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Approach for Harmonization and Quality Control of Environmental Surveillance Methods for Typhoid

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Project Overview

Project aim: Harmonize the diverse aspects of typhoid environmental surveillance activities

Objective 1: Document and review current methodologies and practices for environmental surveillance of bacteria with an emphasis on *Salmonella* spp.

Objective 2: Assess current methods and develop tools (SOPs) for performance testing

Objective 3: Finalize guidelines document for implementation of performance standard-based QA/QC framework

Typhoid Project: External Advisory Committee (EAC)

- Ongoing, independent review and approval by respected global ES experts are necessary
- The EAC will provide critical review and comments on the proposed project inputs and outputs, as these items evolve

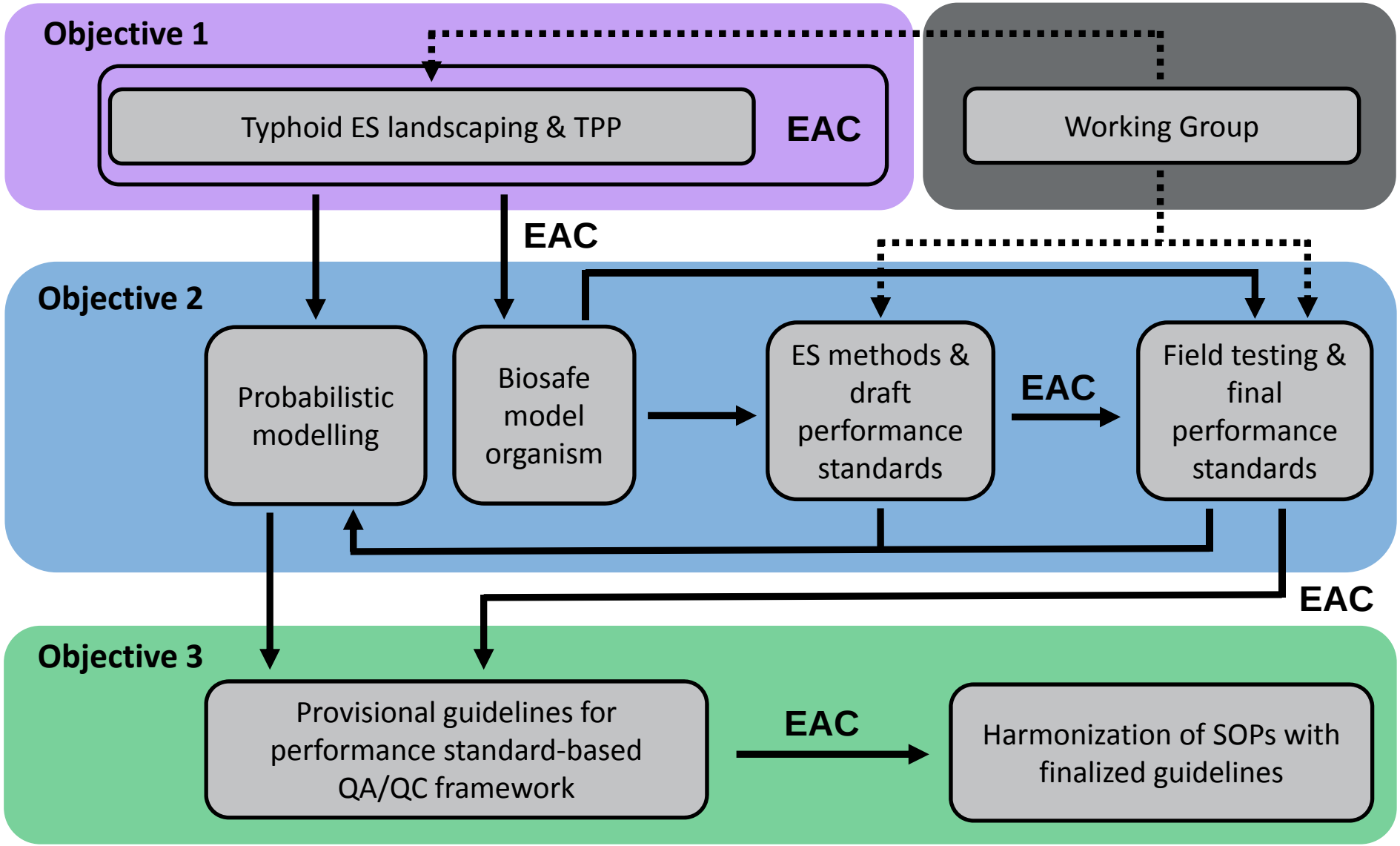
Member	Organization
Maureen Taylor	University of Pretoria
Ananda Bandyopadhyay	BMGF
Adwoa Bentsi-Enchill	WHO
Nicola Elviss	Public Health England
Nicholas Grassly	Imperial College London
Vince Hill	CDC
Andy Pollard	Oxford University
Joan Rose	Michigan State University
Fatima Serhan	WHO

