The Infant Microbiome and Necrotizing Enterocolitis

St. Louis Infant Microbiome Team

HMP

1 September 2010

St. Louis, MO

Baby Boy
26 Weeks gestation, C-section, BW= 720 g

Antibiotics:

- DOLs 1-4: ampicillin, gentamicin (prematurity, choriamnionitis)
- DOLs 5-10: cefotaxime (CSF)
- DOLs 5-7: vancomycin, fluconazole (pathology report, failure to wean from ventilator)
- DOLs 9-18: vancomycin, cefotaxime, fluconazole (leukocytosis, failure to wean from ventilator)

Feeds:

- DOLs 8-21: formula (24 kcal, premature formula)

DOL 21:

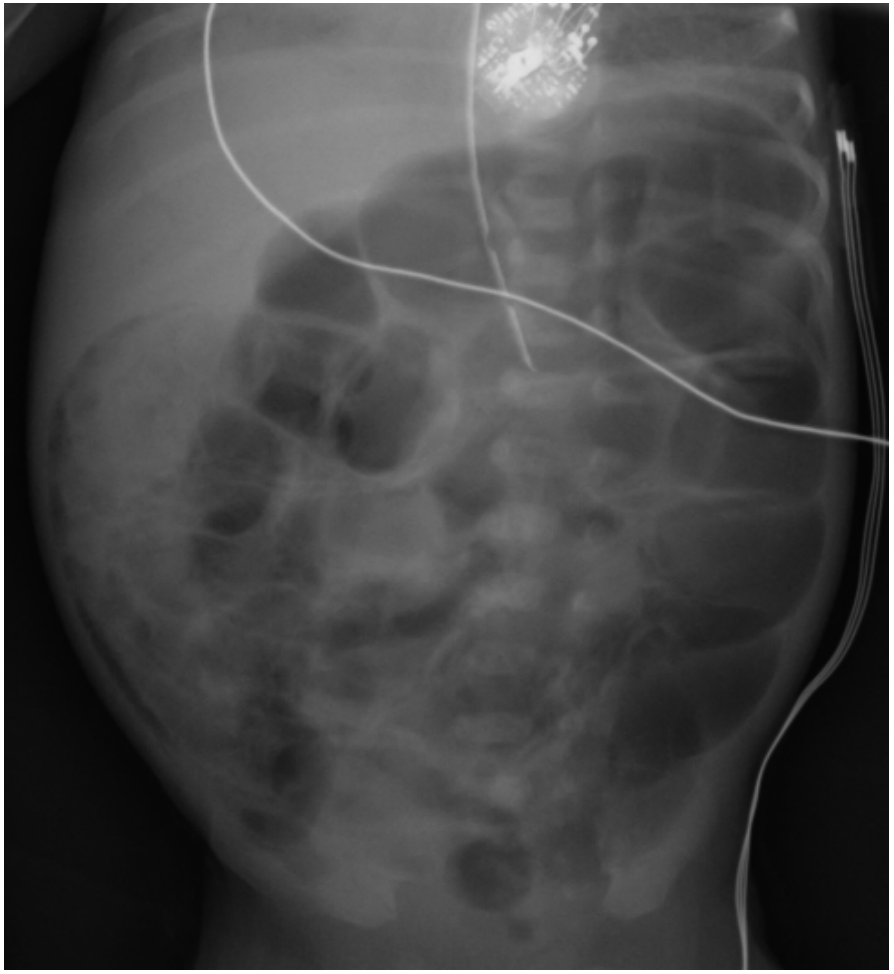
- Transfusion, ventricular tap (intraventricular hemorrhage)
  Increasing ventilator settings: increased opacity; vancomycin, clindamycin

DOL 22:

- Bloody stool

DOL 22

2:58 AM
Gaseous distension



8:15 AM
Extensive right flank pneumatosis.

Exploratory Operation

"… panintestinal necrosis beginning just distal to the Ligament of Treitz and extending to the beginning of the cecum ..."

"... this was not compatible with life and not amenable to any surgical intervention."

Necrotizing Enterocolitis (NEC)

Common: 7-12% of all prematures (<1500 g BW)

Devastating: 20-30% case fatality rate

Almost all cases occur by DOL 35, patients are hospitalized, identifiable risk cohort (BW < 1500)

Occurs postnatally (i.e, after microbial colonization), probiotics might protect, antibiotics might increase risk

The Hypothesis

- One or more identifiable components of the enteric biomass (including bacteria, their genes, transcripts, and gene products)

Study Structure

7 day/week consent

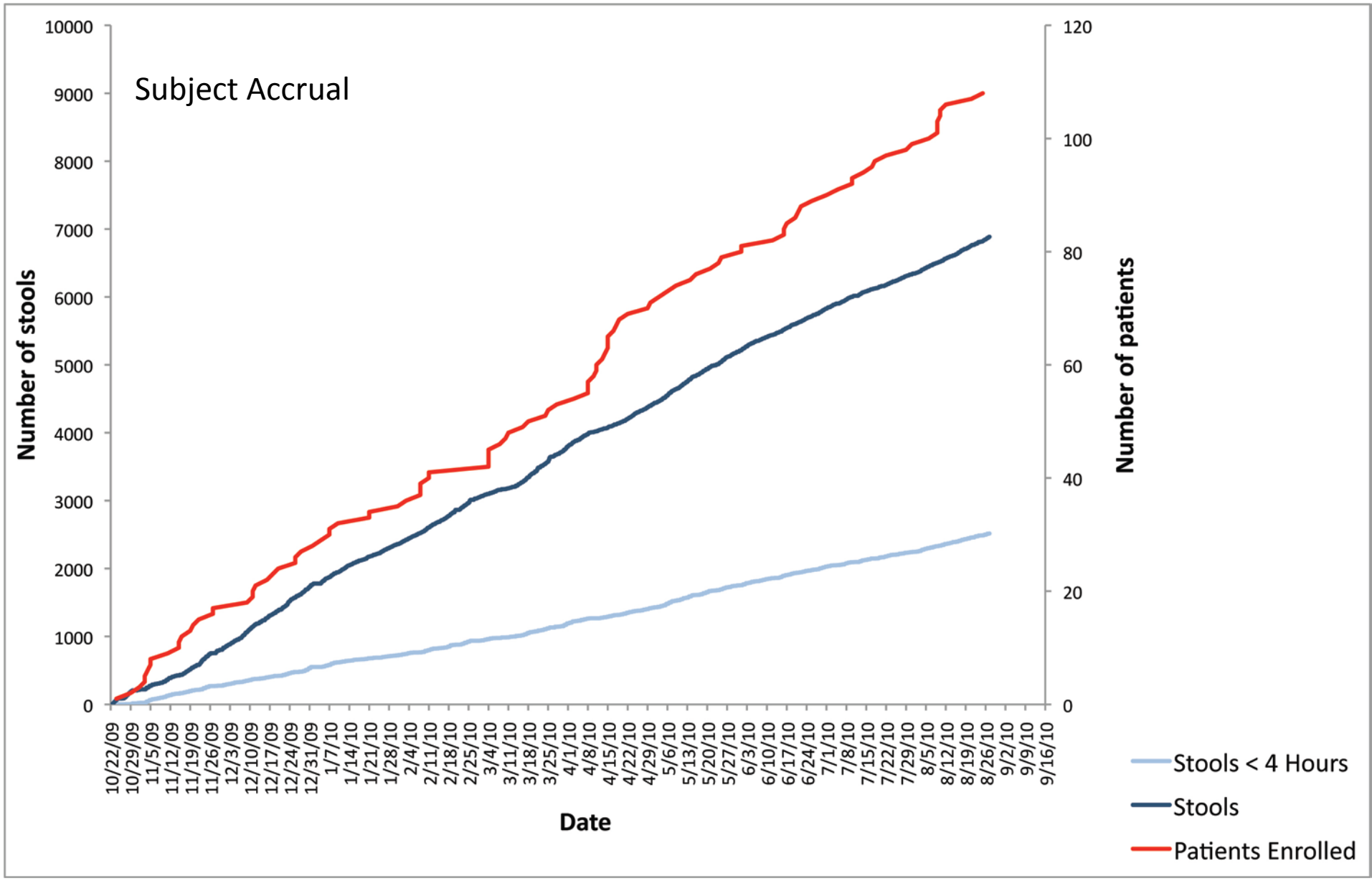
All stools sought (prioritized)

Weekly bloods

Daily ‘real time’ data

(esp. feeds and antibiotics)

