Microbial Source Identification Method Evaluation Study

Presentation to the SCCWRP Commission

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THE PROBLEM

- ***Enterococcus* is a non-specific marker of fecal contamination**
  - Beaches with known sources largely remediated
  - Still some beaches with chronic problems

- **> 50 source-specific genetic methods of fecal identification have been developed**

- **Need to know if they work**

THE SOLUTION

- **SCCWRP Microbial Source Identification Method Evaluation Study**

- **Create blind samples with various sources of fecal material**

- **Use multiple labs to assess repeatability**

SOURCES

- **Human**
  - Individuals, sewage, septage

- **Dog**

- **Gull**

- **Cattle**

- **Pig**

- **Horse**

- **Geese**

- **Deer**

- **Pigeon**

- **Chicken**

Legend

◇ Human Source
◆ Septage
◆ Sewage Source
▲ Dog
▲ Deer
✚ Goose
✚ Pigeon
◆ Gull
▬ Chicken
▮ Horse
● Pig
◆ Cow

Stanford

Monterey, CA

Bakersfield, CA

UCSB

UCLA

SCCWRP

Sacramento

Nevada

Sierra Nevada Mountains

California

Las Vegas

Los Angeles

San Diego

128 mi

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Google earth

35°19'32.69" N 119°03'44.73" W elev 1014 ft

Eye alt 519.48 mi

PARTICIPATING LABS

- Ali Boehm, Stanford
- Jenny Jay, UCLA
- John Griffith, SCCWRP
- Trish Holden, UCSB
- Stefan Wuertz, UC Davis
- Jed Fuhrman, U Southern California
- Chris Sinigaliano, U Miami
- Rachel Noble, U North Carolina
- Mike Sadowsky, U Minnesota
- Jill Stewart, U North Carolina
- Gary Andersen, UC Berkeley
- Jiyoung Lee, Ohio State U
- Joan Rose, Mich State U
- Vijay Kannappan, Wayne State U Michigan
- Scott Reynolds, Environmental Canine Services

- Huw Taylor, U of Brighton, UK
- David Diston, Switzerland
- Melanie Wicki, Federal Office of Health, Switzerland
- Wim Meijer, U of Dublin, Ireland
- Andreas Farnleitner, Vienna U of Technology, Austria
- Michele Gourmelon, Ifremer Laboratoire de Microbiologie Plouzane France
- Raquel Rodriguez, National Institute of Health, Portugal
- Orin Shanks, EPA
- Kelly Goodwin, NOAA
- Jorge Santo Domingo, EPA
- Murulee Byappanahalli, USGS
- Theng Fong, Tetra Tech
- Mauricio Larenas, Source Molecular

STUDY APPROACH

- **Challenge each method with 64 blind samples**
  - Singletons and doubletons of fecal sources
  - High and low concentrations

- **Most methods run by multiple labs**
  - Want to understand method repeatability

