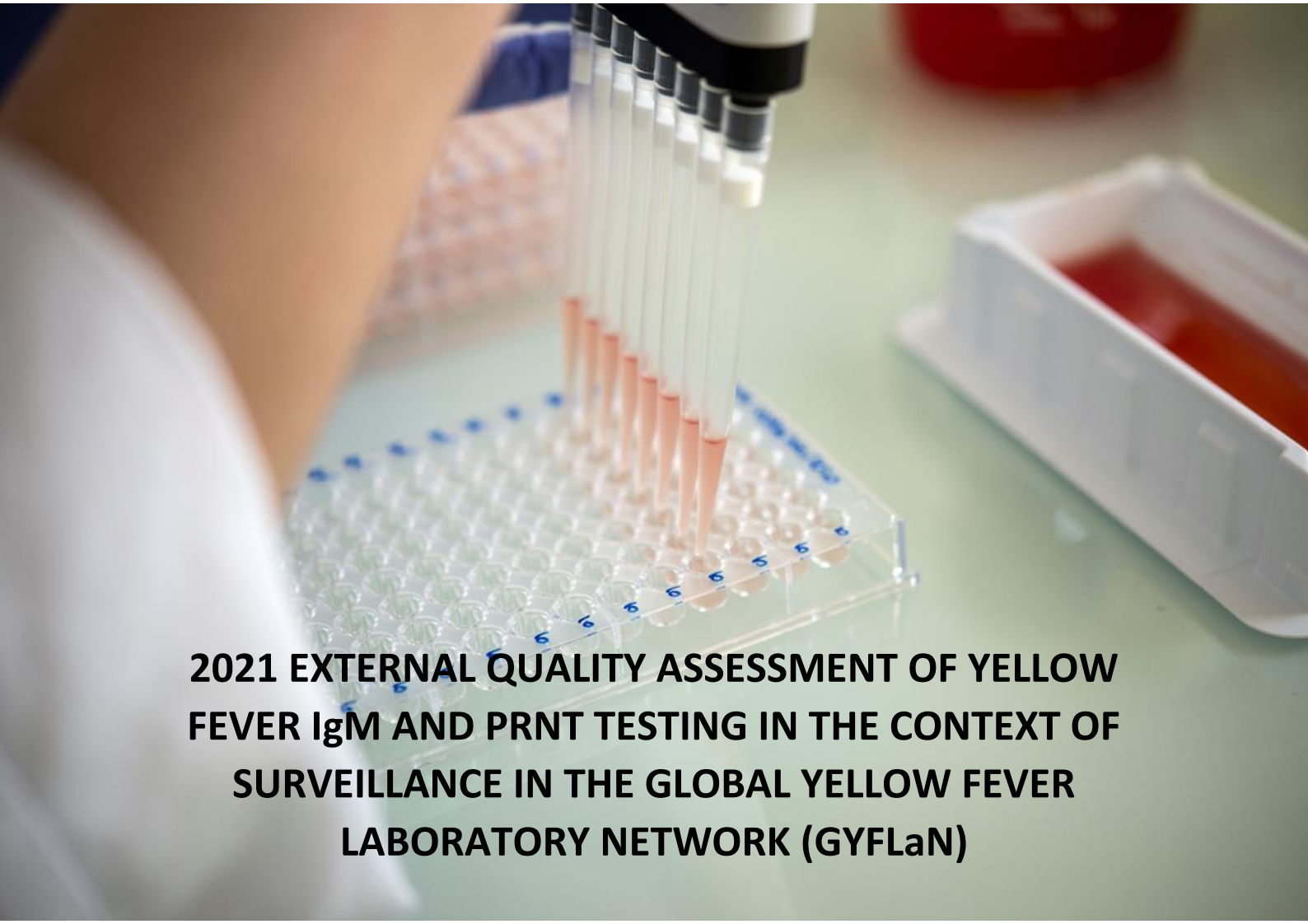
EYE-LTWG Technical Report Series



2021 EXTERNAL QUALITY ASSESSMENT OF YELLOW FEVER IgM AND PRNT TESTING IN THE CONTEXT OF SURVEILLANCE IN THE GLOBAL YELLOW FEVER LABORATORY NETWORK (GYFLaN)

EYE Strategy Laboratory Technical Working group (EYE-LTWG)

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2. Abbreviations

CDC-FC: Centers for Disease Control and Prevention, Fort Collins

DENV: Dengue virus

DX: Diagnostics

ELISA: Enzyme-linked Immunosorbent Assay

Erasmus MC: Erasmus Medical Center

EQA: External quality assessment

EYE: Eliminating Yellow Fever Epidemics

Fiocruz-RJ: Fundacão Oswaldo Cruz-Rio de Janeiro

GYFLaN: Global Yellow Fever Laboratory Network

IEC: Instituto Evandro Chagas

IgM: Immunoglobulin M

IIF: Indirect Immunofluorescence

IPD: Institut Pasteur de Dakar

LTWG: Laboratory Technical Working Group

MAC-ELISA: IgM antibody capture ELISA

MAC-HD ELISA: IgM antibody capture ELISA (half-day testing) provided by CDC-FC

OD: Optical Density

PRNT: Plaque Reduction Neutralization Assay

RKI: Robert Koch Institute

SOP: Standard operating procedure

WHO: World Health Organization

YF: Yellow fever

YFV: Yellow fever virus

3. Introduction

Yellow fever (YF) virus belongs to the *Flavivirus* genus, family *Flaviviridae.* YF is an arboviral disease endemic in 47 countries in tropical areas of sub-Saharan Africa (34 countries) and the Americas (13 countries) (1) transmitted by mosquitoes of the *Aedes*, *Sabethes* and *Hemagogus spp*. Most human infections arise from spill-over from sylvatic mosquitoes that feed on infected non-human primates, the primary reservoirs of the virus.

In most cases, YF virus infection remains asymptomatic or is associated with mild symptoms (85%); however, in symptomatic patients, the lethality varies (20-60%). In humans, YF is a viscerotropic disease affecting the liver and other tissues such as the kidney, spleen, lymph nodes, and heart. After the incubation period (up to six days), an active infection with flu-like illness appears, and at this stage, higher viral loads are present. A remission period of 48 hours appears around seven days from symptom onset, and most individuals recover at this stage. In severe cases, however, this remission phase will be followed by the toxic phase, where haemorrhagic disease and multi-organ failure will take place.

Despite the availability of an efficient and safe attenuated-YF vaccine, YF remains a persistent public health problem and a growing concern in endemic areas. In 2015 Angola experienced the largest outbreak in Africa in the last 20 years, also affecting the neighbouring Democratic Republic of Congo (1). In Nigeria in 2017, and during the outbreak that started in 2016 in Brazil, the virus spread into areas not previously considered at high risk. From January to March 2022, a total of 53 suspected YF cases, including six deaths, have been reported from central Kenya (2), a country that did not report cases since 2011, showcasing the outbreak risk for East Africa in areas with very low vaccination coverage. The resurgence of the disease has been attributed mainly to lapses of continuous vaccination coverage along with waning population-level immunity (3).

Timely laboratory confirmation of suspected YF cases is essential for effective outbreak control and prevention of further spread, and to mobilise the outbreak response adequately around confirmed cases, including vaccination campaigns (1). Non-reliable laboratory data could lead both to overlook the presence of an outbreak until it is too late to mitigate or, on the contrary, the activation of unnecessary countermeasures involving resources and the waste of vaccine doses from the limited YF vaccine stockpile.

Coordinated by WHO, the EYE Strategy, through its advisory Laboratory Technical Working Group (EYE-LTWG), supports the Global YF Laboratory Network (GYFLaN). The GYFLaN is comprised of 68 laboratories in endemic regions (for a complete list see Annex 1). Since its inception, the EYE-LTWG (see list of contributing institutions in Annex 2) is working on improving the laboratory capacity of the GYFLaN since accurate laboratory diagnosis of YF is a key contribution to informing immunization programs (4).