Specimen provenance and curation


Subject Accrual
Number of stools
Number of patients
Date
10000
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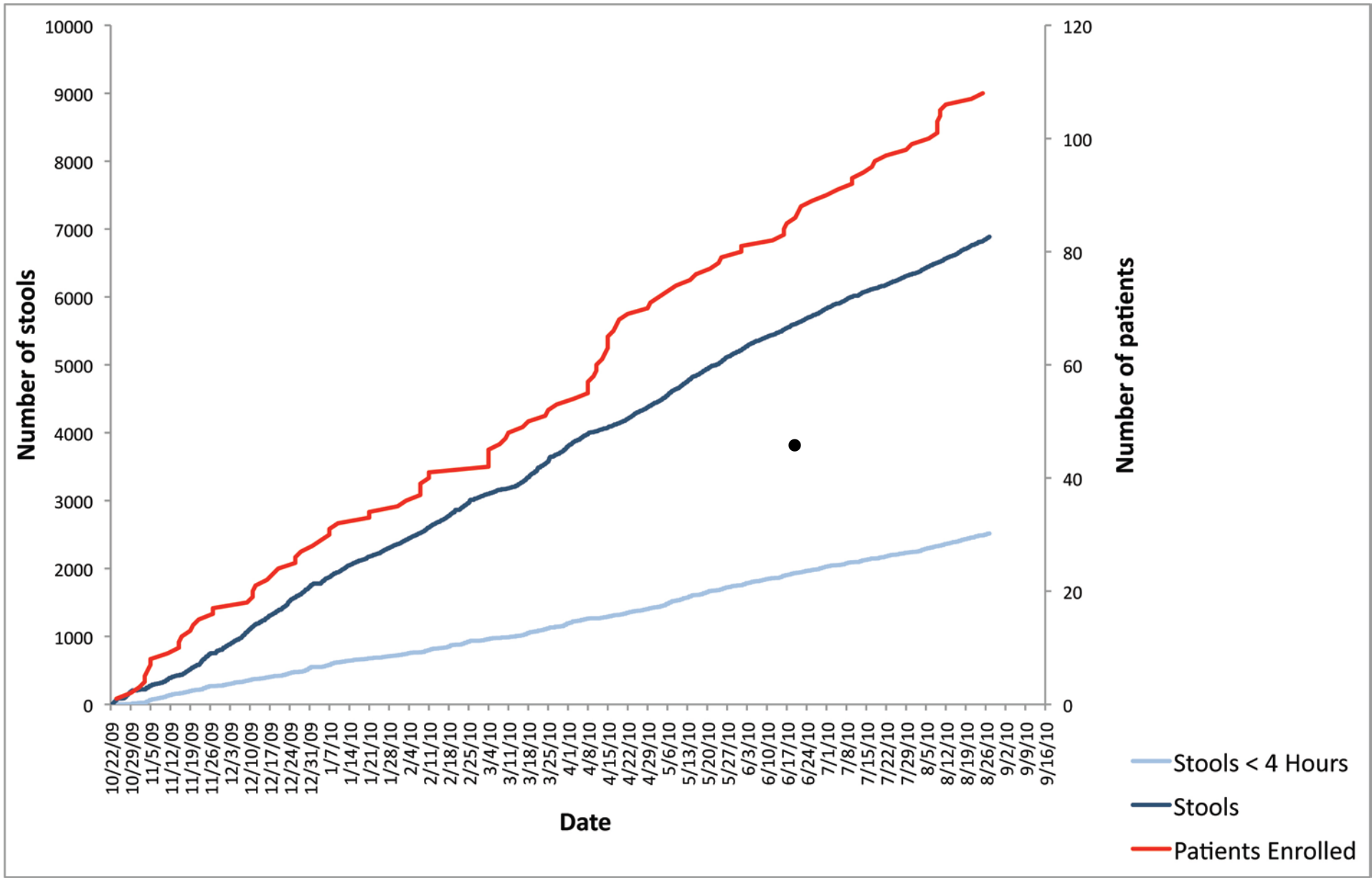
9/16/10
Stools < 4 Hours
Stools
Patients Enrolled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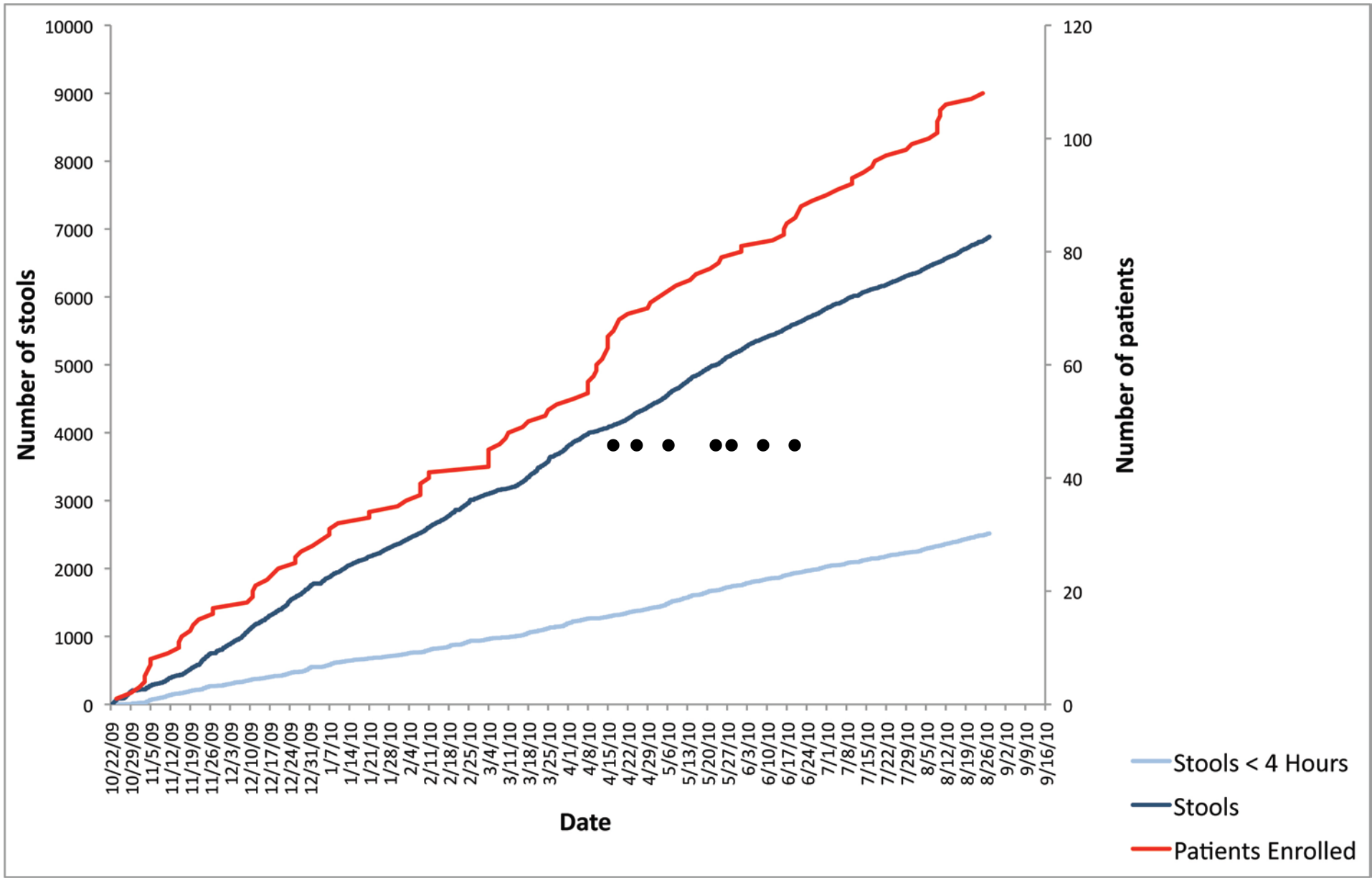
9/9/10
9/16/10
Date
Stools < 4 Hours
Stools
Patients Enrolled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Number of patients
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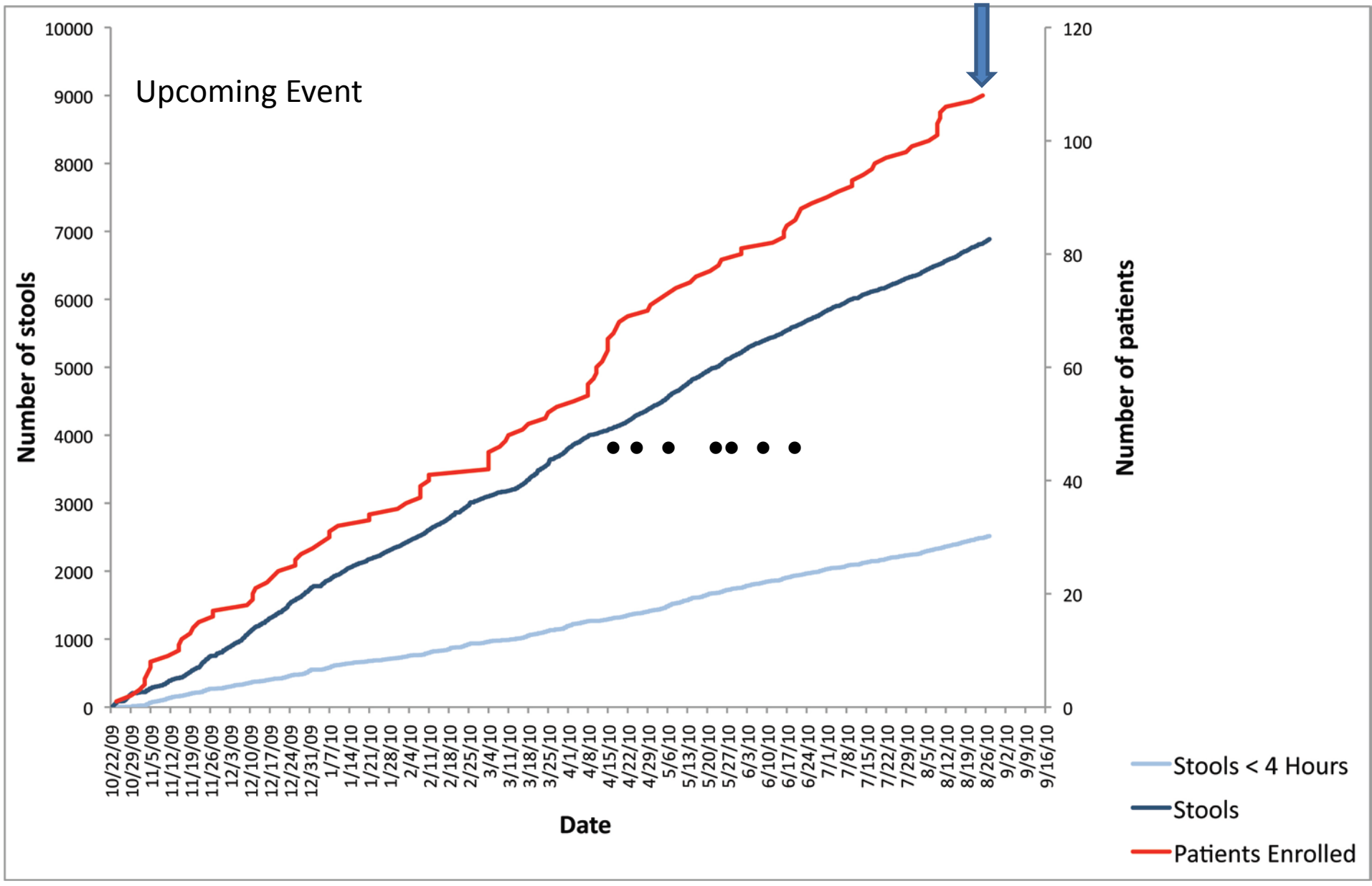
9/16/10
Date
Stools < 4 Hours
Stools
Patients Enrolled


