ELECTRONIC SUPPLEMENTARY MATERIAL

Feasibility and applicability of the bag-mediated filtration system for enhanced environmental surveillance of poliovirus in Kenya using three analysis methods

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MS2 recovery experiments

Methods

Approximately 10^5 PFU of MS2 (ATCC 15597-B1) were seeded to 175 mL 1x phosphate-buffered saline (PBS), agitated using a vortex (30 seconds), and passed through the ViroCap filter inlet using a peristaltic pump. The filtrate was collected and discarded. Filters were stored for 0, 1, 2, 4, or 7 days at 4°C, and two filters were tested for each time point.

Filters were eluted as previously described, using 175 mL sterile 1.5% beef extract, 0.05 M glycine, pH 9.5 (Fagnant et al. 2014). The eluent was added to the filter inlet, let to stand 30 minutes, and recovered via the outlet. The recovered eluate was then pH-adjusted to 7.0-7.5 using 5 M HCl and 5 M NaOH. Infectious MS2 was enumerated by the double agar layer method on *E. coli* F-amp host (ATCC 70081) (Adams 1959). Briefly, 100 µL sample dilutions in PBS and 100 µL log-phase *E. coli* F-amp in nutrient broth were added to 6-8 mL molten bactoagar (0.7% bactoagar, 0.5% sodium chloride) and mixed. The bactoagar was then poured onto a 100 mm tryptic soy agar plate and let to solidify. Plates were incubated at 37°C for 18-20 hours and plaques were counted. Relevant dilutions were plated in duplicate, and recovery was calculated by dividing the recovered count by the known seeded count.

Results and Discussion

MS2 recovery averaged 74.9%, 63.7%, 54.3%, 55.3%, and 69.9% after 0, 1, 2, 4, and 7 days, respectively (Fig. S1). The relatively high MS2 recovery after one day indicates filters could be stored for up to 24 hours after MS2 seeding prior to filtration, while still anticipating greater than 50% recovery of infectious MS2.

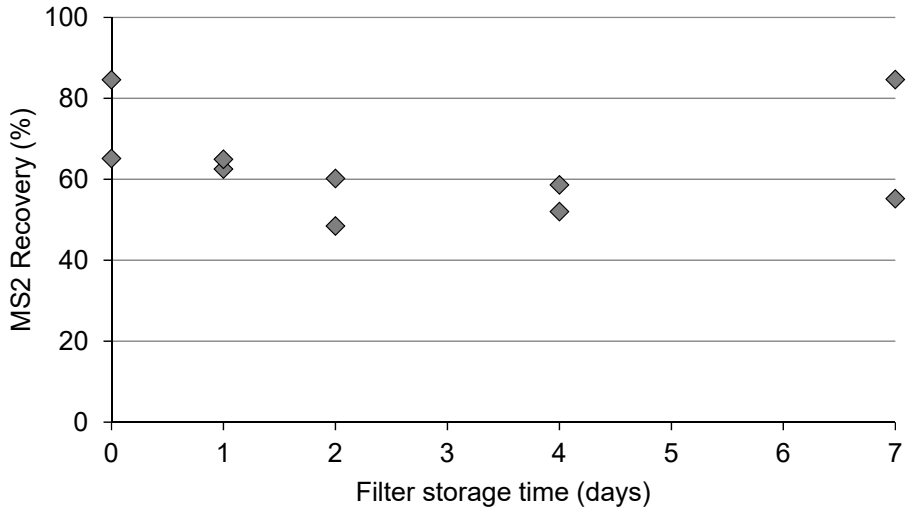

Fig. S1 Infectious MS2 recovery from ViroCap filters after storage at 4°C. *n*=2 per filter storage time.

