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ACTIV INSIGHT018

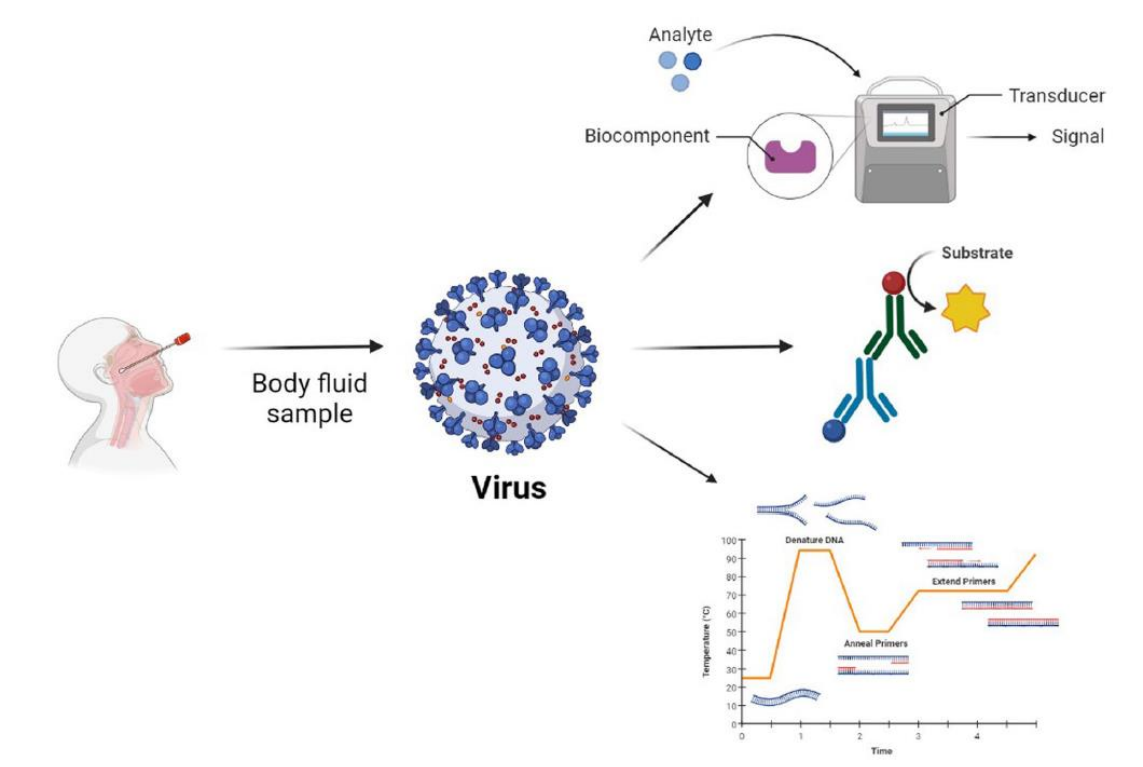
Strategies and Treatments for Respiratory Infections and Viral Emergencies

Diagnostic Challenges in Emerging Pathogens (largely focused on SARS-CoV-2)

HepHIV Conference, Madrid, 15th November 2023

Thanks to Katy Shaw-Saliba, DCR, NIAID

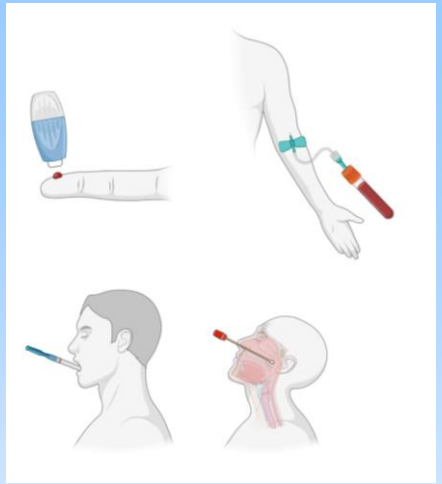
Technologies used for ID diagnosis

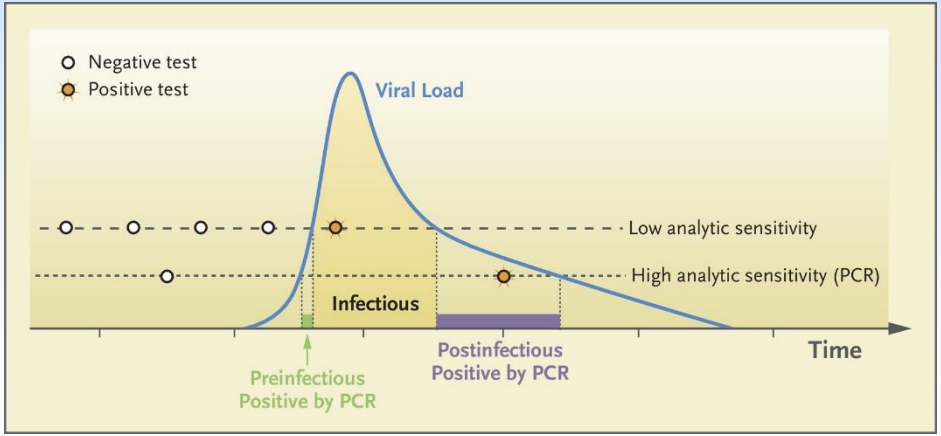


Harsh and Tripathi *Virology Journal (2023) 20:143*

General challenges

- Specimen type choice: least invasive but most likely to have virus
- Testing of asymptomatic contacts
- Antigen tests are more rapid but more prone to false negatives
- Turnaround time for molecular tests
- Scarcity of testing materials (swabs, UTM)

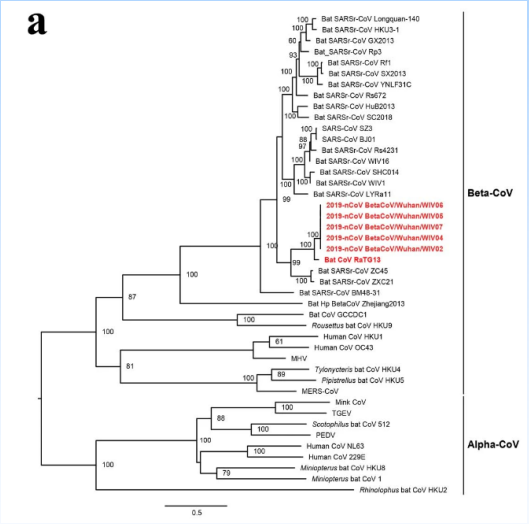


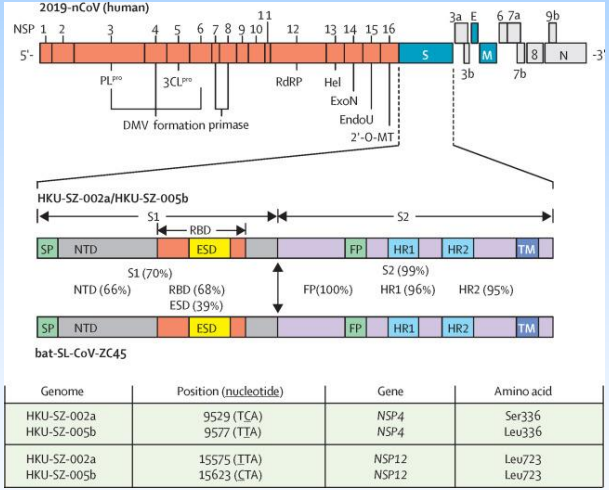


Created with BioRender.com

Mina, M et al., N Engl J Med 2020; 383:e120 DOI: 10.1056/NEJMp2025631

First challenge with a newly emerging pathogen: Identification of target regions for diagnostic assays





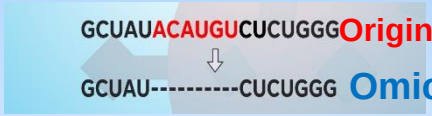
Genome	Position (nucleotide)	Gene	Amino acid
HKU-SZ-002a HKU-SZ-005b	9529 (TCA) 9577 (TTA)	*NSP4* *NSP4*	Ser336 Leu336
HKU-SZ-002a HKU-SZ-005b	15575 (TTA) 15623 (CTA)	*NSP12* *NSP12*	Leu723 Leu723