Upcoming Event
Number of stools
Number of patients
Date
Stools < 4 Hours
Stools
Patients Enrolled
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9000
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2000
1000
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120
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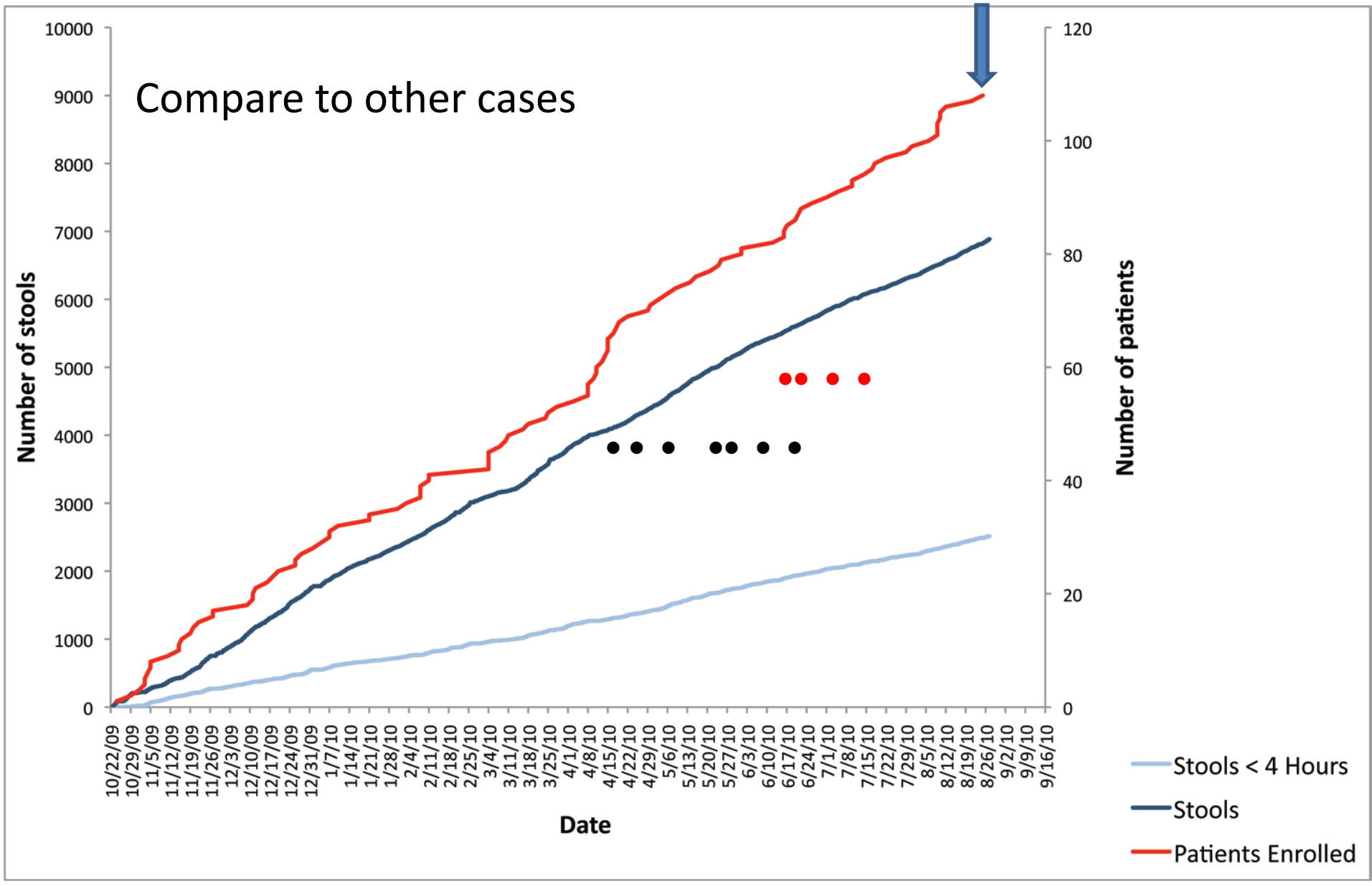
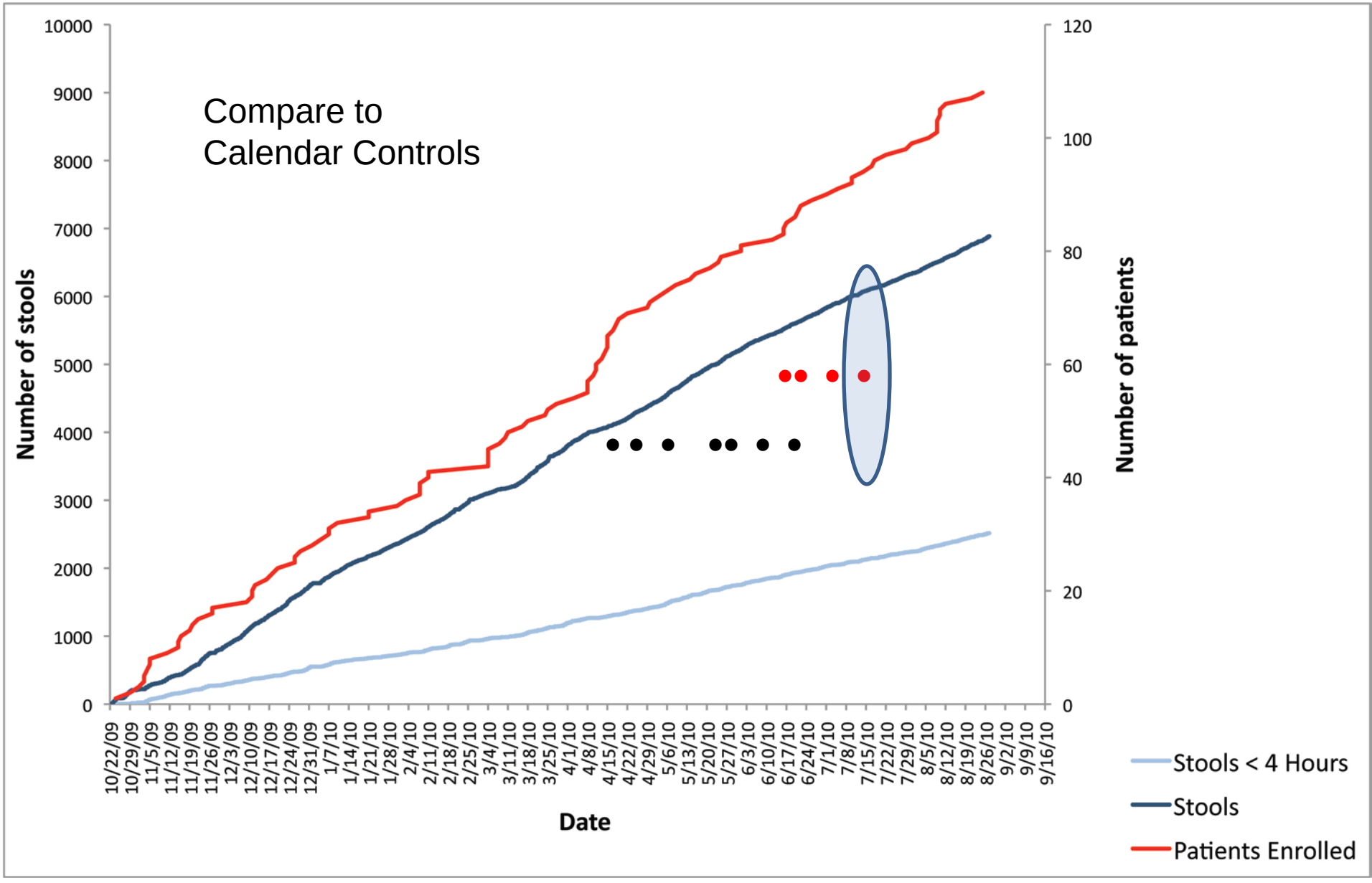
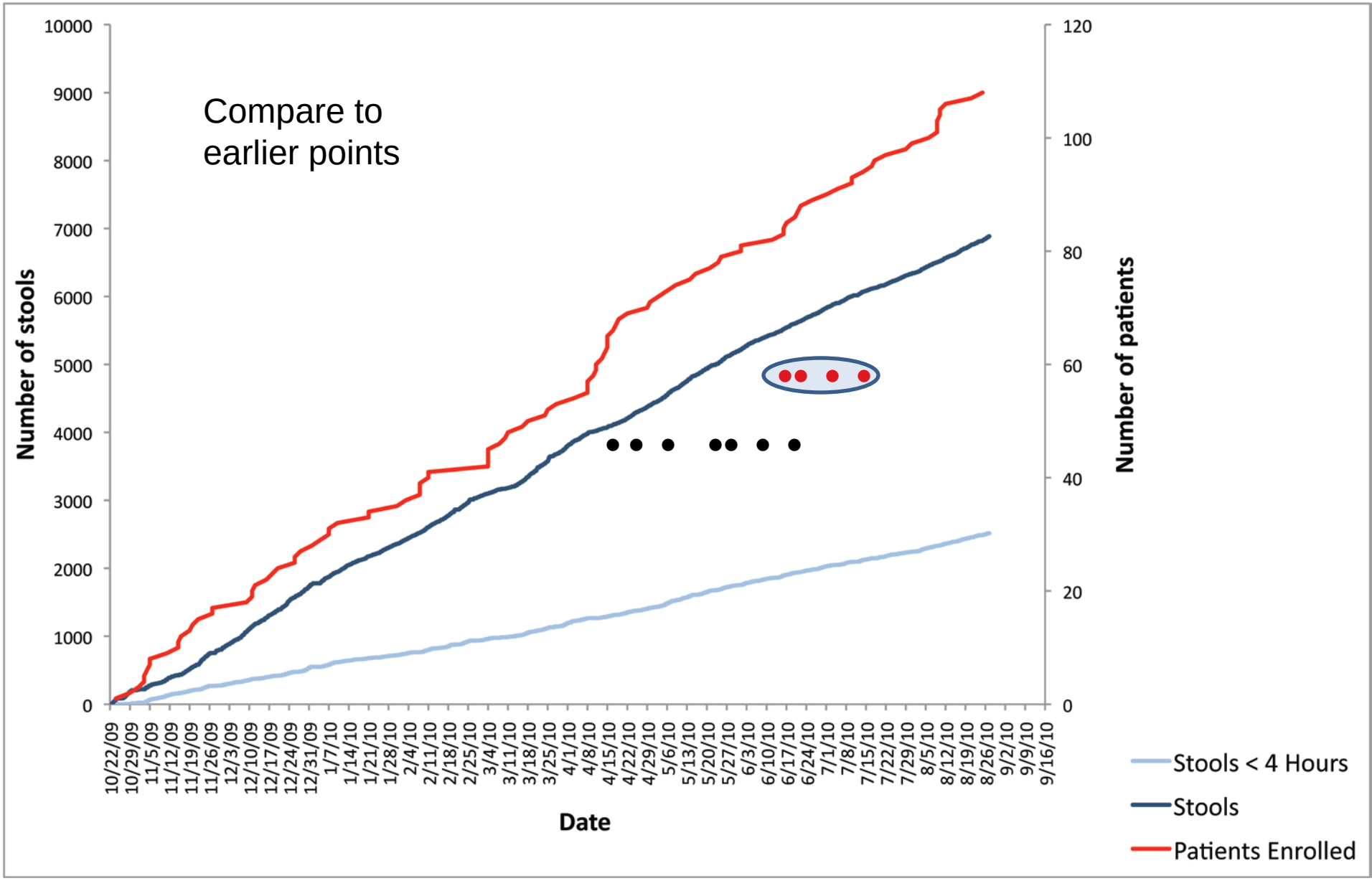
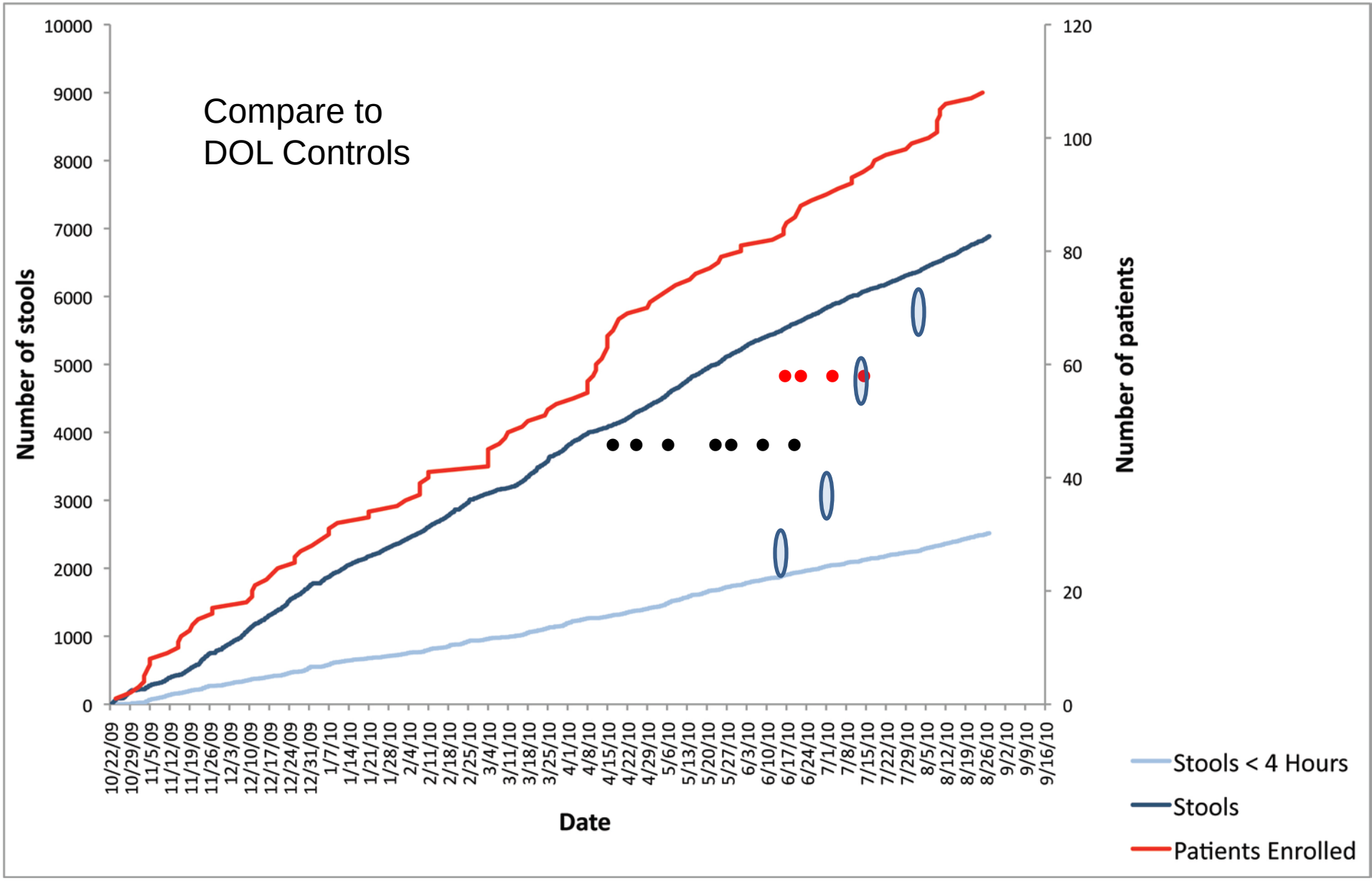
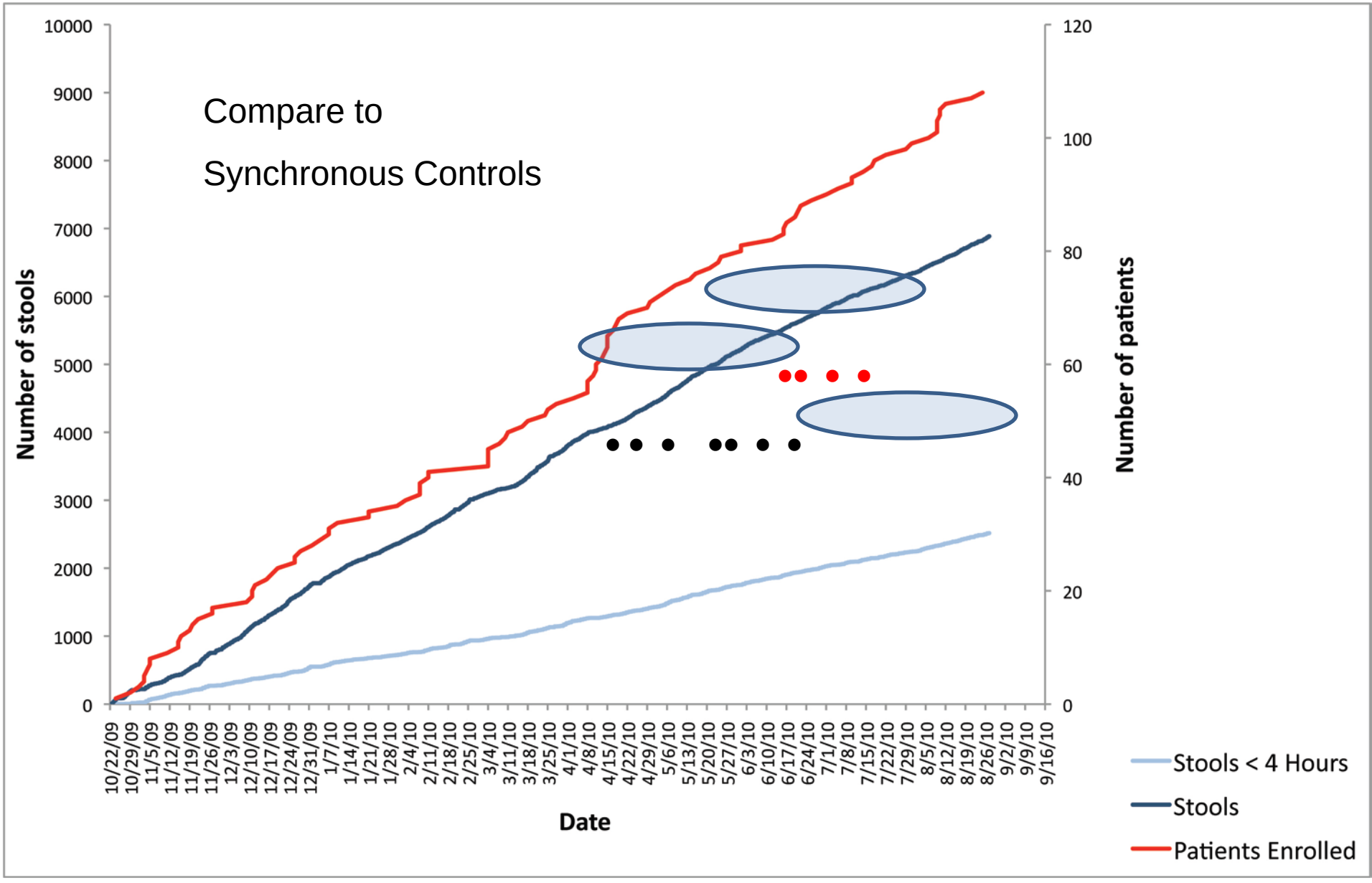
9/16/10