Table S1. Primers and probes used for detection of SL1, SL2, SL3, WPV1, and WPV3

Primer	Sequence (5→3)	Orientation	Position
	Centers for Disease Control and Prevention [a]		
Quadruplex			
Pan-EV PCR-1	GCGATTGTCACCATWAGCAGYCA	Reverse	603–581
Pan-EV PCR-2	GGCCCCTGAATGCGGCTAATCC	Forward	458–480
Pan-EV Probe	VIC-CCGACTACTTTGGGWGTCCGTGT-TAMRA		546–568
Sabin 1 PCR-1	CCACTGGCTTCAGTGTTT	Reverse	2600–2583
Sabin 1 PCR-2	AGGTCAGATGCTTGAAAGC	Forward	2505–2523
Sabin 1 Probe A4	CY5-CGCCCCCACCGTTTCACGGA-BHQ3		2559–2540
Sabin 2 PCR-1	CGGCTTTGTGTCAGGCA	Reverse	2595–2579
Sabin 2 PCR-2	CCGTTGAAGGGATTACTAAA	Forward	2525–2544
Sabin 2 Probe	FAM-ATTGGTTCCCCCGACTTCCACCAAT-BHQ1		2550–2572
Sabin 3 PCR-1	TTAGTATCAGGTAAGCTATC	Reverse	2591–2572
Sabin 3 PCR-2	AGGGCGCCCTAACTTT	Forward	2537–2552
Sabin 3 Probe	ROX-TCACTCCCGAAGCAACAG-BHQ2		2554–2571
Pan Poliovirus			
Pan-PV PCR-1	GGAGCTCCGGGTGGGAYRTACATIATYTGRTAIAC	Reverse	2978–2956
Pan-PV PCR-2	TTGGAGTTCTTCACITAITCIMGITTYGAYATG	Forward	2876–2895
Pan-PV Probe	FAM-TGRTTNARIGCRTGICCRTTRTT-BHQ1		2957–2935
	University of Pretoria [b]		
SABIN1-F	TCCCTTTGACTTAAGTACAAA	Forward	1904-1924
POLIO1-R	GATCCTGCCCAGTGTGTGTAG	Reverse	2083-2063
POLIO1-TM	FAM-AGGGTTCGGTTAAGTGACAAACCACATAC-BBQ		1950-1978
SABIN2-F	AAGGAATTGGTGACATGATTGAGG	Forward	2480-2503
SABIN2-R	CTCGGCTTTGTGTCAGGC	Reverse	2579-2562
SABIN2-TM	FAM-TGGAAGTCGGGGGAACCAATGC-BBQ		2551-2530
SABIN3-F	AATGACCAGATTGGTGATTCCTTG	Forward	3134-3157
SABIN3-R	GTAAATGCGGACTTTGGAGGTTACT	Reverse	3253-3229
SABIN3-TM	FAM-TGTGATCATTGACAACACGAACTGCCAA-BBQ		3218-3191

SL1, Sabin-like poliovirus type 1; SL2, Sabin-like poliovirus type 2; SL3, Sabin-like poliovirus type 3; ITD, intratypic differentiation

[a] The Centers for Disease Control and Prevention used the Poliovirus ITD 4.0/4.1 rRT-PCR Kit

[b] University of Pretoria used the Poliovirus ITD 4.0/4.1 rRT-PCR Kit for detection of WPV1 and WPV3 as described for the Poliovirus ITD 5.0 rRT-PCR Kit by Gerloff et al. 2018, and primers and probes from Nijst et al. 2013 for detection of SL1, SL2, and SL3

Table S2. Real-time RT-PCR programs used for poliovirus detection

	Centers for Disease Control and Prevention [a]		University of Pretoria [b]					
	All primer sets		SL1		SL2		SL3	
	T (°C)	time	T (°C)	time	T (°C)	time	T (°C)	time
reverse transcription	42	45 min	50	45 min	50	45 min	50	45 min
PCR activation	95	3 min	95	15 min	95	15 min	95	15 min
cycles	40 cycles				45 cycles			
denaturation	95	24 sec	95	5 sec	95	5 sec	95	5 sec
annealing	47	30 sec	55	10 sec	58	15 sec	56	15 sec
extension	65	24 sec	72	10 sec	72	5 sec	72	5 sec
cool down	-	-	40	30 sec	40	30 sec	40	30 sec

SL1, Sabin-like poliovirus type 1; SL2, Sabin-like poliovirus type 2; SL3, Sabin-like poliovirus type 3; WPV1, wild poliovirus type 1; WPV3, wild poliovirus type 3; T, temperature; ITD, intratypic differentiation

