APPENDIX for Duintjer Tebbens RJ, Diop OM, Pallansch MA, Oberste MS, Thompson KM. "Characterizing the costs of the Global Polio Laboratory Network: A survey-based analysis" *BMJ Open* 2019;9:e023290, doi:10.1136/bmjopen-2018-023290

A1. Survey instrument

Introduction: Poliovirus Laboratory Survey

The World Health Organization (WHO)-led Global Polio Laboratory Network (GPLN) continues to play an essential role in global polio eradication, and periodic efforts to quantify its overall value provide important information that helps to motivate financial support for GPLN laboratories. Assessing the value of the GPLN is of utmost importance at this stage of the GPEI, as the partners discuss the strategies to maintain polio laboratory functions pre- and post-certification of wild poliovirus eradication and global containment of live polioviruses. This GPLN survey aims to collect data on activities and costs of all of the GPLN laboratories to support an overall synthesis. The objectives of this survey include to: (1) update estimates of the total costs of the GPLN reported based on a similar 2003 survey, (2) better understand the different cost components, including environmental surveillance, and (3) characterize the extent to which the GPLN contributes to surveillance of other diseases. The survey form should take approximately 60 minutes to complete, and we expect that collecting data and calculating some of the costs may take an additional 1-4 hours, depending on the size and complexity of the laboratory. Please start the survey as soon as possible, so if you have any questions or if you need to compile data, you will have time to do so. The survey includes questions about acute flaccid paralysis (AFP) surveillance (i.e., stool samples from AFP cases and contacts) and environmental surveillance (i.e., sewage samples).

Please note:
· we pre-filled some answers based on data collected in GPLNMS annual reports for 2016 as of June 2017, and we ask that you please check the pre-filled answers carefully and correct the information as appropriate.
· please do not leave any answers blank, because we cannot interpret these correctly, so please enter "0" for zero, "unknown" for unknown, "not applicable" for not applicable, or "data not available" or other appropriate text. If you find any question too difficult to answer, please do not quit the entire question or survey, but instead reply with "unable to answer" and please add any information that can help us understand the reason.

We provided a glossary to promote consistent interpretation of survey language. If you have any questions, please contact Dr. Radboud Duintjer Tebbens (Kid Risk) and Dr. Ousmane Diop (WHO). Thank you very much for your time and effort to respond to the survey. We look forward to hearing from you - please complete your response by September 1, 2017. We will share the results with all polio laboratory directors for dissemination once they become available.

1. Please provide information about how to contact you and about your laboratory
Laboratory Name:
Your Name:
Phone number:

Email address:
City:
Country:
WHO Region:
* Total employee full-time equivalents (FTEs) for poliovirus surveillance employed by the laboratory:
Please enter the percent (between 0-100, without the % sign) of FTEs reported for the line with the * above supported by National/internal funds:
Please enter the percent (between 0-100, without the % sign) of FTEs reported for the line with the * above supported by GPEI external funds:
Please enter the percent (between 0-100, without the % sign) of FTEs reported for the line with the * above supported by Other external funds (non GPEI-external funds, including bi-lateral support) - This line should total 100 minus the percents on the prior 2 lines.

2. What role did your laboratory play in the global polio laboratory network in 2016?
Subnational
National
Regional reference
Specialized
Other (please specify)

3. Please list the geographic areas (country, state, region) that your laboratory served in 2016 for each laboratory capacity (enter "None" for any you do not do and please note any special activities by including the word "Special" after the name of the geographic area indicated, for example to help with overflow from another lab, if applicable for 2016):
Virus isolation:
Intratypic differentiation (ITD):
Sequencing:
Serology:
Environmental surveillance:

4. Please estimate what percentages (without including the "%" sign) of polio-supported staff time and equipment your laboratory spends on poliovirus surveillance and research activities (including methods development, serology, clinical trials, next generation or complete genome sequencing, etc.) versus surveillance and research activities for other diseases.
Poliovirus activities (indicate 100 here and 0 on all other answers if your lab supports poliovirus surveillance activities exclusively):
Non-polio enteroviruses:
Measles and/or rubella viruses:
Rotavirus:
Influenza:
Japanese encephalitis:
Yellow fever:
Other arboviruses (e.g., Zika, dengue) or hemorrhagic fever viruses:
Other (please provide percentage here and details about what this includes in Question 9):