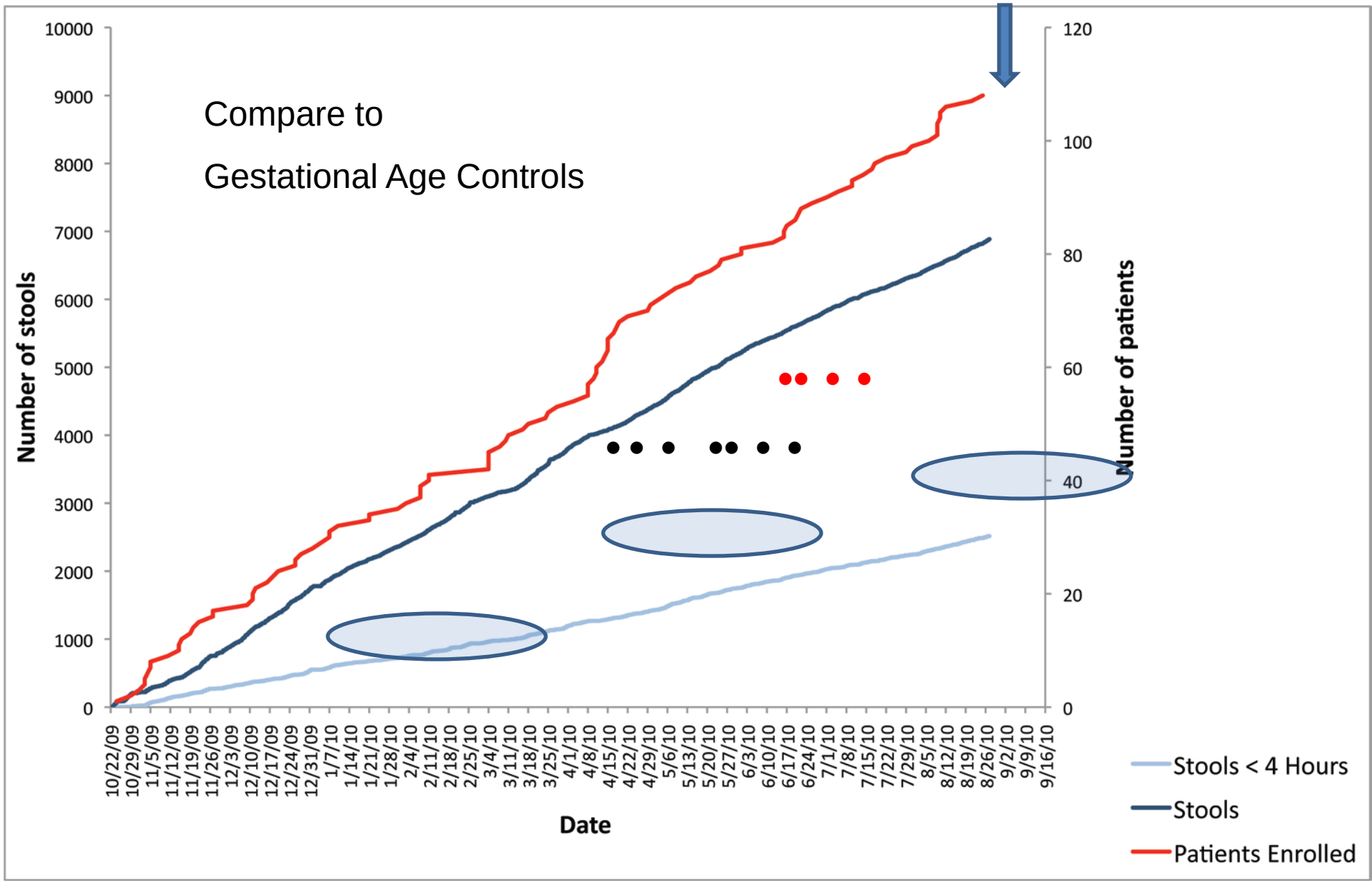

Compare to other cases
Number of stools
Number of patients
Date
Stools < 4 Hours
Stools
Patients Enrolled