- **50 methods evaluated**
  - 28 participating laboratories

CLASSES OF METHODS

- **Presence/ Absence**
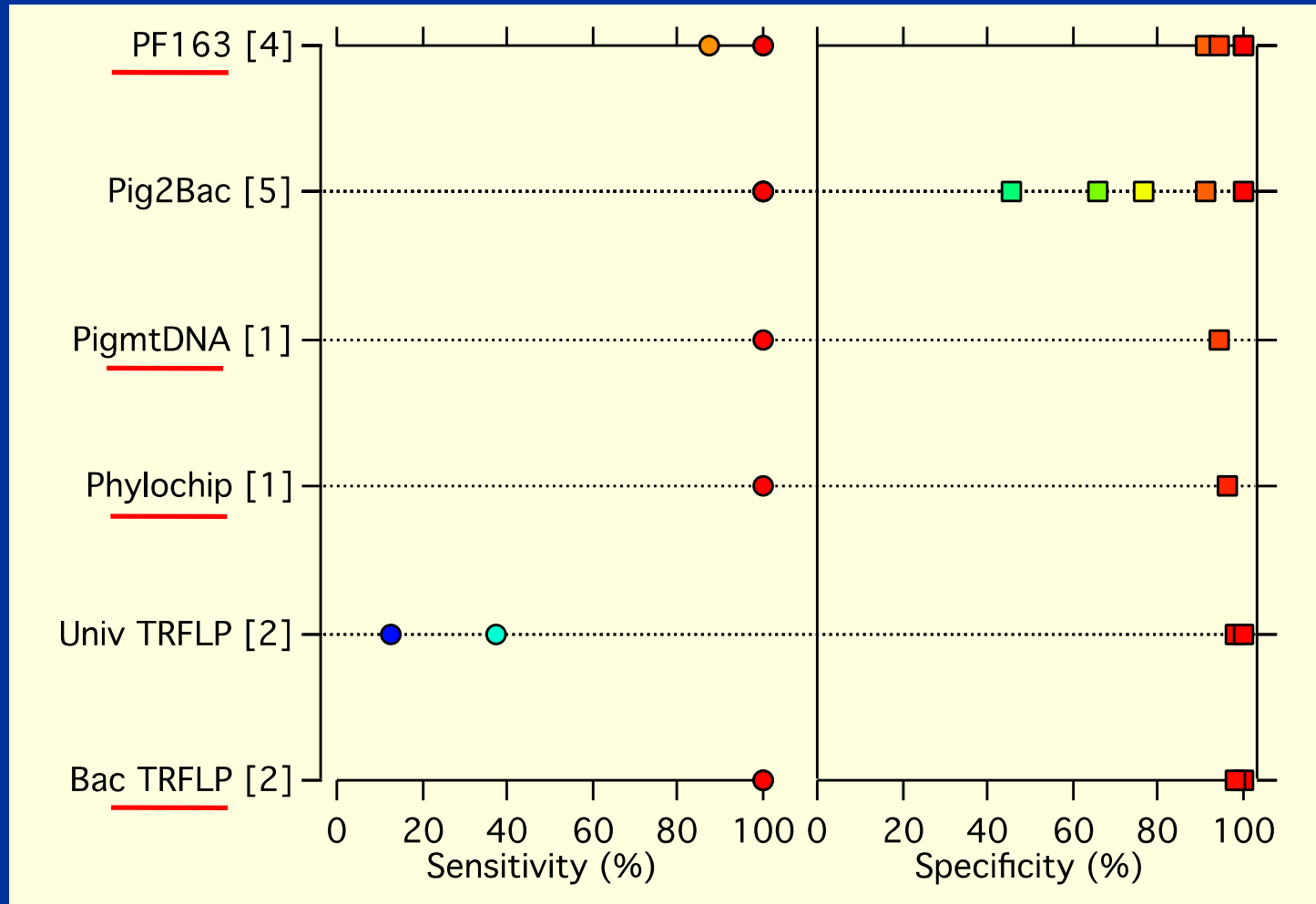 - Detect single source

- **Quantitative**
  - Detect single source
  - Provide information on concentration of source in sample