Typhoid Project: Working Group (WG)

- The WG will provide direct expertise in *S.* Typhi ES, to inform development of the SOPs and performance standards

Member	Organization	Typhoid project site(s)
Eric Alm	MIT	Kathmandu, Nepal
Jason Andrews	Stanford University	Mumbai, India / SEAP sites in Pakistan, Nepal & Bangladesh
Nick Feasey	LSTM	Malawi
Gagandeep Kang	CMC, Vellore	Vellore, India
Christine Moe	Emory University / SaniPath	Kolkata, India
Jennifer Murphy	CDC	Nairobi, Kenya
Muhammad Salman	NIH Pakistan	Pakistan
Mami Taniuchi	University of Virginia	Bangladesh
Scott Meschke, Jeff Shirai, Christa Fagnant-Sperati, Nicky Beck & Nicolette Zhou	University of Washington	NA
David Boyle, Eileen Murphy, Lorraine Lillis & Melissa Atalig	PATH	NA
Supriya Kumar	BMGF	NA

Typhoid Project Structure



Typhoid Project: Objective 1

Objective 1.1: Develop working relationships with current ES field teams

- Visit sites and observe methods
  - UW personnel visited Dhaka, Bangladesh in December 2018
  - Upcoming travels to other ES sites planned for Q2 2019
- Implement a communications plan for frequent discussions and materials & data sharing throughout the project life cycle
  - Monthly WG conference calls scheduled (Next Calls April 16th)
  - In-person meetings planned and/or have occurred
    - EAC Meeting in London (2/21/2019)
    - Side Meeting at Take on Typhoid in Hanoi (3/26/2019)
    - Working Group Convening Planned for September, 2019

NZ44

Typhoid Project: Objective 1

Objective 1.2: Define objective criteria for method evaluation

- Evaluate methods for their potential in ES of *S.* Typhi
  - Completing a comprehensive literature review of published methods
    - Draft circulated for review and comment
  - Some key findings:
    - Several approaches have been described
      - Filtration methods, Moore swabs, Culture and Molecular
    - No consensus on methods
    - Published studies do support successful detection of *S.* Typhi from environmental samples
    - A number of open questions remain
      - Method sensitivity and specificity
      - Reproducibility in different locations
      - Appropriateness of methods for programmatic use cases

Slide 7

NZ44 Placeholder for Graciela's work

Nicolette Zhou, 3/20/2019

Typhoid Project: Objective 1

Objective 1.2: Define objective criteria for method evaluation

- Evaluate methods for their potential in ES of *S.* Typhi
  - Developing surveys in conjunction with EAC and WG members; distributing surveys to understand the methods currently used and other considerations that may impact a sampling plan (*e.g.*, lab capacity, staffing, field sites, etc.)
  - Three surveys developed
  - Survey 1 Initial Survey on environmental surveillance methods (results from 7 groups with 15 methods
  - Survey 2 Collection, concentration, and assay methods for environmental surveillance of *Salmonella* Typhi
  - Survey 3 Site selection