Compare to
Calendar Controls
Number of stools
Number of patients
Date
Stools < 4 Hours
Stools
Patients Enrolled


Compare to earlier points
Number of stools
Number of patients
Date
Stools < 4 Hours
Stools
Patients Enrolled


Compare to
DOL Controls
Number of stools
Number of patients
Date
Stools < 4 Hours
Stools
Patients Enrolled


Compare to
Synchronous Controls
Number of stools
Number of patients
Date
Stools < 4 Hours
Stools
Patients Enrolled


Compare to
Gestational Age Controls
Number of stools
Number of patients
Date
Stools < 4 Hours
Stools
Patients Enrolled

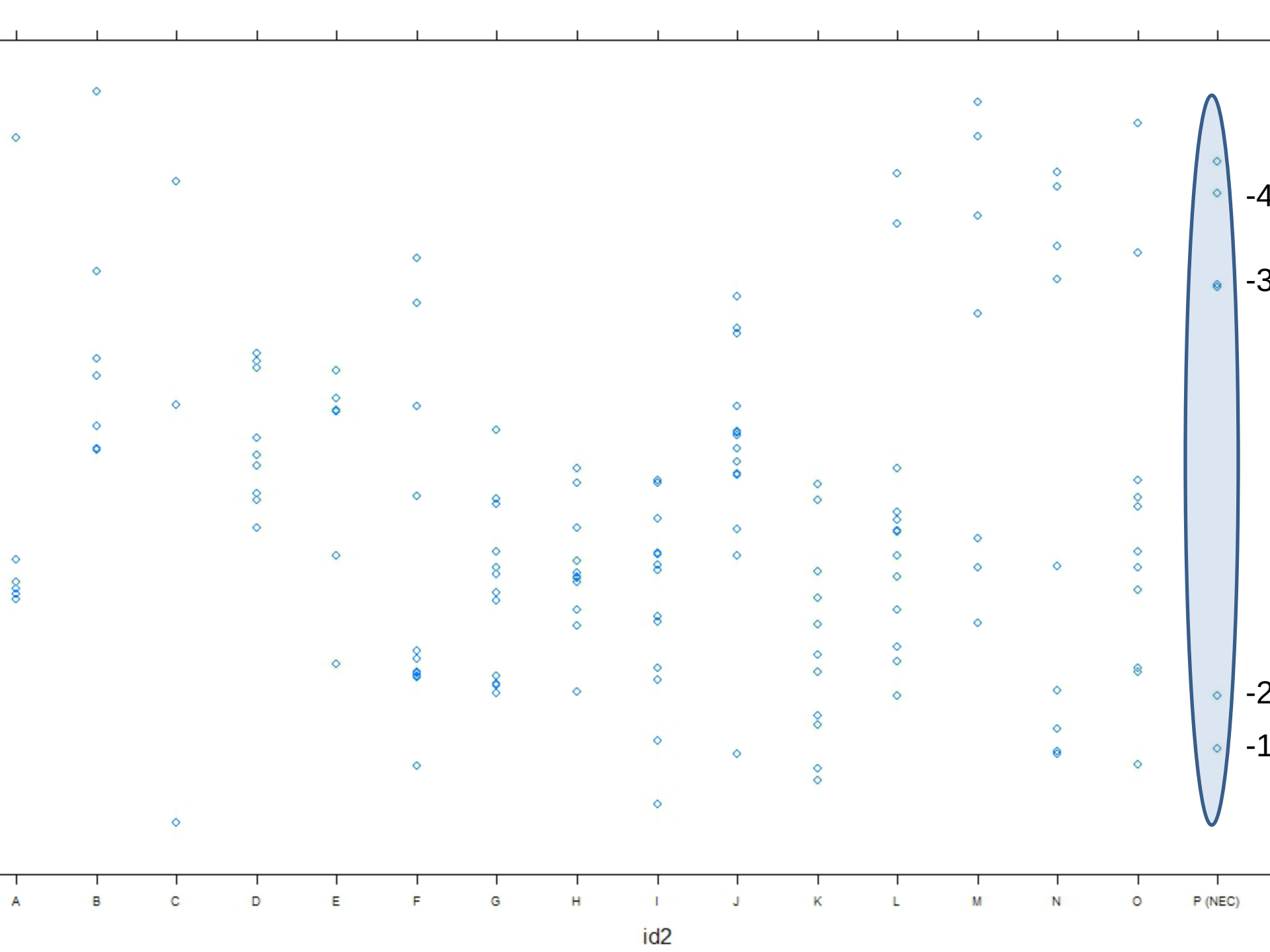
Diminished Progression towards Bacterial Diversification

October – November 2009

Outcome	Model	Day of Life		% of days on antibiotics	
		est	P	est	p
Shannon Index	Continuous predictor, mixed model	0.0096	0.0013	-0.40713	0.0363
Simpson Index	Continuous predictor, mixed model	0.0033	0.0082	-0.2423	0.0027
Gram negative per 1000 reads	GEE Model, Poisson, distribution, continuous predictor	ns	ns	ns	ns
Cocci per 1,000 reads	GEE Model, Poisson distribution	-0.011	0.034	ns	ns
Aerobic per 1000 reads	GEE Model, Poisson distribution	ns	ns	ns	ns

February – June 2010

Day of Life		% of days on antibiotics	
est.	p	est.	p
-.015 + .0049 (D15)	<.0001	ns	ns
- 0.0472+ 0.00174 (D12)	<.0001	ns	ns
-0.067	0.0001	ns	ns
-.041	0.0008	ns	ns
0.0367	0.003	ns	ns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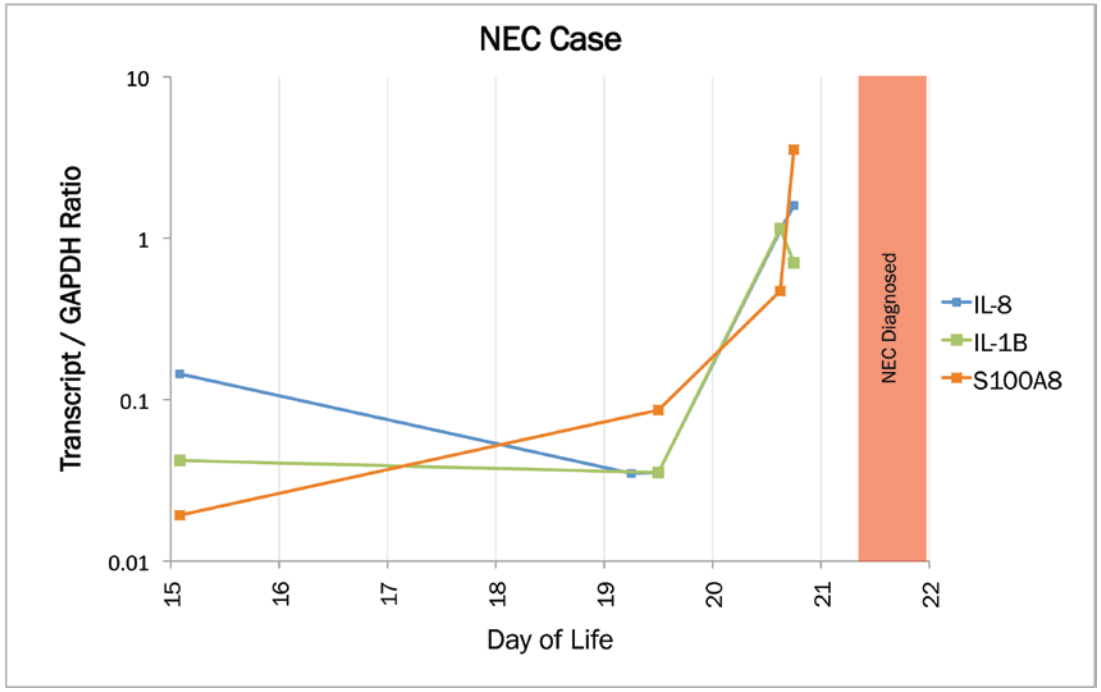
P (NEC)
id2


NEC Case
Transcript / GAPDH Ratio
10
1
0.1
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16
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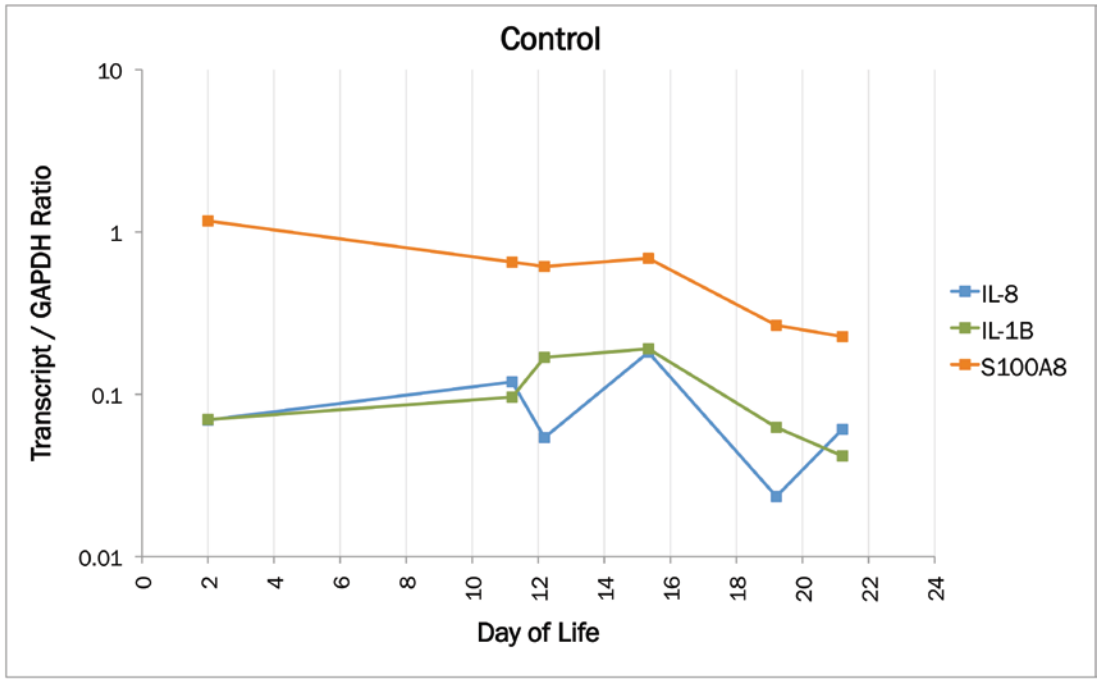
20
21
22
Day of Life
NEC Diagnosed
IL-8
IL-1B
S100A8