[a] Poliovirus ITD 4.0/4.1 rRT-PCR Kit

[b] University of Pretoria used the Poliovirus ITD 4.0/4.1 rRT-PCR Kit for detection of WPV1 and WPV3

Table S3. ICC-RT-PCR cell lines and media used

Cell line	Obtained from	Citation	Propagation media	Wash media	Maintenance media
PLC/ PRF/5	ECACC 85061113	Alexander et al. 1976	E-MEM Pen/Strep 8% FCS Hepes Buffer Tylosin	E-MEM Pen/Strep	E-MEM Pen/Strep Amphotericin B 2% FCS
L20B	CDC via the NICD	Pipkin et al. 1993; Wood and Hull 1999	E-MEM Pen/Strep 5% FCS Hepes Buffer Tylosin	E-MEM Pen/Strep	E-MEM Pen/Strep Amphotericin B 5% FCS
BGM	ECACC 90092601	Dahling et al. 1974	E-MEM Pen/Strep 5% FCS	E-MEM Pen/Strep	E-MEM Pen/Strep Amphotericin B 0.5% FCS

ICC-RT-PCR, integrated cell culture-real-time RT-PCR (reverse-transcription polymerase chain reaction) with PLC/PRF/5, L20B, and BGM (buffalo green monkey) cell lines; ECACC, European Collection of Cell Cultures (Salisbury, UK); CDC, Centers for Disease Control and Prevention; NICD, National Institute for Communicable Diseases (Sandringham, SA); E-MEM, Eagle's minimal essential medium with Earle's salts and L-glutamine (Gibco, Carlsbad, CA, USA); Pen/Strep, 100 U/mL penicillin and 100 µg/mL streptomycin (BioWhittaker® Pen/Strep, Lonza, Verviers, Belgium); FCS, fetal calf serum (FBS Superior, Biochrom, Berlin, Germany); Hepes Buffer, 10 mM Hepes buffer solution (Gibco); Tylosin, 0.008 mg/mL Tylosin solution (Sigma-Aldrich Co., St. Louis, MO, USA); Pen/Strep Amphotericin B, 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.0025 µg/mL amphotericin B (BioWhittaker Pen/Strep Amphotericin B [100x], Lonza)

Table S4. BMFS sampling scheme

Sample collection day (dd/mm/yyyy)	Samples collected	Sequential samples collected	Number of sites sampled	Bucket modification conducted	Collection time (hours) [d]	Time to preservative addition (days) [f]	Time to shipping (days) [h]	Shipping time (days)	Time to elution (days) [k]	Time to secondary concentration (days) [m]	Overall processing time (days) [p]
14/04/2015	4	yes	2 [a]	no	3.5	21	22 [i]		0	4	29 [q]
28/04/2015	6	yes	3 [b]	no	6.2	7	8 [i]	2 [i]	0/3 [l]	4/1 [n]	15
04/05/2015	2	yes	1 [c]	no	1.3	1	2 [i]		3	1	9
13/05/2015	8	yes	4	yes	6.3	5	6	2	0	1	10
26/05/2015	8	yes	4	yes	5.3	2/3 [g]	7	7 [j]	1	4	15
16/06/2015	4	no	4	yes	4.8	2	3	2	0	4	11
30/06/2015	4	no	4	yes	3.6	1	8	2	3	1	15
21/07/2015	4	no	4	yes	4.5	2	6	2	1	1	11
28/07/2015	4	no	4	yes	4.3	1	6	2	2	5	16
11/08/2015	4	no	4	yes	4.4	2	7	2	1	4	15
25/08/2015	4	no	4	yes	5.0	3	8	2	4	1	16
07/09/2015	4	no	4	yes	4.2	1	2	2	0	4	10
12 sampling days	56 BMFS samples collected	Collected during first 5 sampling days	9 sampling days where all 4 sites sampled	Bucket modification used during last 9 sampling days	Average collection time was 4.7 hours when 4 sites sampled on the same day [e]	Occurred within 3 days for 68% of filters	Average hold time was 6 days for 79% of filters	2 days for 90% of shipping events	Occurred within 4 days of filter receipt	Occurred within 5 days of elution [o]	Median time was 15±1.3 days

