



Regional Polio Reference Laboratory Check List for Annual WHO Accreditation

Introduction

Detection and laboratory evaluation of acute flaccid paralysis (AFP) at an annual non-polio rate of $>1/100,000$ in children less than 15 years is the standard for certifying polio eradication for all countries. Tests for virus isolation are performed on two adequate stool specimens collected within 14 days of onset of paralysis, and 24-48 hours apart, from each AFP patient. Supplemental virus surveillance may consist of specimens from contacts of AFP cases, special surveys of healthy children, and the environment, as appropriate. Test results are accepted only from a WHO accredited Poliovirus Laboratory.

Accreditation provides documentation that the Regional Reference Laboratory has the capability and the capacity to detect, identify, and promptly report wild polioviruses and suspected vaccine derived polioviruses (VDPV) that may be present in any specimen. The term “suspected VDPV” in this document refers to poliovirus isolates with discordant results when tested by two intratypic differentiation methods (one antigenic and the other molecular).

Accreditation is reviewed annually by WHO and is based on Laboratory performance during the preceding 12 months with complete data, usually, from 13 months to 1 month prior to evaluation. Accreditation is given for the upcoming calendar year.

Five criteria for accreditation:

1. Intratypic differentiation test results are reported to the Programme and the Regional Laboratory Coordinator on $\geq 80\%$ of all AFP poliovirus isolates within 14 days of receipt or completion of typing if specimens processed in that Laboratory.

This criterion applies to isolates from AFP cases and contacts of AFP cases from investigations. Virus mixtures or viruses re-isolated from referred stool suspensions or original stool samples may require longer than 14 days. ITD tests should be performed on poliovirus isolates from all sources, but those from AFP cases and their contacts should be given highest priority.

2. Wild poliovirus and suspected VDPV isolates from $\geq 90\%$ of AFP cases and contacts are referred for sequencing within 7 days of detection.

This criterion applies to isolates from AFP cases and contacts of AFP cases from investigations. It is essential that the polio eradication programme be able to get information about the characteristics of wild poliovirus or VDPV isolates as soon as possible. Wild polioviruses and suspected VDPV isolates must be forwarded without delay to a Specialized Polio Reference Laboratory for sequencing

3. The score is $\geq 90\%$ on the most recent WHO poliovirus intratypic differentiation proficiency test (PT).

PT results must be reported within 14 days of panel receipt to receive full credit.

4. The score is $\geq 90\%$ on the most recent WHO poliovirus isolation/ identification Proficiency Test (PT). PT results must be reported within 28 days of panel receipt to receive full credit.

5. The score from the annual on-site review of laboratory operating procedures and practices is $\geq 90\%$.

For those that also serve as National Laboratories:

6. Test results are reported to the program on $\geq 80\%$ of AFP specimens within 28 days of receipt.

Viruses that appear late in passage, virus mixtures, or viruses that present typing difficulties may require longer than 28 days.

7. Internal quality control (QC) procedures for L20B and RD cell culture sensitivity are implemented in accordance with the WHO protocol.

Ideally cell line sensitivity should be known for all frozen stocks and evaluated whenever fresh cells are resuscitated or received in the laboratory. It is recommended that cells are evaluated at least midway through their expected use of 15 passages. Assessing sensitivity before discarding at 15 passages can reassure the laboratory that sensitivity has been maintained throughout the period of use, but is not essential for accreditation. Original QC data sheets and summaries of corrective action are retained for documentation and discussion with reviewer.

The annual non-polio enterovirus (NPEV) isolation rate from all stool specimens:

It is not a criterion for accreditation because of the variability of findings influenced by season of the year, elevation, population density or socioeconomic level of geographic areas served by the Laboratory. However, it may be a useful indicator of laboratory performance and should be discussed with the reviewer. The NPEV isolation rate in most tropical countries typically exceeds 10%.