Control
Transcript / GAPDH Ratio
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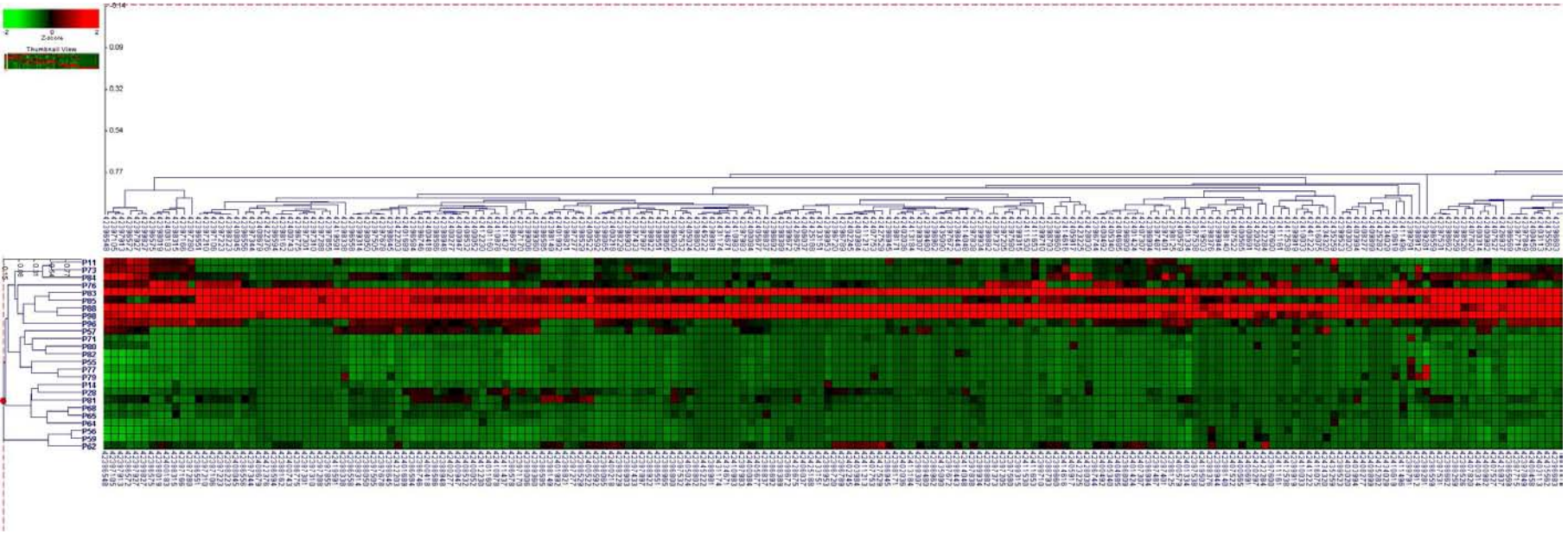
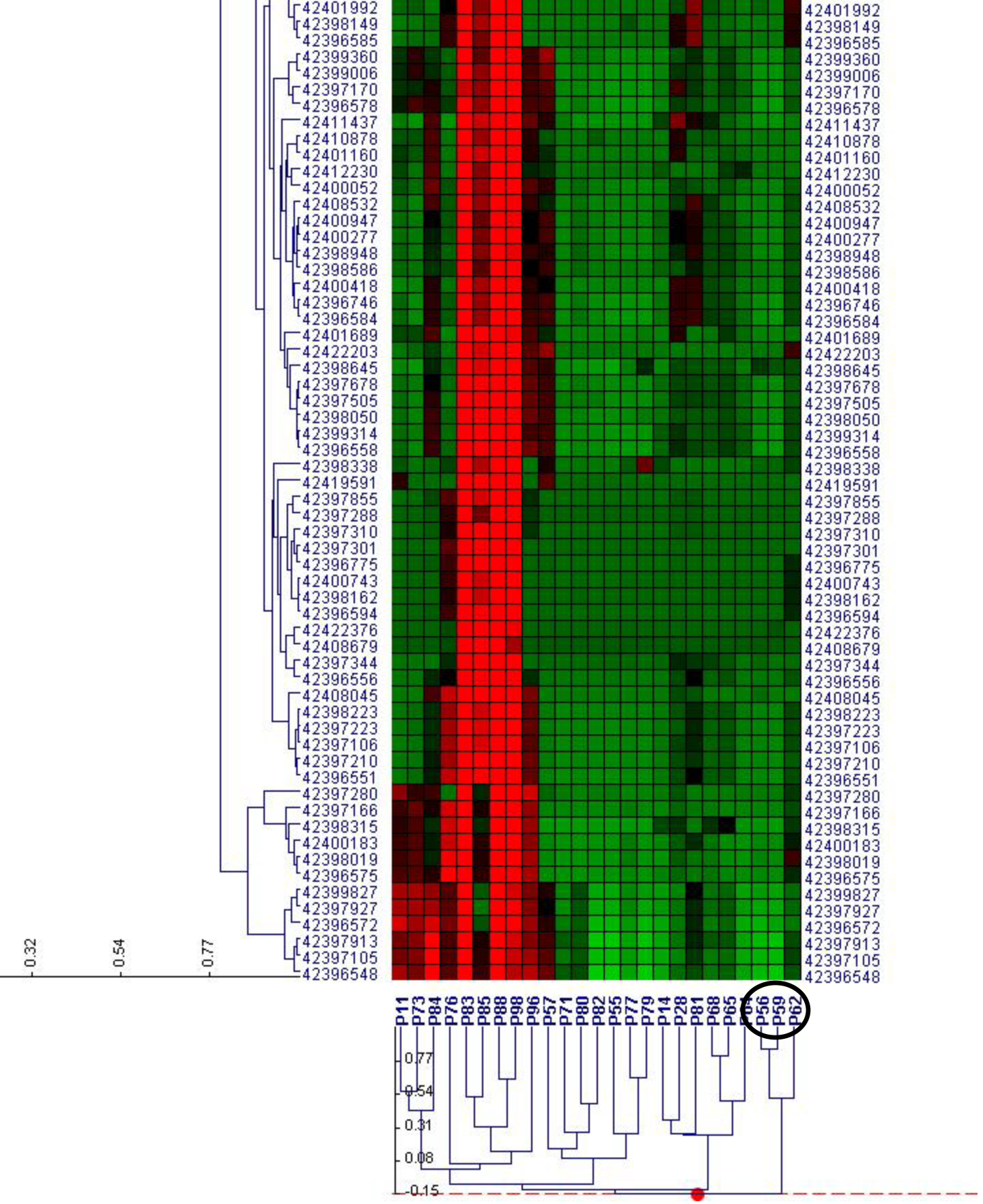
22
24
Day of Life
IL-8
IL-1B
S100A8

Is there a NEC protein signature?

