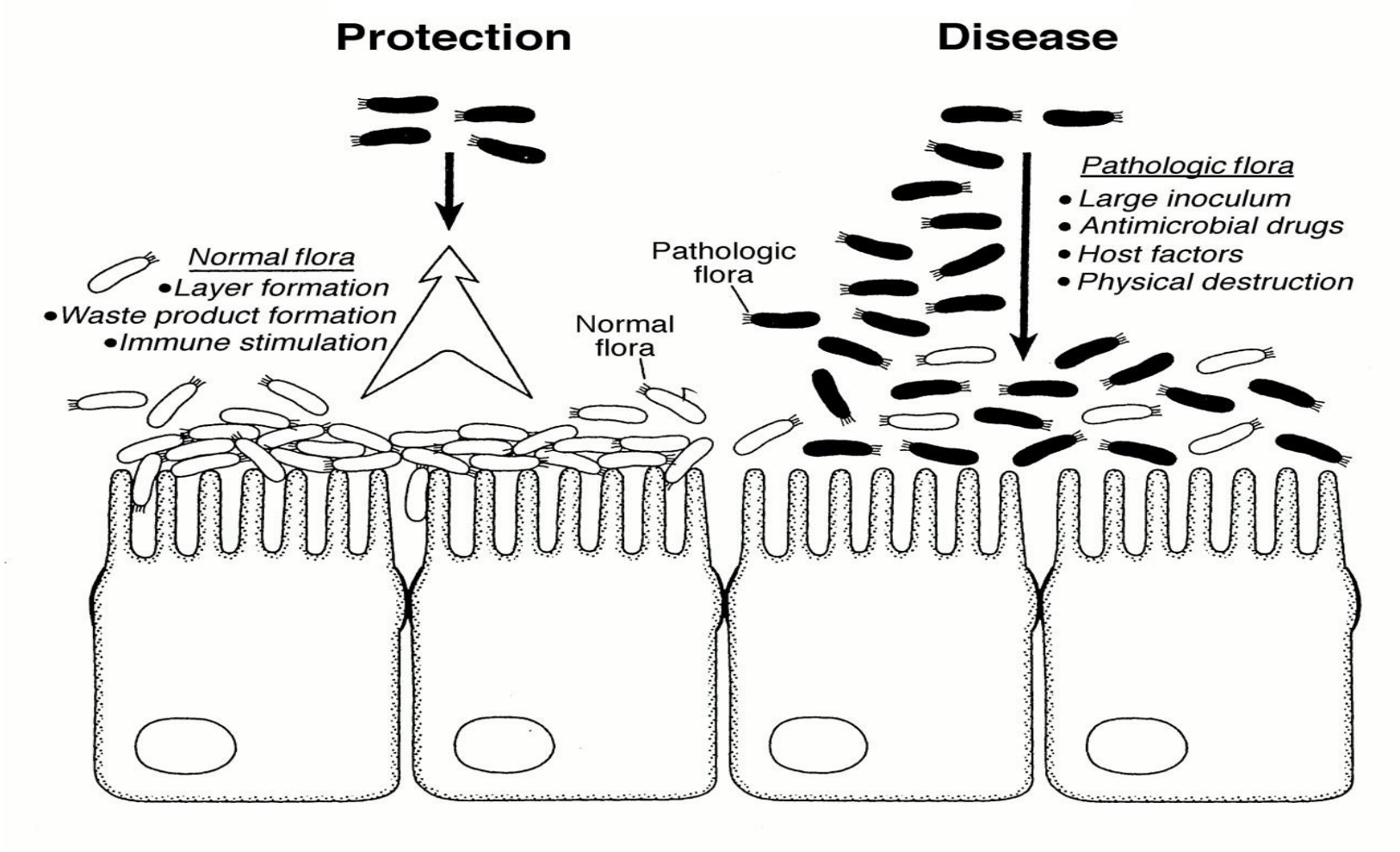
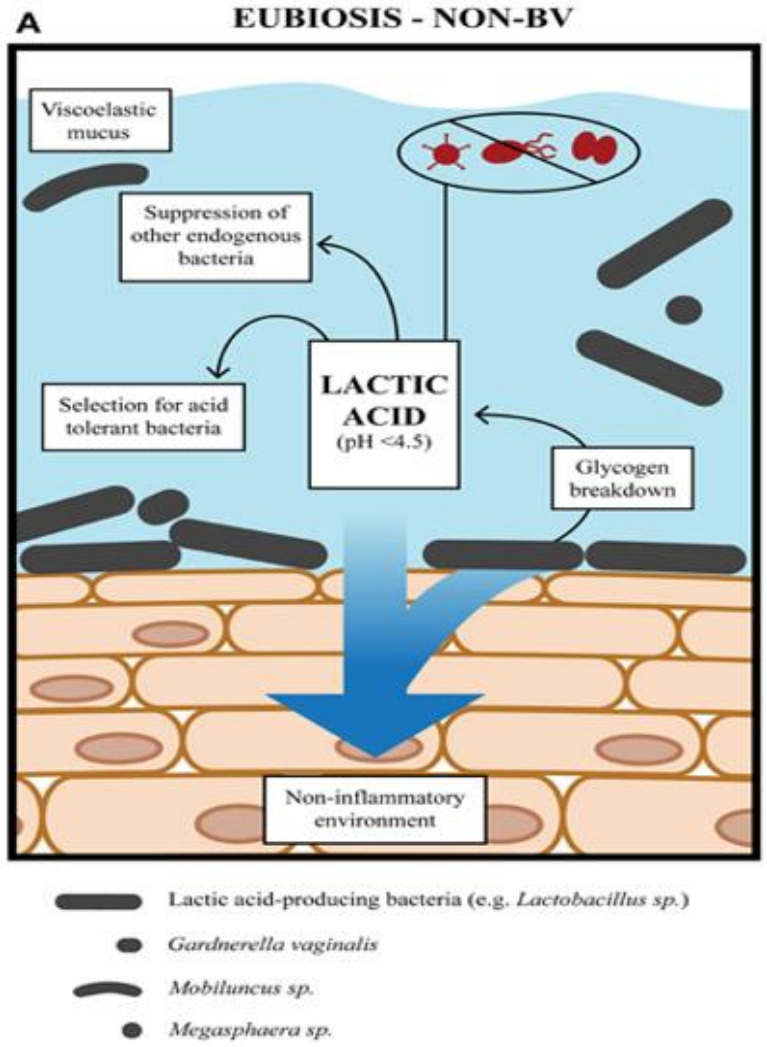
Learning Objectives

- Review the role of both pathogenic and commensal microbiota involved in urogenital infections
- Identify the methods to detect a broad range of vaginal microbiota from a single sample quickly, and cost effectively
- Discuss the practical application of the detection of a broad range of vaginal microbiota from a single sample

The Microflora of Mucosal Surfaces of the Human Body in Health and Disease



Eubiosis of the Female Vaginal Tract

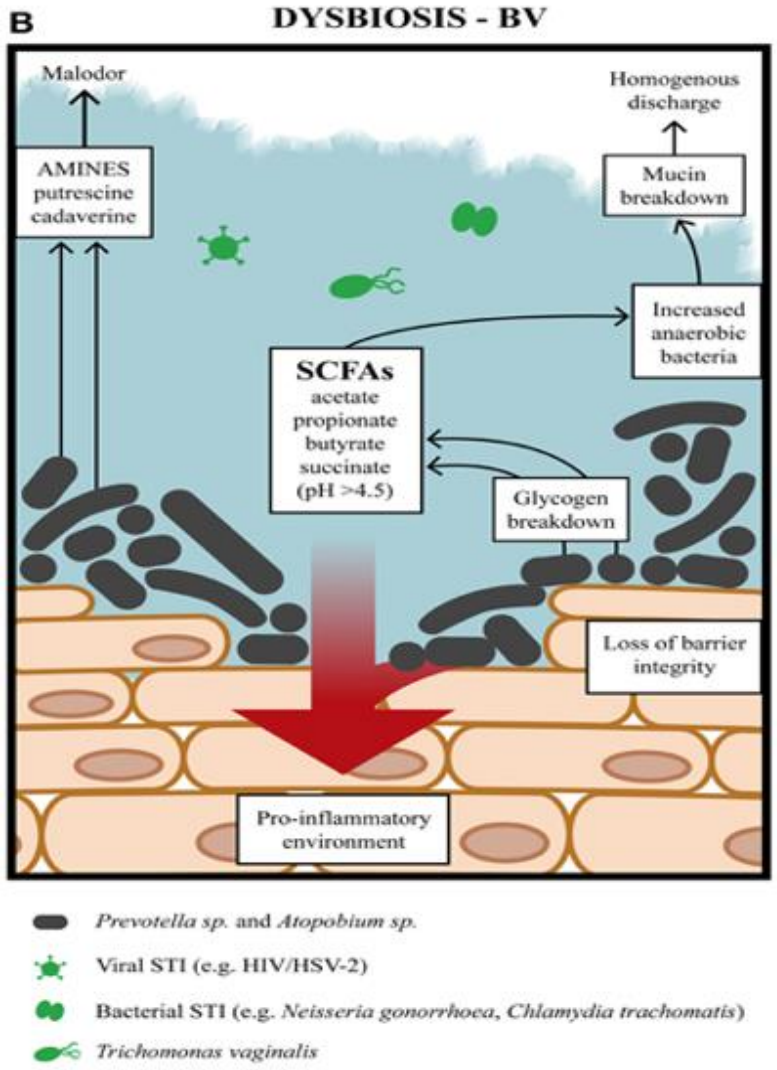


The Normal Microflora of the Vaginal Tract

- Predominant flora of lactobacilli species that colonize and secrete chemical products.
- An ecosystem that harbors a microbiota that protects it from invading pathogens including those that cause urinary tract infections and sexually transmitted diseases.
- Lactobacilli are dominant at concentrations of 10^7 to 10^8 CFU/g of vaginal fluid.
- Potential pathogens are kept at insignificant levels due to the production of large volumes of lactic acid and hydrogen peroxide

Aldunate et al. Antimicrobial and immune modulatory effects of lactic acid and short chain fatty acids produced by vaginal microbiota associated with eubiosis and bacterial vaginosis. Front. Physiol., June, 2015.