For Laboratories with consistently high annual accreditation scores, the Global Laboratory Coordinator may waive the on-site review upon satisfactory completion of the annual checklist by the Laboratory.

Laboratories that perform test procedures other than those recommended by WHO should provide documentation that such procedures, when used in their laboratories, are equivalent in specificity and sensitivity to WHO poliovirus procedures.

A Laboratory that achieves less than the passing score on any one of the applicable five or seven criteria will work with the Global Laboratory Coordinator to:

- Identify areas where improvement is needed.
- Develop and implement a work plan.
- Monitor laboratory progress.
- Provide for additional PT tests where required.
- Provide for re-testing when required.
- Continue after steps to achieve full accreditation.

A Laboratory that fails to achieve passing PT testing scores within 6 months after annual review is deemed non-accredited and must arrange for an accredited Laboratory to perform duplicate tests on all specimens.

The checklist consists of four parts. **Part I** summarizes the findings of the review and the data on which accreditation is based. **Part II** provides a worksheet to calculate and record Laboratory performance for **criteria #1 through #4**. **Criteria #6, #7** and the NPEV isolation rate are for those that also serve as National Laboratories. Selection of the most recent 12-month period, usually from 13 months to one month prior to evaluation, provides an assessment of current performance and permits review of Laboratories at any time during the calendar year. **Part III** provides a profile of the Laboratory. **Part IV** is a checklist for evaluation of laboratory operating procedures and practices for **criterion #5**.

This checklist is intended as a guide. The experienced reviewer is expected to be flexible, ask detailed questions and make recommendations as appropriate to assure high quality laboratory performance.



Regional Polio Reference Laboratory Check List for Annual WHO Accreditation

Laboratories are to be notified in advance of the accreditation review and provided a copy of this form to assist in gathering information

Dates of Review:		Accreditation for Calendar Year:	
Laboratory:			
Address:			
Phone:	Fax:	E-mail:	
Head of Department:			
Head of Laboratory:			
Technical Supervisor:			
Reviewers:			
Name of National Accrediting Authority and current accreditation status:			

Part I: Summary of Review

Recommendations (check one):

- Accredited: Laboratory meets all criteria
- Provisionally accredited: Laboratory passed the most recent proficiency test, but failed to achieve one or more of the remaining criteria
- Do not accredit: Laboratory did not pass the most recent proficiency test

Findings:

1.	ITD test results on $\geq 80\%$ of poliovirus isolates and referrals are reported within 14 days:	%
2.	Wild poliovirus and suspected VDPV isolates from $\geq 90\%$ of AFP cases and contacts are referred for sequencing within 7 days of detection.	%
3.	Score on most recent intratypic differentiation PT is at least 90%:	%
4.	Score on most recent isolation/ identification PT is at least 90%: PT test score for ITD method 1 (Specify method):	%
	PT test score for ITD method 2 (Specify method):	%
5.	Score on annual on-site review is at least 90%:	%

For those that also serve as National Laboratories:

6.	Test results on at least 80% of AFP clinical specimens are reported within 28 days:	%
7.	Internal quality control procedures for cell cultures are implemented:	%

Annual NPEV isolation rate is:	%
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SUMMARY, COMMENTS AND RECOMMENDATIONS:

Part II: Laboratory Performance in Previous 12 Months

Dates from: $\frac{\quad}{d} / \frac{\quad}{m} / \frac{\quad}{y}$ to $\frac{\quad}{d} / \frac{\quad}{m} / \frac{\quad}{y}$

1.	Percentage of intratypic differentiation (ITD) test results on poliovirus isolates from AFP cases and contacts reported to the Programme and the Regional Laboratory Coordinator within 14 days of receipt or completion of typing if specimens processed in this Laboratory:	%
1.1	Number polioviruses received and/or isolated:	
1.2	Number with all ITD test results reported within 14 days:	

COMMENTS AND RECOMMENDATIONS:

2.	Wild poliovirus and suspected VDPV isolates from ≥ 90 % of AFP cases and contacts are referred for sequencing within 7 days of detection.	%
2.1	Number polioviruses received and/or isolated:	
2.2	Number of wild polioviruses detected:	
2.3	Number of suspected VDPV detected:	
2.4	Number of wild polioviruses and suspected VDPV (total of 2.2 and 2.3) referred for sequencing within 7 days of detection:	

COMMENTS AND RECOMMENDATIONS:

3.	Score of the most recent poliovirus ITD proficiency test for Regional Reference Laboratories:			%
ITD method	Date panel received	Date results reported	Score for ITD PT test	
ELISA				
Probe hybridization				
PCR				

Nature of deficiency, if any, and corrective action taken:

COMMENTS AND RECOMMENDATIONS:

4.	Score of the most recent poliovirus isolation/ identification proficiency test:				%
4.1	Date of panel receipt:	/	/		
4.2	Date of test report:	/	/		

Nature of deficiency, if any, and corrective action taken:

COMMENTS AND RECOMMENDATIONS:

For those that also serve as National Laboratories:

5.	Percentage of AFP Test Results Reported within 28 Days:				%
5.1	Number of stool specimens from AFP cases tested:				
5.2	Number with isolation and identification test results reported within 28 days:				

COMMENTS AND RECOMMENDATIONS:

6.	Routine Internal Quality Control Procedures Implemented:				
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COMMENTS AND RECOMMENDATIONS:

7.	Non-polio Enterovirus (NPEV) isolation rate from stools:				%
7.1	Total number of NPEV isolates:				
7.2	Total number of stools tested:				

COMMENTS AND RECOMMENDATIONS:

Part III: Laboratory Profile

1.	Staff
1.1.	Number of scientific and technical staff assigned to poliovirus laboratory: Please list according to function, indicating years of polio laboratory experience and proportion of current working time spent on polio-related activities.

Names of staff	Position Title or Duties	Full-time or Part-time	% of time spent working on polio	Years of experience in Polio Lab

COMMENTS AND RECOMMENDATIONS:

2.	Space (provide floor plan or sketch of laboratories if possible)	
2.1.	Total m ² available:	
2.2.	Number of rooms:	
2.3.	Separate cell culture room:	

COMMENTS AND RECOMMENDATIONS:

3. Reference Laboratory activities
 3.1. Countries/National Laboratories submitting specimens and/or isolates:

Name of Country / National Laboratory	# clinical specimens	# virus isolates

3.2. Training activities performed:

Details of training activities performed:

3.3. Reagents, materials, and supplies provided or distributed:

Details of reagents, materials and supplies provided:

4. Specialized Global Laboratory(s) to which wild poliovirus and suspected VDPV isolates are submitted for confirmation and/or sequencing:

Name of Laboratory	Number of isolates	% Results confirmed

COMMENTS AND RECOMMENDATIONS:

Part IV: Laboratory Operating Procedures and Work Practices

1.	Space (3%)	Score:	
1.1.	Space is used efficiently with appropriate equipment placement:		
1.2.	Space configuration is adequate and consistent with good laboratory practices:		
1.3.	Space is clean and well kept:		

COMMENTS AND RECOMMENDATIONS:

2.	Staff (4%)	Score:	
2.1.	Staff are effectively assigned:		
2.2.	The number of trained staff are adequate to handle the workload:		

COMMENTS AND RECOMMENDATIONS:

3.	Supervision (4%)	Score:	
3.1.	The lines of supervision and accountability are clear:		
3.2.	Supervisor critically reviews test results:		

COMMENTS AND RECOMMENDATIONS:

4.	Cell Lines (8%)	Score:	
4.1.	Written appropriate protocols are available for:		
	a. Freezing and recovery of cells:		
	b. Routine passage of cells:		
4.2.	RD and L20B are in use:		
4.3.	Both cells are available for inoculation weekly:		
4.4.	Cells are obtained from approved WHO stocks:		
4.5.	Low passage stocks are stored in liquid nitrogen:		
4.6.	Cells are routinely replaced at least after 3 months or 15 passages:		
4.7.	Monolayers remain healthy for at least 5 days:		
4.8.	Cells are passaged and maintained in space separate from that used for specimen processing and virus inoculation:		
4.9.	Media and cells are prepared at separate times:		
4.10.	Permanent records are maintained on cell passage and storage histories:		
4.11.	Reagents and stock solutions are labeled correctly, including dates of preparation and expiration, and stored at indicated temperatures:		

COMMENTS AND RECOMMENDATIONS:

5.	Stool Specimens (5%)	Score:	
5.1.	A written protocol for processing specimens is available:		
5.2.	Specimens are processed by chloroform extraction in accord with WHO protocols:		
5.3.	Extracts are stored at -20°C if not inoculated on same day as processed:		
5.4.	All potentially infected clinical materials are processed in a biological safety cabinet:		
5.5.	Original specimens (in original containers) are appropriately labeled and stored at -20°C for at least 12 months:		
5.6.	Stool extracts are discarded within 3 months of confirmation of result:		
5.7.	Specimens, extracts, all virus isolates, and other potentially infectious materials are stored separately from non-infectious materials in designated freezers and refrigerators:		

COMMENTS AND RECOMMENDATIONS:

6.	Virus Isolation (12%)	Score:	
6.1.	Written protocols are available:		
6.2.	Extracts are inoculated within 7 days of processing:		
6.3.	Extracts are inoculated on duplicate RD and L20B cell cultures at the same time:		
6.4.	RD(+)/L20B(-) isolates are passed into L20B cells:		
6.5.	Aerosol resistant tips (ART) with micropipettors, or, cotton plugged sterile pipettes, are used for inoculation and manipulation of cell cultures		
6.6.	The first and second specimens from each patient are processed and inoculated separately, never combined:		
6.7.	Records are maintained on daily observations of inoculated cells:		
6.8.	Two sequential passages of 7-10 days are performed in RD and L20B cell lines before recording as negative (minimum time in culture is 14 days total):		

COMMENTS AND RECOMMENDATIONS:

7.	Virus Identification (12%)	Score:	
7.1.	Appropriate written protocols are available:		
7.2.	WHO approved poliovirus typing sera are used:		
7.3.	WHO typing sera are diluted as recommended, labeled appropriately, and stored in aliquots at -20°C :		
7.4.	Other typing sera, if used, are documented to be equivalent in specificity and sensitivity to WHO sera:		
7.5.	Virus typing worksheets are retained as permanent records:		
7.6.	Isolates are stored at -20°C or lower for at least 12 months:		
7.7.	Storage vials are clearly and permanently labeled:		
7.8.	Permanent records are maintained on the identity and location of all isolates:		

COMMENTS AND RECOMMENDATION:

8.	Intratypic Differentiation (12%)	Score:	
8.1.	All poliovirus isolates and referrals are subjected to two intratypic differentiation methods consisting of:		
	Method 1:		
	Method 2:		
8.2.	Written protocols are available and consistent with laboratory practices:		
8.3.	Appropriate positive and negative controls are used in all tests:		
8.4.	Wild poliovirus reference strains are those that can be readily identified by independent molecular methods:		
8.5.	Work sheets are retained as permanent records:		
8.6.	Isolates giving discordant or indeterminate results are reported to the Regional Laboratory Coordinator within one working day and referred to the appropriate Specialized Reference Laboratory within 7 days:		

COMMENTS AND RECOMMENDATIONS:

9.	Safe handling of wild polioviruses and materials potentially infected with wild polioviruses. (10%)	Score:	
9.1.	An internal control system of permanent records is maintained for all wild polioviruses and materials potentially infected with wild polioviruses, including final disposition:		
9.2.	All wild poliovirus isolates and materials potentially infected with wild polioviruses, not of programmatic importance, are properly disposed of within 1 year:		
9.3.	Wild polioviruses and original materials retained by the laboratory are stored in separate, clearly marked containers with limited (locked) access:		