- **Community**
  - Detect multiple sources
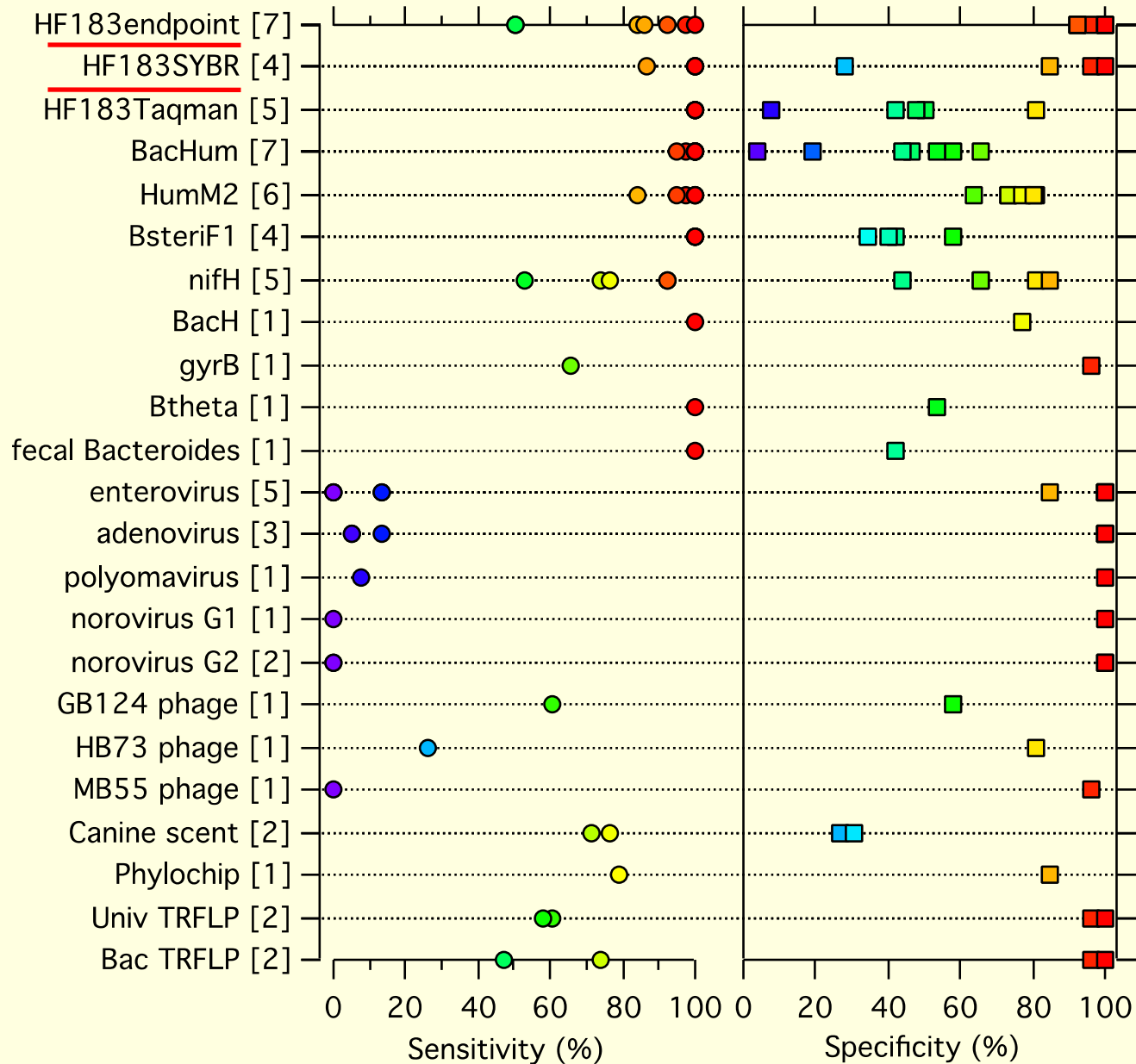 - May provide some information about relative concentration in sample

EVALUATION CRITERIA

- **Correctly identify presence/absence of a host source?**

- **Correctly identify the dominant source?**
  - Relative contribution from each source?