Dysbiosis of the Female Genital Tract



Prominent factors that may predispose patients to BV include:

- Recent antibiotic usage
- Decreased estrogen production of the patient
- Wearing an intrauterine device (IUD)
- Douching
- Sexual activity that could lead to transmission (e.g. having a new sexual partner or a recent increase in the number of sexual partners

Aldunate et al. Antimicrobial and immune modulatory effects of lactic acid and short chain fatty acids produced by vaginal microbiota associated with eubiosis and bacterial vaginosis. Front. Physiol., June, 2015.

Investigating Bacterial Vaginosis

BV is one of the most widely studied obstetric/gynecologic infectious diseases and may affect 1/3 of women at some point in their lives

- Vaginal odor (the most common, and often initial, symptom of BV)
- Mild to moderate increase in vaginal discharge
- Vulvar irritation (not always present)
- Dysuria or dyspareunia are rare
- Disease is more prevalent in certain races and lower socioeconomic classes

These signs and symptoms were summarized by Amsel in 1983 and have become the clinical standard for BV diagnosis

- Vaginal pH > 4.5
- Presence of > 20% per HPF of “Clue cells” on wet mount examination
- Positive amine or “whiff test”
- Homogeneous, non-viscous, milky-white discharge adherent to the vaginal wall

Standardized Microscopic Technique for the Determination of Bacterial Vaginosis

Laboratory examination of vaginal smears and the determination of the Nugent Score

N Score = Sum of the scores for each bacterial morphotypes listed below (Note number of Organisms seen/100 X objective)

Lactobacilli	Score	*Gardnerella, Bacteroides*	Score	Curved Gram-negative bacilli	Score	Sum= Nugent Score
30 or >	0	0	0	0	0	0
5-30	1	<1	1	<1	1	3
1-4	2	1-4	2	1-4	1	5
<1	3	5-30	3	5-30	2	8
0	4	30 or >	4	30 or >	2	10

Interpretation of Nugent Score

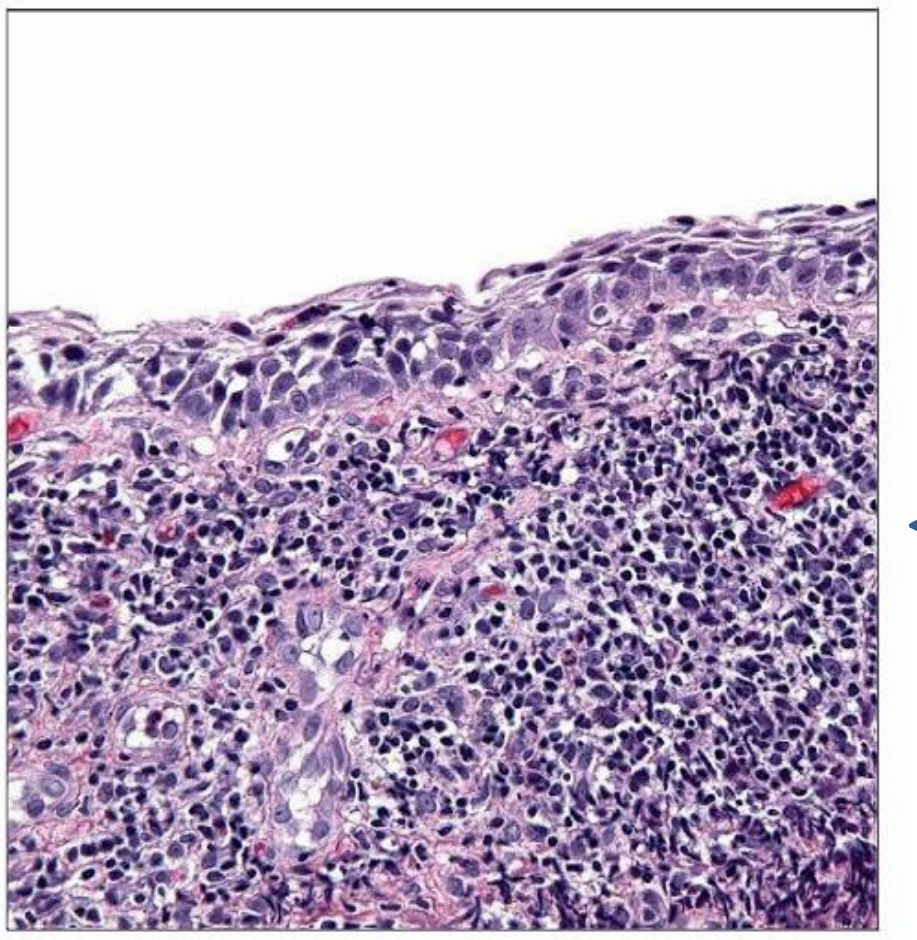
If N Score is:	AND:	Then Report
0-3		Smear NOT consistent with BV
4-6	Clue Cells **NOT** Present	Smear NOT consistent with BV
4-6	Clue Cells **ARE** Present	Smear consistent with BV
4-6		Smear consistent with BV

Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol*. 1991 Feb. 29(2):297-301.

Aerobic Vaginitis

Distinguishing Features and Determination

Aerobic Vaginitis Differs from BV



- Discharge more colored and viscous
- Depletion of normal concentrations of Lactobacilli (very low numbers in more severe cases)
- Discharge is foul smelling (not the typical amine odor with BV)
- Histological appearance of desquamative inflammatory vaginitis (most severe form)
- Significant immune cytokine response (IL-1-β and IL-6)
- Cultures of often purulent discharge show Staph spp, Group B Strep, enterococci, and Gram neg bacilli (often E. coli and Klebsiella)

Nucci, M.R. Desquamative Inflammatory Vaginitis. Pathology & Laboratory Medicine. July, 2016

Presentation and Complications

- Patients complain of burning or painful sensation during intercourse
- Vulvar or vaginal itching
- Ulcerations often associated with moderate to severe case
- Aerobic vaginosis more likely associated with pregnancy complications
- Ascending chorioamnionitis
- Preterm rupture of the membranes
- Preterm delivery

Determination

- Yellow swab test
- Increased pH (> 6)
- Microscopy of wet mount
- Foul smell of discharge without application of KOH
- More esoteric tests include evaluation of cytokines (not feasible for routine practice)
- Other than those mentioned above, point of care testing is limited

The Need for Better Determination in Bacterial Vaginosis and 'Aerobic' Vaginitis

- Both disease entities often rely on subjective criteria of microscopic analysis
- Both diseases are associated with an increased risk of other sexually transmitted diseases
- These clinical entities cannot be treated and managed in the same way (see below)
- Both diseases can affect the outcome of pregnancy; often with severe consequences

Implicated Sexually Transmitted Diseases

- *Chlamydia*
- *Gonorrhoeae*
- *Trichomonas*
- *Mycoplasma*
- *Ureaplasma*
- HIV
- *Herpes simplex* I and II

Treatment Regimens

- Bacterial Vaginosis = Metronidazole or Clindamycin and restoration of Lactobacilli
- Aerobic Vaginosis = Kanamycin ovule, 2% Clindamycin topical, Ampicillin, Fluoroquinolones

Application and Unique Advantages of Molecular Technology

Identification of key organisms associated with vaginosis/vaginitis

Lactobacilli

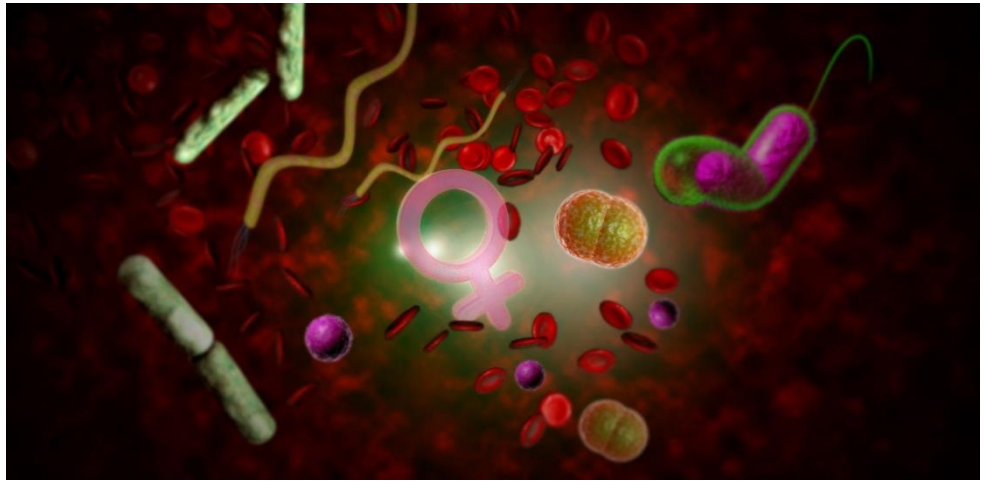
Commensal organisms associated with colonization of the vaginal mucosal surface and are effective producers of H_2O_2 to suppress the growth of invading pathogens include:

- *L. crispatus*
- *L. gasseri*
- *L. jensenii*
- *L. iners*

Organisms that are associated with both BV and Aerobic Vaginitis

- *Clostridiales* order (BVAB 1-3)
- *Atopobium vaginae*
- *Gardnella vaginalis*
- *Sneathia/Leptotrichia*
- *Megasphaera* types 1 and 2
- TM7 type bacteria
- Group B Strep
- Staph sp
- *E. coli*
- *Enterococcus* sp

- Traditional methods are subjective and lack sensitivity/specificity[1]
- Reproducible molecular techniques are more empirical
- Panel-based or multiplex formats permit simultaneous detection of multiple targets associated with various conditions



[1]*Bacterial Vaginosis: An Update on Diagnosis and Treatment;* **Expert Rev Anti Infect Ther**. 2009 Nov;7(9):1109-24

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Vaginal Microbiota Testing using real-time PCR

Doug Rains, Chief Scientific Officer
Quantigen

Introduction

- **Main goals**:
  - To **accurately ID** pathogenic organism as **quickly as possible**.
  - To identify **co-infections** possibly requiring multiple drugs.
  - Ideally: identify any antibiotic resistance (future goal).

Finding a Better Approach

- **Ideal wish list** for future infectious disease test:
  - High level of **specificity** (preferably species-level information) **and sensitivity**.
  - **Broad coverage** of the various microorganisms that can cause a similar presentation.
  - A **single**, minimally invasive **sample collection**.
  - **Rapid** turnaround times.
  - **Low cost** per sample and **easy workflow**.