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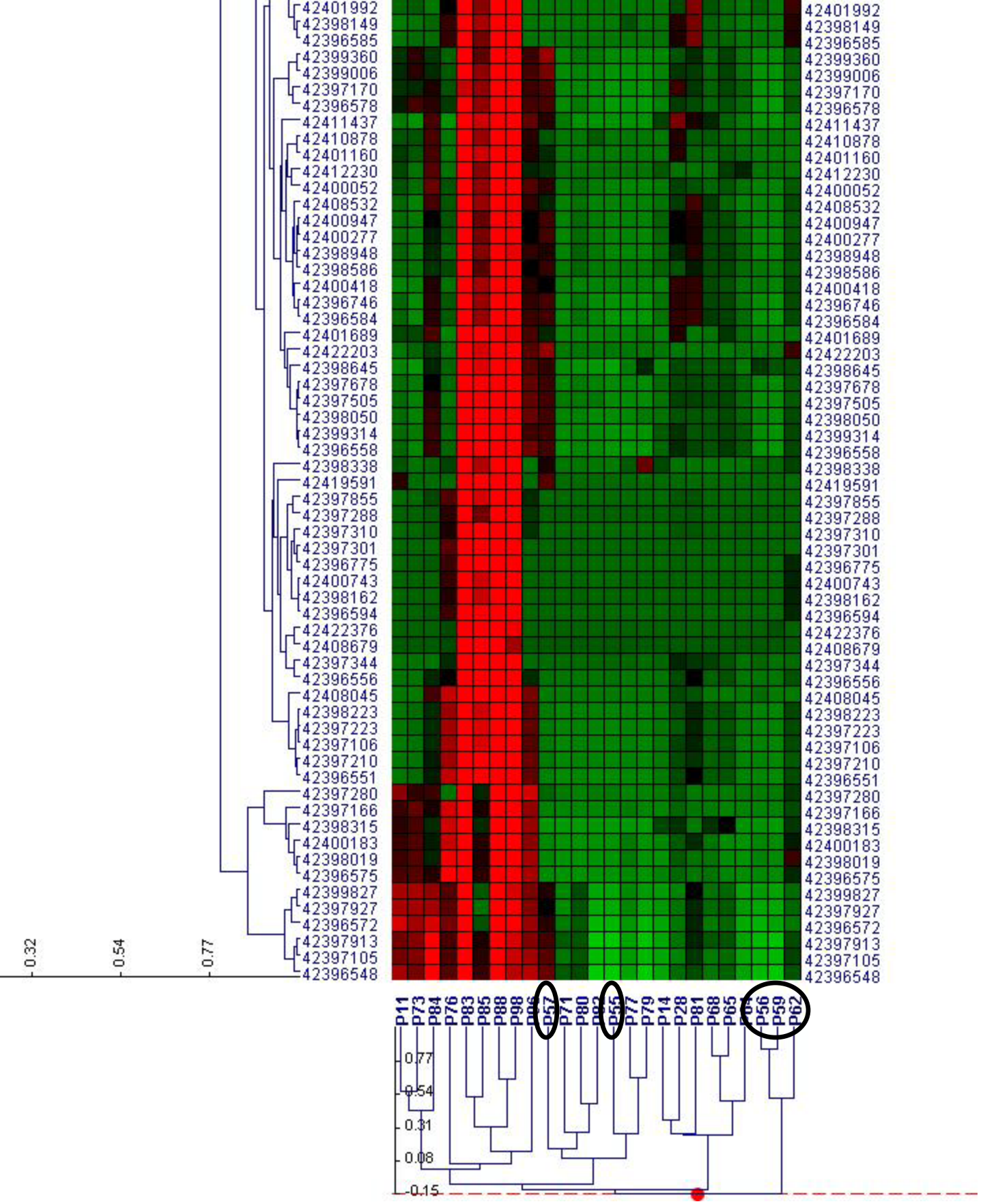
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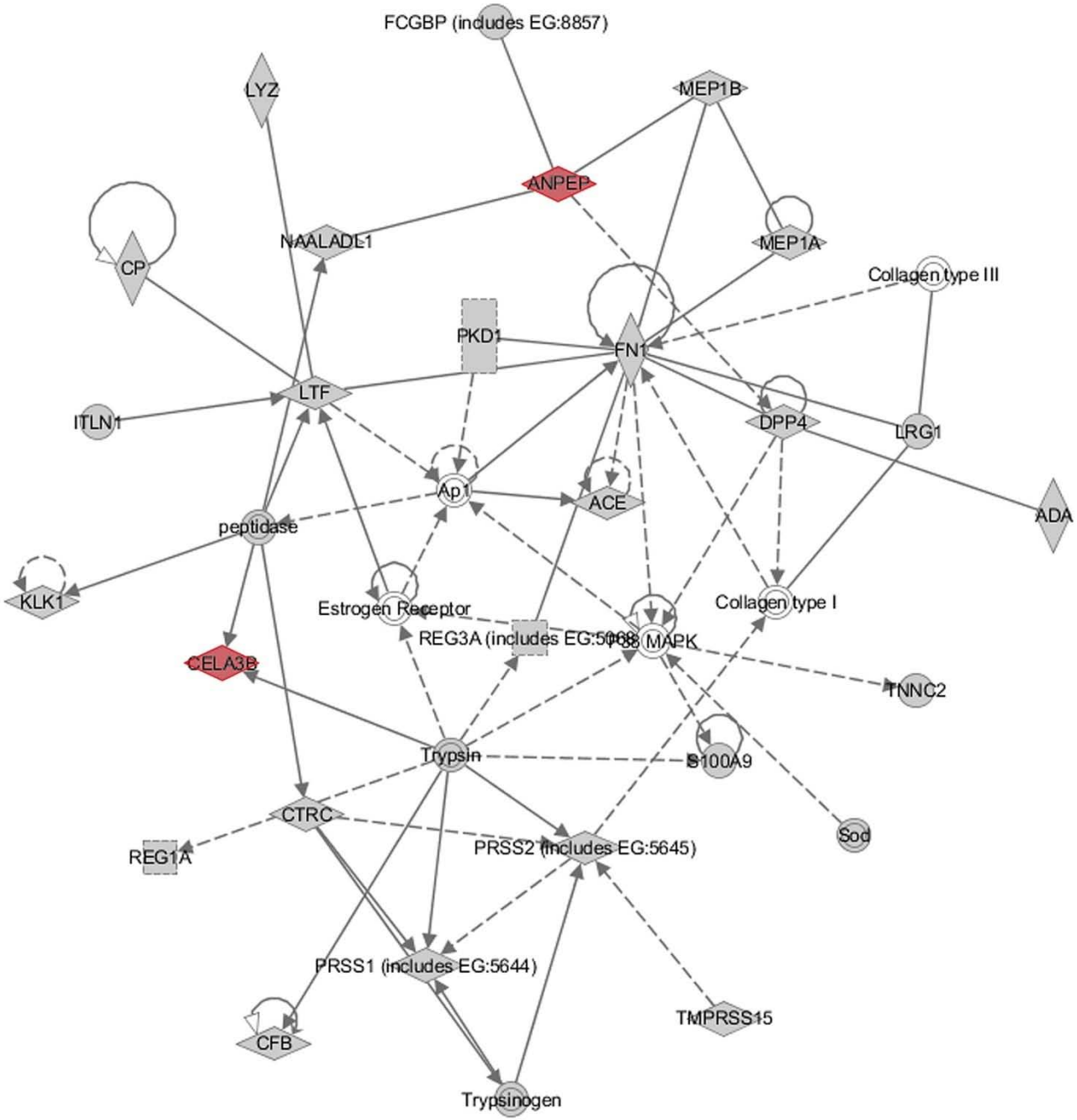
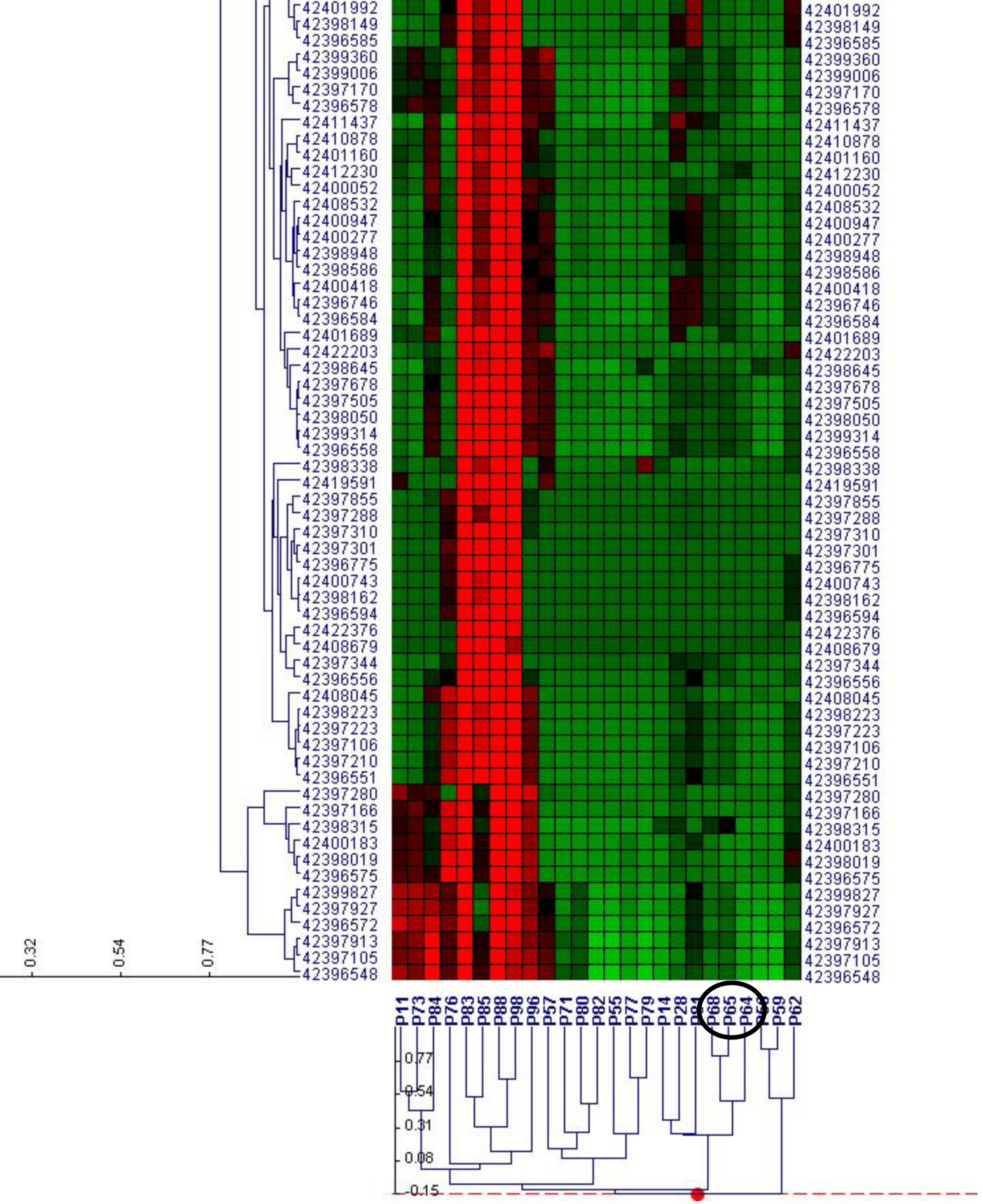

FCGBP (includes EG:8857)
LYZ
MEP1B
ANPEP
NAALADL1
MEP1A
CP
Collagen type III
PKD1
FN1
LTF
ITLN1
DPP4
LRG1
Ap1
ACE
ADA
peptidase
KLK1
Estrogen Receptor
Collagen type I
REG3A (includes EG:5068)
p38 MAPK
CELA3B
TNNC2
Trypsin
S100A9
CTRC
Sod
REG1A
PRSS2 (includes EG:5645)
PRSS1 (includes EG:5644)
TMPRSS15
CFB
Trypsinogen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VRE

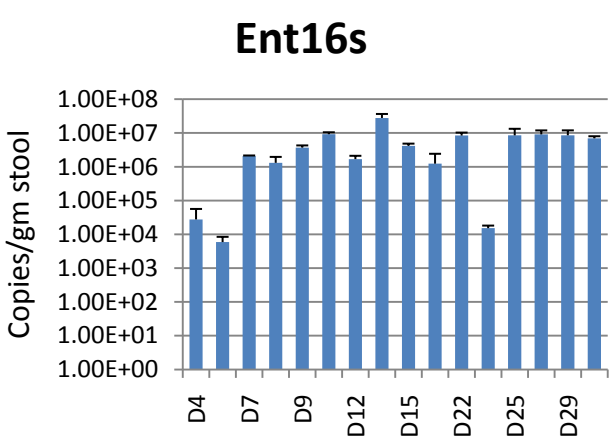
Boy born after 31 weeks gestation (BW = 1250 g)

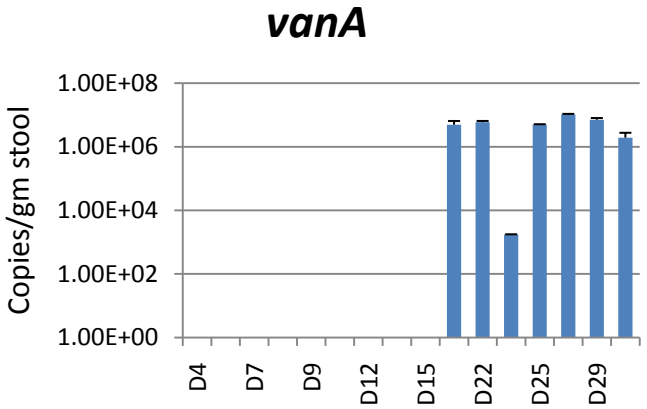
Received antibiotics on days 1-3 of life, gaining and growing

On DOL 21, stool culture screen:

vancomycin resistant enterococci (VRE)

Nine pre-positive and seven post-positive stools available

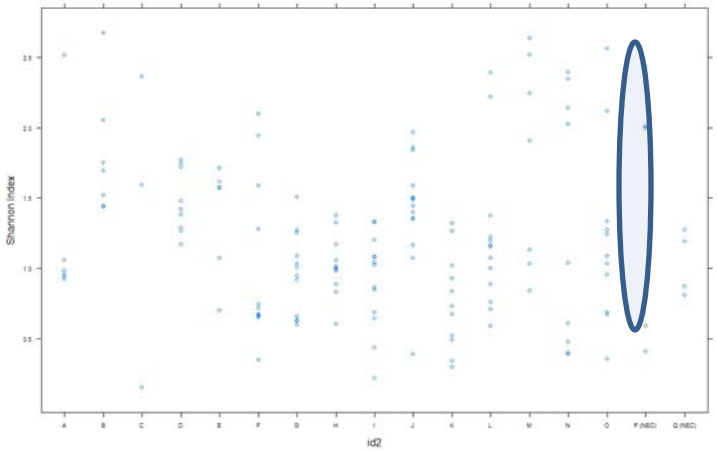




Specimen	Subjects (n)	Specimens (n)	Enterococci (log10)/g (median, range	*vanA* percent of enterococci
Pre VRE	1	9	6.1 (2.7-6.9)	0
Post VRE	1	7	6.3 (4.2-7.1)	71% (11-100%)
All-patient	1	16	6.1 (2.7-7.1)*	0
Controls	8	59	4.5 (2.2-7.1)*	0

* $p<0.0001$

1.