Typhoid Project: Objective 1

Objective 1.2: Define objective criteria for method evaluation

Sample types used	Wastewater (10); Drinking water (5); Surface water (3); Other (1)
Collection method	Grab (12); Composite - Moore Swab (3); Composite - pump (1)
Collection volume	10-100 L (3); 1-10 L (6); 0.5-1 L (2); 0.1-0.5 L (1); N/A - Moore Swab (2); TBD (1)
Primary concentration	Moore Swab (3); Differential centrifuge (1); Membrane filtration (4); BMFS (3); Ultrafiltration - tangential flow (2); Ultrafiltration - dead-end (2); PEG precipitation (1)
Secondary concentration	Skimmed milk flocculation (3); PEG precipitation (2); Ultrafiltration (1); None (8); Not specified (1)
Enrichment	Selenite F broth (3); Pre-enrichment and Selenite cysteine broth (2); Yes, not specified (1); Yes, TBD (1); None (8)
Purification	None (15)
Target organism(s)	*S. typhi* (15); *S. paratyphi* (12); *S. spp*. (6); *S. Typhimurium* (4); *S. Enteritidis* (4); Poliovirus (1); Enteric pathogens (1)
Control organism(s)	External (5); Internal (1); Yes, not specified (5); None (4)
Extraction kit	Qiagen Bacterial DNA Extraction (1); Qiagen DNeasy PowerWater Kit (5); QIAamp DNA Mini Kit (4); QIAamp FastStool Kit (1); Qiagen PowerViral Environmental Isolation Kit (1); Not specified (4)
Molecular method	qPCR (8); Multiplex qPCR (1); TAC qPCR (4); Not specified (2)
Target gene	Baker assay (9); S. Nair quadruplex (1); Not specified (5)
Sequencing used	Yes (3); No (12)
Culture method	Salmonella shigella agar (1); Bismuth sulfate agar (2); XLD agar (5); Yes, TBD (1); None (8)
Detection type	Presence/absence (9); Quantitative (11)
Frequency of sampling	Quarterly (1); Every 2 months (2); Monthly (3); Weekly (4); Every 3 days (1); Once (1); Other (1); Not specified (2)

Typhoid Project: Objective 2

Objective 2.1: Probabilistic modeling

- Initial indicator to understand what performance ranges are necessary for ES tools to survey for *S.* Typhi

Objective 2.2: Create a biosafe microbial model organism

- Organism will serve as the standard reagent to assess performance, qualify, and harmonize ES methods
- Likely candidate: *E. coli* K-12 using CRISPR/Cas9
- Compare to an *S.* Typhi reference strain to ensure direct correlation with molecular detection
- Modified Baker assay target sequence and developed probe targeting this region for use with Baker assay primers

Typhoid Project: Objective 2

Objective 2.3: Assess capture and recovery of field ES methods using biosafe organism in the laboratory

- Compare field ES methods with the biosafe organism and *S.* Typhi
  - Preliminary method evaluation experiments have begun with unmodified *E. coli* and *S.* Typhi, and will include molecular and culture methods
- Develop draft SOPs after performance of tools are established from testing in an ideal *(i.e.,* laboratory*)* setting
- Determine draft performance standards

Objective 2.4: Evaluate method performance using SOPs and biosafe organism at field sites

- Assess the impacts of matrix effects from field sites on ability of methods to meet draft performance standards
- Finalize draft performance standards based on outcomes, data, and user input and feedback

OUTCOMES*: Development of provisional SOPs to use as standards (August 2019); Final SOPs based on final evaluation (TBD)*

Typhoid Project: Objective 3

Objective 3.1: Synthesize and share results

- Pooled data from Objectives 1 & 2 will create specific, qualified metrics on the performance of the current tools at multiple surveillance sites
- Drafts will be shared for expert review

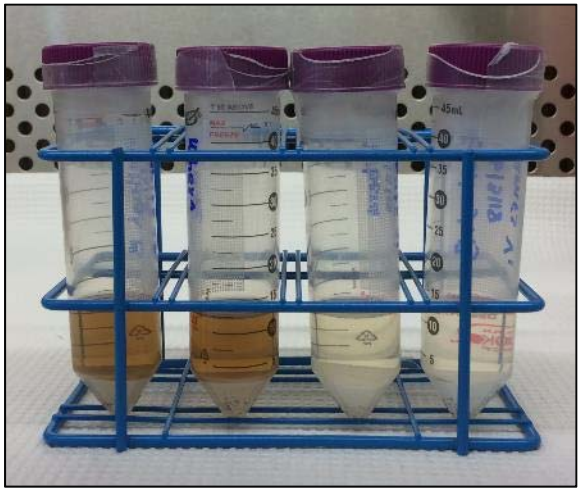
Objective 3.2: Prepare guidelines document

- For implementation of the performance standard-based QA/QC framework for typhoid ES
- Will follow examples from national environmental agencies and standardization organizations

***OUTCOMES**: Development of guidelines for evaluation of provisional SOPs (TBD); Harmonization of SOPs with finalized guidelines (TBD)*

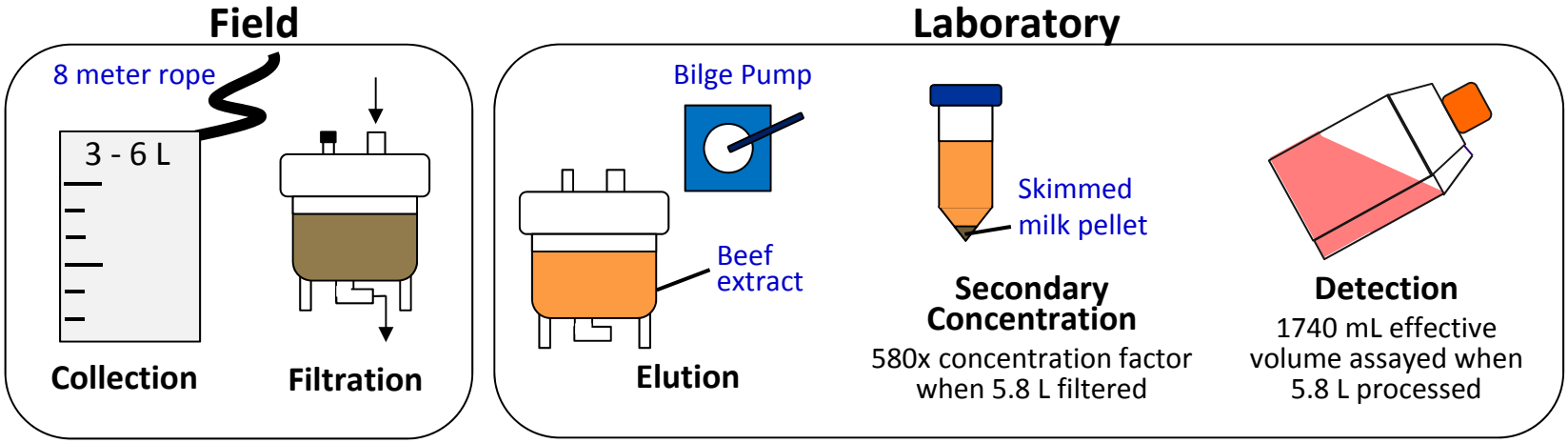
Bag-Mediated Filtration System (BMFS) Overview

Environmental surveillance sampling kit developed with guidance from the WHO, CDC, and Gates Foundation to create an alternative poliovirus environmental surveillance method



Photos: Christine Fagnant-Sperati, Bethel Demeke

BMFS v2 Overview



- In-field gravity filtration using ViroCap cartridge filters
- Cartridge filters transported on cold chain to laboratory for elution and secondary concentration
- Sample processing concentrates 3-6 L to 10 mL

Advantages

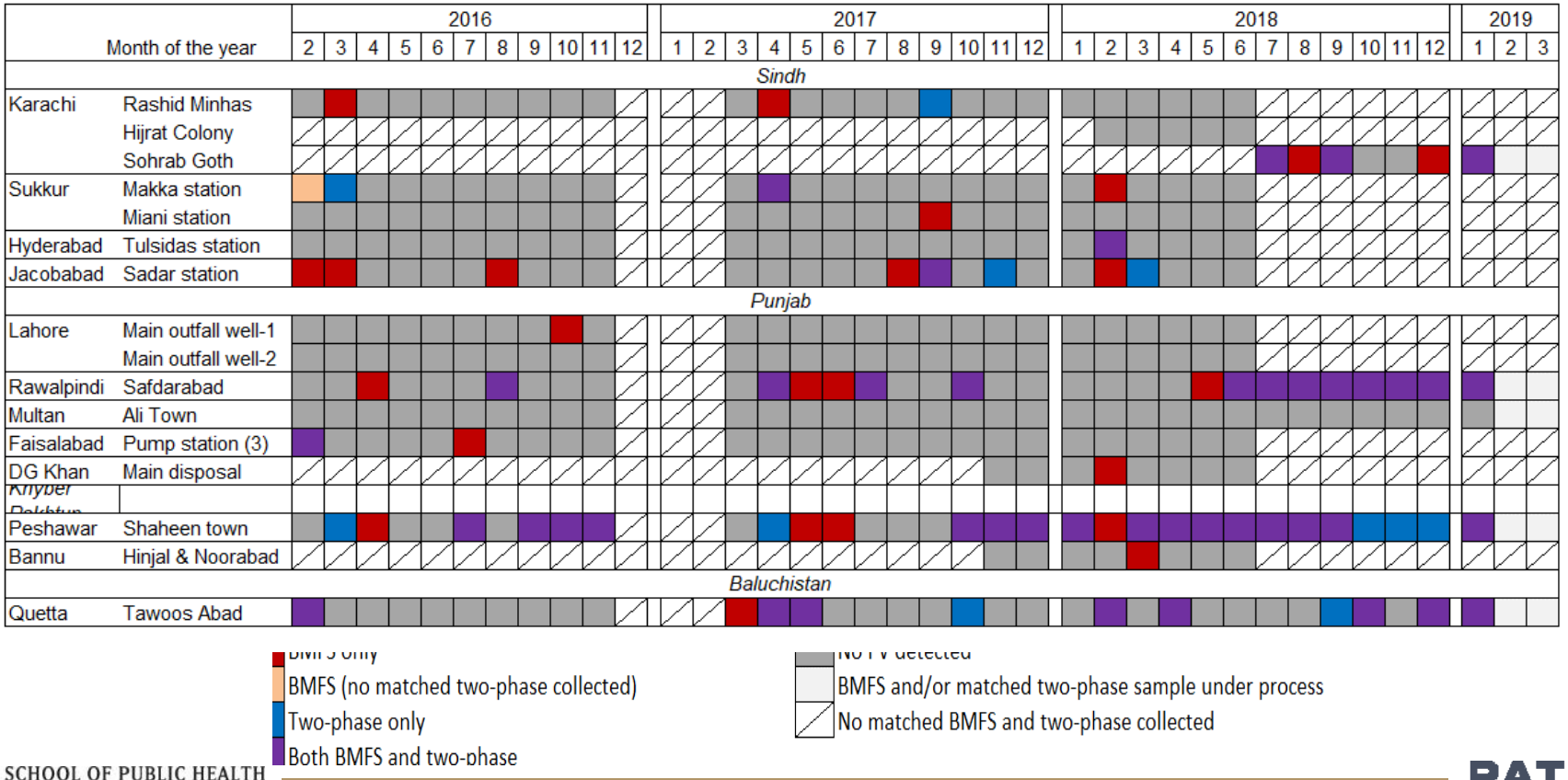
- High sensitivity
- Initial processing is electricity-free
- Cartridge filters ship easily

Used in Kenya, Pakistan, India, and Bangladesh

BMFS v1 use in Pakistan: Feb 2016-present

Objective: Compare poliovirus detection in BMFS v1 samples with the WHO 'grab' method (two-phase separation) (365 sets)

- BMFS detected WPV1 during **24** sampling events in which the two-phase method did not
- Two-phase detected WPV1 during **11** sampling events in which the BMFS did not
- WPV1 was detected more frequently in BMFS than two-phase samples (p=0.029)



BMFS v2 Verification in Pakistan: Jan-Mar 2019

Objective: Compare poliovirus detection in BMFS v1, BMFS v2, and two-phase samples (21 sets)

- Samples collected at 7 sites
- Collection began January 2019
- Results available from 3 sampling events (Faisalabad, Lahore, & DG Khan)
  - WPV1 detected in BMFS v1 sample from Faisalabad
  - WPV1 detected in all samples from Lahore

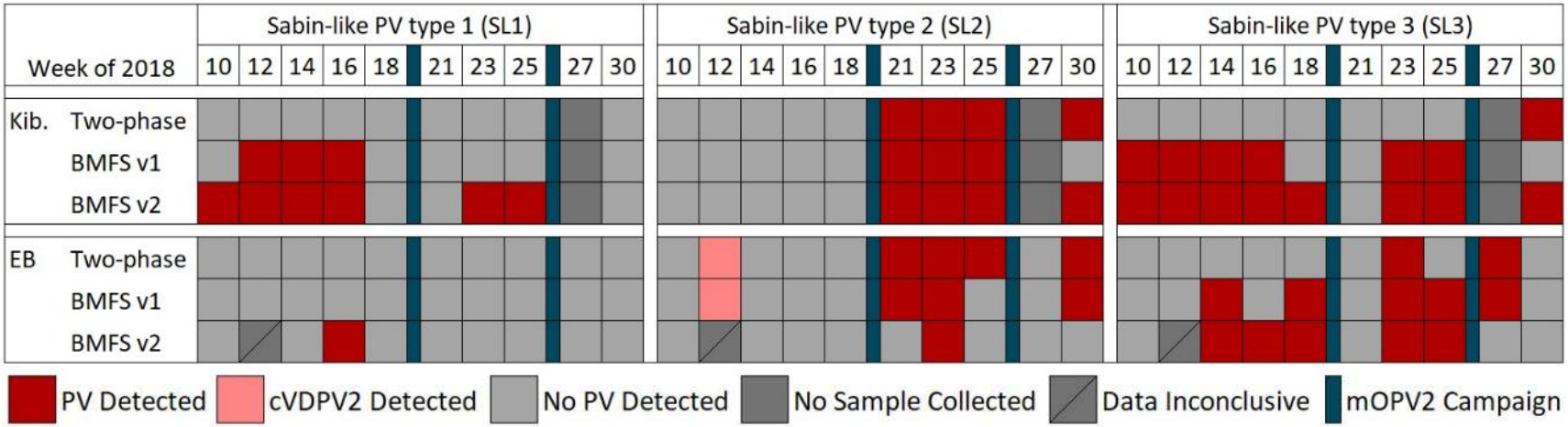
BMFS v2 Verification in Kenya: Mar-July 2018

Objective: Compare PV detection in BMFS v1, BMFS v2, and two-phase samples (18 sets)

- SL1 & SL3 detected significantly more in BMFS v2 than two-phase samples (*p*=0.008 & 0.003, respectively)
- VDPV2 detected in BMFS v1 & two-phase samples; BMFS v2 inconclusive
- SL2 detection not statistically different between two-phase, BMFS v1, and BMFS v2 samples

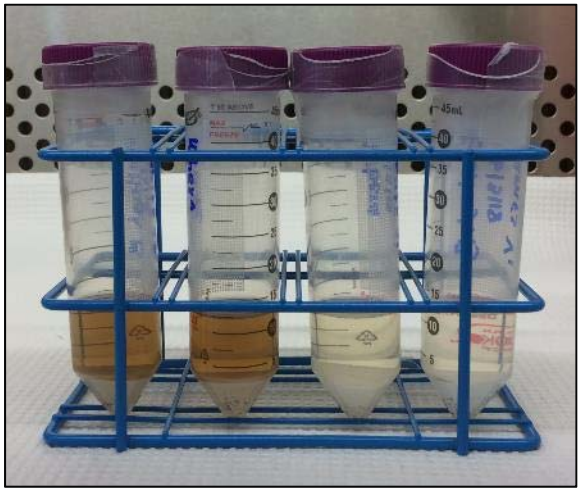
BMFS v2 is a verified alternative to the field-validated BMFS v1 for environmental surveillance of poliovirus

	SL1 % (n)	SL2 % (n)	SL3 % (n)
Two-phase	**0.0 (18)**	**88.8 (9)**	**16.7 (18)**
BMFS v1	**16.7 (18)**	**66.6 (9)**	**61.1 (18)**
BMFS v2	**38.9 (18)**	**55.6 (9)**	**72.7 (18)**
p-value (McNemar mid-p test)			
Two-phase vs. BMFS v2	**0.008**	0.125	**0.003**
BMFS v1 vs. v2	0.063	0.625	0.375



Bag-Mediated Filtration System (BMFS) Overview

- *Proven utility for environmental surveillance*
- *Allows 3-5 liter samples*
- *Adaptable to composite sampling*
- *Allows multiplexing of target pathogen*
  - *Though NOT fully evaluated for bacteria (yet)*



Photos: Christine Fagnant-Sperati, Bethel Demeke

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KEMRI: Peter Borus, James Nyangao, Evans Komen, Benlick Mwangi, Rosemary Nzunza
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Oxford University: Andrew Pollard
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University of Michigan: Joan Rose
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