Real-time PCR Solution

- **Panel Testing Using Real-Time PCR**
  - Many benefits:
    - **Specificity** – Real-time PCR reports precisely which microorganism(s) are present.
    - **Sensitivity** – Numerous CDC-acknowledged studies have demonstrated the **improved detection rates** of PCR-based methodologies over more traditional tests, especially for STIs.*

*Source: *Morbidity and Mortality Weekly Report,* Centers for Disease Control; March 14, 2014 / 63(RR02); 1-19.

Real-time PCR Solution

- **Panel Testing Using Real-Time PCR**
  - Many benefits:
    - **Broad coverage** – Real-time PCR comprehensive test covers 27 microorganisms from four major areas:
      - ✓ Sexually-transmitted infections (STIs)
      - ✓ Aerobic vaginitis (AV), including Group B Strep
      - ✓ Candidiasis
      - ✓ **Bacterial vaginosis** (details to follow)

Real-time PCR Solution

- **Panel Testing Using Real-Time PCR**
  - Many benefits:
    - **Flexibility** – One can choose which **individual panel(s)** to run, or perform a **comprehensive screen** in cases where either (a) presentation profile is vague or ambiguous, or (b) multiple infections are suspected.

Real-time PCR Solution

- **Panel Testing Using Real-Time PCR**
  - Many benefits:
    - **Single sample collection** – Panel-based real-time PCR performs a fully comprehensive screen across all 27 organisms using a single vaginal swab.

Real-time PCR Solution

- **Panel Testing Using Real-Time PCR**
  - Many benefits:
    - **Rapid turnaround**– The ability to report full-panel results within 12-24 hours of sample receipt.

Real-time PCR testing system

- **Utilizing Real-Time PCR System:**
  - Uses **5' nuclease chemistry** (sensitive and specific).
  - All assays on this panel were **pre-designed**.
  - **Low cost** per data point / sample
  - **Easy workflow** and **quick TAT**.
  - Max. **throughput**: about 180 samples/8-hour shift.

Developing a real-time PCR bacterial vaginosis test

- **Studying Bacterial vaginosis: background**
  - A condition in which the vaginal microflora, normally dominated by lactobacillus spp., is overtaken by an array of **anaerobic species**.
  - Often **asymptomatic**; when symptoms are present, most are non-specific (e.g., itching, discharge).

Developing a real-time PCR bacterial vaginosis test

- **Big challenges:**
  - **No single microbe** is exclusively associated with BV.
  - Heavy reliance on presentation: **Amsel criteria** most common approach.
    - Questionable sensitivity, no accounting for *lactobacilli*.
  - Alternatively, experienced labs can prepare a Gram-stained sample: **Nugent testing**.
    - Requires experience, can be time-sensitive, and is blind to species with no cell wall (e.g., mycoplasma).

Developing a real-time PCR bacterial vaginosis test

- **Better approach:**
  - Characterize vaginal microflora using a highly **specific and sensitive method**, such as real-time PCR / 5' nuclease chemistry.
  - Survey a **large collection of microbes** known to associate with BV.
  - Determine which **microbial signatures** associate with BV (using Nugent scoring as the benchmark).
  - Develop an **interpretive algorithm** that is as independent of sample collection technique as possible.

Microbes assessed

- *Gardnerella vaginalis*
- *Atopobium vaginae*
- Megasphera Type 1
- Megashphera Type 2
- *Mycoplasma hominis*
- *Mobiluncus curtisii*
- *Mobiluncus mulieris*
- *Ureaplasma urealyticum*
- BVAB2
- *Prevotella bivia*
- *Lactobacillus iners*
- *Lactobacillus jensenii*
- *Lactobacillus gasseri*
- *Lactobcillus curtisii*
- Broad-range 16s (for monitoring sample collection)

Real-time PCR BV Study Design

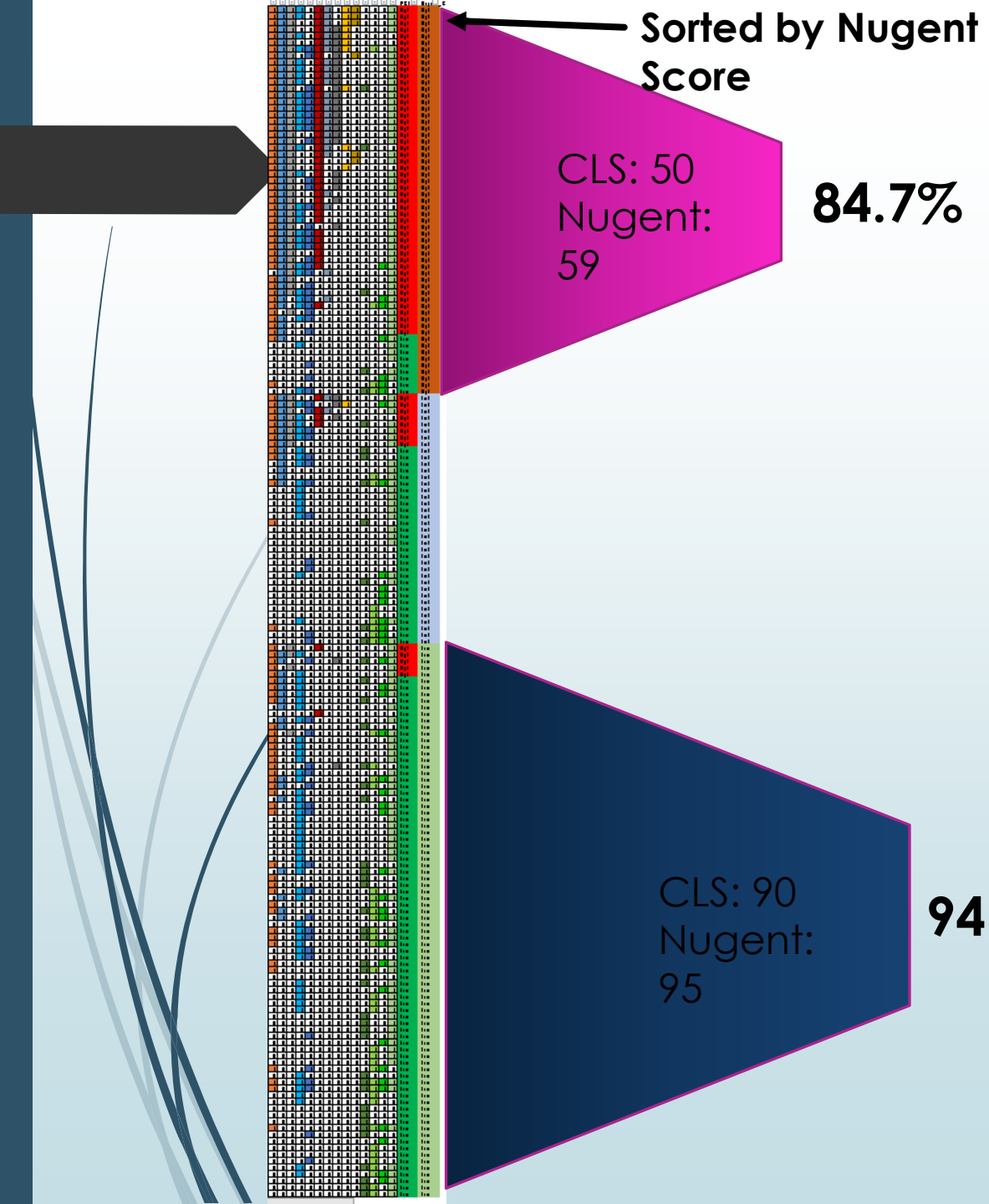
- **Phase 1a: algorithm training**
  - Collect 200 duplicate study swab samples – one for Nugent, one for real-time PCR using large BV panel.
  - Data → Bioinformatics partner for statistical analysis / development of a **PCR-based interpretive algorithm** that predicts BV status.

Real-time PCR BV Study Design

- **Phase 1b: algorithm validation**
  - Collect an additional 200 study samples for Nugent / real-time PCR.
  - Apply algorithm developed in Phase 1a.
  - Calculate **specificity and sensitivity** relative to Nugent scores.

Example of Results

Nugent Score		*G. vaginalis*	*A. vaginae*	Megasph. 1	Megasph. 2	*M. hominis*	*U. urealyt.*	*M. curtisii*	BVAB2	*M. mulieris*	*P. bivia*	*L. gasseri*	*L. iners*	*L. crisp.*	*L. jensenii*
10	QG-1134-1 1														
8	QG-1189-2														
8	QG-1190-2														
9	QG-1197-2														
8	QG-1151-2														
8	QG-1152-2														
1	QG-1142-1 1														
1	QG-1133-1 1														
1	QG-1149-1 1														
1	QG-1150-2 1														
1	QG-1157-2														
1	QG-1158-2														
2	QG-1166-2														

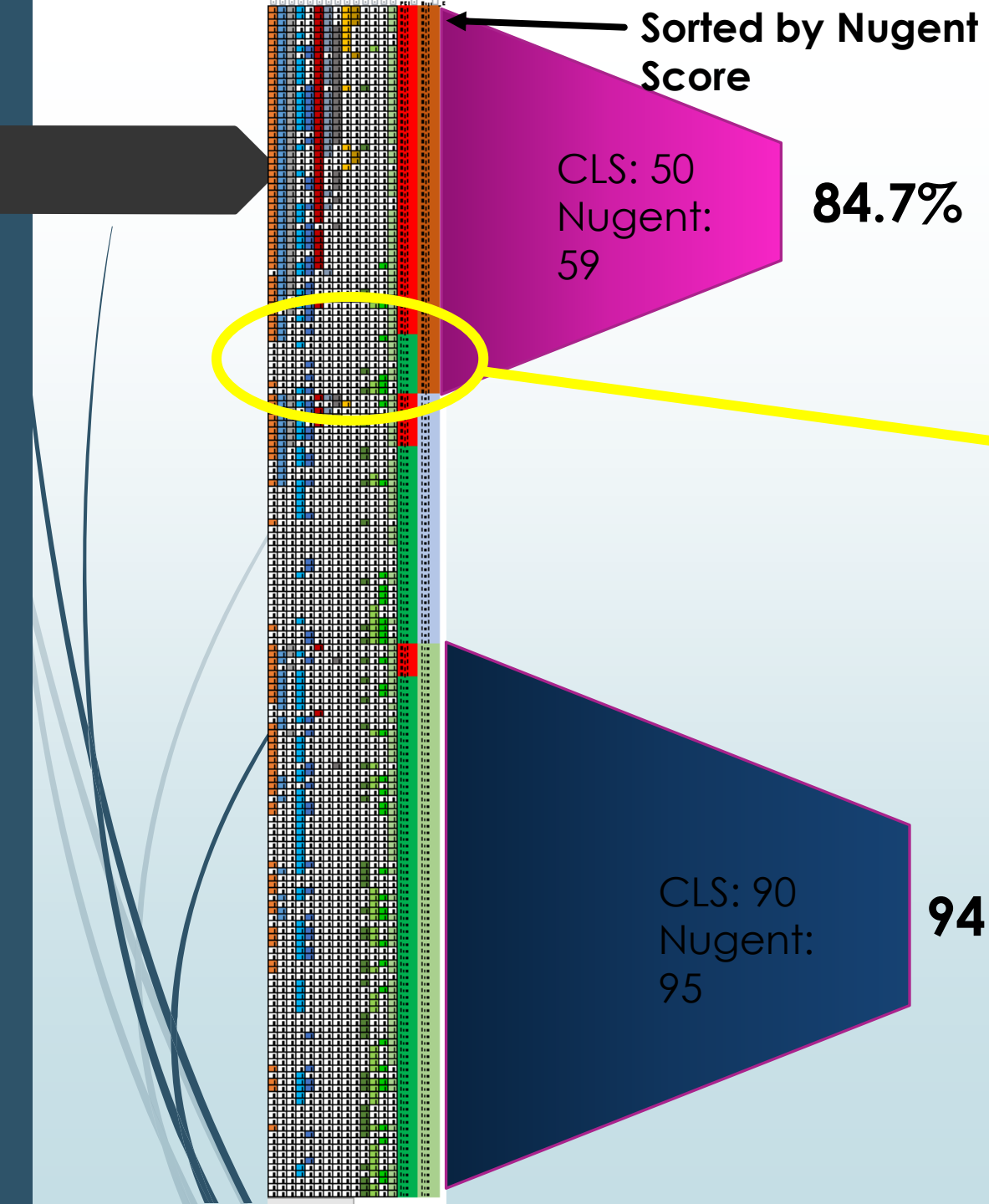


84.7%

Sensitivity
= percentage of samples correctly identified as BV+

94.7%

Specificity
= percentage of samples correctly identified as BV-



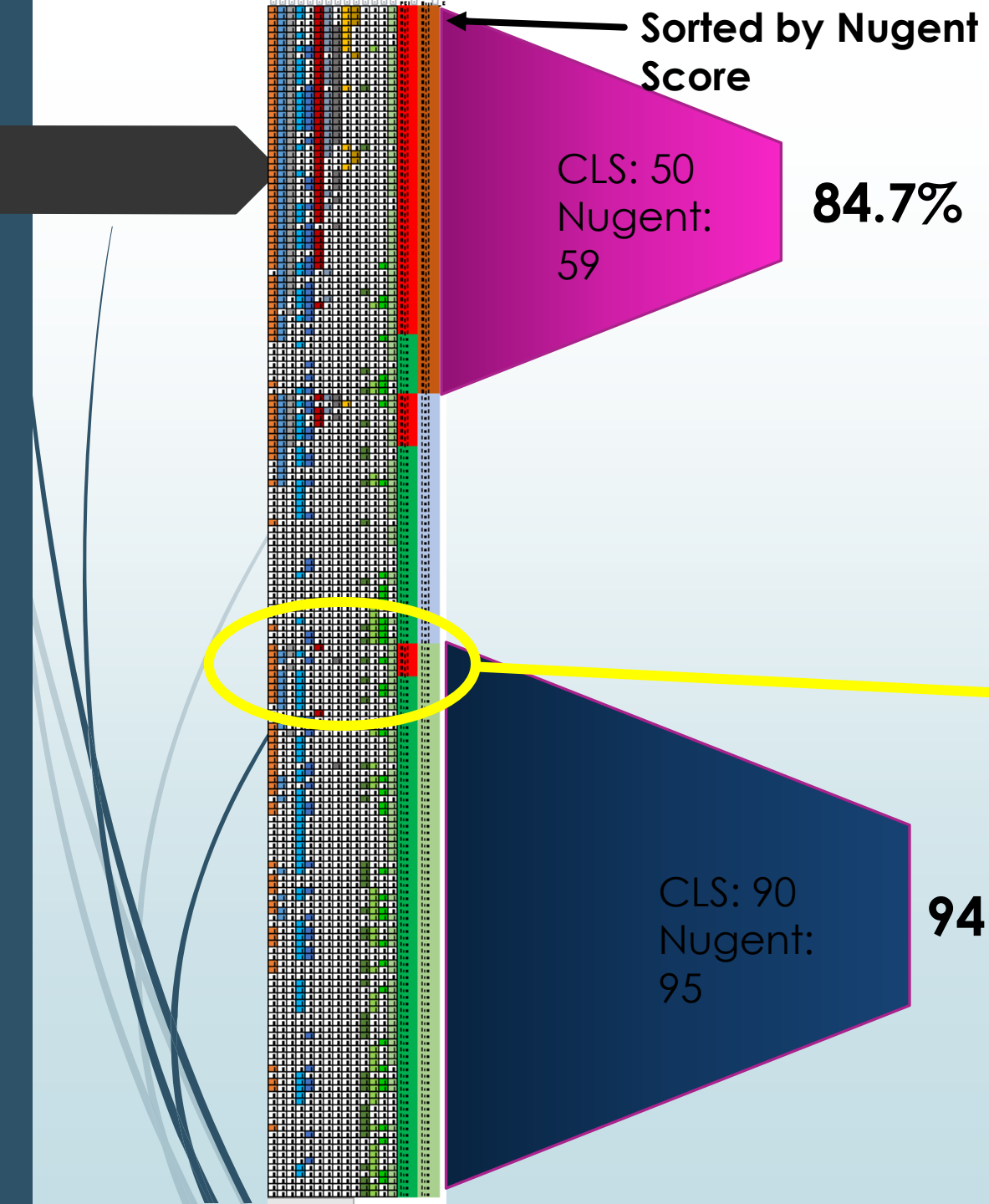
Sensitivity

= percentage of samples correctly identified as BV+

1	1	0	0	0	0	0	0	0	0	0	0	1	1
0	0	0	1	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0	0	1
0	0	0	0	1	0	0	0	0	0	0	0	1	1
1	0	0	0	0	0	0	0	0	0	0	1	1	0
0	0	0	1	1	0	0	0	0	0	1	1	1	0

Specificity

= percentage of samples correctly identified as BV-

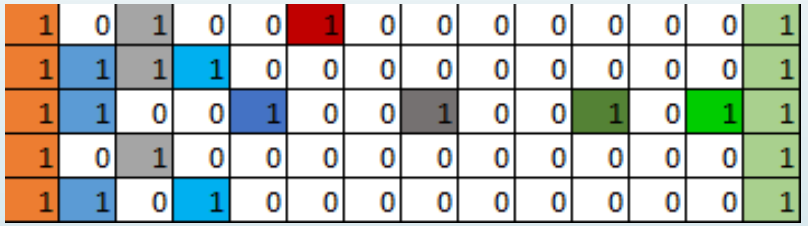


84.7%

94.7%

Sensitivity

= percentage of samples correctly identified as BV+



Specificity

= percentage of samples correctly identified as BV-

Real-time PCR BV Study: Phase 2

- **Phase 2 (ongoing)**
  - Collect an additional 200 study samples for Nugent / real-time PCR, including 50 negative subjects.
  - In addition to Nugent scoring, attain putative BV status using both Amsel criteria and a secondary molecular method.
  - Refine algorithm in an effort to achieve greater specificity and sensitivity.

Real-time PCR BV Study: Collaborators

A collaboration between Quantigen Laboratory in Fishers, IN, PrimeX Laboratories in Van Nuys, CA, and the Research and Development Institute (RDI) in Van Nuys, CA.

Acknowledgments:

Erik Avaniss-Aghajani, PrimeX Laboratories
Zohrab Bostanian, RDI
Coriell Institute for Medical Research

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Developing a Molecular Assay Algorithm for Bacterial Vaginosis Determination

Jeffrey Shaman, PhD, Dir., Business Development, Coriell Life Sciences

Women's Health Reporting

Data

1. Results from Real-time PCR Women's Health microorganism assay Infection Information
2. Infection details and demographics from the Study

Report Requirements

- Results of assays
- Microorganism Details
- Interpretation
- Determination of BV

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- Focused on empowering those who use genetics
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Women's Health Reporting

Goal

- Identify an Algorithm that can Recognize Patterns in the WH microorganism assay results from Real-time PCR

Success Metric

- The algorithm's output matches the Study's assessment of positive Bacterial Vaginosis

Report Requirements

- Results of assays
- Microorganism Details
- Interpretation
- Determination of BV

Women's Health Reporting

Report Requirements

- Results of assays
- Microorganism Details
- Interpretation
- Determination of BV

Women's Health Reporting

Algorithm Development Process

- Ingest raw data
  - 400 samples
- Convert raw data into usable form
  - Linearize Ct
- Apply statistical models to a training subset
  - (linear, logarithmic, orthogonal transformations, etc.)
- Evaluate wide variety of models– apply algorithm to validation set of data
- Choose model that meets success metrics

Women's Health Reporting

Algorithm Development Process Raw Data

DIL_04_27_2016_VGL46.eds

```
* Experiment Name = VGL46
* Experiment Run End Time = Not Started
* Experiment Type = Gene Expression
* Instrument Name = 285880760
* Instrument Serial Number = 285880760
* Instrument Type = QuantStudio 12K Flex
* Passive Reference =
* Quantification Cycle Method = Ct
* Signal Smoothing On = true
* Stage/ Cycle where Analysis is performed = Stage 2, Step 3
* User Name = NA

[Results]
```