COMMENTS AND RECOMMENDATIONS:

10.	Biosafety (8%)	Score:	
10.1.	Employees have been instructed in biosafety:		
10.2.	Written instructions are available to all employees:		
10.3.	Biosafety practices are enforced, including:		
	a. Hand washing:		
	b. Pipetting with aid of mechanical device:		
	c. Routine use of gloves and lab coats		
	d. No eating, drinking, smoking, or storage of food in laboratory		
	e. Decontaminating all infectious or clinical waste before discarding:		
	f. Decontaminating lab work surfaces:		
	g. Immunizing staff against polio:		
10.4.	Biosafety cabinets and clean air cabinets are used for potentially infected and clean materials, respectively:		
10.5.	Safety cabinets are maintained as recommended, including filter changes, and dates recorded:		

COMMENTS AND RECOMMENDATIONS:

11.	Cooperation with Regional Offices (8%)	Score:	
11.1.	Test results are reported to the Regional office and the submitting Laboratory within one working day of satisfactory completion, or as requested by the Regional Office:		
11.2.	Summary data are reported to the Regional office with the agreed frequency and format:		

COMMENTS AND RECOMMENDATIONS:

12.	Cooperation with EPI Staff by those serving as National Laboratories (8%)	Score:	
12.1.	Lab and EPI staff communicate/meet at least monthly:		
12.2.	EPI staff are contacted if specimens arrive without adequate information or EPID numbers:		
12.3.	Lab staff member(s) serve on:		
	a. AFP review committees:		
	b. NID planning committees:		

COMMENTS AND RECOMMENDATIONS:

13.	Virus isolation data base (6%)			Score:	
13. 2	The following data are available on all polio and non-polio enterovirus isolates:				
Variable description		Virus Isolation Lab		Intratyptic Differentiation Lab	
		Needed	Available	Needed	Available
Epidemiology and case identification					
EPID no.		✓		✓	
Specimen no. from virus isolation lab		✓		✓	
Specimen no. from intratyptic differentiation lab				✓	
Name of patient		✓		✓	
District/municipality code of patient		✓		✓	
Province/state code of patient		✓		✓	
Country code of patient		✓		✓	
Date of last OPV		✓			
Specimen number (i.e. 1 st or 2 nd)		✓		✓	
Specimen source		✓			
Date of paralysis onset		✓		✓	
Date of specimen collection		✓			
Lab doing virus isolation				✓	
Virus isolation					
Condition of stool upon arrival		✓			
Date stool inoculated		✓			
P1 isolated		✓			
P2 isolated		✓			
P3 isolated		✓			
Non-polio enterovirus isolated		✓			
Poliovirus(es) isolated but type(s) unknown		✓		✓	
Date typing results available		✓			
Date typing results reported to EPI		✓			
Intratyptic differentiation					
Date isolate inoculated				✓	
Date isolate sent for intratyptic differentiation		✓*			
Date isolate received in lab for intratyptic differentiation				✓*	
Name of intratyptic differentiation lab		✓*			
P1 intratyptic differentiation		✓		✓	
P2 intratyptic differentiation		✓		✓	
P3 intratyptic differentiation		✓		✓	
Date intratyptic differentiation results available		✓		✓	
Date intratyptic differentiation reported to EPI				✓	
Sequencing information					
Poliovirus isolate sent for sequencing				✓	
Date poliovirus referred for sequencing				✓	
Name of sequencing laboratory				✓	
Date sequencing results available				✓	
Date sequencing results reported to EPI				✓	

*Not applicable if virus isolation and intratyptic differentiation performed in same lab

Note: For those Laboratories that do not serve as National Laboratories, 100% equals 92 out of 92.

Total Score: