Differentiating between human and non-human sources using source-associated markers

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There are a bunch of source-associated assays!

- There are over 10 human-associated assays
  - Sometimes primers different by a few bp, some are Taqman, some are SYBR…
- Which ones perform best?
- Which ones should we use for source tracking?

Source Identification Protocol Project (SIPP) method evaluation study

- Assess performance of 43 popular (or new!) assays
- Most assays were non-library, culture-independent methods (lots of PCR!)
- 27 labs participated
- Each assay tested run by 1-7 labs
- Labs used their own SOP in many cases
- 64 blinded samples containing feces from 1 or 2 sources in an artificial freshwater
  - No background microbial community
- Sensitivity and specificity of assays calculated

Participating Labs

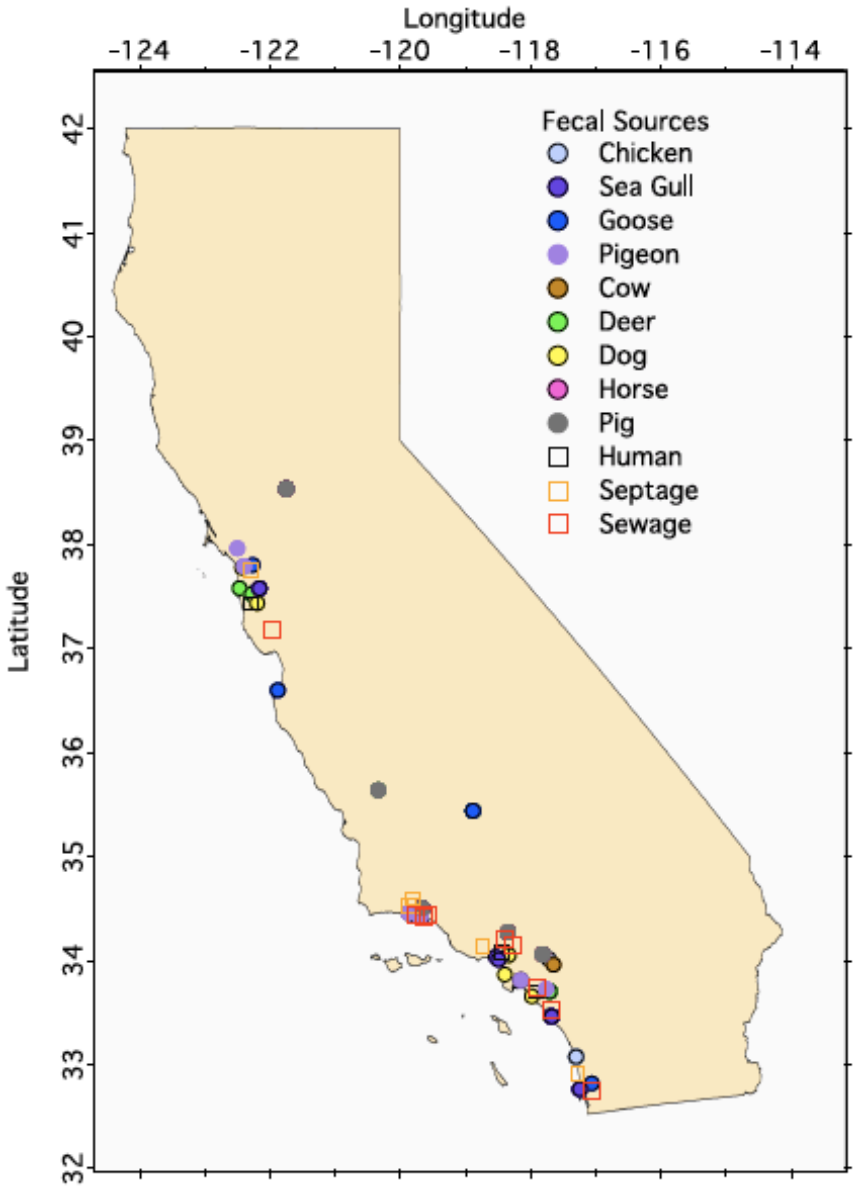
- Ali Boehm, Stanford
- Jenny Jay, UCLA
- John Griffith, SCCWRP
- Trish Holden, UCSB
- Orin Shanks, US EPA
- Jed Fuhrman, U Southern California
- Chris Sinigalliano, U Miami
- Rachel Noble, U North Carolina
- Mike Sadowsky, U Minnesota
- Jill Stewart, U North Carolina
- Gary Anderson, UC Berkeley
- Jiyoung Lee, Ohio State U
- Joan Rose, Mich State U
- Vijay Kannappan, Wayne State U Michigan
- Jody Harwood, U South Florida
- S. Wuertz, UC Davis
- J. Ebon and H. Taylor, U of Brighton, UK
- Wim Meijer, U of Dublin, Ireland
- Andreas Farnleitner, Vienna U of Technology, Austria
- D. Diston and M. Wicki, Federal Office of Health, Switzerland
- Michele Gourmelon, Ifremer Laboratoire de Microbiologie Plouzane France
- R. Rodriguez & J. Brandao , National Institute of Health, Portugal
- Kelly Goodwin, NOAA
- Jorge Santo Domingo, US EPA
- Murulee Byappanahalli, USGS
- Theng Fong, Tetra Tech
- T. Madi, Source Molecular
- Scott Reynolds, Environmental Canine Services
- D. Kashian, Ottawa County Health