Well	Well Position	Omit	Sample Name	Target Name	Task	Reporter	Quencher	Ct	Ct Mean	Ct SD	Amp Score	Cq	Cq Conf	CRTAMPLITUDE	HIGHSD	CRTNOISE	ROX	Signal
1	A1a1	false	QG-1001	AICSW7C	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	6,882.794							
2	A1a2	false	QG-1001	AICSW7C	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	5,844.265							
3	A1a3	false	QG-1001	AICSW7C	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	5,817.441							
4	A1a4	false	QG-1001	AID1VDK	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	5,667.353							
5	A1a5	false	QG-1001	AID1VDK	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	5,684.471							
6	A1a6	false	QG-1001	AID1VDK	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	5,204.382							
7	A1a7	false	QG-1001	AIN1GRC	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	5,643.294							
8	A1a8	false	QG-1001	AIN1GRC	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	5,255.324							
9	A1b1	false	QG-1001	AIN1GRC	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	7,852.353							
10	A1b2	false	QG-1001	AIQJCRB	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	7,296.853							
11	A1b3	false	QG-1001	AIQJCRB	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.692	0.000	Y	N	Y	6,611.441							
12	A1b4	false	QG-1001	AIQJCRB	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	6,273.706							
13	A1b5	false	QG-1001	AIGJRP0	UNKNOWN	FAM	NFQ-MGB	25.424	25.022	0.360	1.575	0.967	N	N	N	6,436.265						
14	A1b6	false	QG-1001	AIGJRP0	UNKNOWN	FAM	NFQ-MGB	24.728	25.022	0.360	1.599	0.980	N	N	N	6,607.941						
15	A1b7	false	QG-1001	AIGJRP0	UNKNOWN	FAM	NFQ-MGB	24.913	25.022	0.360	1.588	0.983	N	N	N	6,336.294						
16	A1b8	false	QG-1001	AIHSPV8	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	6,247.441							
17	A1c1	false	QG-1001	AIHSPV8	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	6,434.382							
18	A1c2	false	QG-1001	AIHSPV8	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.548	0.000	Y	N	Y	6,021.059							
19	A1c3	false	QG-1001	AI0IXJR	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.674	0.000	Y	N	Y	5,976.471							
20	A1c4	false	QG-1001	AI0IXJR	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	5,604.529							
21	A1c5	false	QG-1001	AI0IXJR	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	6,067.794							
22	A1c6	false	QG-1001	AIRSAXK	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.781	0.000	Y	N	Y	5,869.029							
23	A1c7	false	QG-1001	AIRSAXK	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	5,888.941							
24	A1c8	false	QG-1001	AIRSAXK	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	5,675.529							
25	A1d1	false	QG-1001	AIMSH8N	UNKNOWN	FAM	NFQ-MGB	15.966	15.936	0.262	1.591	0.964	N	N	N	6,407.441						
26	A1d2	false	QG-1001	AIMSH8N	UNKNOWN	FAM	NFQ-MGB	15.661	15.936	0.262	1.591	0.958	N	N	N	6,139.412						
27	A1d3	false	QG-1001	AIMSH8N	UNKNOWN	FAM	NFQ-MGB	16.182	15.936	0.262	1.590	0.971	N	N	N	5,955.941						
28	A1d4	false	QG-1001	AII1NRX	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	5,802.765							
29	A1d5	false	QG-1001	AII1NRX	UNKNOWN	FAM	NFQ-MGB	Undetermined		0.000	0.000	Y	N	Y	6,034.471							

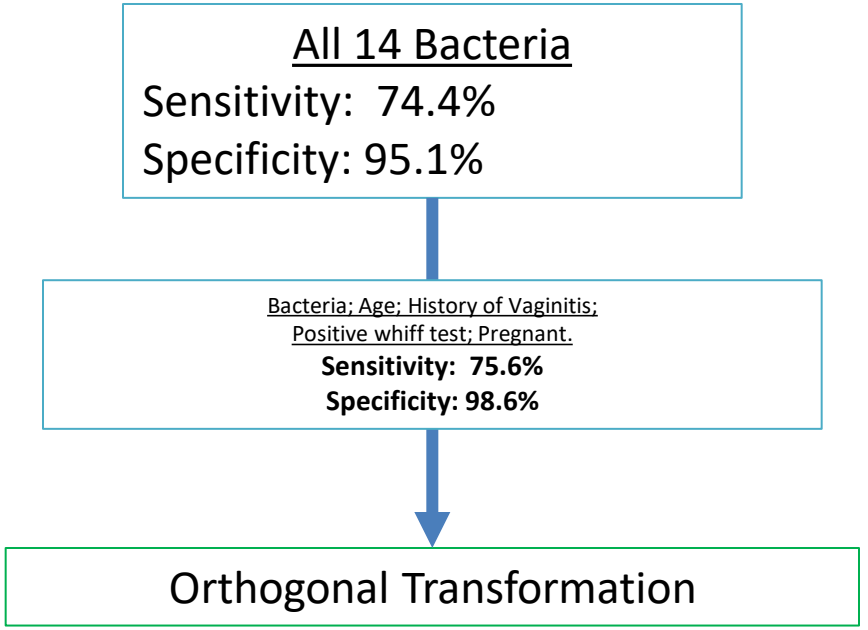
Normal text file | length : 282171 lines : 3099 | Ln : 6 Col : 149 Sel : 0 | 0 | Dos\Windows | UTF-8 | INS

Women's Health Reporting

Algorithm Development Process Raw Data

Evaluated 53 Distinct Models for the way they clustered the samples

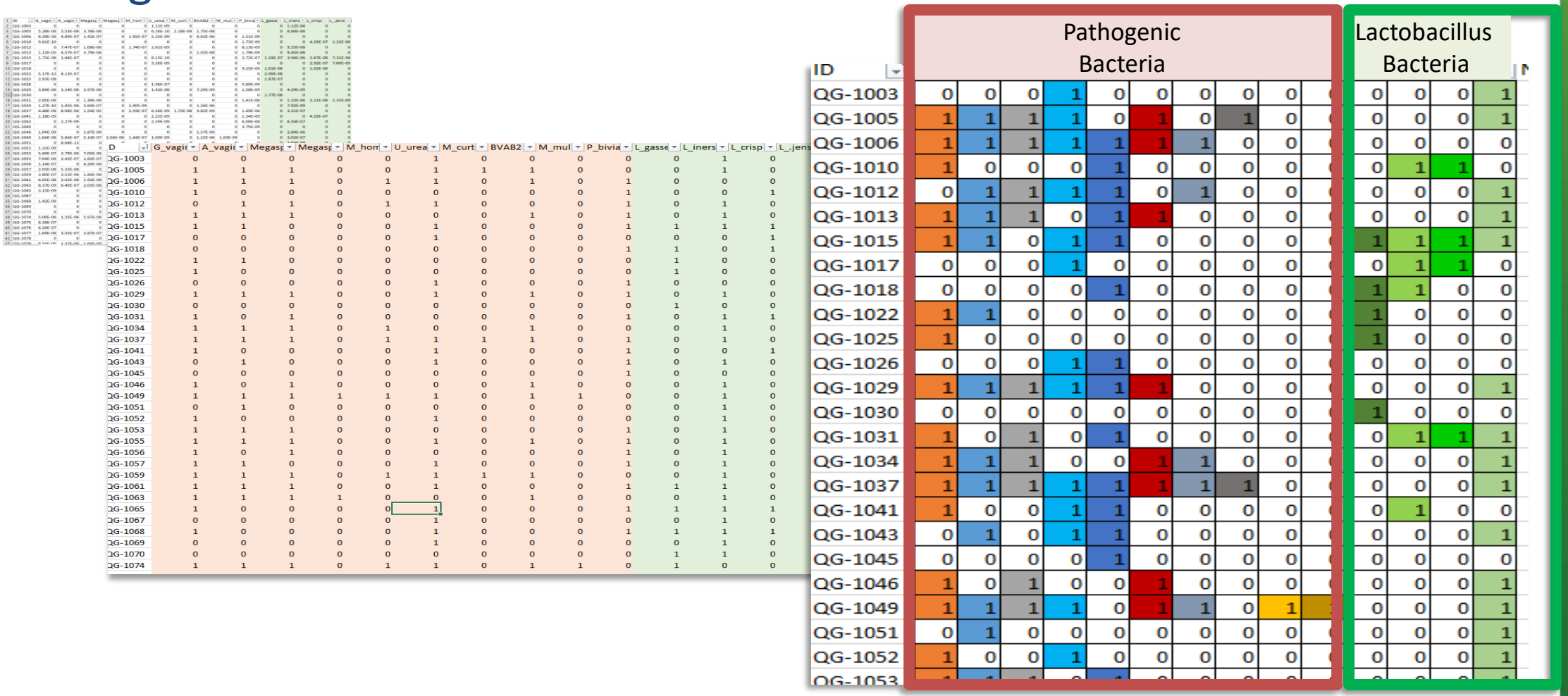
			sen = sensitivity. mip = min_pos. spe = specificity. man = max_neg.				
subset of	number o	highlight about the feature subset	sensitivity > 7 => BV+	sensitivity > 6 => BV+	sensitivity > 5 => BV+	specificity < 4 => BV-	specificity < 5 => BV-
feature	num_fe	note_feature	sen_mip_	sen_mip_	sen_mip_	spe_man_	spe_man_
set16	30	pos_whiff, Pregnant; 14 bac log ^2, ^1	0.4634	0.6951	0.7439	0.8732	0.9789
set18	31	History_of_Vaginitis_bin, pos_whiff, Pregnant; 14 bac l	0.4634	0.6951	0.7439	0.8873	0.9859
set13	30	Age, Pregnant; 14 bac log ^2, ^1	0.4878	0.6829	0.7439	0.8662	0.9859
set15	29	Pregnant; 14 bac log ^2, ^1	0.4756	0.6829	0.7439	0.8662	0.9859
set17	30	History_of_Vaginitis_bin, Pregnant; 14 bac log ^2, ^1	0.4634	0.6829	0.7317	0.8803	0.9859
set19	32	Age, History_of_Vaginitis_bin, pos_whiff, Pregnant; 14	0.4512	0.6829	0.7439	0.8803	0.9859
set20	28	12 bac log ^2, ^1; Age, History_of_Vaginitis_bin, pos_w	0.4878	0.6829	0.7561	0.8873	0.9859
set23	26	pos_whiff, Pregnant; 12 bac log ^2, ^1	0.5	0.6829	0.7561	0.8803	0.9789
set30	30	13 bac log ^2, ^1; Age, History_of_Vaginitis_bin, pos_w	0.4756	0.6829	0.7439	0.8803	0.9859
set31	30	13 bac log ^2, ^1; Age, History_of_Vaginitis_bin, pos_w	0.4878	0.6829	0.7439	0.8873	0.9859
set32	29	1 inter; 12 bac log ^2, ^1; Age, History_of_Vaginitis_bin	0.4634	0.6829	0.7561	0.8944	0.9859
set01	29	14 bac log ^1; all others	0.4024	0.6707	0.7439	0.8591	0.9507
set01	29	14 bac log ^1; all others	0.4024	0.6707	0.7439	0.8591	0.9507
set04	33	9 bac log ^2, ^1; all others	0.5244	0.6707	0.7439	0.8451	0.9437
set05	42	9 bac log ^3, ^2, ^1; all others	0.5244	0.6707	0.7439	0.8451	0.9437
set06	44	1 inter; 14 bac log ^1, ^2; all others	0.4512	0.6707	0.7439	0.8662	0.9648
set11	28	no others; 14 bac log ^2, ^1	0.4878	0.6707	0.7317	0.8732	0.9859
set12	30	History_of_Vaginitis_bin, pos_whiff; 14 bac log ^2, ^1	0.4878	0.6707	0.7317	0.8732	0.9859
set21	27	12 bac log ^2, ^1; History_of_Vaginitis_bin, pos_whiff,	0.4756	0.6707	0.7439	0.9014	0.9859
set22	26	12 bac log ^2, ^1; History_of_Vaginitis_bin, Pregnant	0.4878	0.6707	0.7317	0.8873	0.9859
set24	26	11 bac log ^2, ^1; Age, History_of_Vaginitis_bin, pos_w	0.4756	0.6707	0.7439	0.8944	0.9789
set25	29	1 bac log^3, 12 bac log ^2, ^1; Age, History_of_Vaginitis	0.4878	0.6707	0.7561	0.8803	0.9789
set26	26	11 bac log ^2, ^1; Age, History_of_Vaginitis_bin, pos_w	0.4878	0.6707	0.7561	0.8873	0.9859
set27	24	10 bac log ^2, ^1; Age, History_of_Vaginitis_bin, pos_w	0.4878	0.6707	0.7439	0.8873	0.9789
set28	29	1 bac log^3, 12 bac log ^2, ^1; Age, History_of_Vaginitis	0.4878	0.6707	0.7439	0.8873	0.9859
set29	30	2 bac log^3, 12 bac log ^2, ^1; Age, History_of_Vaginitis	0.4878	0.6707	0.7439	0.8803	0.9789
set33	29	1 inter; 12 bac log ^2, ^1; Age, History_of_Vaginitis_bin	0.4756	0.6707	0.7561	0.9014	0.9859
set34	30	2 inter; 12 bac log ^2, ^1; Age, History_of_Vaginitis_bin	0.4634	0.6707	0.7561	0.8944	0.9859
set01	29	14 bac log ^1; all others	0.4024	0.6585	0.7439	0.8591	0.9507
set07	42	13.5 bac log ^2, ^1; all others	0.4634	0.6585	0.7439	0.8732	0.9648
set08	41	13 bac log ^2, ^1; all others	0.4756	0.6585	0.7439	0.8732	0.9648
set09	39	12 bac log ^2, ^1; all others	0.4878	0.6585	0.7439	0.8732	0.9648
set14	29	Age; 14 bac log ^2, ^1	0.4878	0.6585	0.7317	0.8662	0.9859
set02	43	14 bac log ^2, ^1; all others	0.4634	0.6463	0.7439	0.8732	0.9648
set03	57	14 bac log ^3, ^2, ^1; all others	0.4634	0.6463	0.7195	0.8521	0.9437
set10	39	no ethnicity; 14 bac log ^2, ^1; all others	0.4512	0.6463	0.7439	0.8662	0.9648
set01	29	14 bac log ^1; all others	0.2979	0.5957	0.7447	0.7882	0.9059