5. Did your laboratory perform the following for poliovirus environmental surveillance in 2016 (if none indicate no for all)?
Site selection: Y/N
Sample collection: Y/N
Sample transportation: Y/N
Concentration: Y/N
Virus isolation: Y/N
Intratypic differentiation: Y/N
Sequencing: Y/N
Other (please specify): Y/N

6. Please tell us about any poliovirus serology testing you did in 2016 (if none, then enter "None" for this question).
How many serum samples did you test for poliovirus antibodies in 2016?
Approximately how many employee hours did your laboratory spend in 2016 for poliovirus serum sample processing?
What laboratory method do you use for poliovirus serology testing?
Please indicate the purpose(s) for the poliovirus serology sampling (e.g., seroprevalence assessment, support for vaccine trials, etc.)

7. Please tell us the number of samples your laboratory processed in 2016 related to other activities (i.e., non-AFP, non-poliovirus environmental surveillance, and non-poliovirus serology activities) for the following (please specify details about the methods used and your role in sample collection in Question 9)
Non-polio enterovirus surveillance:
Healthy children / adult surveys (e.g., stool surveys) that are not part of AFP surveillance:
Clinical trial support:
Other (please specify the nature of these samples in Question 9):

8. What currency do you use to track laboratory costs and will you use to report costs in this survey?

9. Please specify details here if you answered "other" for Question 4 and/or 7, please also describe any research activities conducted by your laboratory in 2016 related to polioviruses, and please use this space to enter any other comments you would like to make related to the questions on this page.

10. How many samples/isolates from AFP cases and their contacts did you process in 2016?
Acute flaccid paralysis (AFP) surveillance:
Virus isolation:
Intratypic differentiation:
Sequencing:
Other (please enter the number here and specify the type of processing in Question 14):

11. How many people (full-time equivalents) worked on the different steps of processing AFP samples in 2016?

Cell culture:
Virus isolation:
Intratypic differentiation:
Sequencing:
Management (including supervisors, data management, analytics, recording, and reporting):
Other (please enter number here and specify the type of processing in Question 14):

12. How much did your laboratory spend (in the currency you specified in Question 8) for analysis of AFP samples in 2016 for each cost category?
Personnel (costs should correspond to number of people in Question 11 plus any staff not on payroll):
Training (please exclude any costs counted in the personnel row above):
Equipment, please estimate the amortized annual cost, see Excel worksheet:
Durable supplies, please estimate the amortized annual cost, see Excel worksheet:
Consumable supplies attributable to each sample:
Shared consumable supplies purchased by laboratory not easily attributable to each sample:
Donated supplies provided by your lab to other labs (please specify the other labs you provide these to in Question 14):
Operations:
Shipping/transport:
Technical support (not otherwise captured):
Other (please specify in Question 14):

13. Please indicate the approximate percents of the amounts spent in Question 12 for each c ost category by contribution type: 1. National/internal; 2. GPEI external; and 3. Bilateral and non-GPEI external. For example, if all support came from national sources then indicate "100; 0; 0" OR if all contributions came from the GPEI indicate "0; 100; 0" OR if approximately equal support came from each indicate "33.4; 33.3; 33.3" and please verify that the totals of all three components of the answer for each row add to 100)
Personnel
Training
Equipment
Durable supplies
Consumable supplies
Shared consumable supplies
Donated supplies
Operations
Shipping/transport
Technical support
Other

14. Please specify details here about Questions 10-13 for which you answered "other" or enter any comments you would like to make related to the questions on this page.

15. Did your laboratory support any poliovirus environmental surveillance or research activities in 2016 (please verify)?

No
Yes

16. Did your laboratory first establish its capacity to process poliovirus environmental samples between 2010 and 2016 (i.e., relatively recently)? (If yes, the survey will ask you to estimate set up costs. If your laboratory established its capacity to process environmental samples before 2010, but made significant investments in 2016 to expand its capacity, then answer yes and estimate the costs for expanding the capacity in 2016 in Question 18).
No
Yes

17. Please enter the dates your laboratory first began to develop the capacity to support poliovirus environmental surveillance efforts and became fully operational (if exact date unknown, please estimate month and enter "14" for day)?
Date laboratory began to develop the poliovirus ES capacity: MM/DD/YYYY
Date your lab became fully operational to support poliovirus environmental surveillance: MM/DD/YYYY

Environmental surveillance SET UP questions (for capacity established AFTER 2009 OR expanded during 2016 ONLY):

18. Please estimate the costs your laboratory spent to SET UP poliovirus ES capacity between the dates you reported in Question 17 (in the currency you specified in Question 8) for each cost category.
Facility (purchase/renovation of physical facility)
New personnel for laboratory set up
Training
New equipment for concentration (e.g., centrifuge, refrigerators, funnels, filtration devices, etc.)
New equipment for expanded poliovirus processing capacity
Durable supplies for start up
Consumable supplies for start up
Operations for start up
Technical support for start up
Other (please specify in Question 20)

19. If you included estimates of SET UP costs in Question 18, please indicate the approximate percents of the amounts for each cost category by contribution type: 1. National/internal; 2. GPEI external; and 3. Bilateral and non-GPEI external. For example, if all support came from national sources then indicate "100; 0; 0" OR if all contributions came from the GPEI indicate "0; 100; 0" OR if approximately equal support came from each indicate "33.4; 33.3; 33.3" and please verify that the totals of all three components of the answer for each row add to 100)
Facility
New personnel for laboratory set up
Training for start up
New equipment for concentration
New equipment for expanded poliovirus processing capacity

Durable supplies for start up
Consumable supplies for start up
Operations for start up
Technical support for start up
Other (please specify in Question 20)

20. Please specify details here about Questions 18-19 for which you answered "other" or enter any comments you would like to make related to the questions on this page.

21. Which organization(s) collect the poliovirus environmental samples that your laboratory receives?

22. Please enter the total number of environmental samples your laboratory received in 2016 from each of the following types of water source(s) sampled (if known). If only unknown water source(s) sampled, then please indicate the total number of environmental samples for 2016 in the second-to-last row.
Wastewater treatment plant
Pumping station
Open drains or canals
Streams, rivers, or other flowing surface water
Lakes, ponds or other standing surface water
Access point from sewage system
Unknown
Other (please indicate type in Question 27)

23. Please enter the number of environmental samples for which your laboratory took the indicated number of days between the time of sample collection and starting the process of virus isolation. Your internal data for all poliovirus ES samples should provide the sample collection date and the date your lab started sample processing.
Less than 2 days
3 to 5 days
6 to 10 days
11 to 15 days
16 to 20 days
21 to 25 days
26 to 30 days
31 to 35 days
More than 35 days

24. How many environmental samples did your laboratory process in 2016 for each of the following?
Concentration using
WHO-recommended two-phase separation
Concentration using other methods (please specify method(s) used in Question 27)
Virus isolation
Intratypic differentiation

Sequencing
Research
Direct detection
Other (please specify type of processing in the comment field at the bottom of this page in Question 27)

25. How much did your laboratory spend (in the currency you specified in Question 8) for analysis of environmental samples in 2016 (excluding any costs for SET UP that occurred in 2016, which you should have reported in Question 18) and excluding any costs already reported in Question 12 related to AFP processing that applied to processing environmental samples.
Personnel (FTEs for environmental surveillance activities)
Training
Equipment, please estimate the amortized annual cost, see Excel worksheet
Durable supplies, please estimate the amortized annual cost, see Excel worksheet
Consumable supplies
Shared consumable supplies
Donated supplies (please specify the other labs you provide these to in Question 27)
Operations
Shipping/transport
Technical support
Other (please specify in Question 27)