Assays Tested

Cow/Ruminant/Pig	Gull / Other	Human	Human / commun.
Cow M2	Gull2SYBR	HF183SYBR	HF183 endpt
Cow M3	Gull2Taqman	HF183Taqman	Human EV
BacCow	LeeSeaGull	BacHum	Human AdV
BacR	Gull2Endpt	HumM2	Norovirus G1
Rum2Bac	DogBac	BsteriF1	Norovirus GII
Bovine AdV	BacCan	nifH	Human PyV
Bovine PyV	HoF597	BacH	
CF128	Omvito	gyrB	Phylochip
CF193		Btheta	Bac. TRFLP
Pig2Bac		GB124 phage	Gen TRFLP
PF163		HB73 phage	Canine Scent
mtPigDNA		MB55 phage	

Challenge Samples

Singletons	Doubletons
chicken	sewage/chicken
deer	sewage/gull
dog & dog 1:10	sewage/pig
goose	human/cow
gull & gull 1:10	human dog
horse	human/goose
pig & pig 1:10	human/gull
pigeon	septage/horse
cow & cow 1:10	
human & human 1:10	
septage & septage 1:10	
sewage & sewage 1:10	



Presence/Absence Analysis

BLOQ = positive

Sensitivity & Specificity Definitions

%sensitivity = 100*(true positive/(true positive + false negative))

%specificity = 100*(true negative/(true negative + false positive))

Assays with greater than 80% for both are highlighted for all labs if n≤3; one lab outlier allowed for n>3

