Consistent and Reproducible Production of a Microbiota-based Drug for Recurrent *C. difficile* Infection: Application of a Novel Diagnostic for Dysbiosis

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Disclosure

- Program Manager, Rebiotix Inc., Roseville, MN USA

Background

- Perturbation of the gut microbiota by antibiotics is a major risk factor for both primary and recurrent *Clostridium difficile* infection (CDI).
- Restoration of the gut microbiota protects against CDI recurrence.[1,2]
- RBX2660 is manufactured from live human-derived microbes using standardised, quality controlled-processes.
- Tested in >300 patients in 3 robust, FDA-regulated clinical trials including a randomized, double-blind placebo controlled Phase 2 trial (PUNCH CD 2).

1. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile*-associated diarrhea. *J Infect Dis*. 2008;197(3):435-8.

2. Orenstein R, Dubberke E, Hardi R, et al. Safety and durability of RBX2660 (microbiota suspension) for recurrent *Clostridium difficile* Infection: Results of the PUNCH CD study. *Clin Infect Dis*. 2016;62(5):596-602.

Objectives

- Use a novel platform (GA-map Dysbiosis Test, Genetic Analysis, Oslo, Norway) to assess if the RBX2660 manufacturing process alters the normal microbiome.
- Determine, using this unique platform, whether or not a normal microbiome is maintained by healthy donors over time.

Methods

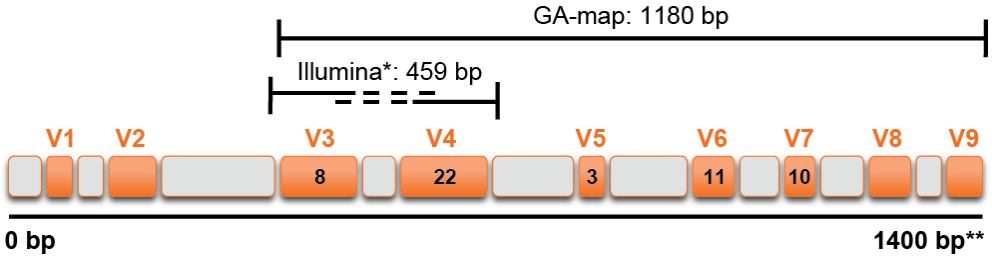
- A total of 70 drug substance samples sourced from 17 unrelated donors from August 2014 to February 2016 were compared with 70 matched samples of finished drug product using the GA-map Dysbiosis Test.
  - Donor characteristics: median age: 23 years; range 18 to 57 years; 94% male
- Includes a small sub-set of 4 donors with longitudinal data
- Product tested was collected over 4-13 months.
  - Stored at -80°C to preserve samples
  - Drug substance and drug product were collected and frozen on the same day

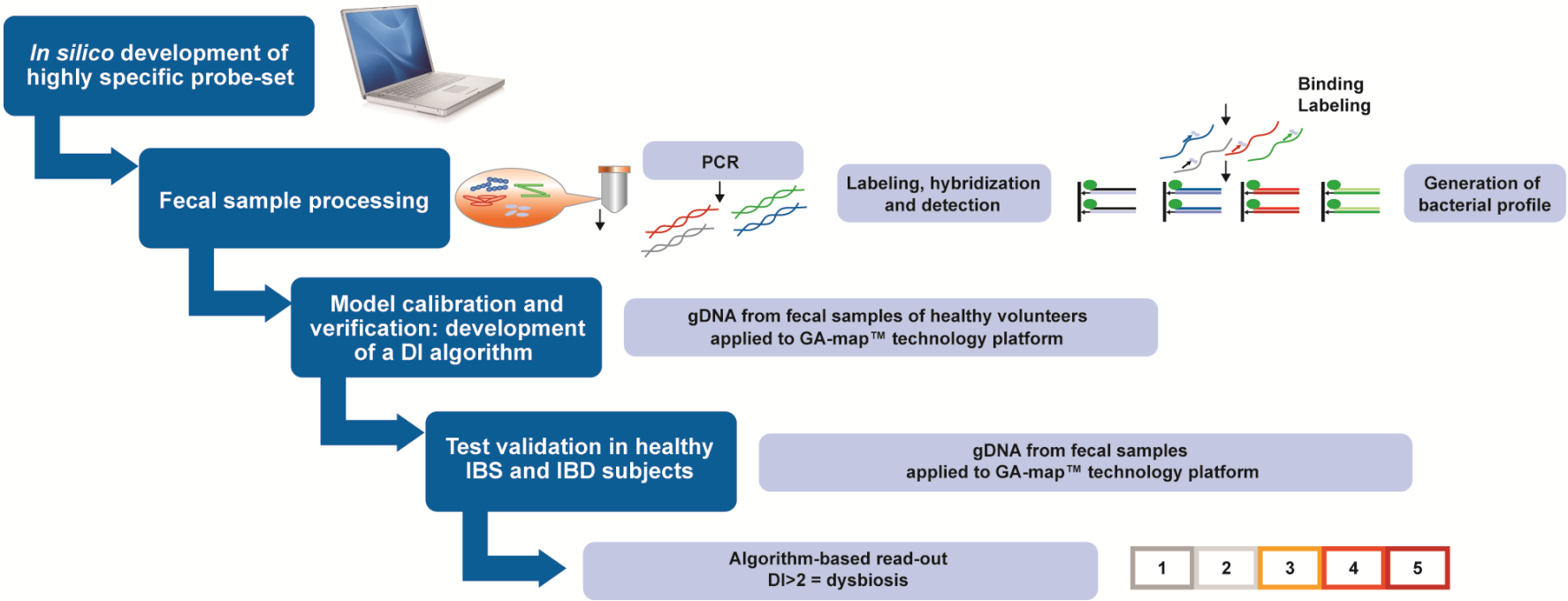
Methods

- The GA-map Dysbiosis Test was developed as a diagnostic tool for IBS/IBD.
- Validated in accordance with EU requirements to identify and characterize dysbiosis.[1]
- Uses 54 probes targeting V3 to V7 of the bacterial 16s rRNA gene to characterize and identify bacteria present.
- Covers approximately 300-400 bacteria at different taxonomic levels and provides an assessment of the “normal” or “dysbiotic” microbial community by using multiple variable regions.
- Enables serial assessment of fecal bacterial community abundance profile and potentially clinically relevant alterations in the microbiota over time.

1. Casén C, Vebø HC, Sekelja M, et al. Deviations human gut microbiota: A novel diagnostic test for determining dysbiosis in patients with IBS or IBD. *Aliment Pharmacol Ther*. 2015;42:71-83.

Methods


GA-map: 1180 bp
Illumina*: 459 bp
V1
V2
V3
V4
V5
V6
V7
V8
V9
8
22
3
11
10
0 bp
1400 bp**


In silico development of highly specific probe-set
Fecal sample processing
PCR
Labeling, hybridization and detection
Binding
Labeling
Generation of bacterial profile
Model calibration and verification: development of a DI algorithm
gDNA from fecal samples of healthy volunteers applied to GA-map™ technology platform
Test validation in healthy IBS and IBD subjects
gDNA from fecal samples applied to GA-map™ technology platform
Algorithm-based read-out
DI>2 = dysbiosis
1
2
3
4
5

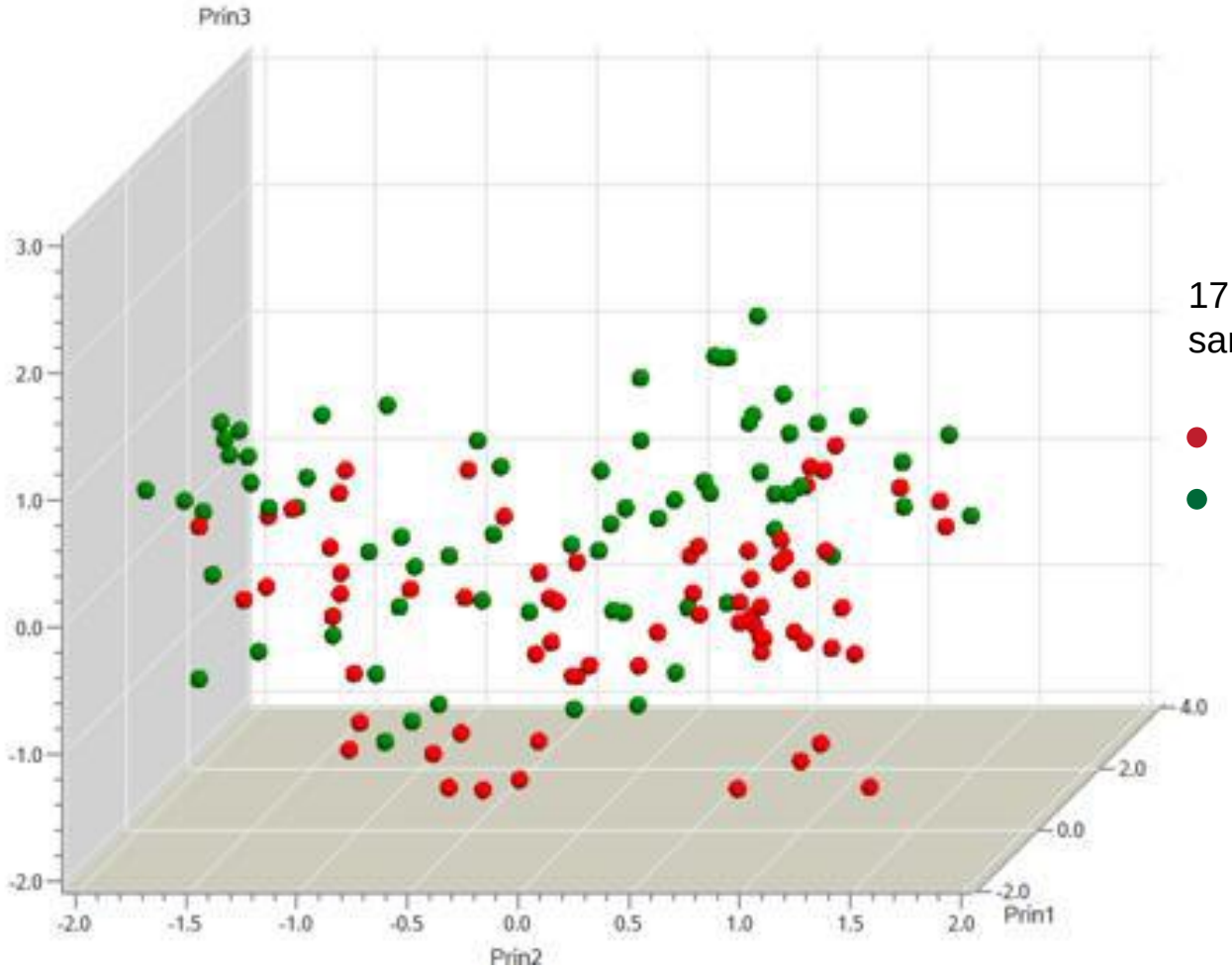
Comparative Signal Strength of Bacteria: DP vs. DS

Bacteria	Signal Strength in DP vs. DS	Mean Difference (95% CIM)
Bacteroidetes		
Bacteroides fragilis	Increased	0.07 (0.03, 0.11)
Parabacteroides	Increased	0.12 (0.07, 0.17)
Alistipes	Increased	0.17 (0.11, 0.23)
Firmicutes		
Lachnospirae	Decreased	-0.13 (-0.15, -0.11)
Streptococcus	Decreased	-0.16 (-0.20, -0.13
Negativicutes	Increased	0.03 (0.01, 0.06)
Clostridia	Decreased	-0.18 (-0.20, -0.16)
Actinobacteria		
Bifidobacterium	Decreased	-0.33 (-0.38, -0.28)

DP = drug product; DS = drug source, CIM = confidence interval of mean

- High accuracy: 83.4% at cross validation
- Drug substance and drug product profiles largely conserved.

Principal Component Analysis Score Plots

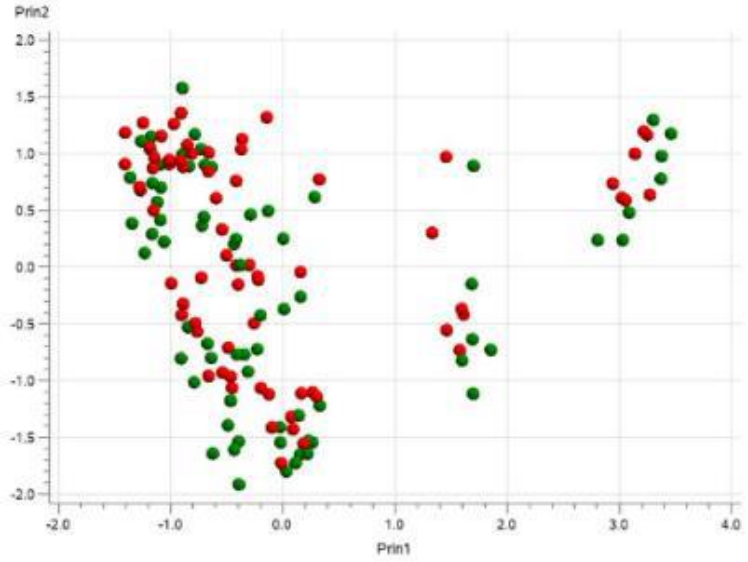


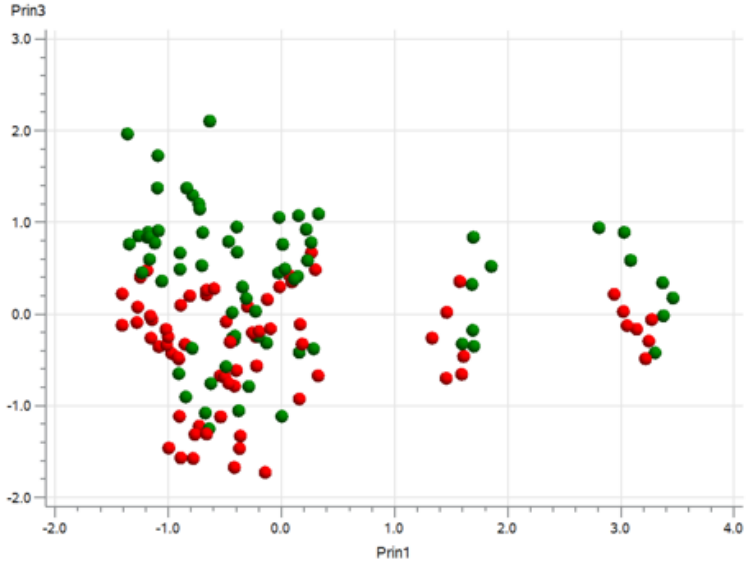
17 donors, N=140 samples

- Drug product
- Drug substance

log 10 transformed data of 54 probes in GA-map Dysbiosis Test

Principal Component Analysis Score Plots





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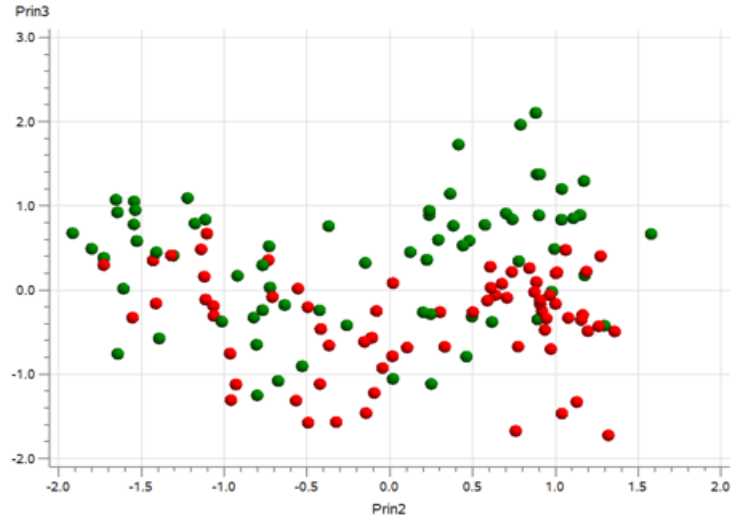
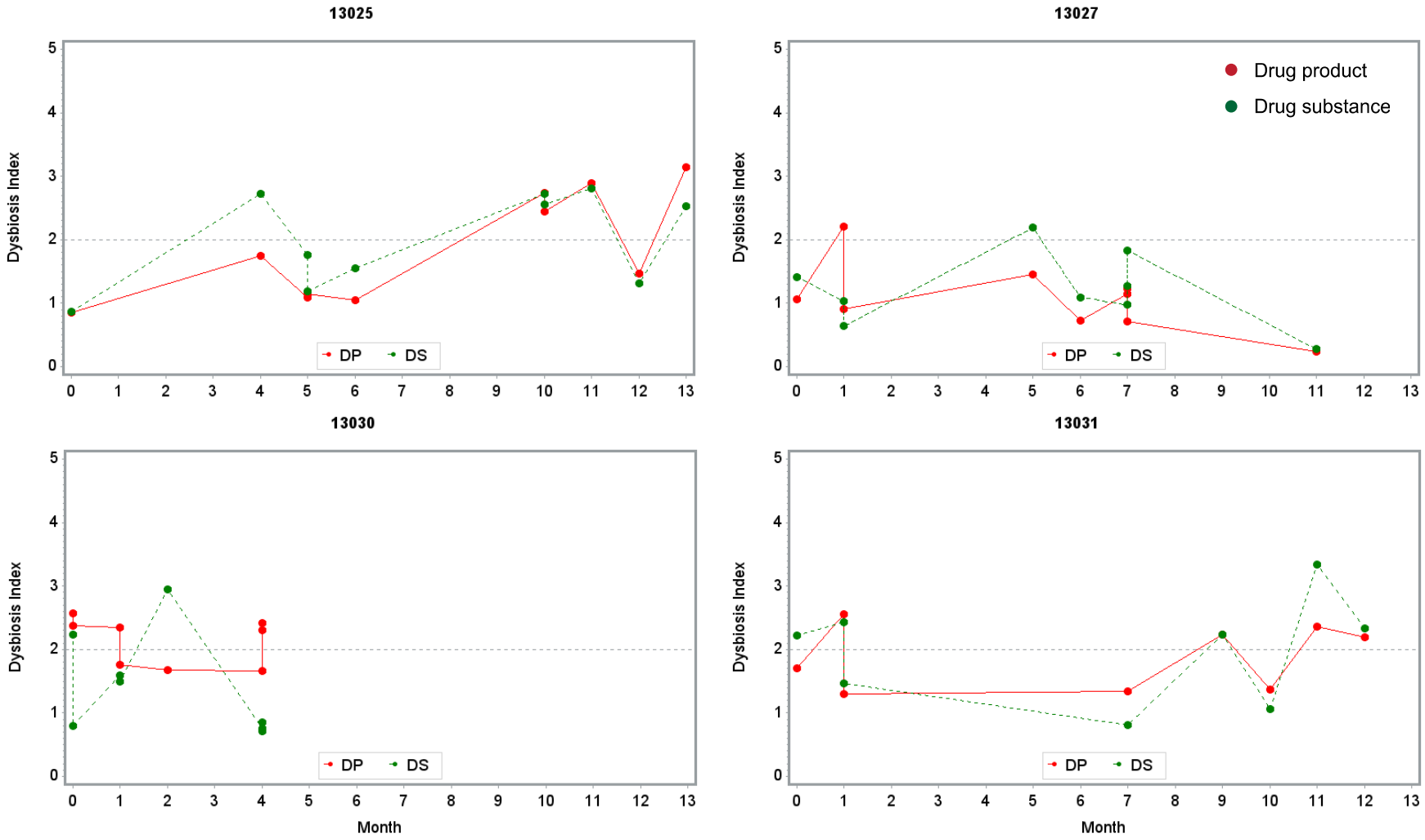


log 10 transformed data of 54 probes in GA-map Dysbiosis Test

Longitudinal Analysis



Drug substance shows slightly more variance than the drug product: SD=0.76 vs. SD=0.82

Summary

- The GA-map Dysbiosis Test confirms that bacterial community abundance in the drug substance is conserved in manufactured RBX2660. Dysbiosis is not instigated with the standardized manufacturing process.
- The GA-map Dysbiosis Test also demonstrated that donors maintain a consistent, normal microbiome over time, corresponding with previous findings.[1]

1. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature*. 2012;489:220-230.

Discussion

- Validated using healthy controls and patients with IBS and IBD from Norway, Sweden, Denmark and Spain.
  - Validity of test when used in different populations (North America, Asia, Middle East, etc.) for diagnosis or identification of a “normal” microbiome?
- Ability to monitor alterations in the microbiome of patient’s with CDI to potentially predict therapeutic outcome or relapse?
  - Longitudinal patient samples – how do they change compared to dysbiotic index?
- Further exploration of healthy donors over time – how long do donors maintain a stable microbiome?

Acknowledgements

- Genetic Analysis Team
  - Christina Casén
  - Hans Fagerun
- Rebiotix Technical Team

Thank you!