The EYE-LTWG seeks a coordinated and standardized YF diagnostic approach within the GYFLaN. To that purpose, in recent years, an effort has been made to implement uniform molecular and serological diagnostics for the detection of YF cases. As such, WHO in consultation with the LTWG and other partners, recently led the revision of the recommended testing algorithm, as well as the independent kit performance evaluation programme for new commercial YF assays, including both serologic and molecular assays. The LTWG also plays an important role in technically supporting various regional and national trainings on YF diagnostics with a broad curriculum covering all basic aspects of YF molecular and serological diagnosis including intensive practical sessions.

To better understand the diagnostic capacities, gaps and needs of the GYFLaN laboratories in Africa, WHO-commissioned laboratory assessments were carried out in 2018 in 25 African countries at high risk for YF outbreaks (5). During these assessments, it was apparent that there was a significant variation regarding quality assurance and quality control procedures within the laboratory network. In some laboratories, the only quality control indicator of proficiency was the referral of specimens with positive IgM results, and 10% of specimens with negative results to the regional reference laboratories (RRLs) for confirmation. However, the GYFLaN labs have not participated regularly in external quality assessment (EQA) schemes using YF proficiency panels to allow them to evaluate their performances.

Since YF serological diagnosis is widely implemented in the whole network, an EQA exercise for the serological detection of YF IgM in the GYFLaN laboratories was organized and facilitated by the EYE-LTWG. The main objective of this EQA was to provide an objective measure and overview of the quality of the serological diagnosis and capacities within the GYFLaN. Through this activity, the laboratories were provided with qualified materials for the evaluation of their performances. This EQA exercise reflects the impact of the specific trainings for YF diagnostics in the GYFLaN and will aid in identifying current weaknesses and gaps that will need to be addressed in the near future to assure a solid and homogeneous quality of YF serological diagnosis in the network. It also will provide baseline data to assess any changes to quality serological testing with the introduction of kit-based assays. The exercise will guide future actions for improvement of YF IgM detection in the GYFLaN laboratories performing routine YF diagnosis.

4. Methodology

4.1 EQA organisation and participation summary

The 2021 WHO YF EQA programme was organised by WHO, RKI, and CDC-FC. A special subgroup of experts in the EYE-LTWG was convened to discuss the design and development of the exercise and set up the parameters to be measured. The agreed-upon aim was to produce an IgM proficiency panel of 10-12 samples including YF positives of varying titers, negatives, and a few of non-yellow fever virus origin, plus a PRNT proficiency panel of 5-6 YF PRNT positive, negative, and other flavivirus PRNT-positive samples.

Two different panels were offered depending on the laboratories' capacities; an IgM panel (for detection of probable acute cases) and a PRNT panel for differential diagnosis in laboratories that offer confirmatory testing. The IgM and PRNT panels were prepared at CDC-FC. Before delivering to the laboratories, the panels were externally validated by three network laboratories (see Panel preparation and composition section) and consensus results were generated from the validation testing.

All laboratories in the GYFLaN that routinely perform IgM testing were invited to participate in the YF Serological EQA. Affirmative responses were received from 29 laboratories in 24 African countries and 21 laboratories in 15 countries in the Americas. Two of the 50 laboratories that replied were ultimately unable to accept panel shipment. A total of 48 IgM panels were shipped to 27 laboratories in Africa and 20 laboratories in the Americas (panels were sent twice to one laboratory). PRNT panels were sent to four laboratories in Africa and nine laboratories in the Americas. Instructions and results workbooks were provided to all laboratories, and all EQA results were due to the EYE-LTWG three weeks after receipt of the panels.

A total of 40 laboratories (24 in Africa and 16 in the Americas) submitted results for the EQA. All 40 laboratories[1] sent results for the IgM panel and only five laboratories sent results for the PRNT panel (1 out of 4 labs in Africa, and 4 out of 9 labs in the Americas).

A list of laboratories that sent results for the EQA can be found in Annex 3.

Results were analysed and individually-prepared responses and scores were emailed to the submitting laboratories and WHO regional coordinators in March 2022.

4.2 Panel preparation and composition

To prepare the EQA panels, samples were gathered from YF acute cases and YF-vaccinated individuals. Archived patient samples from Burkina Faso, the Democratic Republic of Congo, Nigeria, Senegal, Cameroon and Uganda were provided by the national yellow fever laboratories in these countries[2]. Erasmus MC and CDC-FC provided serum samples from YF

[1] One of the 24 African labs did not use the required results workbook and results were included in the analysis.

[2] The following institutions kindly provided IgM-positive samples: Institut+ Pasteur de Dakar (Senegal), Nigeria Centre for Disease Control (Nigeria), Centre Pasteur du Cameroun (Cameroon), Uganda Virus Research Institute (Uganda), Centre Muraz (Burkina Faso), Institut National de Recherche Biomédicale (DRC)

vaccinees. As specificity controls for the EQA, samples positive for dengue, Zika and Leptospira were provided by Erasmus MC, CDC-FC and National Institute for Communicable Diseases-NHLS in South Africa, respectively. A pan-flavivirus-positive sample containing chimeric antibodies against a conserved region of the envelope protein of flaviviruses was considered for the panel (6). Negative sera were included as a negative sample. The samples were inactivated by heat (56°C for one hour) and stored frozen before shipment to CDC-FC. Sample quality and IgM titer were estimated on arrival at CDC-FC, and only those providing unequivocal results were used to prepare the panels under CDC IRB protocol #6773.

Archived residual diagnostic samples were used for this EQA and only small volumes were available. Sample pooling was, therefore, necessary to prepare enough panels for the participating laboratories, the pre- and post-exercise testing and validation, and some spares for each WHO region. A total of 79 IgM panels and 21 PRNT panels were prepared. Before panel preparation, pooled samples were tested at CDC-FC to ensure the absence of infectivity using CDC Laboratory Safety Review Board protocols that included plaque assays and 3 blind passages in tissue culture. Some samples were treated again at 56°C for an additional hour to remove residual infectivity and were retested in tissue culture. To reach the needed volume and depending on the IgM titer, samples were diluted using commercially sourced normal human serum (Sigma-Aldrich).

Draft panel samples in their final dilutions were pre-tested before lyophilization to confirm the presence of desired reactivity. Panels were then freeze-dried using 2X Lyophilization Reagent (OPS Diagnostics, Lebanon, NJ) and VirTis Genesis 25EL Pilot Lyophilizer (model: 446496 from SP Scientific, Warminster, Pa), and tested again for confirmation of successful preparation. The in-house CDC MAC ELISA (7) was used for IgM testing. For the PRNT panel, six different neutralisation assays were done (YF, DENV 1-4, Zika) using the CDC-FC protocol based upon Beaty *et al* (8).

The lyophilized IgM and PRNT panels were externally validated at FioCruz-RJ (Brazil), IEC (Brazil) and IPD (Senegal). For the IgM panel, IPD used the CDC IgM MAC ELISA, and IEC and Fiocruz-RJ used in-house ELISAs. For PRNT, Fiocruz-RJ used YF, DENV 1-4 and Zika viruses, and IEC and IPD used YF, DEN 2 and Zika viruses. The results from the external validation were added to those of CDC-FC and a consensus set of results was agreed upon, which formed the reference results for the EQA. The final composition and reference results for the IgM and PRNT panels are provided in Tables 1 and 2 respectively.

An IgM and a PRNT lyophilized panel were kept at -20°C and were tested at CDC-FC at the end of the EQA exercise to confirm sample stability throughout the entire procedure.