Used singletons and doubletons

Sensitive and Specific Assays

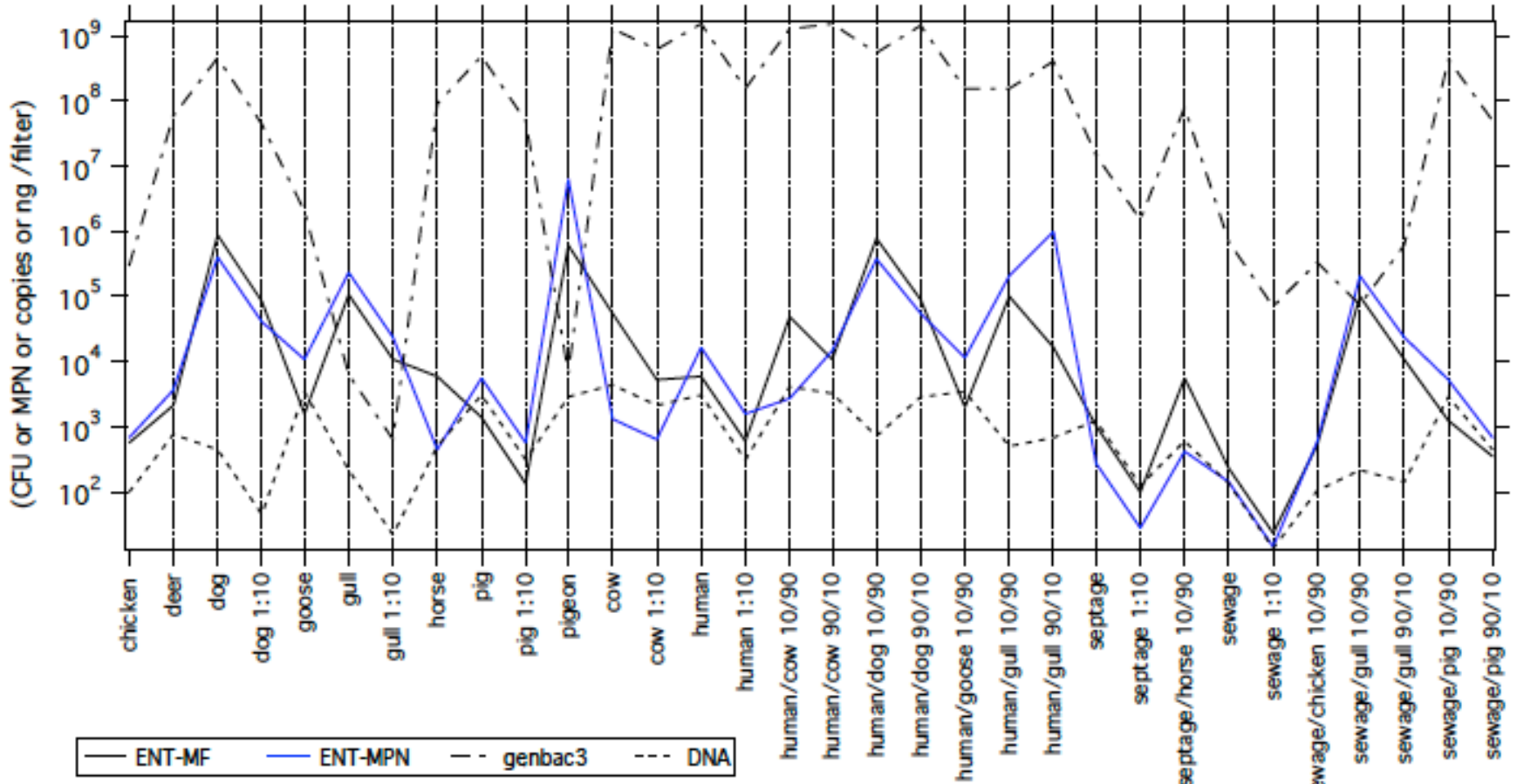
Human	HF183 endpoint, HF183SYBR
Cow	CowM2, CowM3**
Ruminant	Rum2Bac, CF193*
Dog	BacCan**
Gull	Gull2SYBR, LeeSeaGull**
Pig	PF163, mtPigDNA**, PhyloChip**, Bac TRFLP
Horse	HoF597, PhyloChip**, Bac TRFLP
Chicken	PhyloChip**
Deer	PhyloChip**, Bac TRFLP, Univ TRFLP
Goose	-

* run by 1 lab

**run by 1 lab that developed the assay

Quantitative Analysis

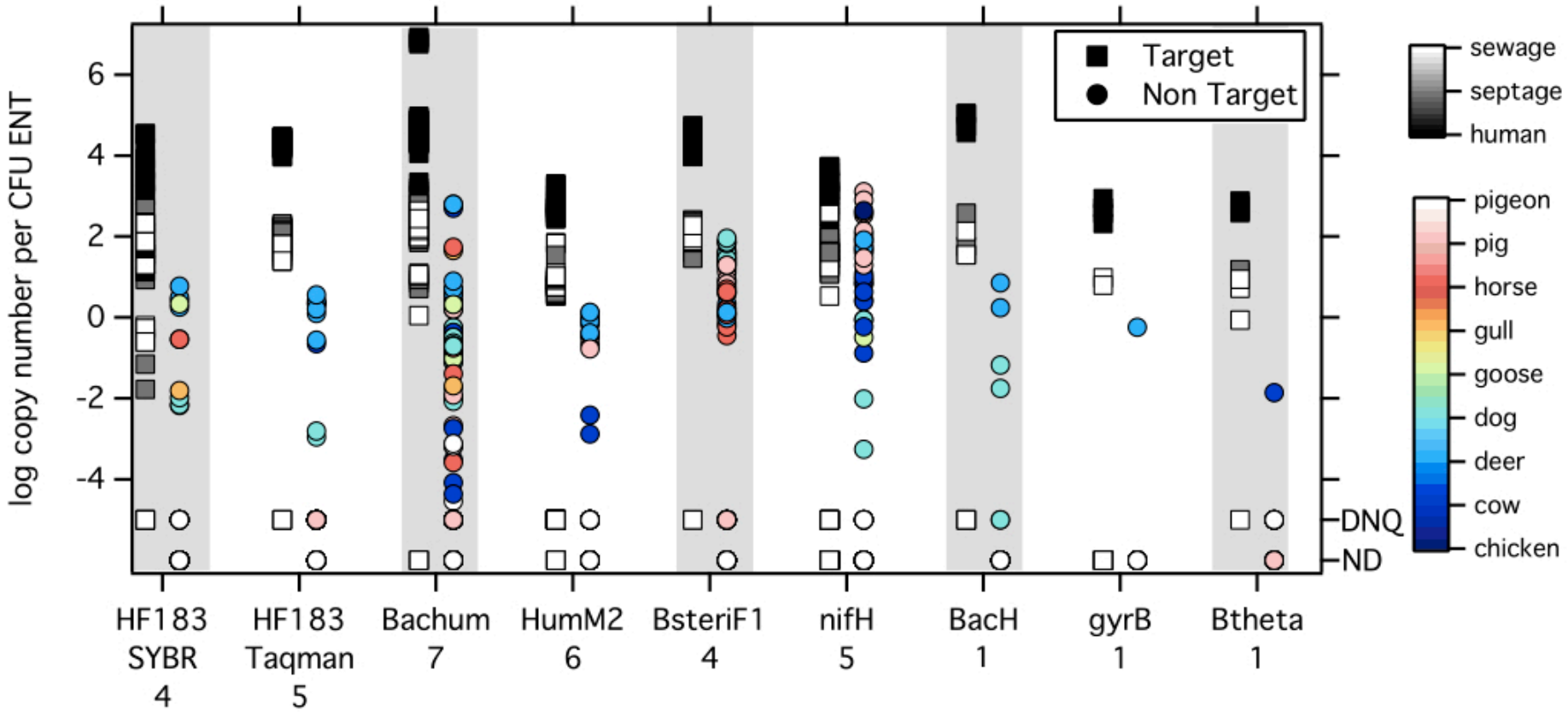
How do we measure the amount of fecal material in each sample?