26. Please indicate the approximate percent of the amounts spent in Question 25 for each c ost category by contribution type: 1. National/internal; 2. GPEI external; and 3. Other external. For example, if all support came from national sources then indicate "100; 0; 0" OR if all contributions came from the GPEI indicate "0; 100; 0" OR if approximately equal support came from each indicate "33.4; 33.3; 33.3" and please verify that the totals of all three components of the answer for each row add to 100)
Personnel
Training
Equipment
Durable supplies
Consumable supplies
Shared consumable supplies
Donated supplies
Operations
Shipping/transport
Technical support
Other (please specify in Question 27)

27. Please specify details here about Questions 21-26 for which you answered "other" or enter any comments you would like to make related to the questions on this page.

28. Please list and indicate the nature and source of all in-kind contributions your laboratory receives that support AFP and/or ES sample processing (please provide a brief description that

includes the amount, source, and purpose of the in-kind support). If your laboratory provides in-kind support to other laboratories, please provide details about this.
No
Yes (please specify)

29. Did your laboratory experience any significant changes in its workload/workflow in 2016 compared to 2015, if so please describe reasons (e.g., increased/decreased AFP, contact samples, special surveys, serology or clinical trials, introduction of environmental surveillance, implementation of polio laboratory containment and GAP III requirements or other activities, and impacts of changes in financials support, etc.)?
No
Yes (please specify)

30. Does your laboratory expect to make any significant changes in its workload/workflow in the future compared to 2016, if so please describe reasons (e.g., increased/decreased AFP, contact samples, special surveys, serology or clinical trials, or other activities, introduction of environmental surveillance)?
No
Yes (please specify)

31. What other costs or issues related to poliovirus laboratories do you think we should consider? What questions should we ask that we did not ask? Please use this space to make any final comments on the survey. Thank you very much for your responses.

32. Are you ready to submit your completed survey?
No (if not, please make sure to select "Prev" below to go back to the prior questions)
Yes (if so, and only if so, select "Done" below, because you will not be able to make any changes after selecting "Done")

A2. Responding laboratories

We received responses from the following 131 Global Polio Laboratory Network (GPLN) laboratories, organized by World Health Organization (WHO) region, laboratory type, and country of laboratory location:

African Region (15 of 16)
Regional reference laboratories in Central African Republic, Ghana, and South Africa
National laboratories in Algeria, Cameroon, Cote d'Ivoire (note: this lab also serves as the National lab for Mali, Burkina Faso, Liberia, and Sierra Léone), Democratic Republic of the Congo, Ethiopia, Kenya, Madagascar, Nigeria (2: Ibadan, Maiduguri), Uganda (note: this lab also serves as the National lab for Burundi, Rwanda, and the Republic of Tanzania, South Sudan), Zambia, and Zimbabwe (note: this lab also serves as the National lab for Malawi)

Region of the Americas (9 of 11)
Global specialized laboratory in the United States of America
Regional reference laboratory in Brazil

National laboratories in Canada, Columbia, Cuba, Mexico, Trinidad and Tobago, and Venezuela
Subnational laboratory in Brazil

Eastern Mediterranean Region (12 of 12)
Regional reference laboratories in Egypt, Kuwait, Pakistan, and Tunisia
National laboratories in Iran, Iraq, Jordan, Morocco, Oman, Saudi Arabia, Sudan, and the Syrian Arab Republic

European Region (39 of 48)
Global specialized laboratories in France and the Netherlands
Regional reference laboratories in Finland, Italy, and the Russian Federation
National laboratories in Albania, Austria, Belarus, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, France, Georgia, Greece, Hungary, Ireland, Israel, Kazakhstan, Latvia, Lithuania, Moldova, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Switzerland, Turkey, Ukraine, United Kingdom, and Uzbekistan
Subnational laboratories in Russian Federation (Khabarovsk), Turkey, and Ukraine (Odessa)

Note: At the time of the survey, we did not contact the National lab in the Democratic People's Republic of Korea because it was considered dormant (i.e., no active or known contact)

South East Asia Region (14 of 16)
Global specialized laboratory in India
Regional reference laboratories in Sri Lanka and Thailand
National laboratories in Bangladesh, India (6 – Bangalore, New Delhi, Ahmedabad, Kasauli, Kolkata, and Lucknow), Indonesia (3 - Bandung, Jakarta, Surabaya), and Myanmar

Western Pacific Region (42 of 43)
Global specialized laboratory in Japan
Regional reference laboratories in Australia and China
National laboratories in China (Hong Kong), Malaysia, Mongolia, New Zealand, Philippines, Republic of Korea, Singapore, and Viet Nam (2 – Hanoi, Ho Chi Minh)
Subnational laboratories in China (30 – Anhui, Beijing, Chongqing, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Hainan, Hebei, Heilongjiang, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Jilin, Liaoning, Neimengu, Ningxia, Qinghai, Shaanxi, Shandong, Shanghai, Shanxi, Sichuan, Tianjin, Xinjiang, Yunnan, and Zhejiang)

In addition to these GPLN laboratories, we received a response from the Concentration-only laboratory in Niger.

A3. Technical details for analysis

Adjustment for under-reporting of (shared) consumable costs

When both the (shared) consumable supply costs per reported virus isolation test equaled less than $20 and the absolute (shared) consumable supply costs equaled less than $400, we multiplied the reported costs by the reported number of virus isolation tests. The second

condition served to ensure no undue multiplication by the number of virus isolation tests for some laboratories with very large numbers of reported virus isolation tests but modest reported (shared) consumable supplies. This approach resulted in multiplication by the number of virus isolation tests of the reported consumable and shared consumable supplies for AFP sample processing for 59 and 25 laboratories, respectively. The results remained robust to choices of the thresholds of $20 and $400. With the exception of two laboratories that clearly reported (shared) consumable supplies per sample for ES sample processing, we did not adjust any of the reported (shared) consumable supply costs for ES sample processing.

Adjustments to account for missing data

As described in the main text, we separated the cost categories into non-zero categories (NZCs) and possible zero categories (PZCs). Some respondents indicated challenges in separating AFP and ES sample costs, and others explicitly indicated that they reported only the combined costs. This led us to pre-process the data from these laboratories. Based on the average total costs per sample processed for virus isolation reported among all laboratories that provided separate costs for AFP and ES, we assume that, on average, ES samples require seven times the cost per virus isolation test as AFP samples. Specifically, for costs in the NZCs, if a laboratory reported non-zero costs for AFP processing and either indicated that they combined AFP and ES costs or reported zero recurring or set-up ES costs for the cost category, then we estimated the portion of reported AFP costs attributable to ES based on the number of ES samples processed for virus isolation times seven, divided by the total samples (i.e., the number of ES samples times seven plus the number of AFP samples processed for virus isolation). We then subtracted the estimated ES-attributable costs from the reported AFP costs. For PZCs, we estimated and subtracted the ES-attributable costs only if the laboratory reported non-zero AFP costs and explicitly indicated that they combined ES and AFP costs (i.e., not if they reported 0 ES costs for the category). Recognizing uncertainty about the true ratio of costs per sample processed for virus isolation for ES compared to AFP samples, we explored the impact of varying this ratio from three to ten.

In addition to making assumptions to separate combined cost estimates, we further treated the data differently depending on the type of cost category. For NZCs, we interpreted any response not corresponding to a positive number as a missing estimate requiring estimation (i.e., even if a laboratory responded with 0, we interpreted this as an indication that the laboratories did not have access to the data required to estimate the costs). For PZCs, we interpreted zeroes, blanks, or any text indicating an inability to estimate the costs (e.g., not applicable, unknown, unable to estimate) as a true zero. For these categories, we only estimated costs for non-responding laboratories or laboratories that did not provide an estimate for any of the cost categories in the corresponding question according to the logic shown in Table A1 for AFP and Table A2 for ES.

Table A1: Logic for interpretation of AFP cost responses (after any subtractions as a result of logic in Table A2)

Value	Type of cost category	Interpretation	Treatment
Non-response or no cost provided for entire question	Any	No information available	Estimate based on regression
Positive number	Any	Laboratory-estimated value available	Keep response (influence regression)
Zero	PZC	True zero	Keep as 0 (influence regression)
	NZC	Costs not actually zero	Estimate based on regression
Other text (e.g., unknown)	PZC	Costs actually zero	Set to 0 (influence regression)
	NZC	Non-zero costs, but unknown	Estimate based on regression

NZC, non-zero (cost) category; PZC, possible zero (cost) category

Table A2: Logic for interpretation of ES recurring cost responses

Value	Type of cost category	Corresponding set-up cost category	Corresponding AFP cost category	Interpretation	Treatment
Non-response or no cost provided for entire question	Any	Any	Any	No information available	Estimate based on regression
Positive number	Any	Any	Any	Laboratory-estimated value available	Keep response (influence regression)
Zero	PZC	Any	Any	True zero	Keep as 0 (influence regression)
	NZC	Positive number	Any	Assume cost included in set-up costs	Keep as 0 to avoid double-counting (influence regression)
	NZC	Not a positive number	Positive number	Assume costs included in AFP costs	Estimate based on ES-attributable costs, then subtract from corresponding AFP cost category
	NZC	Not a positive number	Not a positive number	Non-zero costs, but unknown	Estimate based on regression
Respondent indicated cost included in AFP costs	PZC	Any	Positive number	Assume included in AFP costs	Estimate based on ES-attributable costs, then subtract from corresponding AFP cost category
	PZC	Any	Not a positive number	Costs actually zero	Set to 0 (influence regression)
	NZC	Any	Positive number	Assume included in AFP costs	Estimate based on ES-attributable costs, then subtract from corresponding AFP cost category
	NZC	Any	Not a positive number	Non-zero costs, but unknown	Estimate based on regression (but do not subtract from corresponding AFP cost category)
Other text (e.g., unknown)	PZC	Any	Any	Costs actually zero	Set to 0 (influence regression)
	NZC	Any	Any	Non-zero costs, but unknown	Estimate based on regression

NZC, non-zero (cost) category; PZC, possible zero (cost) category

A4. Other findings

Other diseases

Table A3 show the breakdown of polio-supported staff time spent on polio and non-polio diseases by WHO region. Only 1 of 132 (1%) of laboratories that responded to the survey did not provide estimates for the total number of polio-supported FTEs or the percentages spent on polio and other diseases. Overall, polio-supported staff spent approximately 30% of time supporting activities for other diseases or viruses, including non-polio enteroviruses (11%), measles and/or rubella viruses (7%), and a wide range of other diseases not specifically asked about in the survey (5%). The American (41%) and European (46%) regions reported the lowest percentages of staff time spent on polio. The Eastern Mediterranean region (87%), which includes one laboratory serving two polio-endemic countries (i.e., Afghanistan and Pakistan), reported the highest percentage.

Respondent laboratories collectively reported spending 41 FTEs on diseases/conditions not specifically listed in Table A3. The laboratories reported that these other diseases/conditions included TORCH, exanthemal infections, urogenital, immunology, intestinal and parasitic infection groups, human immunodeficiency virus, hepatitis, acute respiratory viral infections, teratogenic infections, mycoplasma, chlamydophyll, transgenic organisms control, astrovirus, norovirus, sapovirus, adenovirus, rabies, non-influenza respiratory diseases, non-rotavirus acute gastroenteritis, herpes group viruses, mumps, rhinovirus, parainfluenza virus, respiratory syncytial virus, metapneumovirus, parechovirus, polyomavirus, varicella virus, diphtheria, tetanus, pertussis, cytomegalovirus, crystalli, parotitis, severe fever with thrombocytopenia syndrome, meningitis, and encephalitis.

Table A3: Staff time spent on polio and non-polio diseases by World Health Organization Region for staff supported by funding for polio (i.e., polio-supported staff)

Disease/virus	Number (%) of employee full-time equivalents, by World Health Organization region (N=number of responses)						
	European (N=39)	Western Pacific (N=42)	Southeast Asian (N=14)	African (N=15)	Eastern Mediterranean (N=12)	American (N=8)	All (N=130)
Polio	59 (46)	83 (60)	171 (82)	137 (83)	83 (87)	25 (41)	558 (70)
Non-polio enteroviruses	30 (23)	24 (18)	11 (5)	5 (3)	3 (3)	15 (24)	88 (11)
Measles and/or rubella viruses	7 (5)	13 (9)	22 (10)	14 (9)	3 (3)	1 (1)	59 (7)
Rotavirus	5 (3)	4 (3)	3 (1)	2 (1)	2 (2)	1 (2)	16 (2)
Influenza	12 (9)	3 (2)	1 (0)	2 (1)	1 (1)	1 (1)	20 (3)
Japanese encephalitis	0 (0)	4 (3)	0 (0)	0 (0)	0 (0)	0 (0)	4 (1)
Yellow fever	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	2 (0)
Other arboviruses or hemorrhagic fever viruses	2 (2)	1 (0)	0 (0)	1 (0)	0 (0)	1 (1)	4 (1)
Other	15 (11)	5 (4)	2 (1)	1 (1)	4 (4)	14 (22)	41 (5)
All diseases	129	137	209	164	95	57	792

Other types of polio laboratory tests

Laboratories reported performing several other types of laboratory tests, including ELISA, PCR, RT-PCR, HBsAg, microtitration, genotyping, and serology for numerous viruses and on various sample types (i.e., sera, nasopharyngeal washings, blood, feces, urine, urogenital scrapings, sectional material, mites, spinal fluid, rectal swab and vomitus from diarrhea and food poisoning cases, ice and drinking water, soil) as well as virus isolation on fecal samples from AFP cases over age 15, AFP samples from provinces outside of the areas normally served by the laboratory, fecal samples from non-AFP patients not part of a survey, and research activities.

Table A4 summarizes the reported number of samples or isolates processed in the context of different activities. The difference between the number of concentrates and the number of isolates for ES probably comes from laboratories that (re)tested samples already concentrated by another laboratory, including third-party laboratories not part of the GPLN. A much larger fraction of isolates from ES samples compared to AFP samples underwent Intratypic differentiation (ITD) testing (54%) and sequencing (15%), probably because ES samples comprise a composite from potentially thousands of individuals and they often yield complex mixtures of viruses. This results in higher costs on a per-sample basis for ES than AFP, with ES sample processing additionally requiring three times as many cell cultures as the AFP sample processing. As shown in Table A4, laboratories also reported analyzing almost 2,000 ES samples in the context of research activities and 82 ES samples using direct detection methods.

Forty responding laboratories further reported analyzing over 50,000 serum samples for the presence of antibodies, which they estimated took almost 13,000 employee hours (i.e., 12.7 FTEs assuming 2,000 employee hours per year). Laboratories analyzed almost 40,000 samples in the context of non-polio enterovirus surveillance and approximately 150,000 other samples, reflecting the reality that many GPLN laboratories perform non-polio services (not necessarily funded by polio surveillance), particularly in countries with no recent polio outbreaks. While 49 laboratories reported testing other samples, 3 of these laboratories accounted for 83% of the 150,000 samples and indicated that their reported numbers included routine diagnostic services. Laboratories also reported analyzing approximately 6,900 and 4,300 samples in the context of healthy children or adult stool surveys and clinical trials, respectively.

Table A4: Reported number of samples/isolates processed for different activities

Activity	Nature of testing/activity	Number of samples/isolates
Acute flaccid paralysis surveillance	Virus isolation	243,897
	Intratypic differentiation	10,380
	Sequencing	751
	Other[a]	925
Environmental surveillance	Concentration (two-phase method)	5,509
	Concentration (other methods)	2,703
	Virus isolation	12,170
	Intratypic differentiation	6,638
	Sequencing	1,847
	Research	1,971
	Direct detection	82
Serology	Serum antibody testing	52,020
Other	Non-polio enterovirus surveillance	38,589
	Healthy children/adults surveys	6,907
	Clinical trial support	4,337
	Other[b]	149,345