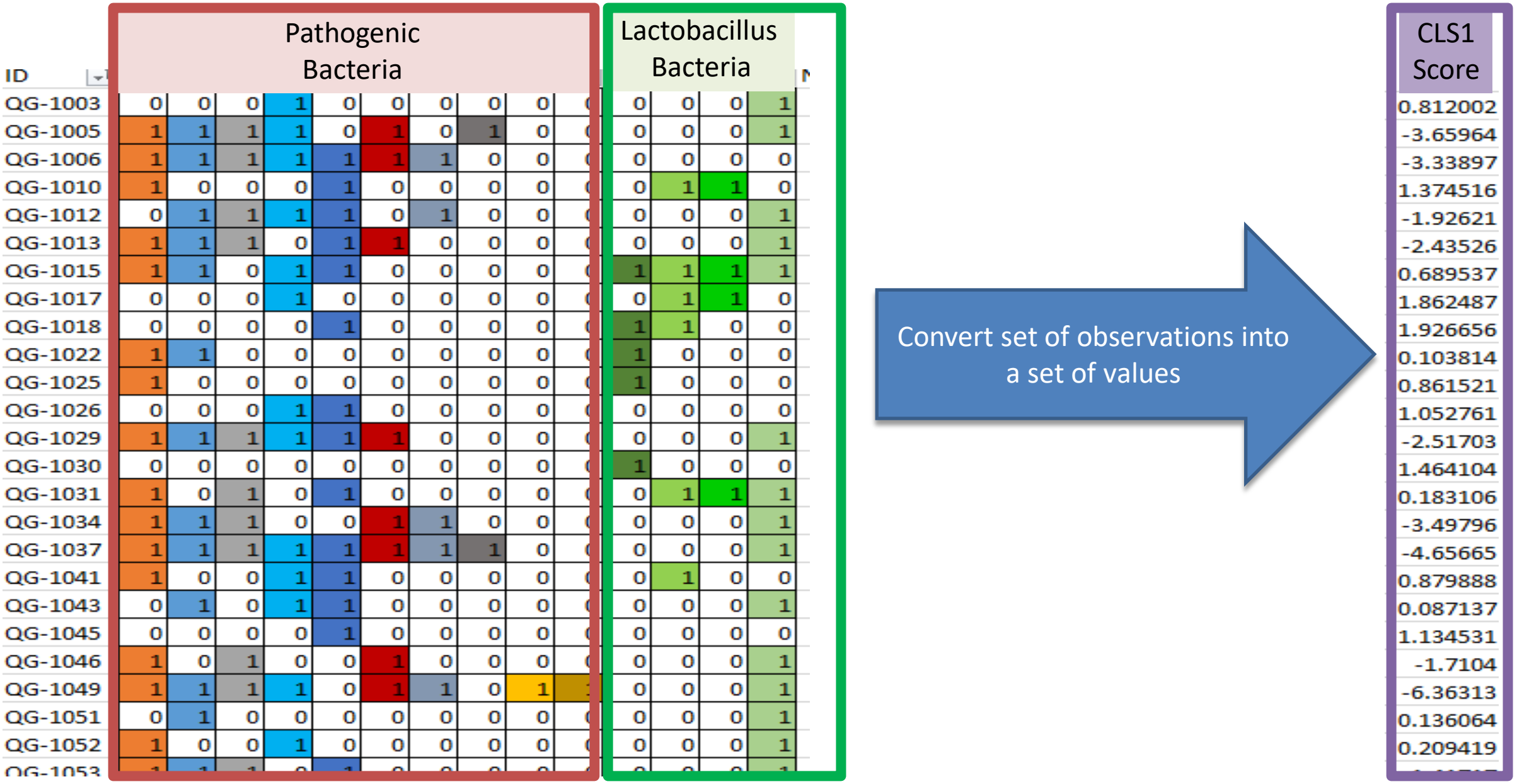
Women's Health Reporting

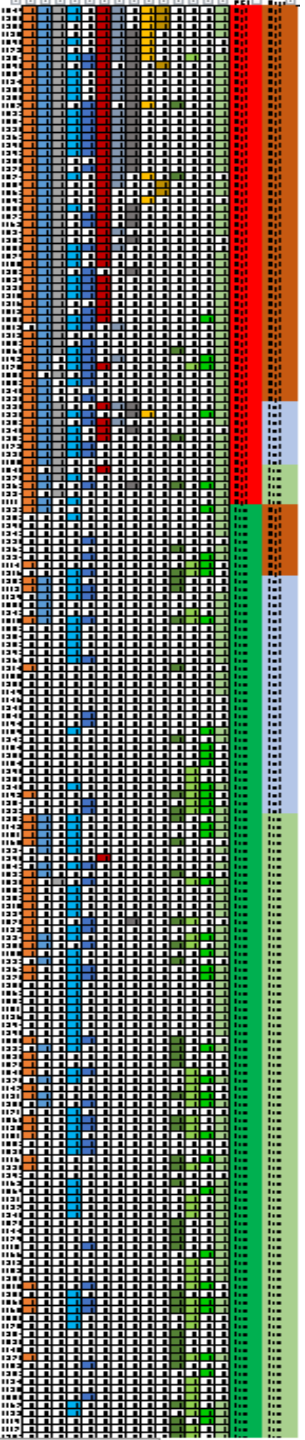
Algorithm Development Process Raw Data

Convert Data for Evaluation of Orthogonal Transformation Model



ID	Pathogenic Bacteria										Lactobacillus Bacteria			
QG-1003	0	0	0	1	0	0	0	0	0		0	0	0	1
QG-1005	1	1	1	1	0	1	0	1	0		0	0	0	1
QG-1006	1	1	1	1	1	1	1	0	0		0	0	0	0
QG-1010	1	0	0	0	1	0	0	0	0		0	1	1	0
QG-1012	0	1	1	1	1	0	1	0	0		0	0	0	1
QG-1013	1	1	1	0	1	1	0	0	0		0	0	0	1
QG-1015	1	1	0	1	1	0	0	0	0		1	1	1	1
QG-1017	0	0	0	1	0	0	0	0	0		0	1	1	0
QG-1018	0	0	0	0	1	0	0	0	0		1	1	0	0
QG-1022	1	1	0	0	0	0	0	0	0		1	0	0	0
QG-1025	1	0	0	0	0	0	0	0	0		1	0	0	0
QG-1026	0	0	0	1	1	0	0	0	0		0	0	0	0
QG-1029	1	1	1	1	1	1	0	0	0		0	0	0	1
QG-1030	0	0	0	0	0	0	0	0	0		1	0	0	0
QG-1031	1	0	1	0	1	0	0	0	0		0	1	1	1
QG-1034	1	1	1	0	0	1	1	0	0		0	0	0	1
QG-1037	1	1	1	1	1	1	1	1	0		0	0	0	1
QG-1041	1	0	0	1	1	0	0	0	0		0	1	0	0
QG-1043	0	1	0	1	1	0	0	0	0		0	0	0	1
QG-1045	0	0	0	0	1	0	0	0	0		0	0	0	0
QG-1046	1	0	1	0	0	1	0	0	0		0	0	0	1
QG-1049	1	1	1	1	0	1	1	0	1		0	0	0	1
QG-1051	0	1	0	0	0	0	0	0	0		0	0	0	1
QG-1052	1	0	0	1	0	0	0	0	0		0	0	0	1
QG-1053	1	1	1	0	1	0	0	0	0		0	0	0	1


