





United Nations Office for Disarmament Affairs

CIRCB

International Centre for Genetic Engineering and Biotechnology NORWEGIAN MINISTRY OF FOREIGN AFFAIRS

'Reducing Biological Risks by Promoting the Peaceful Use of Biology'

13:00 -14:45hrs 12 December 2023 Room XXV, Palais des Nations Geneva, Switzerland

ICGEB Capacity-Building Project

in the Republic of Cameroon

Dr FOKAM Joseph,

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Virologist

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"To enhance detection capacity to respond to SARS-CoV-2 and other emerging infections in Sub-Saharan Africa by assessing and transferring cost-effective technology for rapid viral identification and surveillance"





OUTLINE



I. CAMEROON IN A NUTSHELL

CIRCB

- II. INFECTIOUS DISEASE BURDEN
- III. ICGEB CONTRIBUTION IN DIAGNOSTICS
- IV. ICGEB CONTRIBUTION IN GENOMIC SURVEILLANCE
- V. PANDEMIC PREPAREDNESS WITH ICGEB
- VI. TAKE HOME MESSAGE

I. CAMEROON IN A NUTSHELL

Location: A country at the Centre of Africa

Capital cities : Yaoundé (political) & Douala (economical)

Overall surface : 466 050 km²

Population : 28.6 million in 2023 (2.63% annual increase)

Official languages : French and English

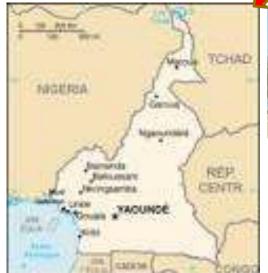


Yaounde (Capital)





Wourri river (Douala sea port)







ameroun

Kribi (Beach)

Limbe (Beach)



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II. INFECTIOUS DISEASE BURDEN (1/4)

Disease surveillance week 48	New Cases	New Deaths	Cumul Cases	Cumul Deaths
Anthrax (" <i>charbon</i> " in french)	0	0	0	0
Chikungunya	0	0	0	0
Cholera	47	3	4238	153
Dengue	0	0	0	0
Dracunculiasis	0	0	0	0
Viral haemorrhagic fever (last was Lyell syndrome)	0	0	0	0
Yellow fever	35	00	1322	17
Typhoid fever (all 10 regions)	13376	20	609307	472
Meningitis	13	1	1057	46
Malaria (all 10 regions)	45 333	86	2099920	2673
Poliomyelitis	7	0	633	11

II. INFECTIOUS DISEASE BURDEN (2/4)

Disease surveillance week 48	New Cases	New Deaths	Cumul Cases	Cumul Deaths
Plague (" <i>Peste</i> " in french)	0	0	0	0
Dog bites	150	0	5994	19
Snake bites	160	15	8873	103
Rabies	0	0	6	2
Measles	16	0	9135	57
SRAS	3	0	590	22
Flu syndrome	4934	7	215951	102
Мрох	0	0	89	0
Smallpox	0	0	0	0
Diphtheria	2	1	2	1
COVID-19	1	0	808	11

II. INFECTIOUS DISEASE BURDEN (3/4)

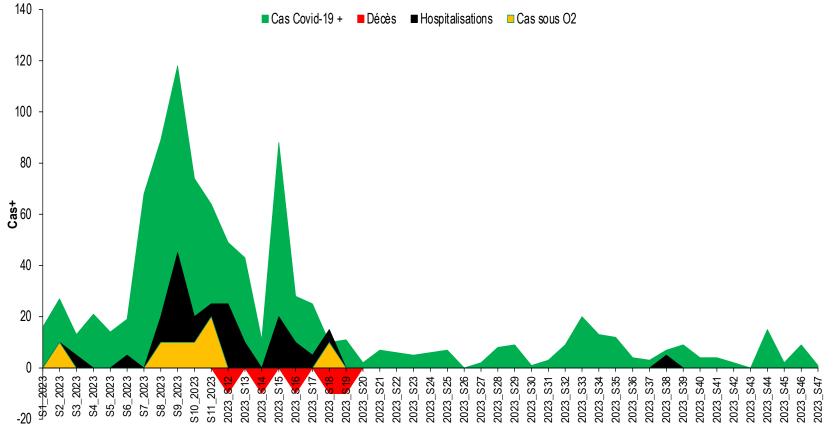
Dynamics of COVID-19 burden in Cameroon: 2020 – 2022

Legend: Order of wave First wave Second wave Third wave Fourth wave Duration in weeks 16 21 11 8 Period (in weeks), Year W18-33, 2020 W2-22, 2021 W36-W46, 2021 W50, 2021 - W5, 2022 Date start – Date end 27/04/2020-16/08/2020 1/11/02-06/06/2021 15/06/09-21/11/2021 06/09/2022 to 06/02/2022 Nber confirmed cases 16,948 52,271 21,753 10,803 Nber hospitalised 1.847 4,675 2.230 809 Nber deaths 386 835 426 79 Case Fatality rate 2.3% 1.6% 2.0% 0.73 - Viral strains - Viruses of the lineage - Non-variants of - Non-variants of - Omicron variant (90%) isolated by of origin (100%) concern (70%) concern (60%) - Non-variants of sequencing (%) - Alpha variant (20%) - Delta variant (40%) concern (10%) - Beta variant (10%) 40,000 30,000 20,000 10,000 5.000 4.000 3.000 2.000 1,000 500 543 544 545 545 547 547 548 549 549 5333. 535.5 S: Epidemiological weeks Confirmed cases Hospitalised cases Deaths Cases on oxygen therapy

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II. INFECTIOUS DISEASE BURDEN (4/4)

COVID-19 under control in Cameroon since mid-2023 (Jan-Dec)



Semaines épidémiologiques

III. ICGEB CONTRIBIUTION IN DIAGNOSTICS (1/6)

National Laboratory capacity in response to COVID-19

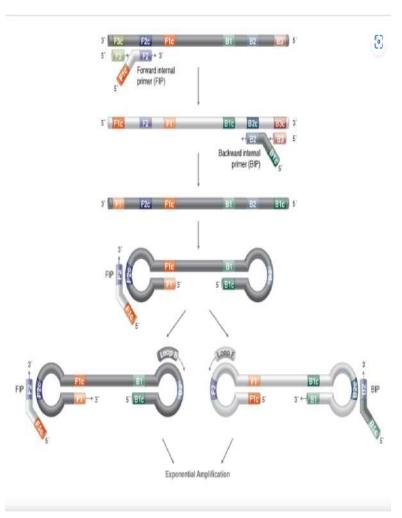
N°	Key indicators	March 2020, (n)	March 2022 (n)	Specific comments
i.	Number of frameworks for the genomic surveillance platform	0	1	Strong governmental engagement (ministerial decision, April 12, 2021)
ii.	Number of national strategies for SARS-CoV-2 genomic surveillance	0	2	The first plan has been revised as per changes in the pandemic.
iii.	Number of laboratories with the capacity for COVID-19 molecular testing	1 (45	24 public laboratories and 21 private laboratories
iv.	Number of laboratories with the capacity for variant screening by PCR point mutation assay	0	16	These are laboratories with open real-time PCR systems for SARS-CoV-2
V.	Number of laboratories with the capacity/network for SARS-CoV-2 sequencing	0	6	5 public labs and 1 private lab (performing targeted and/or whole- genome sequencing)
vi.	PCR-positive samples successfully processed for SARS-CoV-2 genomic surveillance	0	3,881	1,509 PCR-mutation assays, 1,612 targeted sequencing, 760 whole-genome sequencing

III. ICGEB CONTRIBIUTION IN DIAGNOSTICS (2/6)

First experience with the RT-LAMP technology for COVID-19

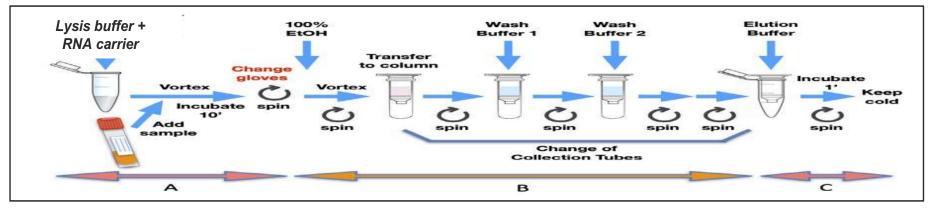
Loop-Mediated Isothermal Amplification:

- Loop-mediated isothermal amplification (LAMP) uses 4-6 primers recognizing 6-8 distinct regions of target DNA for a highly specific amplification reaction.
- A strand-displacing <u>DNA</u> <u>polymerase</u> initiates synthesis and 2 specially designed primers form "loop" structures to facilitate subsequent rounds of amplification through extension on the loops and additional annealing of primers.

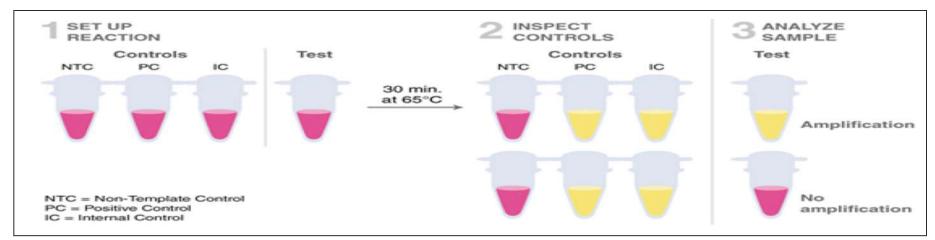


III. ICGEB CONTRIBIUTION IN DIAGNOSTICS (3/6)

First experience with the RT-LAMP technology for COVID-19



First step: Viral RNA isolation using a standard commercial kit (QIAamp® Viral RNA Mini Kit, Qiagen).



Second step: Amplification using a thermocycler at a single temperature of 65°

III. ICGEB CONTRIBIUTION IN DIAGNOSTICS (4/6)

Results of COVID-19 RT-LAMP on nasopharyngeal swabs

Acceptable performance of RT-LAMP on nasopharyngeal specimens (excellent outcome with high viral loads)

	CAMEROON		
Stratification	N Sensitivity		
		(CI 95%)	
CT<25	129	98 (93-100)	
CT≥25	93	63 (53-73)	

EClinicalMedicine 40 (2021) 101101





journal homepage: https://www.journals.elsevier.com/eclinicalmedicine

Research Paper

Diagnostic performance of a colorimetric RT -LAMP for the identification of SARS-CoV-2: A multicenter prospective clinical evaluation in sub-Saharan Africa

Marycelin Mandu Baba^{a,1}, Molalegne Bitew^{b,1}, Joseph Fokam^{c,d,1}, Eric Agola Lelo^{e,1}, Ahmed Ahidjo^a, Kominist Asmamaw^b, Grace Angong Beloumou^c, Wallace Dimbuson Bulimo^e, Emanuele Buratti^f, Collins Chenwi^c, Hailu Dadi^b, Pierlanfranco D'Agaro^{g,h}, Laura De Conti^f, Nadine Fainguem^c, Galadima Gadzama^a, Paolo Maiuriⁱ, Janet Majanja^e, Wadegu Meshack^e, Alexis Ndjolo^c, Celine Nkenfou^c, Bamidele Soji Oderinde^a, Silvanos Mukunzi Opanda^e, Ludovica Segat^g, Cristiana Stuani^f, Samwel L. Symekher^e, Desire Takou^c, Kassahun Tesfaye^b, Gianluca Triolo^f, Keyru Tuki^b, Serena Zacchigna^f, Alessandro Marcello^{f,*}

III. ICGEB CONTRIBIUTION IN DIAGNOSTICS (5/6)

Results of COVID-19 RT-LAMP on saliva without extraction

Overall acceptable performance of RT-LAMP on saliva (n= 970)

CT< 37 (National threshold)

RT PCR (REFERENCE: Gold standard)				
		+	-	TOTAL
RT-LAMP	+	44	33	77
(Under	-	11	798	809
evaluation)	TOTAL	55	831	886

Statistic	Value	95% CI
Sensitivity	80.0%	68.4% to 88.6%
Specificity	96.0%	95.3% to 96.6%
Positive Predictive Value (*)	57.1%	48.8% to 63.3%
Negative Predictive Value (*)	98.6%	97.9% to 99.2%

Kappa= 0.64 CI : 0.54 to 0.74

III. ICGEB CONTRIBIUTION IN DIAGNOSTICS (6/6) Capacity-building on RT-LAMP for other laboratories

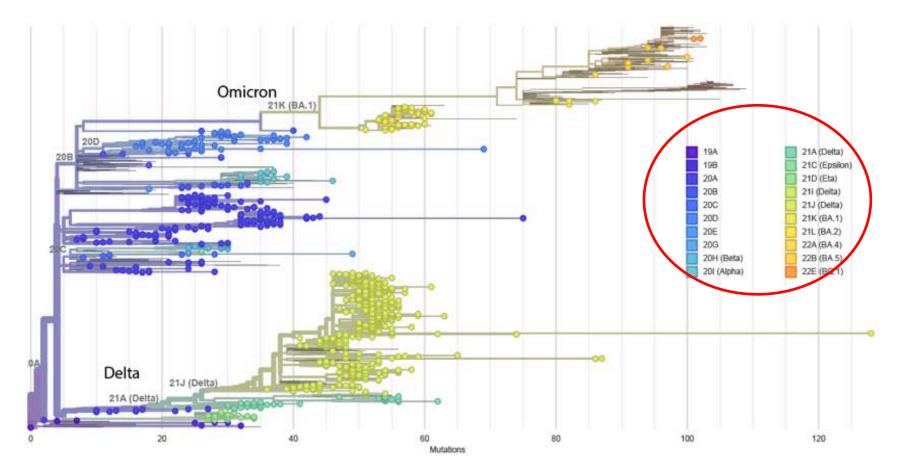


- Successful implementation in all labs: thermocycler, heating block, or water bath
- Result performance: 100% concordance trained lab versus reference lab (CIRCB)

IV. ICGEB CONTRIBIUTION IN GENOMIC SURVEILLANCE IN CAMEROON (1/5)

Genomic surveillance of SARS-CoV-2 reveals highest severity and mortality of delta

over other variants: evidence from Cameroon. Fokam et al., Nat Sc. Reports 2023



With contribution from ICGEB for whole genome sequencing (Bill & Melinda Gates sponsorship)

IV. ICGEB CONTRIBIUTION IN GENOMIC SURVEILLANCE IN CAMEROON (2/5)

Targeted sequencing of SARS-CoV-2 of positive samples

Sanger-sequencing and interpreted of variants using Stanford db.v9.5

- Site: Virology Laboratory of the "Chantal BIYA" International Reference Centre (CIRCB), Yaoundé, Cameroon
- Laboratory technique: design of Sanger sequencing of the spike region of SARS-CoV-2 positive specimens.
- Training: Laboratory sequencing testing with reference to ICGEB whole genome sequences.

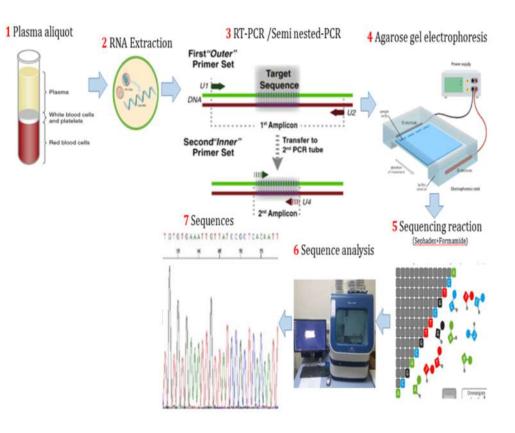
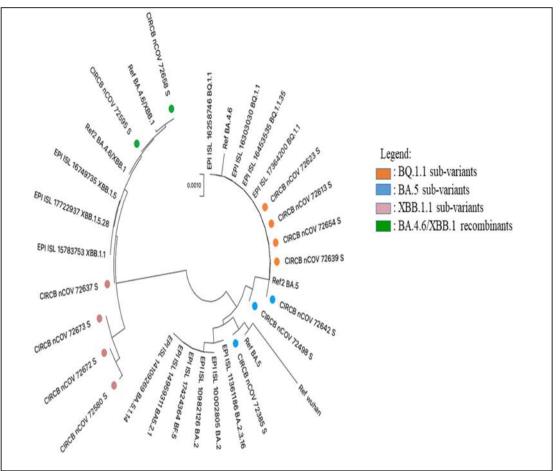


Figure : Sanger sequencing pipeline

IV. ICGEB CONTRIBIUTION IN GENOMIC SURVEILLANCE IN CAMEROON (3/5) Real-time sequencing of sub-variants in 2023



• Current trend of COVID-19: New

Omicron sub-variants and recombinants with mild symptoms.

• Genomic surveillance: Atypical recombinants (BA.4.6/XBB.1), timely detect and track novel strains, related disease severity and risk of transmission for optimal pandemic control.

Figure: Phylogenetic tree of SARS-CoV-2 sequences obtained

IV. ICGEB CONTRIBIUTION IN GENOMIC SURVEILLANCE IN CAMEROON (4/5) WORKSHOP ONT SEQUENCING FOR SARS-CoV-2 in ITALY & CAMEROON



ICGEB-Italy



PORTABLE SEQUENCING DEVICE CE MARKED: YES Dimensions Size: W 105 mm, H 23 mm, D 33 mm Weight: 87 g



Primers: SARS-CoV-2 (ref. Diatheva)

Primers: SARS-CoV-2 (ref. Diatheva) 5 kit for 100 reactions



Fluorometer, benchtop

Qubit[™] 4 Fluorometer, with WiFi



SuperScript™ IV First-Strand Synthesis System Catalog number: 18091050





CIRCB-Cameroon









Incubators, laboratory, thermocycler

qTOWER3 G touch

qTOWER³ G touch (230 V), incl. color module 1





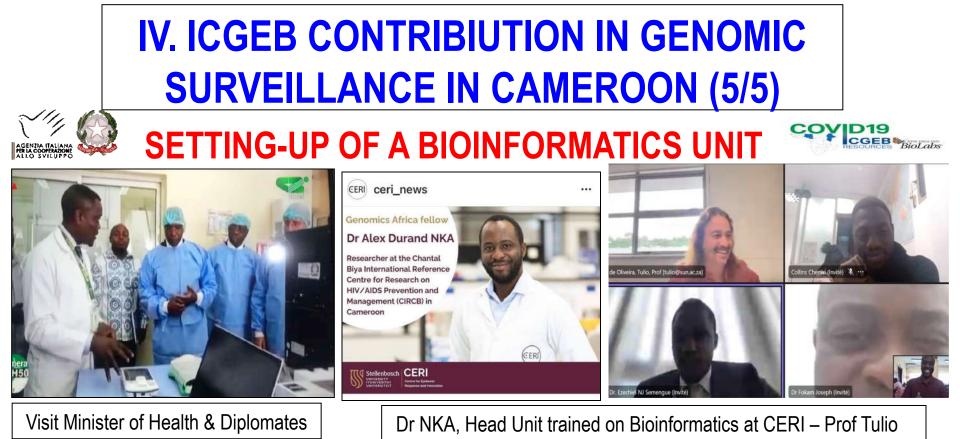
R9 Flowcells (Oxford Nanopore)

R9 Flowcells (Oxford Nanopore)

Flow Cells for MinION and MinION Mk1C Supplier: Oxford Nanopore Technologies Nanopore sequencing flow cells for use w

PC workstation

ESPRIMO P5011 Personal computer with licensed operating system a



- ✓ **Bioinformatics unit**: Office workspace provided to the team;
- ✓ **Staff**: Head of unit & staff designated in the Virology Laboratory;
- ✓ Basic equipment: High throughput computers already in place;
- ✓ Staff training: further opportunities identified (ICGEB, CERI, Africa CDC);
- ✓ pending needs: a server/cluster for sequence data storage & sharing;
- ✓ Vision: become a bioinfo Ref. centre for surveillance in Central Africa.

V. PANDEMIC PREPAREDNESS WITH ICGEB (1/3)

National workshop to scale-up RT-LAMP in district laboratories of Cameroon for the surveillance of emerging pathogens



Diagnostic moléculaire rapide et simplifié

Les Laborantins s'abreuvent au Centre international de référence Chantal Biya

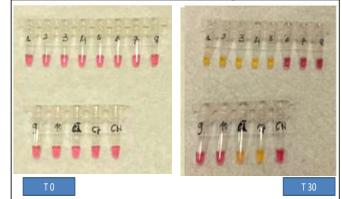
- C'est à la faveur d'un atelier qui a débuté hier, et s'achève ce jour, auquel prend part les responsables des laboratoires au niveau des districts en vue du renforcement de leurs capacités au diagnostic moléculaire rapide et simplifié de la Covid-19, pour l'appliquer à toute future pandémie.
- Ledit atelier est organisé par Le Centre international de référence Chantal Biya pour la recherche sur la prévention et la prise en charge du VIH/sida (CIRCB), dirigé par le Pr. Alexis Ndjolo, en partenariat avec le Centre de Coordination des Opérations d'urgence de la Santé publique (CCOUSP) et le Laboratoire National de Santé Publique (LNSP). Page 4



RT-LAMP laboratory training



RT-LAMP Result interpretation



Collaboration with the National Public Health Lab & the Public Health Emergencies Centre



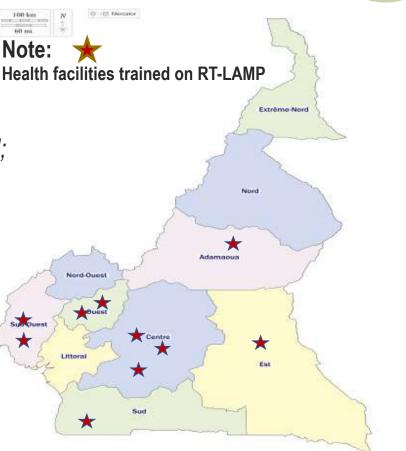
Phase 2+

V. PANDEMIC PREPAREDNESS WITH ICGEB (2/3)

Rollout of RT-LAMP in 10 Laboratories of Cameroon for community-based surveillance of emerging pathogens

Established RT-LAMP Lab network:

- 1. CIRCB: Lead reference lab.
- 2. NPHL: Yaoundé urban;
- 3. Ekoumdoum district hosp: Yaoundé rural;
- 4. Dang district hosp: Northern region;
- 5. Olamze district hosp: Southern boarder;
- 6. EUC laboratory: urban western region;
- 7. DREAM laboratory: rural western region;
- 8. FHS laboratory: urban southwest region;
- 9. Limbe Hosp: southwest boarder region;
- 10. Abong-Mbang district hosp: East region.





Phase 2+

V. PANDEMIC PREPAREDNESS WITH ICGEB (3/3)

Rollout of RT-LAMP in 10 African countries and in community

laboratories for front-line surveillance of emerging pathogens Sudan Burkina Faso 71 Senegal Ethiopia Ivory Coast Nigeria Cameroon Kenya Angola Zimbabwe

- The Project started officially on 20 August 2021 in five (05) African countries with nasopharyngeal swabs;
- Project expanded to 10 African countries with saliva testing and extraction-free;
- Project extended at the level of district laboratories for community-based surveillance of pathogens;
- Project network established for both epidemiological and genomic surveillance of other emerging pathogens (arboviral diseases) in sub-Saharan Africa.



Phase 2+

VI. TAKE HOME MESSAGE

ICGEB Capacity-building in Cameroon:

- **RT-LAMP technology**: user-friendly across laboratories;
- RT-LAMP implementation: successful both with nasopharyngeal swabs and with saliva samples in across laboratories;
- Genomic surveillance: effective with sequencing of variants;
- Extension of RT-LAMP technology to district laboratories: an addedvalue for disease surveillance within the local communities;
- Established network: an effective system contributing for optimal pandemic preparedness and surveillance in sub-Saharan Africa.











3RD INTERNATIONAL



COVID-19 team (at start)











COVID-19 team (with ICGEB)

IG AFRICA IN THE GLOBAL HEALTH