[a] Includes serotyping and polymerase chain reaction analysis of non-polio enteroviruses identified in acute flaccid paralysis cases, Sanger sequencing, and next generation sequencing of complete genomes

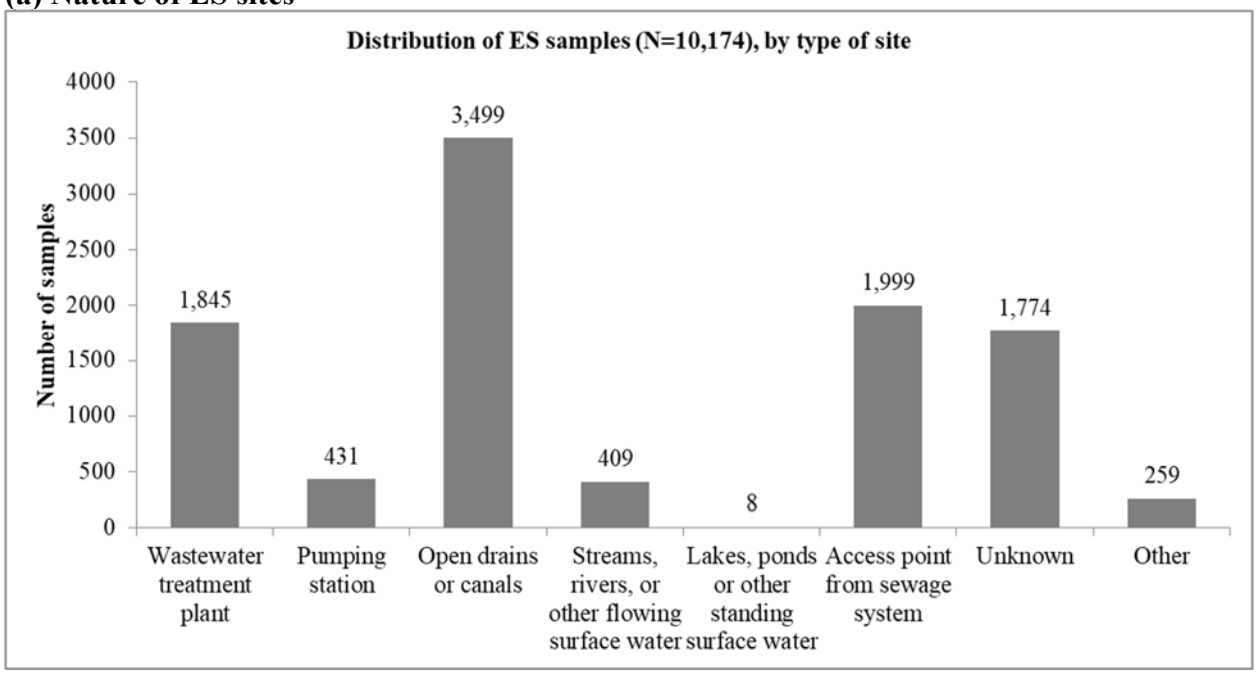
[b] See text

Additional results related to ES sampling

Figure A1 summarizes characteristics of the ES systems based on reported results for approximately 10,000 ES samples (the total numbers of samples differ from Table A4 due to incomplete responses for some (sub)questions and possible double-counting of samples analyzed by multiple laboratories through the referral system). The majority of ES samples came from open drains or canals (34%), followed by other access points from sewage systems (19%), wastewater treatment plants (18%), and unknown sources (18%). Eighty percent of samples started processing for virus isolation within 5 days of sample collection, which likely reflects the routine handling of ES samples collected in the context of ongoing ES (see Figure A1b). However, the reported 6% of samples taking more than 35 days until virus isolation began suggests a long tail of the distribution of transportation and processing delays (Figure A1b). The delays may relate to a supply shortage situation during the rapid global expansion of ES, which efforts to streamline quality assurance and quality control may limit as the system become more established. Moreover, ES conducted in the context of research activities may follow different timelines.

Figure A1: Reported results related to the ES systems

(a) Nature of ES sites



(b) Distribution of duration from sample collection to beginning of processing for virus isolation