Pathogenic Bacteria
Lactobacillus Bacteria
CLS1 Score
Convert set of observations into a set of values



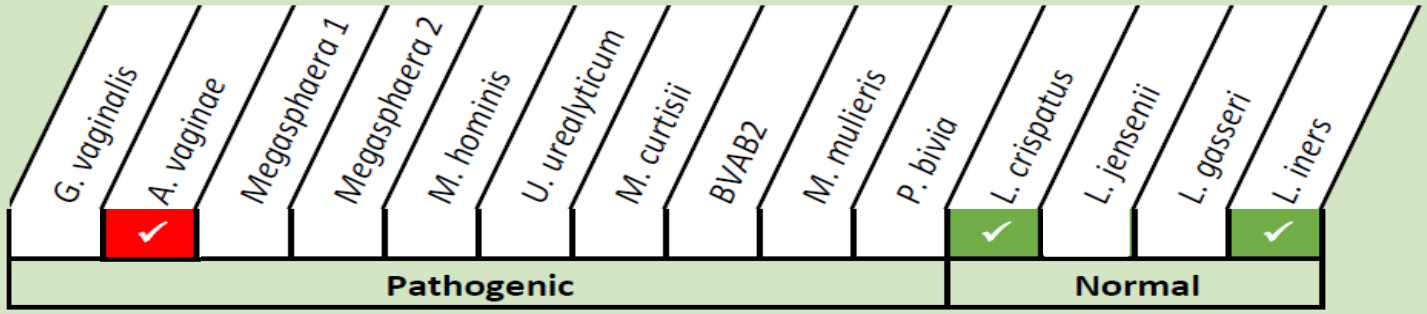
CLS1 Algorithm sensibly groups specimens according to bacterial presence

- The CLS1 High group contains only 5% of Nugent Lows; those 5 samples “look” pathogenic
  - **CLS1 High = BV+**
- The CLS1 Lows are Nugent Lows with a mix of additional Nugent categories (Intermediates, Highs)
  - **CLS1 Low = Not conclusive for Bacterial Vaginosis**
- “Exceptions” to the CLS1 Algorithm make biological sense

CLS1 Algorithm Result = LOW

Results:

Pathogenic Bacteria Detected

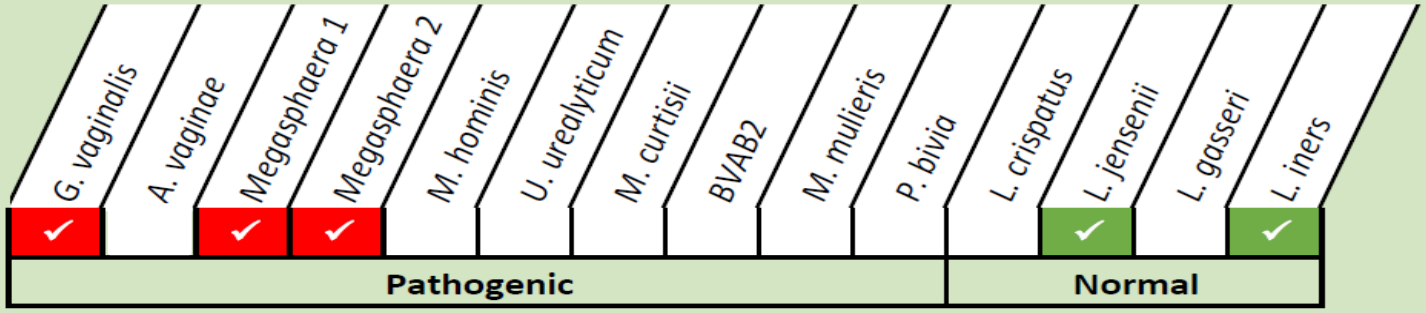


- Investigation outcomes: The pattern of pathogenic and normal bacterial flora does not suggests bacterial vaginosis.

CLS1 Algorithm Result = HIGH

Results:

Pathogenic Bacteria Detected

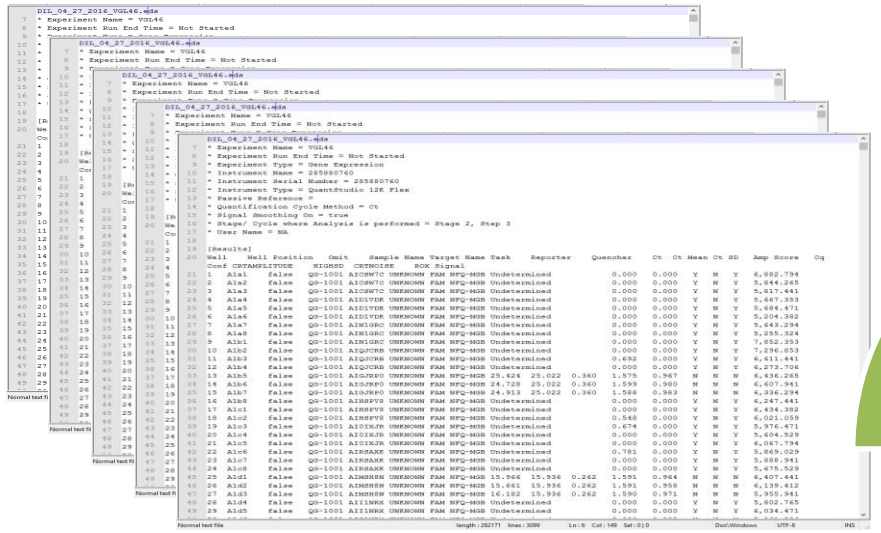


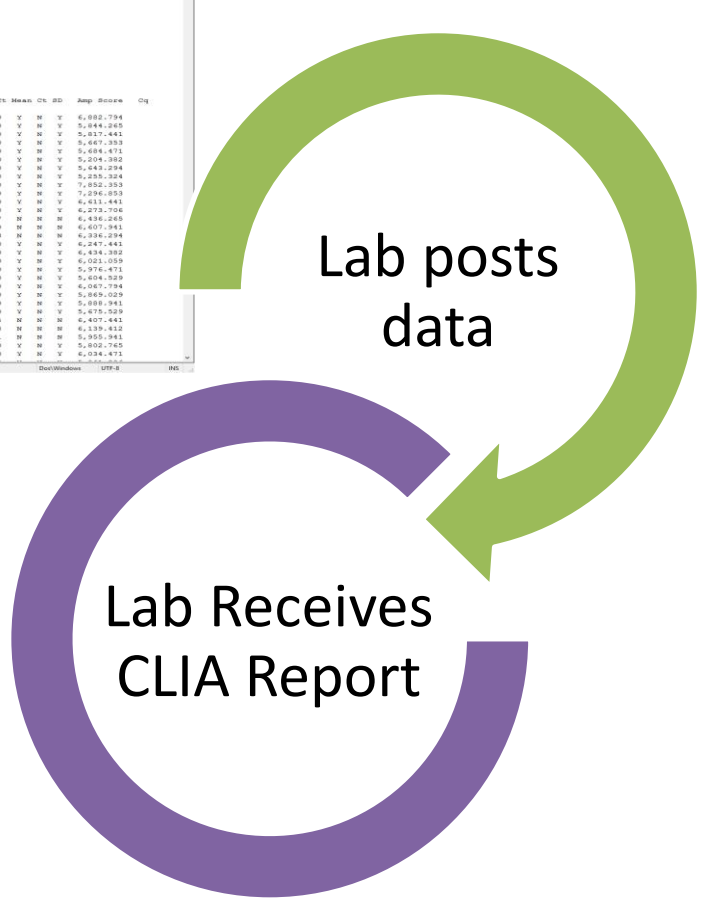
- Interpretation: The pattern of pathogenic and normal bacterial flora suggests bacterial vaginosis.