Approach

- Plot copy number per CFU enterococci for each quantitative assay for singletons only
- Good performance
  - Sensitive assay has HIGH copy number per CFU in target host feces
    - Metric: Median in target host higher than 50 copies / CFU
  - Specific assay has LOW copy number per CFU in nontarget host feces
    - Metric: Non-target feces have copies/CFU all lower than those measured in target feces – for ROQ measures only!

Human Assays



Assay	Host	N	% non-target in target range	Median in target (copies/ CFU)	Specific	Sensitive	Spec & Sens
BacH	human	1	0	87	Y	Y	Y
BacHum	human	7	11	331	N	Y	N
BsteriF1	human	4	10	123	N	Y	N
Btheta	human	1	0	11	Y	N	N
gyrB	human	1	0	0.003	Y	N	N
HF183SYBR	human	4	7	52	N	Y	N
HF183Taqman	human	5	0	138	Y	Y	Y
HumM2	human	6	0	7	Y	N	N
nifH	human	5	19	33	N	N	N
BacCow	cow	5	7	13490	N	Y	N
CowM2	cow	5	0	15	Y	N	N
CowM3	cow	1	0	1	Y	N	N
BacR	ruminant	2	0	955	Y	Y	Y
Rum2Bac	ruminant	1	0	832	Y	Y	Y
Gull2SYBR	gull	4	10	0.4	N	N	N
Gull2Taqman	gull	6	10	7	N	N	N
LeeSeaGull	gull	1	0	55	Y	Y	Y
DogBact	dog	5	15	145	N	Y	N
BacCan	dog	1	6	5495	N	Y	N
Pig2Bac	pig	5	0	107152	Y	Y	Y

Assay	Host	N	% non-target in target range	Median in target (copies/ CFU)	Specific	Sensitive	Spec & Sens
BacH	human	1*	0	87	Y	Y	Y
BacHum	human	7	11	331	N	Y	N
BsteriF1	human	4	10	123	N	Y	N
Btheta	human	1	0	11	Y	N	N
gyrB	human	1	0	0.003	Y	N	N
HF183SYBR	human	4	7	52	N	Y	N
HF183Taqman	human	5	0	138	Y	Y	Y
HumM2	human	6	0	7	Y	N	N
nifH	human	5	19	33	N	N	N
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CowM3	cow	1	0	1	Y	N	N
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LeeSeaGull	gull	1*	0	55	Y	Y	Y
DogBact	dog	5	15	145	N	Y	N
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Pig2Bac	pig	5	0	107152	Y	Y	Y

Concluding Remarks

- There are a number of good assays for the State of California for detecting human and non-human feces
- Next challenge – interpretation of environmental measurements
- Need to develop methods for source allocation (Ratio approach)
  - X% of enterococci are from human
- Need to develop methods for assessing whether a waterbody is human or non-human
  - there is a 50% chance this waterbody is impacted by human feces