BMFS, bag-mediated filtration system; [a] samples only collected from Kibera and Starehe, as the method was new to the field technicians and a scaled sampling was desired; [b] planned sampling event at Kibera was postponed to one week later due to the time of day and traffic considerations; [c] make-up sampling day for the postponed sample from Kibera; [d] time from the beginning of first sample collection to arrival back at KEMRI; [e] collection time does not include filtration time for these 9 sampling days; [f] between sample collection and preservative addition; [g] preservatives added to Kibera samples within 2 days, while preservatives were added to Starehe, Eastleigh A, and Eastleigh B samples within 3 days; [h] between sample collection and shipment to the University of Pretoria; [i] samples from the first three sampling days shipped together. The delay in shipping was due to incorporation of new routines, procurement of shipment boxes, and coordination with the courier; [j] shipping time was seven days due to a courier mistake in which the filters were detoured rather than flown directly from Kenya to South Africa; [k] between sample receipt at the University of Pretoria and filter elution; [l] filter elution was completed for Starehe samples on the day of receipt, while elution of Kibera, Eastleigh A, and Eastleigh B samples was completed three days after filter receipt; [m] between filter elution and initiation of secondary concentration; [n] secondary concentration was completed on the same day for samples from all four sites, though filter elution was completed for Starehe samples three days prior to filter elution for the samples from the other sites; [o] delays in completion of secondary concentration was due to the overnight step required. Therefore, when elution occurred on a Thursday, secondary concentration was delayed until Monday; [p] between sample collection and completion of secondary concentration; [q] the time delay was due to logistical challenges with shipping (7% of filters)

Table S5. Comparison of PV and MS2 detection in matched two-phase and BMFS samples.

Sample collection day (dd/mm/yyyy)	Site	Two-phase			BMFS			
		SL1[a]	SL2[a]	SL3[a]	SL1[a]	SL2[a]	SL3[a]	MS2[b]
14/04/2015	Kibera	0	0	0	1	1	1	478
14/04/2015	Kibera				0	1	1	33
14/04/2015	Starehe	0	0	1	0	1	1	128
14/04/2015	Starehe				0	1	1	316
28/4/2015	Starehe	1	0	1	0	0	1	3.8
28/4/2015	Starehe				0	1	1	247
28/4/2015	Eastleigh A	0	0	0	1	1	0	77.3
28/4/2015	Eastleigh A				1	0	0	74.3
28/4/2015	Eastleigh B	0	0	0	0	0	1	0.7
28/4/2015	Eastleigh B				0	0	1	0.7
05/04/2015	Kibera	0	1	1	1	1	1	430
05/04/2015	Kibera				1	1	1	244
13/05/2015	Kibera	0	0	0	0	0	1	0.7
13/05/2015	Kibera				0	0	1	1.4
13/05/2015	Starehe	1	1	0	1	0	1	9.5
13/05/2015	Starehe				0	1	0	23.4
13/05/2015	Eastleigh A	0	1	0	1	1	1	134
13/05/2015	Eastleigh A				0	1	0	74.3
13/05/2015	Eastleigh B	1	0	1	0	1	0	113
13/05/2015	Eastleigh B				0	1	0	29.1
26/05/2015	Kibera	1	1	1	0	1	1	0
26/05/2015	Kibera				1	1	1	0.7
26/05/2015	Starehe	1	1	0	0	1	1	16.2
26/05/2015	Starehe				0	1	1	4
26/05/2015	Eastleigh A	0	0	0	0	1	0	73.6
26/05/2015	Eastleigh A				0	1	0	47.9
26/05/2015	Eastleigh B	0	0	0	1	1	1	8.8
26/05/2015	Eastleigh B				0	1	1	2.02
16/06/2015	Kibera	0	1	0	1	0	1	0.7
16/06/2015	Starehe	0	1	0	1	1	0	0
16/06/2015	Eastleigh A	0	0	0	0	1	1	0.7
16/06/2015	Eastleigh B	0	1	1	0	0	0	1.4
30/06/2015	Kibera	0	1	0	1	1	1	6.8
30/06/2015	Starehe	0	0	1	0	1	1	0.7
30/06/2015	Eastleigh A	0	1	0	0	1	0	0.7
30/06/2015	Eastleigh B	1	1	0	1	1	0	0.7
21/07/2015	Kibera	0	1	1	0	1	1	25
21/07/2015	Starehe	0	1	0	0	1	1	499
21/07/2015	Eastleigh A	0	0	0	0	1	0	4.05
21/07/2015	Eastleigh B	0	1	1	0	1	1	0.7
28/07/2015	Kibera	0	1	0	0	1	0	0.1
28/07/2015	Starehe	0	1	1	0	1	1	23
28/07/2015	Eastleigh A	0	1	0	0	0	0	16
28/07/2015	Eastleigh B	0	0	0	0	0	0	120
08/11/2015	Kibera	0	1	0	0	1	0	31
08/11/2015	Starehe	0	1	1	1	1	1	11
08/11/2015	Eastleigh A	1	1	0	0	1	0	2.7
08/11/2015	Eastleigh B	0	1	0	0	1	1	8.8
25/08/2015	Kibera	0	1	0	1	1	0	6
25/08/2015	Starehe	1	1	1	0	1	1	0
25/08/2015	Eastleigh A	0	1	0	0	1	0	1
25/08/2015	Eastleigh B	0	1	1	0	1	1	0
09/07/2015	Kibera	0	1	0	0	1	0	1.4
09/07/2015	Starehe	0	1	1	0	1	0	50
09/07/2015	Eastleigh A	0	0	0	0	0	0	0
09/07/2015	Eastleigh B	1	0	0	0	1	1	10