CLS REPORTING SOLUTIONS - EXAMPLES

Women’s Health Reporting

Simple Reporting Process



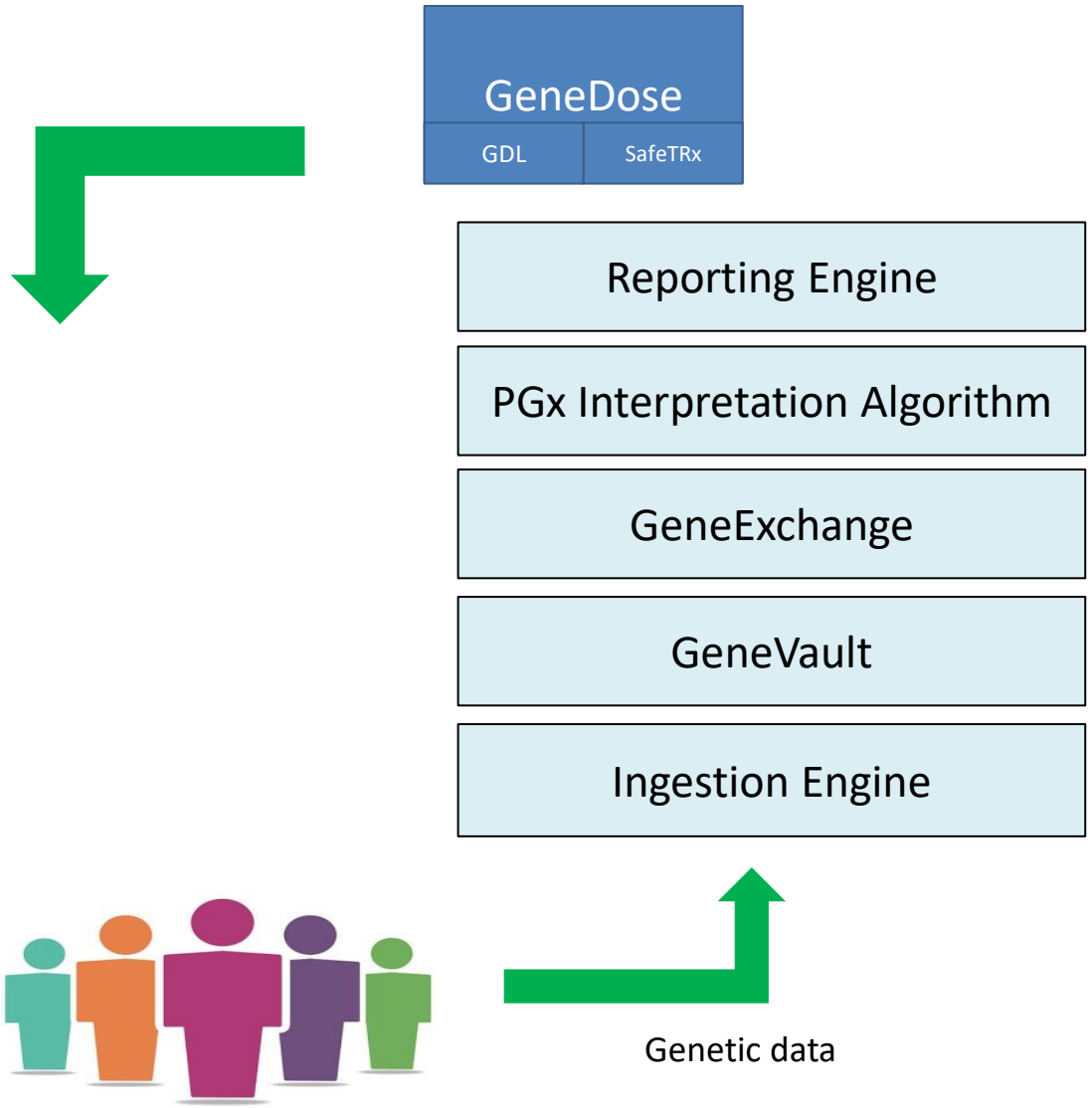


Process and Architecture

Reports

Infrastructure

Customers

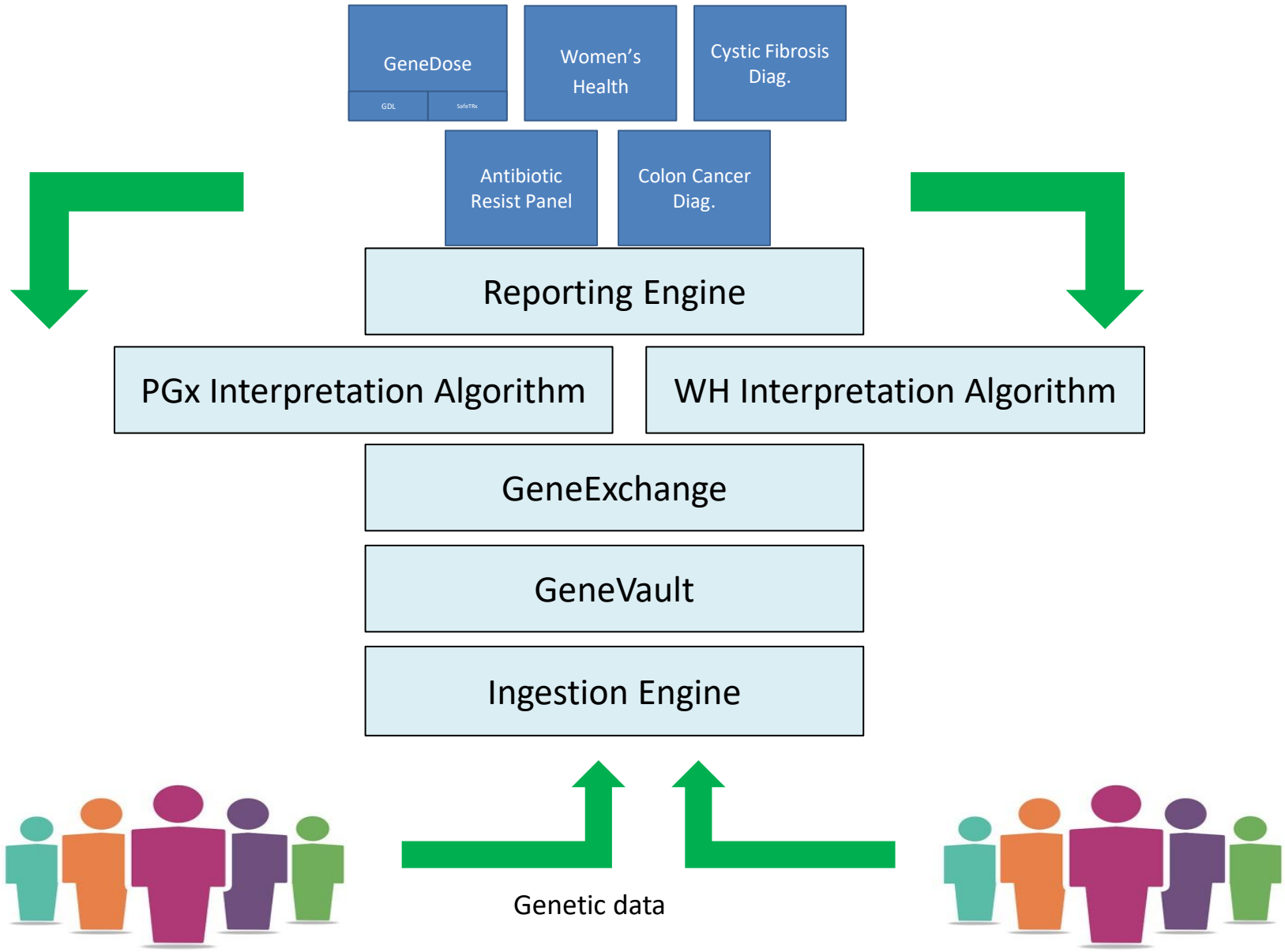


Process and Architecture

Reports

Infrastructure

Customers

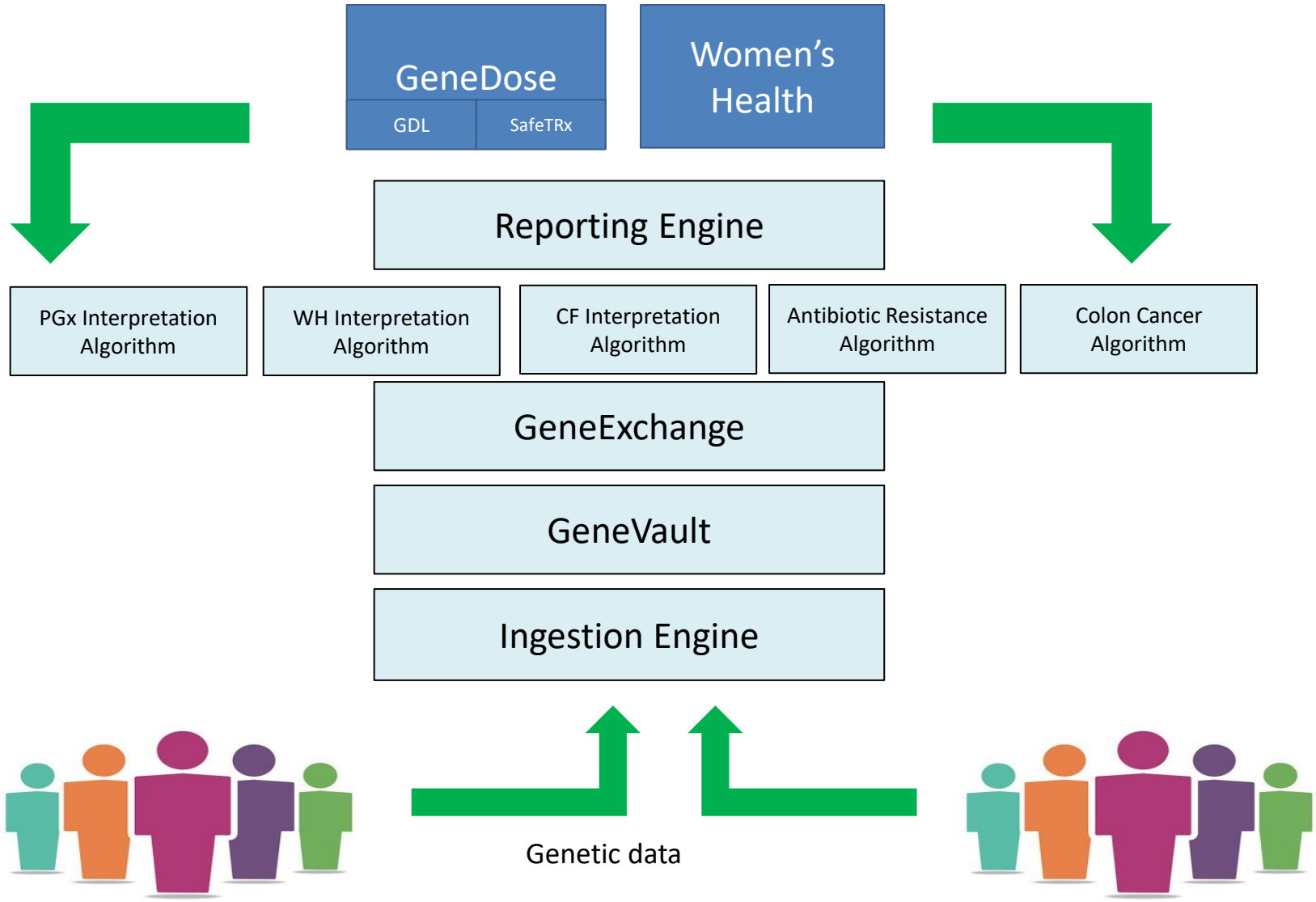


Process and Architecture

Reports

Infrastructure

Customers



Women’s Health Reporting

- Support laboratories in their missions to deliver world-class diagnostic solutions
- Complete Decision Support
- Actionable based on Study with Quantigen and ThermoFisher Scientific
- Customizable reports – logos, branding, and panels