Table 1. IgM Panel composition

Sample ID	YF IgM goal	YF IgM dilution titer*	Source of sera	YF IgM consensus result
S#1	NEG	NA	Negative control	NEG
S#2	HIGH POS	>1:12800	YF vaccinee	POS
S#3	NEG	NA	Zika infection	NEG
S#4	NEG	NA	YF vaccinee – IgG	EQ/NEG
S#5	MED/LOW POS	1:3200	YF wild-type infection	POS/EQ
S#6	HIGH POS	>1:12800	YF wild-type infection	POS
S#7	MED POS	6400	YF wild-type infection	POS
S#8	NEG	NA	Leptospira infection	NEG
S#9	MED POS	>1:12800	YF vaccinee	POS
S#10	LOW POS	3200	YF vaccinee	POS/EQ
S#11	HIGH POS	12800	Flavivirus chimera**	POS

* Titer at CDC-FC using the YF CDC MAC ELISA assay

**This was used as a training sample and was not included in the EQA analysis

POS: positive, NEG: negative; EQ: equivocal

Table 2. PRNT Panel Composition

Sample ID	PRNT Titer (1:x)*						Source of sera	Final Interpretation
	YF	Zika	DENV1	DENV2	DENV 3	DENV 4		
S#1	160	<10	<10	<10	<10	<10	YF POS (vaccinee)	YF
S#2	40	<10	<10	<10	<10	<10	YF POS (vaccinee)	YF
S#3	<10	1280	5120	640	640	320	Zika infection** POS	Flavivirus (DENV1)
S#4	320	80	160	20	20	20	YF/Zika infection** POS	Flavivirus
S#5	80	20	40	20	20	10	YF/Zika infection** POS	Flavivirus
S#6	<10	<10	<10	<10	<10	<10	NEGATIVE Control	Negative

*Titers at CDC-FC; **likely secondary to dengue infection

4.3 Shipping and submission of results and accompanying data

The IgM and PRNT panels were shipped at 4°C with instructions on resuspending the samples for use in the assays. Laboratories received an Excel-based workbook as a standardized template for reporting results obtained in the EQA, which also included a copy of the instructions. The laboratories were requested to report results within three weeks of receiving the panel. Both instructions and reporting tables were provided in English, French and Spanish. The participating laboratories were requested to provide the sample results and additional information to help understand the overall performance and capabilities of the laboratories for the serological diagnosis of YF cases.

Additional information included contact information, dates of arrival and testing of the panels, quality of the samples on arrival, the specific assay used, control values for the assay, and use of in-house positive controls. The laboratories were requested to provide the valid SOP used to perform the assay and the raw data obtained to assess testing accuracy and consistency of results. The results were submitted via e-mail to capture the accurate dates of submission. Upon submission, the laboratories received an automatic email confirming the data receipt.

4.4 Results evaluation

Each laboratory received an identification number to ensure anonymity.

For both the IgM and PRNT panels, the scoring system was divided into two parts: technical and post-analytical proficiency. For technical evaluation, the numerical laboratory results and their qualitative interpretations were captured. For post-analytical evaluation, other quality control indicators such as timeliness of reporting, the inclusion of controls, proper validation of assays using validation criteria, and provision of SOP and raw data were considered.

For the technical evaluation of the IgM panel, 0.5 points were given for each correct laboratory result of the 10 samples (maximum 5 points), 0.5 points for each correct interpretation of the 10 samples (maximum 5 points) and 1 point for each concordance with the expected reference results (maximum 10 points), for a total of 15 points.

For the post-analytical scoring of the IgM panel detection, points were awarded for providing dates of reception and testing, recording the method name, providing results of the negative control, and positive and negative controls meeting the assay validity cut-off criteria. An extra point was granted for using in-house controls. Points were deducted for late reporting, lack of raw data documentation and/or SOP (Figure 1A).

For the technical evaluation of the PRNT panel, three points were given for each correct interpretation of YF PRNT results of the 6 samples (maximum 18 points), 4.5 points for each concordance with the expected YF PRNT results (maximum 27 points), one point for each correct differential diagnostic interpretation of the 6 samples (maximum 6 points), and 1.5 points for each concordance with the expected differential PRNT results (maximum 9 points), for a total of 60 points.

For the post-analytical evaluation of the PRNT panel, one point each was awarded for the recording of the receipt and testing dates, the recording of positive and negative control data, and valid positive and negative controls. Points were deducted for late reporting, lack of raw data documentation or/and SOP (Figure 1B).

The final score of the laboratory was weighted where for both the IgM and PRNT panels, technical proficiency accounted for 75% and post-analytical proficiency accounted for 25%. Examples of the scoring system for the YF IgM panel (Figure 1A) and for the YF PRNT panel (Figure 1B) are depicted below. In the scoring for both IgM and PRNT panels, scores for each sub-part or final combined results of ≥90% = "pass", 80% to <90% = "provisional pass", and <80% = "fail" grades. The tables in Figures 1A and 1B below use the convention of green = pass, orange = provisional pass, and red = fail.

Figure 1A. Scoring System used for the YF IgM panel evaluation

PART 1: YF IgM - TECHNICAL PROFICIENCY

Sample Results	O.D. or IFA titer	P/N or index	NBR	Reported Result	correct interp.	Correct Interpret. (0.5pt/result)		WHO Result	Correct Concordance (1.0pt/result)	
YF IgM 1	0,053			Negative	Negative	0,5		Negative	1	
YF IgM 2	1,376			Positive	Positive	0,5		Positive	1	
YF IgM 3	0,359			Positive	Positive	0,5		Negative	0	
YF IgM 4	0,139			Negative	Negative	0,5		Negative	1	
YF IgM 5	0,879			Positive	Positive	0,5		Positive	1	
YF IgM 6	3,558			Positive	Positive	0,5		Positive	1	
YF IgM 7	2,147			Positive	Positive	0,5		Positive	1	
YF IgM 8	0,035			Negative	Negative	0,5		Negative	1	
YF IgM 9	0,458			Positive	Positive	0,5		Positive	1	
YF IgM 10	0,239			Equivocal	Equivocal	0,5		Equivocal	1	
YF IgM 11	2,409			Positive	Positive	N/A		Positive	N/A	
Sub-Score (%)						100%			90%	
Sub-total pts/max						5	5		9	10
Total Score (%)						93%				
Total pts/max						14			15	

PART 2: YF IgM - POST ANALYTICAL PROFICIENCY

Post-analytical criteria	Score obtained	Max Score
Panel receipt and test dates recorded	2	2
Assay method name recorded	1	1
Negative control data recorded	1	1
Neg. controls meets criteria	1	1
Pos. controls meets criteria	1	1
Use of inhouse ctrl (bonus pt)	1	0
Timeliness of reporting	2	2
SOP missing (-1 pt)	-1	0
Total Score (%)	100%	
Total pts/max	8	8

YF IgM FINAL SCORE (PART 1 + PART 2)

Post-analytical criteria	Weighted Score	Max Score
Total Part 1 (75%)	70%	75%
Total Part 2 (25%)	25%	25%
Total Score (%)	95%	

Figure 1B. Score System used for the YF confirmatory diagnosis PRNT panel evaluation