PV, poliovirus; BMFS, bag-mediated filtration system; SL1, Sabin-like poliovirus type 1; SL2, Sabin-like poliovirus type 2; SL3, Sabin-like poliovirus type 3; [a] presence (1) or absence (0); samples were measured by WHO (World Health Organization) algorithm, using virus isolation on L20B and RD (human rhabdomyosarcoma) cells followed by ITD (intratypic differentiation); [b] percent recovery; samples were measured by double agar layer, and compared with 100% recovery of 10^5 PFU seeded MS2

Table S6. Comparison of PV detection in BMFS samples as measured by three different detection methods

Comparison of PV detection in WHO algorithm and direct RT-PCR								
SL1	WHO +	WHO -	SL2	WHO +	WHO -	SL3	WHO +	WHO -
direct +	0	0	direct +	6	0	direct +	12	1
direct -	8	34	direct -	26	10	direct -	10	19
OR (CI)	nd		OR (CI)	nd		OR (CI)	10 (1.3, 78)	
p-value	0.0039		*p*-value	$1.49x10^{-8}$		*p*-value	0.0063	
Comparison of PV detection in WHO algorithm and ICC-RT-PCR								
SL1	WHO +	WHO -	SL2	WHO +	WHO -	SL3	WHO +	WHO -
ICC +	5	9	ICC +	31	5	ICC +	19	9
ICC -	3	25	ICC -	1	5	ICC -	3	11
OR (CI)	0.33 (0.090, 1.2)		OR (CI)	0.2 (0.023, 1.7)		OR (CI)	0.33 (0.090, 1.2)	
p-value	0.092		*p*-value	0.125		*p*-value	0.092	
Comparison of PV detection in direct RT-PCR and ICC-RT-PCR								
SL1	ICC +	ICC -	SL2	ICC +	ICC -	SL3	ICC +	ICC -
direct +	0	0	direct +	6	0	direct +	13	0
direct -	13	29	direct -	28	8	direct -	15	14
OR (CI)	nd		OR (CI)	nd		OR (CI)	nd	
p-value	0.00012		*p*-value	$3.73x10^{-9}$		*p*-value	$3.05x10^{-5}$	

PV, poliovirus; BMFS, bag-mediated filtration system; WHO, World Health Organization; WHO algorithm measured by virus isolation on L20B and RD (human rhabdomyosarcoma) cells followed by ITD (intratypic differentiation); direct, direct real-time RT-PCR (reverse-transcription polymerase chain reaction); ICC, integrated cell culture-real-time RT-PCR with PLC/PRF/5, L20B, and BGM (buffalo green monkey) cell lines; SL1, Sabin-like PV type 1; SL2, Sabin-like PV type 2; SL3, Sabin-like PV type 3; nd, not determined; OR, odds ratio; CI, 95% confidence intervals; *p*-value, calculated by the McNemar mid-p test

Table S7. Comparison of PV detection in BMFS samples analyzed by ICC-RT-PCR

Comparison of PV detection after amplification on PLC/PRF/5 and L20B cell lines								
SL1	PLC +	PLC -	SL2	PLC +	PLC -	SL3	PLC +	PLC -
L20B +	4	6	L20B +	22	7	L20B +	14	5
L20B -	3	29	L20B -	4	9	L20B -	6	17
OR (CI)	0.5 (0.13, 2.0)		OR (CI)	0.57 (0.17, 2.0)		OR (CI)	1.2 (0.37, 3.9)	
p-value	0.344		*p*-value	0.388		*p*-value	0.774	
Comparison of PV detection after amplification on PLC/PRF/5 and BGM cell lines								
SL1	PLC +	PLC -	SL2	PLC +	PLC -	SL3	PLC +	PLC -
BGM +	1	0	BGM +	12	1	BGM +	4	3
BGM -	6	35	BGM -	14	15	BGM -	16	19
OR (CI)	nd		OR (CI)	14 (1.8, 106)		OR (CI)	5.3 (1.6, 18)	
p-value	0.016		*p*-value	5.2×10^{-4}		*p*-value	0.0026	
Comparison of PV detection after amplification on L20B and BGM cell lines								
SL1	L20B +	L20B -	SL2	L20B +	L20B -	SL3	L20B +	L20B -
BGM +	1	0	BGM +	12	1	BGM +	5	2
BGM -	9	32	BGM -	17	12	BGM -	14	21
OR (CI)	nd		OR (CI)	17 (2.3, 128)		OR (CI)	7 (1.6, 31)	
p-value	0.0020		*p*-value	7.63×10^{-5}		*p*-value	0.0024	

PV, poliovirus; BMFS, bag-mediated filtration system; ICC-RT-PCR, integrated cell culture-real-time RT-PCR (reverse-transcription polymerase chain reaction) with PLC/PRF/5, L20B, and BGM (buffalo green monkey) cell lines; SL1, Sabin-like PV type 1; SL2, Sabin-like PV type 2; SL3, Sabin-like PV type 3; nd, not determined; OR, odds ratio; CI, 95% confidence intervals; *p*-value, calculated by the McNemar mid-p test

Table S8. Comparison of PV detection in matching BMFS and two-phase samples as measured by WHO algorithm

Comparison of PV detection in BMFS-1 and single BMFS samples and two-phase samples								
SL1	Two-phase +	Two-phase -	SL2	Two-phase +	Two-phase -	SL3	Two-phase +	Two-phase -
BMFS +	2	10	BMFS +	23	10	BMFS +	11	13
BMFS -	7	23	BMFS -	4	5	BMFS -	3	15
OR (CI)	1.4 (0.54, 3.8)		OR (CI)	2.5 (0.78, 8.0)		OR (CI)	4.3 (1.2, 15)	
p-value	0.481		*p*-value	0.118		*p*-value	0.013	
Comparison of PV detection in BMFS-2 and single BMFS samples and two-phase samples								
SL1	Two-phase +	Two-phase -	SL2	Two-phase +	Two-phase -	SL3	Two-phase +	Two-phase -
BMFS +	2	7	BMFS +	24	10	BMFS +	11	11
BMFS -	7	26	BMFS -	3	5	BMFS -	3	17
OR (CI)	1.0 (0.35, 2.9)		OR (CI)	3.3 (0.92, 12)		OR (CI)	3.7 (1.0, 13)	
p-value	1.0		*p*-value	0.057		*p*-value	0.035	

PV, poliovirus; BMFS, bag-mediated filtration system; WHO algorithm, virus isolation on L20B and RD (human rhabdomyosarcoma) cells followed by ITD (intratypic differentiation); BMFS-1, first collected BMFS sample of the two sequentially collected BMFS samples; BMFS-2, second collected BMFS sample of the two sequentially collected BMFS samples; SL1, Sabin-like PV type 1; SL2, Sabin-like PV type 2; SL3, Sabin-like PV type 3; nd, not determined; OR, Odds ratio; CI, 95% confidence intervals; *p*-value, calculated by the McNemar mid-p test

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