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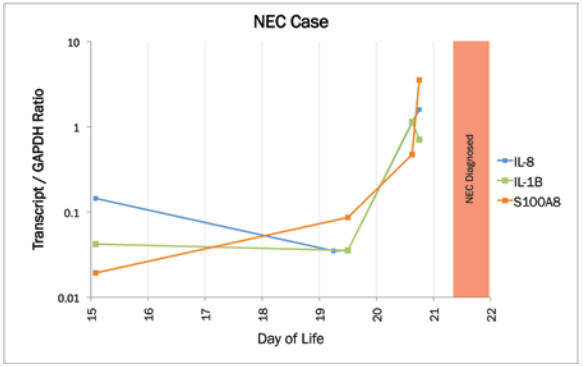
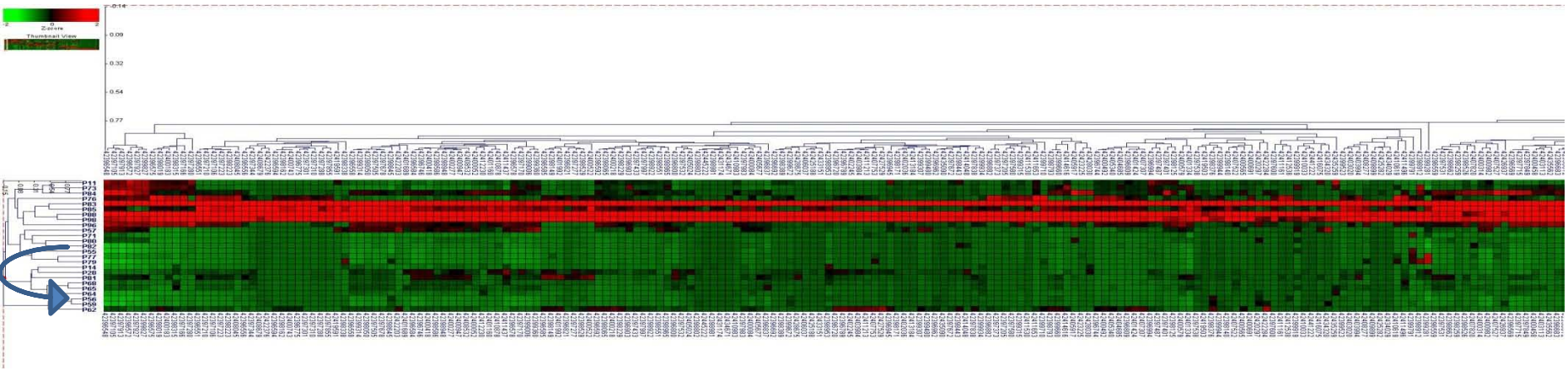
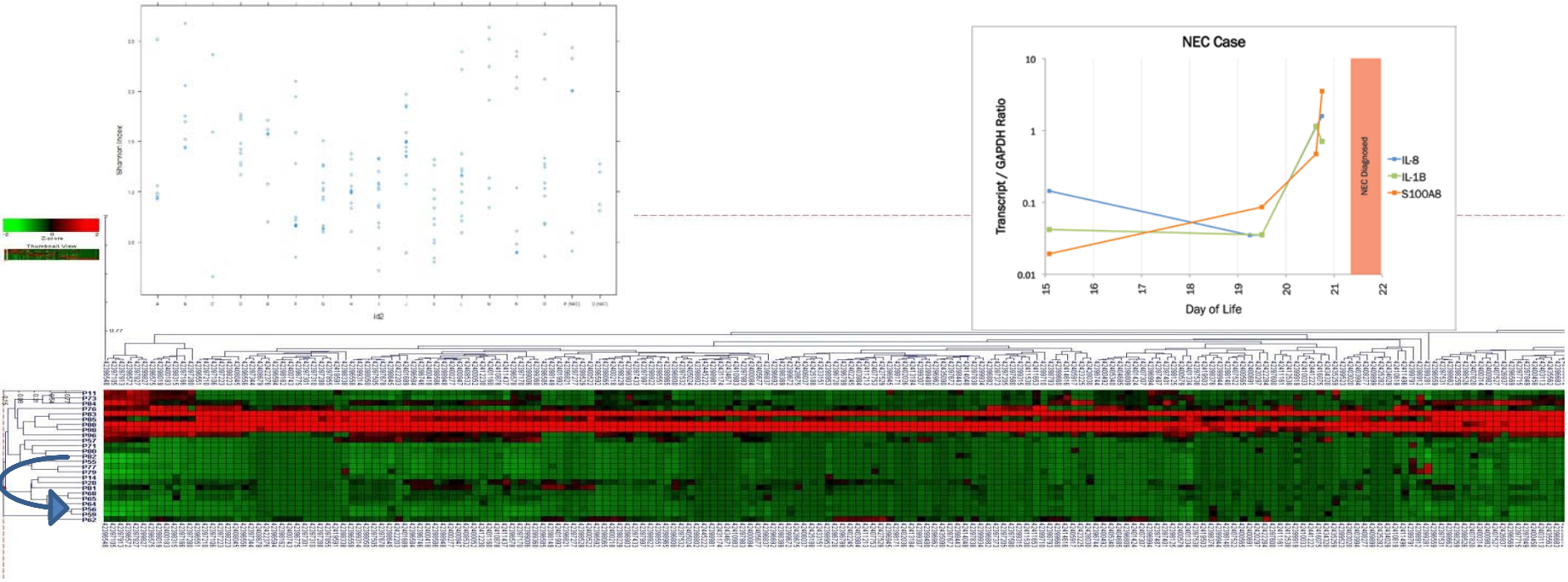
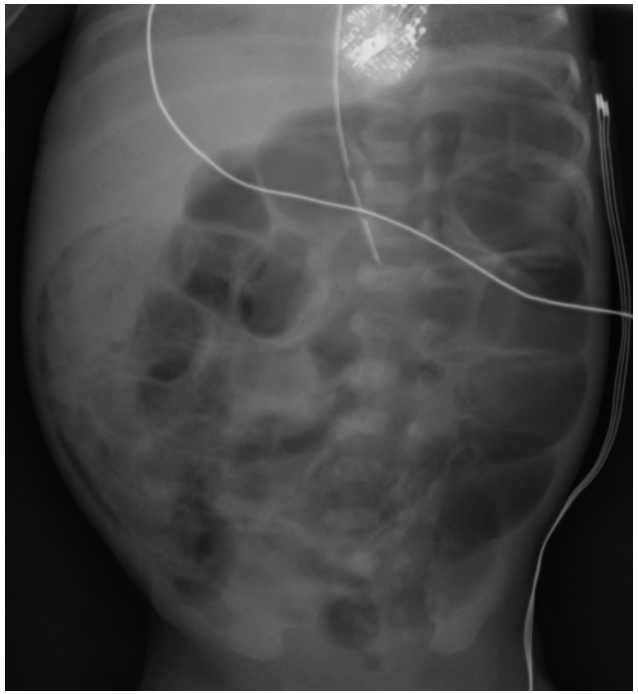

NEC Case
Transcript / GAPDH Ratio
10
1
0.1
0.01
15
16
17
18
19
20
21
22
Day of Life
NEC Diagnosed
IL-8
IL-1B
S100A8

3.



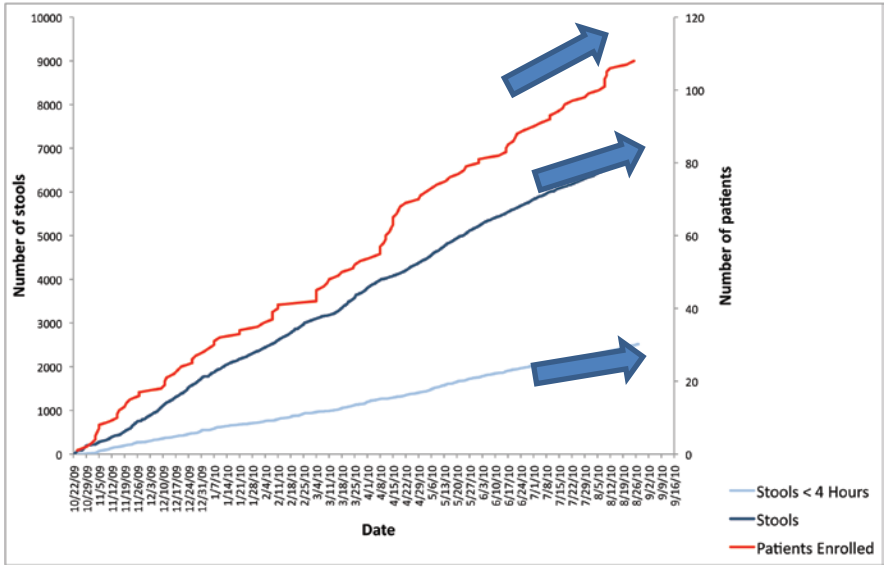

NEC Case
Transcript / GAPDH Ratio
10
1
0.1
0.01
15
16
17
18
19
20
21
22
Day of Life
NEC Diagnosed
IL-8
IL-1B
S100A8



8:15 AM, DOL22

Future Directions

- Extended accrual
- Shotgun sequencing
- Metatranscriptomics
- Viral discovery
- Proteomics



Thanks!

NICU Families and Nurses

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