PART 1: YF PRNT - TECHNICAL PROFICIENCY

Sample Results	Reported YF titer	Reported YF Result	Correct Interpret. (3pts/result)	pts	WHO YF Result	Correct YF Concordance (4.5pts/each)	Reported different. diagnosis	Correct Different. Dx Interpretation (1pt/result)	pts	WHO different. Result	Correct Different. Concord. (1.5pts/result)
YF PRNT 1	1:40	Positive	**Positive**	**3**	Positive	**4,5**	YF	**YF**	**1**	YF	**1,5**
YF PRNT 2	Negative	Negative	**Negative**	**3**	Positive	**0**	Negative	**Negative**	**1**	YF	**0**
YF PRNT 3	Negative	Negative	**Negative**	**3**	Negative	**4,5**	Flavi	**Flavi**	**1**	Flavi	**1,5**
YF PRNT 4	1:20	Positive	**Positive**	**3**	Positive	**4,5**	Flavi	**Flavi**	**1**	Flavi	**1,5**
YF PRNT 5	1:40	Positive	**Positive**	**3**	Positive	**4,5**	YF	**Flavi**	**0**	Flavi	**0**
YF PRNT 6	Negative	Negative	**Negative**	**3**	Negative	**4,5**	Negative	**Negative**	**1**	Negative	**1,5**

			YF PRNT interpret sub-score			YF PRNT Concord. sub-score			Diff. PRNT interpret sub-score			Diff. PRNT Concord. sub-score	
Sub-Score (%)			**100%**			**83%**			**83%**			**67%**	
Sub-total pts/max			**18**	**18**		**23**	**27**		**5**	**6**		**6**	**9**
Part 1 Score (%)			**85,8%**										
Part 1 pts/max			**51,5**						**60**				

Notes:

Correct interpretation based on positive titer and titer difference (if applicable) values listed in columns CE and CF of the PRNT Results tab on viruses used.
Correct YF concordance is as compared to WHO YF reference results.
Correct Differential concordance is as compared to WHO differential results based on reference results for YF, Zika and DEN (any serotype).
1pt is deducted from Part 2 score for each 1 week (or part thereof) of late reporting (>21 days post reception)
Legend for colour coding of scores: Red= FAIL, Orange=PROVISIONAL PASS, Green= PASS

PART 2: YF PRNT - POST ANALYTICAL PROFICIENCY

Post-analytical criteria	Lab Score	Max Score
Panel receipt and test dates	2	2
Positive YF control data recorded	1	1
Negative control data recorded	1	1
Pos. control(s) meet criterion	1	1
Neg. control meets criterion	1	1
Timeliness of reporting	2	2
SOP missing	-1	0
Part 2 Score (%)	**87,5%**	
Part 2 pts/max	**7**	**8**

YF PRNT FINAL SCORE (PART 1 + PART 2)

Score (relative weight)	Weighted Score	Max Score
Total Part 1 (75%)	64,4%	75%
Total Part 2 (25%)	21,9%	25%
Total Score (%)	**86,3%**	

The scores, results and documentation of the participating laboratories were reviewed independently by WHO, CDC-FC and RKI, and discussed for homogeneity of criteria before informing the laboratories. The individual results were reported back to the participants in

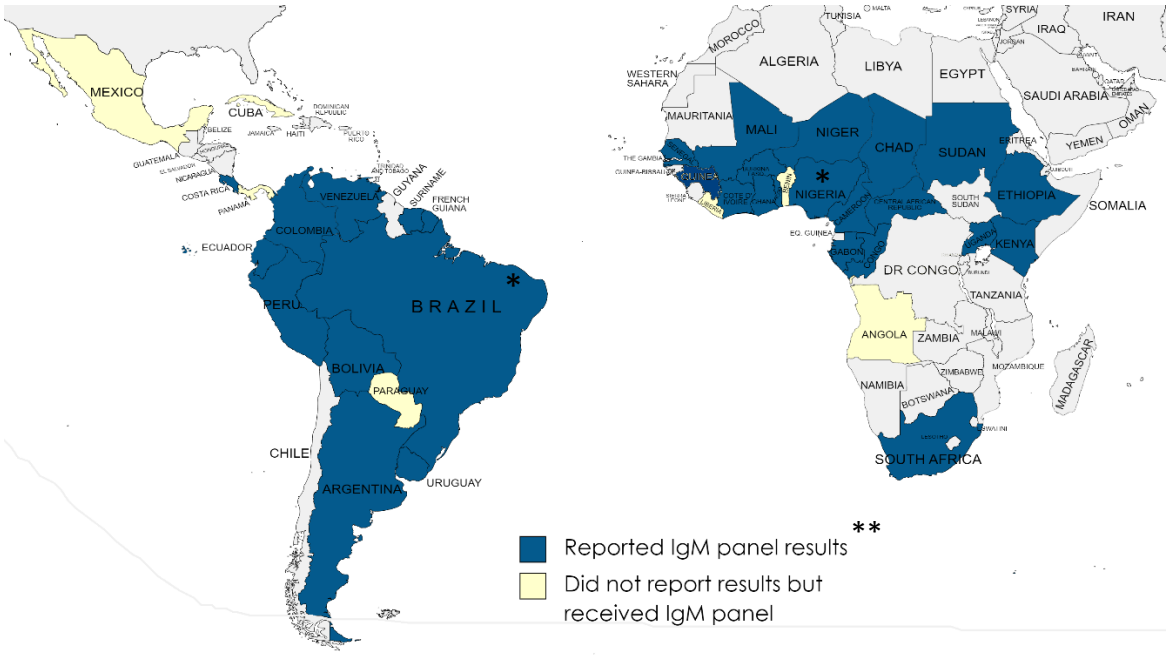
April 2022. The AFRO, EMRO, and PAHO YF laboratory coordinators were informed of the individual results of the laboratories to assure follow-up.

5. Results and Analysis

5.1. Participation

A total of 50 laboratories from 39 countries were contacted to request participation in the YF Serological EQA. Two countries did not provide clearance to ship the panels (the Democratic Republic of Congo and South Sudan) and were therefore excluded as participants in the 2021 EQA programme. Of the 48 laboratories that received an IgM EQA panel, results were received from 40 laboratories (16 from the Americas and 24 from Africa), and eight laboratories did not report results. One African laboratory was excluded due to submitting results in an incorrect format. Countries included in the analysis are shown in Figure 2.

Figure 2. Countries participating in the YF IgM Serological Panel EQA

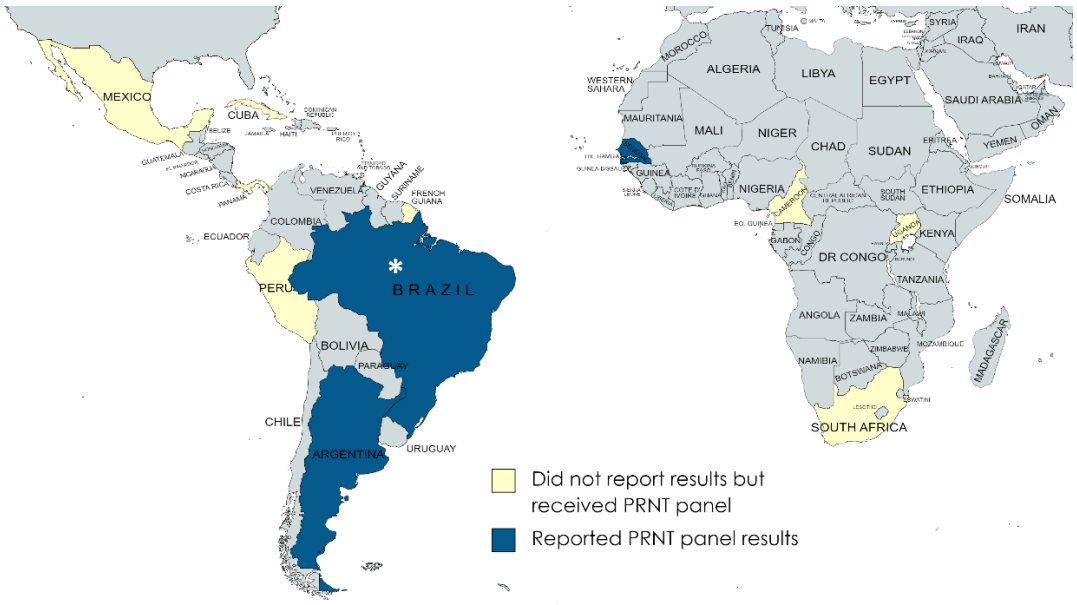


6 Laboratories in Brazil and 6 Laboratories in Nigeria

*** 1 laboratory sent result using incorrect format (results not included in the final analysis)*

Of the 13 laboratories (4 in Africa, 9 in the Americas) that received the PRNT EQA panel, only 5 reported results (1 in Africa, 4 in the Americas) as seen in Figure 3. Two laboratories reported experiencing problems with the cell cultures hindering the testing of the panel.

Figure 3. Laboratories participating in the PRNT Serological Panel EQA



* 3 Laboratories in Brazil received PRNT panels

5.2. Results

All laboratories reported the arrival of the panels in good condition, but during testing, two laboratories reported troubles with the visual aspect of the samples. In one case, the laboratory discarded some samples of the panel that exhibited haemolysis. However, haemolysis, lipemia and other blood alterations are expected in patients with viral haemorrhagic fever infection. Therefore, the lack of results submission for these samples was penalised. Another laboratory found difficulties with the viscosity of the samples. Since no other laboratory reported similar issues this was not considered a generalized technical problem of the panel.

5.2.1 IgM panel

Nine of the laboratories reporting IgM panel results used an in-house ELISA assay, three laboratories used expired YF MAC-HD (half-day) ELISA kits, and 27 laboratories used the YF CDC YF MAC-ELISA or an adapted version of it. One laboratory also reported results obtained using the commercial Euroimmun YF IgM Immunofluorescence assay. The overview of the technical results reported by the laboratories for the IgM panel is depicted in Table 3. Incorrect results are marked in red. Since each in-house assay is different, and data from a small number of laboratories was available, conclusions could not be drawn on the benefit of one assay over the other, nor was this the purpose of this programme. In Table 4 the results reported by the laboratories are classified according to the assay used.

A total of 28 laboratories (one with two sets of results; L7A and L7B) passed the IgM EQA exercise with final ≥90% pass results (21 laboratories with 100% correct technical results), seven laboratories received a final provisional pass (two of them experienced important deficiencies in the technical part), and four laboratories failed the exercise (Table 5). This indicates there is a need for improvement in 13 laboratories of the GYFLaN with 11 laboratories not passing the overall exercise and 2 laboratories failing the post-analytical proficiency part.

For laboratory L7, values generated using method B were used for the classification of the laboratory as this was their routine method. False-negative results were reported by 12 laboratories, while false-positive or false-equivocal results were present in ten laboratories. Samples S#5, S#7 and S#10 had the lowest IgM titers and represented the more challenging samples (Table 1). Failure in the detection of these samples indicates a need for improved sensitivity of the assays used (Table 3).

During this first YF IgM Serological EQA, some common themes were identified in the post-analytical performance of the laboratories that deserve to be addressed in the future. This is reflected in the post-analytical proficiency scores of the laboratories (Table 5).

Ten laboratories did not provide a valid SOP for the assay used. Although some of these laboratories may have a standardised protocol in place, we either did not receive it or we considered the document sent insufficient; therefore, these laboratories were penalised in the scoring. The implementation and use of SOPs in the laboratory routine are critical to a successful quality system. The SOP provides information to perform the assay properly and consistently to achieve quality results. The working SOPs that were provided were thoroughly reviewed and any inconsistencies identified were communicated as part of the individual feedback to the laboratories.

Second, 13 laboratories did not include in-house controls in the IgM detection assay. The inclusion of in-house controls, both in serology and molecular diagnosis, assures a higher degree of quality control of the assay. However, for laboratories in countries where YF cases are not detected regularly, obtaining samples in volumes that are adequate to serve a positive control might be challenging.

During the analysis, some inconsistencies in the laboratory results were identified, such as poor reproducibility, low OD in the positive controls, or elevated background. The laboratories were invited to correct them and revise their standardization by making use of the guides provided at the end of the exercise.

Worryingly, in three laboratories, the IgM assay control results were not interpreted correctly or were not used to validate/invalidate the tests. Adequate controls and validation rules are an important part of quality control in diagnostic laboratories to ensure the results produced are reliable and accurate. A more adequate selection of the negative and/or positive control was suggested to ten laboratories to support a correct evaluation of the assay. ELISA positive controls with OD values around 1.0 were recommended, as well as the use of human sera instead of buffer for the negative control. Some laboratories applied the cut-off validation values of an older CDC YF MAC-ELISA protocol, and once identified, these laboratories were advised to adapt their protocols to the new recommended values.

Table 3. Overview of laboratory results obtained by the GYFLaN laboratories in the YF IgM panel

	Expected Results	L1	L2	L3	L4	L5	L6	L7/A	L7/B	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19
S#1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	EQV	NEG	NEG	NEG
S#2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
S#3	NEG	NEG	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	EQV	NEG	NEG	NEG	NEG	NEG	POS	NEG
S#4	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	EQV	NEG	NEG	NEG	NEG	NEG	EQV	NEG
S#5	POS	POS	POS	POS	POS	NEG	POS	POS	NEG	NEG	POS	POS	EQV	POS	EQV	POS	NEG	POS	POS	POS	EQV
S#6	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
S#7	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
S#8	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
S#9	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS
S#10	POS	POS	EQV	POS	POS	NEG	POS	POS	NEG	NEG	POS	EQV	NEG	POS	NEG	POS	POS	POS	POS	POS	POS

	Expected Results	L20	L21	L24	L25	L26	L27	L28	L29	L30	L31	L32	L33	L34	L35	L36	L37	L38	L39	L40	L41
S#1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
S#2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS	EQV	EQV
S#3	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS	EQV	NEG	NEG	NEG	NEG	NEG	NEG	POS	ND
S#4	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	EQV	ND
S#5	POS	EQV	NEG	EQV	POS	NEG	EQV	EQV	POS	EQV	EQV	ND	EQV	EQV	EQV	EQV	NEG	NEG	EQV	POS	POS
S#6	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	ND	POS	POS	POS	POS	POS	POS	POS	POS	POS
S#7	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	ND	POS	POS	POS	POS	NEG	EQV	POS	POS	POS
S#8	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	ND	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
S#9	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS	NEG	NEG
S#10	POS	EQV	EQV	EQV	POS	EQV	EQV	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS	NEG	NEG

Positive: POS; Negative: NEG; Equivocal: EQV, Non-interpretable: N.I.; not done: ND. Incorrect results are marked in red

Table 4 Results obtained by the laboratories using different IgM assays

S #1						
	Assays reported:	Total	Positive	Equivocal	Negative	Correct result (%)
	CDC-Fort Collins YF IgM MAC ELISA	27	0	1	26	96.3
	CDC-Fort Collins YF IgM HD ELISA	3	0	0	3	100
	Euroimmun Anti-YF IIFT (IgM)	1	0	0	1	100
	In house ELISA	9	0	0	9	100
	TOTAL	**40**	**0**	**1**	**39**	**97.5**
S #2						
	Assays reported:	Total	Positive	Equivocal	Negative	Correct result (%)
	CDC-Fort Collins YF IgM MAC ELISA	27	26	0	1	96.3
	CDC-Fort Collins YF IgM HD ELISA	3	3	0	0	100
	Euroimmun Anti-YF IIFT (IgM)	1	1	0	0	100
	In house ELISA	9	6	2	1	66.7
	TOTAL	**40**	**36**	**2**	**2**	**90**
S #3						
	Assays reported:	Total	Positive	Equivocal	Negative	Correct result (%)
	CDC-Fort Collins YF IgM MAC ELISA	27	1	0	26	96.3
	CDC-Fort Collins YF IgM HD ELISA	3	0	2	1	33.3
	Euroimmun Anti-YF IIFT (IgM)	1	0	0	1	100
	In house ELISA	8	3	0	5	62.5
	TOTAL	**39**	**4**	**2**	**33**	**84.6**
S #4						
	Assays reported:	Total	Positive	Equivocal	Negative	Correct result (%)
	CDC-Fort Collins YF IgM MAC ELISA	27	1	1	25	92.6
	CDC-Fort Collins YF IgM HD ELISA	3	0	1	2	66.7
	Euroimmun Anti-YF IIFT (IgM)	1	0	0	1	100
	In house ELISA	8	0	1	7	87.5
	TOTAL	**39**	**1**	**3**	**35**	**89.7**
S #5						
	Assays reported:	Total	Positive	Equivocal	Negative	Correct result (%)
	CDC-Fort Collins YF IgM MAC ELISA	27	9	13	5	81.5
	CDC-Fort Collins YF IgM HD ELISA	3	2	1	0	100
	Euroimmun Anti-YF IIFT (IgM)	1	0	0	1	0
	In house ELISA	8	6	0	2	75
	TOTAL	**39**	**17**	**14**	**8**	**79.5**
S #6						
	Assays reported:	Total	Positive	Equivocal	Negative	Correct result (%)
	CDC-Fort Collins YF IgM MAC ELISA	27	27	0	0	100
	CDC-Fort Collins YF IgM HD ELISA	3	3	0	0	100
	Euroimmun Anti-YF IIFT (IgM)	1	1	0	0	100
	In house ELISA	8	8	0	0	100
	TOTAL	**39**	**39**	**0**	**0**	**100**
S#7						
	Assays reported:	Total	Positive	Equivocal	Negative	Correct result (%)
	CDC-Fort Collins YF IgM MAC ELISA	27	25	0	2	92.6
	CDC-Fort Collins YF IgM HD ELISA	3	3	0	0	100
	Euroimmun Anti-YF IIFT (IgM)	1	1	0	0	100
	In house ELISA	8	7	1	0	87.5
	TOTAL	**39**	**36**	**1**	**2**	**92.3**
S #8						
	Assays reported:	Total	Positive	Equivocal	Negative	Correct result (%)
	CDC-Fort Collins YF IgM MAC ELISA	27	0	0	27	100
	CDC-Fort Collins YF IgM HD ELISA	3	0	0	3	100
	Euroimmun Anti-YF IIFT (IgM)	1	0	0	1	100
	In house ELISA	8	0	0	8	100
	TOTAL	**39**	**0**	**0**	**39**	**100**
S#9						
	Assays reported:	Total	Positive	Equivocal	Negative	Correct result (%)
	CDC-Fort Collins YF IgM MAC ELISA	27	23	0	4	85.2
	CDC-Fort Collins YF IgM HD ELISA	3	3	0	0	100
	Euroimmun Anti-YF IIFT (IgM)	1	1	0	0	100
	In house ELISA	9	6	0	3	66.7
	TOTAL	**40**	**33**	**0**	**7**	**82.5**
S #10						
	Assays reported:	Total	Positive	Equivocal	Negative	Correct result (%)
	CDC-Fort Collins YF IgM MAC ELISA	27	17	5	5	81.5
	CDC-Fort Collins YF IgM HD ELISA	3	3	0	0	100
	Euroimmun Anti-YF IIFT (IgM)	1	0	0	1	0
	In house ELISA	9	4	2	3	66.7
	TOTAL	**40**	**24**	**7**	**9**	**77.5**
S #11						
	(informative sample)					
	Assays reported:	Total	Positive	Equivocal	Negative	Correct result (%)
	CDC-Fort Collins YF IgM MAC ELISA	26*	25	0	1	96.3
	CDC-Fort Collins YF IgM HD ELISA	3	3	0	0	100
	Euroimmun Anti-YF IIFT (IgM)	1	1	0	0	100
	In house ELISA	9	9	0	0	100
	TOTAL	**39***	**38**	**0**	**1**	**97.43**
	*one result was non-interpretable					

Note: The CDC-Fort Collins YF IgM HD ELISAs used in the EQA exercise were all used beyond their expiration date

Table 5. Scores obtained by the laboratories in the YF IgM serology panel

	Technical Proficiency			Postanalytical Proficiency	Final Score IgM Serology
	Interpretation of results	*Concordance with reference*	*Technical score*		
L1	100	100	100	100	100
L2	100	90	93	100	95
L3	100	100	100	100	100
L4	100	100	100	88	97
L5	100	80	87	100	90
L6	100	100	100	100	100
L7A	90	100	97	100	98
L7B	100	80	87	100	90
L8	100	70	80	88	82
L9	100	100	100	100	100
L10	100	100	100	88	97
L11	100	90	93	100	95
L12	80	80	80	100	85
L13	100	80	87	63	81
L14	100	100	100	100	100
L15	100	90	93	100	95
L16	100	100	100	100	100
L17	100	100	100	88	97
L18	100	80	87	88	87
L19	100	100	100	100	100
L20	100	100	100	63	91
L21	100	80	87	88	87
L24	100	100	100	100	100
L25	100	100	100	88	97
L26	100	80	87	100	90
L27	100	100	100	100	100
L28	100	100	100	100	100
L29	100	90	93	100	95
L30	100	100	100	100	100
L31	100	100	100	100	100
L32	60	50	53	100	65
L33	100	90	93	63	86
L34	100	100	100	88	97
L35	100	100	100	100	100
L36	100	100	100	63	91
L37	100	50	67	88	72
L38	100	50	67	100	75
L39	100	100	100	100	100
L40	100	50	67	100	75
L41	100	63	75	100	81

Green: PASS; Yellow: PROVISIONAL PASS; Red: FAIL

Thirteen laboratories reported their results late. The presence of the SARS-CoV-2 pandemic, however, made not only the execution of the panels difficult but created a delay for the whole EQA exercise. It is necessary, however, to mention that suspected cases of YF must be tested immediately to clarify if preventive public health measures are to be implemented, considering that one confirmed YF case could indicate an outbreak scenario.

An individualized analysis of the reported results was sent back to all laboratories in April 2022. In this analysis, specific weaknesses, as well as strengths of the performing laboratory, were remarked on and actions to be taken were suggested. Background documents, tools, and guidelines (troubleshooting guide for addressing technical issues in the serological diagnosis of YF using the CDC YF MAC-ELISA), PowerPoint presentations with notes on troubleshooting and optimization of the assay, an updated version of the assay protocol prepared by CDC-FC, and a calculations spreadsheet for the CDC MAC-ELISA were provided to the laboratories with feedback information for self-paced troubleshooting. Among the actions proposed were the calibration of the ELISA reader and optimization of the protocol set-up in the laboratory for increasing sensitivity.

5.2.2 PRNT

For the PRNT, each laboratory used its own protocol, differing in the viral strains and cell lines used. All laboratories used plaque reduction of 90% but with different cut-offs. For the differential diagnosis, only two laboratories out of five included all dengue (DENV) serotypes in the testing, while dengue serotype-2 (DENV2) was the only strain included in the testing for the three other laboratories. The overview of the PRNT panel results is shown in Table 6.

Only five of 13 laboratories (38.5%) receiving PRNT panels sent testing results (Figure 3). The laboratories were requested to test not only for anti-YF reactivity, but to follow a differential diagnosis scheme including other relevant flaviviruses that could resemble symptomatology and share the geographical distribution of YF. In this case, a minimum of Zika and the four DENV serotypes were suggested. Only two laboratories included the four DENV serotypes in the testing, whereas the rest of laboratories used DENV2 serotype as the DENV representative. This led to a false interpretation of results in sample S#5 in those laboratories using only DENV2 as the DENV representative. It is recommended to include the four different DENV serotypes in the PRNT testing for reliable differential diagnosis. Only one laboratory reported 100% correct results, three laboratories passed the exercise with acceptable results, and one laboratory received a provisional pass (Table 8).

Table 6. PRNT panel laboratory results reported by the laboratories

	Sample #1	**Sample #2**	**Sample#3**	**Sample#4**	**Sample#5**	**Sample#6**
Expected	YF	YF	FLAVI	FLAVI	FLAVI	NEG
L1	YF	YF	Flavi	YF	YF	NEG
L2	YF	NEG	Flavi	Flavi	YF	NEG
L11	YF	YF	Flavi	Flavi	DENV is unspecific.	NEG
L18	YF	NEG	Flavi	Flavi	Flavi	NEG
L41	YF	YF	Flavi	Flavi	Flavi	NEG

Although all laboratories used a PRNT 90% assay, the cut-off for positive results differed among them (Table 7), ranging from titers ≥1:10 to titers ≥1:40. The difference in the cut-off selection did not associate with differences in sensitivity. All the laboratories used a minimum of 4-fold difference in the titer obtained between the different flavivirus PRNT results to identify the probable infecting virus.

Although samples were correctly identified as positive or negative in most cases, two laboratories did not identify sample S#2 as positive, suggesting this sample was the most challenging in the PRNT panel. This indicates a lack of sensitivity in the assay that must be addressed. One laboratory reported a YF titer of 1:10 for samples S#3 and S#6. Since this laboratory used a higher titer cut-off the samples remain correctly interpreted but this higher cut-off might suggest a high background in the assay (Table 7). The titers obtained for the samples in the different laboratories varied greatly, therefore the qualitative discerning capacity is more reliable than the quantitative results of the assays.

Table 7. PRNT panel results. YF titers obtained and cut-off values used

	Sample #1	**Sample #2**	**Sample#3**	**Sample#4**	**Sample#5**	**Sample#6**	**Cut-off**
Expected	160	40	<10	320	80	<10	**≥10**
L1	160	80	<10	160	320	<10	**≥10**
L2	40	<10	<10	20	40	<10	**≥10**
L11	80	80	<10	80	40	<10	**≥10**
L18	40	<10	<10	160	40	<10	**≥20**
L41	160	40	10	80	40	10	**≥40**

Despite these drawbacks, four laboratories out of five passed the PRNT exercise, with only one laboratory getting a provisional pass. The overview of the laboratory scores in the PRNT EQA panel is depicted in Table 8. While all YF-positive samples were identified as YF positives, issues were observed in the discrimination between other flaviviruses. Since the presence of serological cross-reactivity among flaviviruses like YF and DENV is frequent and different DENV serotypes circulate in YF endemic areas, special attention is needed in the correct use of the PRNT and the inclusion of other flaviviruses for confirmation of YF cases. This is especially important to prevent the initiation of unnecessary reactive vaccination campaigns.

Table 8. Scores obtained by the laboratories in the YF PRNT (confirmatory diagnosis) panel

	YF PRNT		Differential PRNTs		Technical Score	Postanalytical performance	Final Score PRNT (confirmatory YF Dx)
	YF PRNT	*Concordance with reference*	*Differential PRNT*	*Concordance with reference*			
L1	100	100	100	67	95	100	96.3
L2	100	83	83	67	85.8	87.5	86.3
L11	100	100	83	83	95.8	87.5	93.8
L18	100	83	100	100	90	62.5	83.1
L41	100	100	100	100	100	100	100

YF SEROLOGICAL EQA CONCLUSIONS AND RECOMMENDATIONS

The YF serological EQA consisted of two panels, a YF IgM panel and a YF PRNT panel. This is the first YF serological EQA performed under the activities of the EYE Strategy and the coordination of the EYE-LTWG.

The high participation level of the GYFLaN laboratories (96% of contacted laboratories finally received the IgM panels and 83.3% of laboratories receiving the YF IgM panels reported their results) indicates that this exercise was well-perceived and there is a genuine interest from the laboratories to participate in such activities. By participating in the EQA exercise, the laboratories can not only check their performances but can also have access to well-characterized materials and compare their results objectively with those of other laboratories. The laboratories can use their participation in the EQA as part of their QC/QA activities.

Overall, the results show there is a good capacity within the network for the serological diagnosis of yellow fever, with most laboratories successfully passing the exercise; however, some issues where improvement is necessary have been identified. Provision of in-house positive controls for all laboratories would be advantageous and would improve the general quality of testing. QC/QA approaches including the use of SOPs, calibration of readers, and optimization of the assays are all critical and should be applied in all the network laboratories. From the results and the reported associated data, it seems that some laboratories implemented the CDC YF MAC-ELISA protocol as it was received, without further optimization or validation in their working conditions. Laboratories must be encouraged to optimize and validate their tests, and to request support or additional training from their regional coordinators in case of need.

A total of 13 laboratories need direct support to improve their performance. Twinning activities or specific training sessions for these laboratories can be coordinated by the WHO regional coordinators with the involvement of regional reference laboratories, reference centers, and WHO collaborating centres (CC) to quickly elevate their performances.

The poor level of participation for the PRNT panel (only 38.5% of laboratories receiving the PRNT panel reported results), and the observed results indicate this is a weakness within the GYFLaN. The PRNT is the confirmatory assay for samples with positive YF serology in absence of a positive RT-PCR result, or samples taken more than one week after symptom onset. Considering the importance of routine disease surveillance programmes and how case confirmation directly informs and impacts strategic rapid outbreak immunization responses in individual countries, future participation in the YF PRNT EQA programme will be mandatory for all regional reference laboratories and WHO CCs to maintain their statuses. PRNT capacity-building activities will be critical to improve the overall performance of the network and to expand the number of laboratories with the capacity to perform this YF confirmatory assay. Regardless of whether each laboratory has access to different YF strains and cell culture lines, it would be advantageous to bring PRNT testing to comparable levels among the WHO CC and RRLs.

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Annex 1. The Global Yellow Fever Laboratory Network (GYFLaN)

The **Global Yellow Fever Laboratory Network (GYFLaN)** is constituted by:

- 3 African Regional Reference Laboratories (RRL):
  - Institut Pasteur de Dakar, Senegal (IPD)[3]
  - Uganda Virus Research Institute, Uganda (UVRI)
  - Centre Pasteur du Cameroun, Cameroon (CPC)
- 5 WHO Collaborating Centers (CC):
  - Instituto Nacional de Enfermedades Virales Humanas „Dr. Julio I. Maiztegui“ (INEVH), Argentina
  - Instituto Evandro Chagas (IEC), Brazil
  - Instituto de Diagnóstico y Referencia Epidemiológicos (INDRE), Mexico
  - Instituto de Salud Pedro Kouri (IPK), Cuba
  - Centers for Disease Control and Prevention Fort Collins (CDC-FC), USA
- 61 National Laboratories:

  34 National Laboratories in 29 countries in the WHO AFRO region:
  - Instituto Nacional de Investigação em Saúde (INIS), Angola
  - Institut National de Santé Publique (INSP), Benin
  - Centre Muraz - Laboratoire National des fièvres hémorragiques virales (CM), Burkina Faso
  - Institut Pasteur de Bangui (IPB), Central African Republic
  - Hôpital General de Référence Nationale (HGRN), Chad
  - Institut Pasteur of Côte d'Ivoire (IPCI), Côte d'Ivoire
  - National Institute of Biomedical Research (INRB), Democratic Republic of Congo
  - Centro Médico La Paz, Equatorial Guinea
  - National Health Laboratory (NHL), Eritrea
  - Ethiopian Public Health Institute (EPHI), Ethiopia
  - Université des Sciences de la Santé (USS), Gabon
  - National Public Health Laboratory (NPHL), Gambia
  - National Public Health and Reference Laboratory (NPHRL), Ghana
  - Laboratoire des Fièvres Hémorragiques, Guinea
  - Laboratório nacional da Saúde Pública (LNSP) Guinea-Bissau
  - Kenya Medical Research Institute (KEMRI), Kenya
  - National Public Health Institute (NPHI), Liberia
  - Institut National de Recherche en Santé Publique (INRSP), Mali
  - Institut National de Recherches en Santé Publique (INRSP), Mauritania
  - Laboratoire de Biologie Medicale - Hopital National de Niamey, Niger
  - University of Benin Teaching Hospital (UBTH), Nigeria
  - University of Nigeria Teaching Hospital (UNTH), Nigeria
  - Central Public Health Laboratory - Nigerian Centre for Disease Control (CPHL – NCDC), Nigeria
  - Maitama District Hospital (MDH), Nigeria
  - NCDC National Reference Laboratory (NRL), Nigeria
  - Yususf Dansoho Memorial Hospital (YDMH), Nigeria
  - Laboratoire National de Santé Publique (LNSP), Republic of the Congo
  - Rwanda Biomedical Center - National Reference Laboratory Division (RBC), Rwanda

[3] IPD is also a WHO collaborative Centre on Arboviruses and other hemorrhagic fever viruses

- Central Public Health Reference Laboratory (CPHRL), Sierra Leone
- Centre for Emerging Zoonotic and Parasitic Diseases - National Institute for Communicable Diseases (NICD), South Africa
- Public Health Laboratory (PHL), South Sudan
- Institut National d'Hygiène (INH), Togo
- National Public Health Laboratory (NPHL), Tanzania
- Zambia National Public Health Institute (ZNPHI), Zambia

1 National Laboratory in the WHO EMRO region
- National Public Health Laboratory (NPHL), Sudan

26 National Laboratories in 23 countries in the WHO American región
- Centro Nacional de Enfermedades Tropicales (CENETROP), Bolivia
- Fiocruz - Fundação Oswaldo Cruz (FIOC-RE), Brazil
- Fiocruz - Fundação Oswaldo Cruz (FIOC-RIO), Brazil
- Fiocruz - Fundação Oswaldo Cruz (Carlos Chagas Institute), Brazil
- Instituto Adolfo Lutz (IAL), Brazil
- Instituto de Salud Publica (ISP), Chile
- Instituto Nacional de Salud (INS), Colombia
- Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA), Costa Rica
- Instituto Pedro Kourí (IPK), Cuba
- Laboratorio de Virología del Laboratorio Nacional de Salud Pública Dr. Defilló (LNSPDD), Republica Dominicana
- Instituto Nacional de Investigación en Salud Pública (INSPI), Ecuador
- Laboratorio Central Dr. Max Bloch, El Salvador
- Laboratorio Nacional de Salud (LNS), Guatemala
- Institute Pasteur, French Guyane
- Laboratoire National de Santé Publique, Haiti
- Laboratorio Nacional de Vigilancia de la Salud (LNVS), Honduras
- Arbovirus Laboratory, Department of Microbiology, University of West Indies, Jamaica
- Centro Nacional de Diagnóstico y Referencia, Nicaragua
- Instituto Conmemorativo Gorgas de Estudios de la Salud (ICGES), Panamá
- Laboratorio Central de Salud Pública (LCSP), Paraguay
- Instituto Nacional de Salud (INS), Peru
- CDC - Puerto Rico, Puerto Rico
- Academic Hospital Paramaribo, Suriname
- Caribbean Public Health Agency (CARPHA), Trinidad y Tobago
- Departamento de Laboratorio de Salud Pública (DLSP), Uruguay
- Instituto Nacional de Higiene Rafael Rangel (INHRR), Venezuela

Annex 2. The EYE Laboratory Technical Working Group (EYE-LTWG)

The **EYE Strategy's advisory Laboratory Technical Working Group (EYE LTWG)** is constituted of stakeholders and experts from the following institutions:

- Global and Regional Lab Coordinators from WHO HQ (Geneva), and regional Offices: WHO-AFRO, WHO-EMRO, WHO-PAHO
- EYE Secretariat and EYE.OPS (hosted by WHO)
- Erasmus MC, The Netherlands
- National Institute for Public Health and the Environment (RIVM), The Netherlands
- CDC Fort Collins, USA
- Robert Koch Institute (RKI), Germany
- Global Alliance for Vaccine Initiative (Gavi), Switzerland
- Institut Pasteur de Dakar, Senegal (IPD)
- Uganda Virus Research Institute, Uganda (UVRI)
- Centre Pasteur du Cameroun, Cameroon (CPC)
- Nigeria CDC, Nigeria
- Fiocruz - Fundação Oswaldo Cruz (FIOC-RIO), Brazil
- National Institute for Communicable Diseases (NICD), South Africa
- UNICEF Supply Division, Denmark
- Independent expert consultants hired by WHO

Annex 3. List of YF laboratories that submitted results

African Regional Reference Laboratories (RRL):

- Institut Pasteur de Dakar, Senegal (IPD) (also WHO CC)
- Uganda Virus Research Institute, Uganda (UVRI)
- Centre Pasteur du Cameroun, Cameroon (CPC)

WHO Collaborating Centers (CC):

- Instituto Nacional de Enfermedades Virales Humanas „Dr. Julio I. Maiztegui“ (INEVH), Argentina
- Instituto Evandro Chagas (IEC), Brazil

National Laboratories in the WHO AFRO region:

- Centre Muraz - Laboratoire National des fièvres hémorragiques virales (CM), Burkina Faso
- Institut Pasteur de Bangui (IPB), Central African Republic
- Hôpital General de Référence Nationale (HGRN), Chad
- Institut Pasteur of Côte d'Ivoire (IPCI), Côte d’Ivoire
- Ethiopian Public Health Institute (EPHI), Ethiopia
- Université des Sciences de la Santé (USS), Gabon
- National Public Health and Reference Laboratory (NPHRL), Ghana
- Laboratoire des Fièvre Hémorragiques, Guinea
- Kenya Medical Research Institute (KEMRI), Kenya
- Institut National de Recherche en Santé Publique (INRSP), Mali
- Laboratoire de Biologie Medicale - Hopital National de Niamey, Niger
- University of Benin Teaching Hospital (UBTH), Nigeria
- University of Nigeria Teaching Hospital (UNTH), Nigeria
- Central Public Health Laboratory - Nigerian Centre for Disease Control (CPHL – NCDC?), Nigeria
- Maitama District Hospital (MDH), Nigeria
- NCDC National Reference Laboratory (NRL), Nigeria
- Yususf Dansoho Memorial Hospital (YDMH), Nigeria
- Laboratoire National de Santé Publique (LNSP), Republic of the Congo
- Centre for Emerging Zoonotic and Parasitic Diseases - National Institute for Communicable Diseases (NICD), South Africa
- Institut National d'Hygiène (INH), Togo

National Laboratories in the WHO EMRO region:

- National Public Health Laboratory (NPHL), Sudan

National and Subnational Laboratories in the WHO American region:

- Centro Nacional de Enfermedades Tropicales (CENETROP), Bolivia
- Fiocruz - Fundação Oswaldo Cruz (FIOC-RIO), Brazil
- Fiocruz - Fundação Oswaldo Cruz (Carlos Chagas Institute), Brazil

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- Instituto Nacional de Salud (INS), Colombia
- Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA), Costa Rica
- Instituto Nacional de Investigación en Salud Pública (INSPI), Ecuador
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