- **How repeatable are the assays?**

- **Do assay combinations provide more information than a single assay?**

HUMAN ASSAYS

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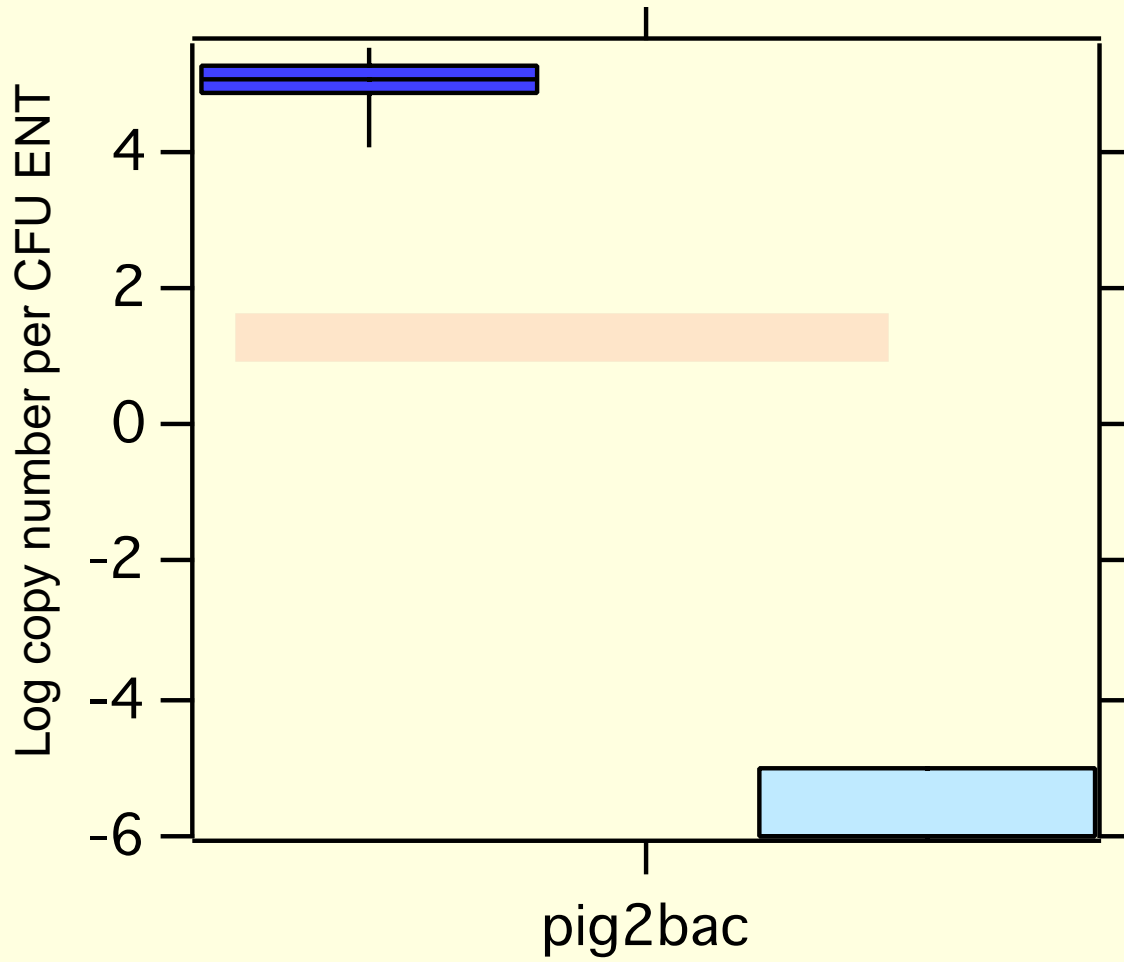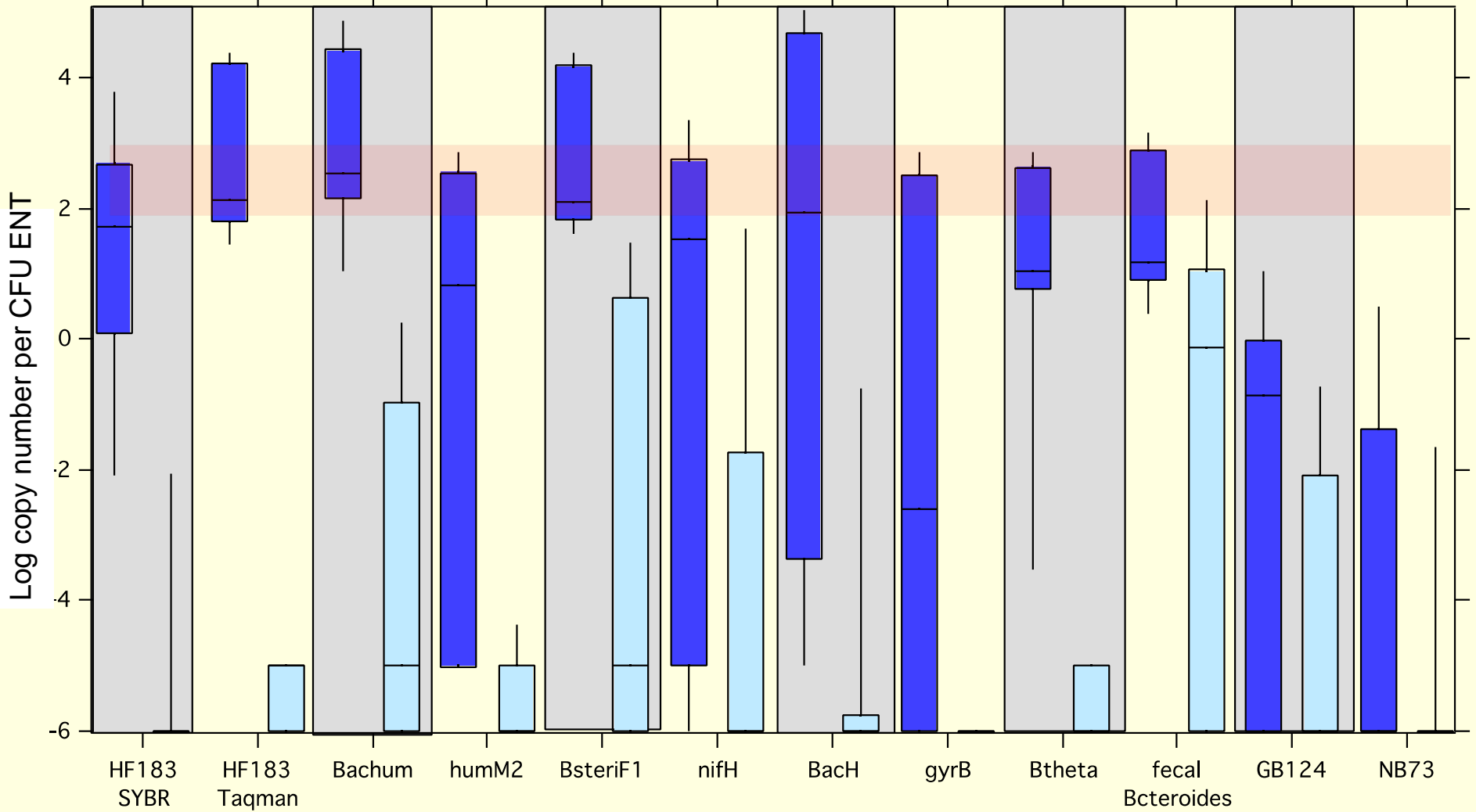

Box plot titled "HUMAN ASSAYS" showing Log copy number per CFU ENT on the y-axis (ranging from -6 to 4) for various human assays: HF183 SYBR, HF183 Taqman, Bachum, humM2, BsteriF1, nifH, BacH, gyrB, Btheta, fecal Bcteroides, GB124, NB73.

BOTTOM LINE

	Human	Cow	Dog	Gull	Pig	Horse
Binary	HF183endpt, HF183SYBR	CF193 CowM2 CowM3 Rum2bac	BacCan	Gull2EndPt Gull2SYBR LeeSeaGull	PF163 mtPigDNA Phylochip Bac TRFLP	HoF597 Phylochip Bac TRFLP
Quant.	HF183Taqman BacH	BacR Rum2bac BacCow*	BacCan	LeeSeaGull	pig2bac	n.a.

In addition, all community methods were excellent for deer, and phylochip was excellent for chicken.

NEXT STEPS

- **We now have a suite of reliable source-specific markers**
  - Plan to use in field studies this year

- **Develop a *Microbial Source Identification Study Manual* for the State**

- **Special Issue of *Water Research* devoted to this study**