- Rapid sequence analysis and deposit in a public database of the SARS-CoV-2 genome sequence was key
- Pathogen target region decision:
  - Specific for the emerging pathogen but also conserved enough to detect variants
  - For antigen tests: expression level of the protein matters

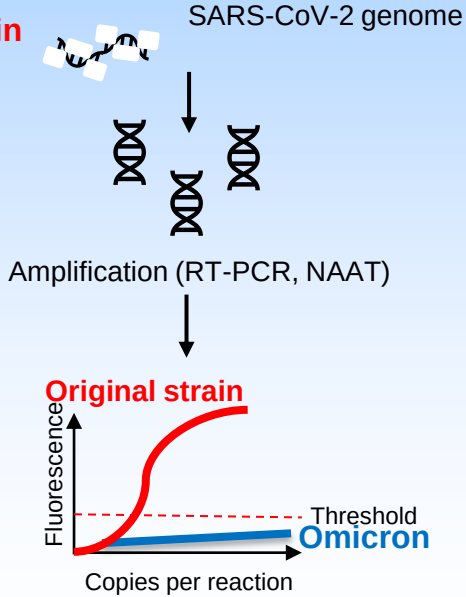
Zhou, P., Yang, XL., Wang, XG. et al. Nature 579, 270–273 (2020).;
Chan, Jasper Fuk-Woo et al. The Lancet, Volume 395, Issue 10223, 514 - 523

Impact of viral variants on molecular tests: deletions and point mutations can result in target amplification failure so it is important to have multiple targets

Spike: Δ69-70



Primer and/or probes no longer bind



Negative

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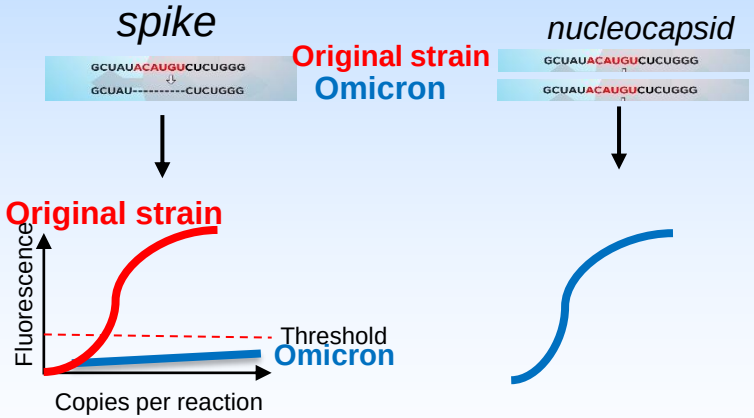
If multiple gene targets (TaqPath)



Positive

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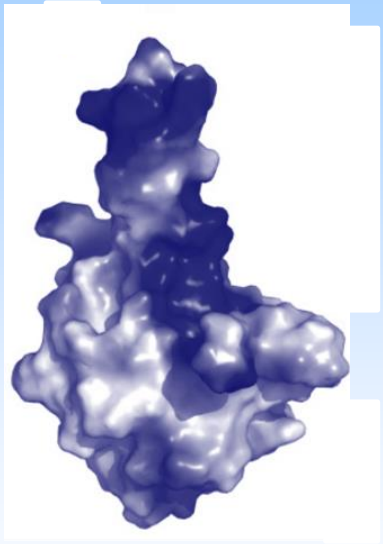
Turn-around time of the essence:

case example of 3 emergency departments using cohort isolation until test results became available

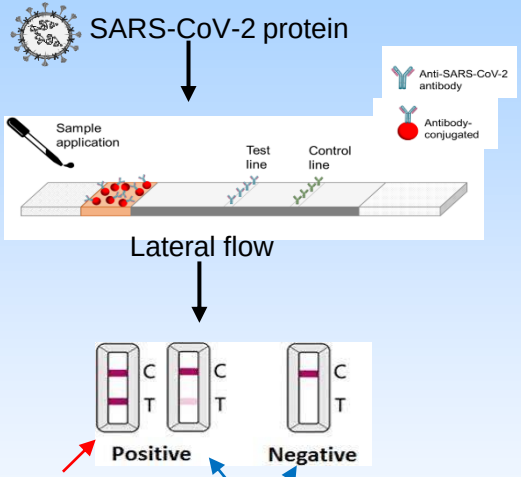
	Standard platform	Rapid return	P-value
Order to result time - median	7.8 hrs (IQR) 3.71–11.68]	1.9 hrs (IQR 1.40–2.82)	<0.0001
% with available result before departure from ED	51%	92%	<0.0001
Exposure time for uninfected - median	19.2 hrs (IQR 9.45–44.59)	6.6 hrs (IQR 4.13–13.57)	<0.001

Hinson et al, J Hosp Inf 2021

Impact of viral variants on antigen assays



Nucleocapsid protein



Original strain

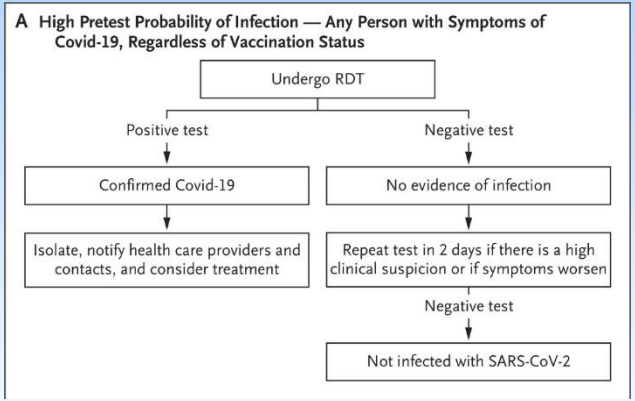
Variant (reduced sensitivity)

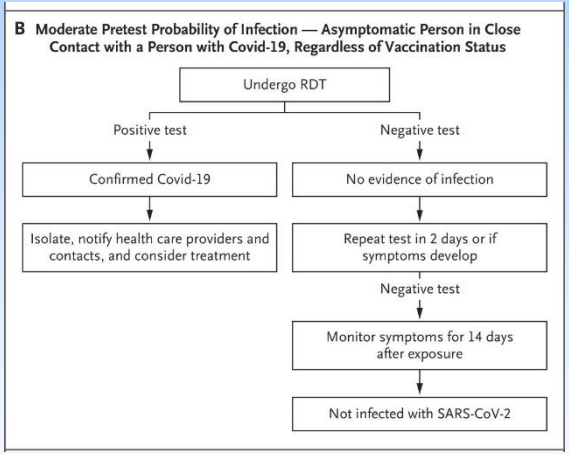
- Antigen assays recognize a tertiary structure
- For SARS-CoV-2: most antigen assays target nucleocapsid (tends to be more conserved than spike)
- Mutations in nucleocapsid could result in reduced sensitivity
- The limit of detection in antigen assays is lower than PCR so decreased viral load can impact performance

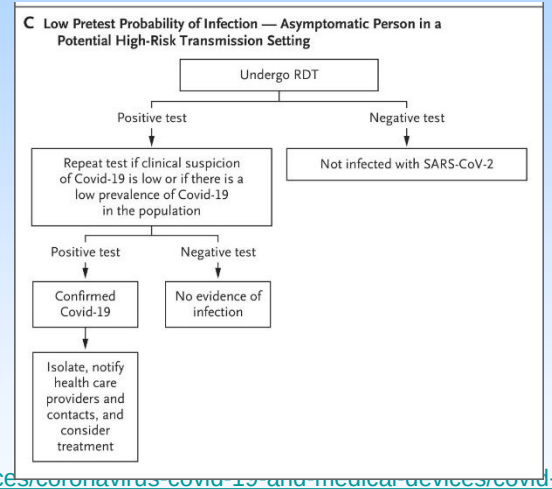
Image: https://www.sciencedirect.com/science/article/pii/S2211383520305505

Antigen assays: other considerations

- Sensitivity on antigen assays is lower than molecular assays
  - Particularly when a person is asymptomatic/pre-symptomatic or viral load is low
  - Testing algorithm depends on the pretest probability



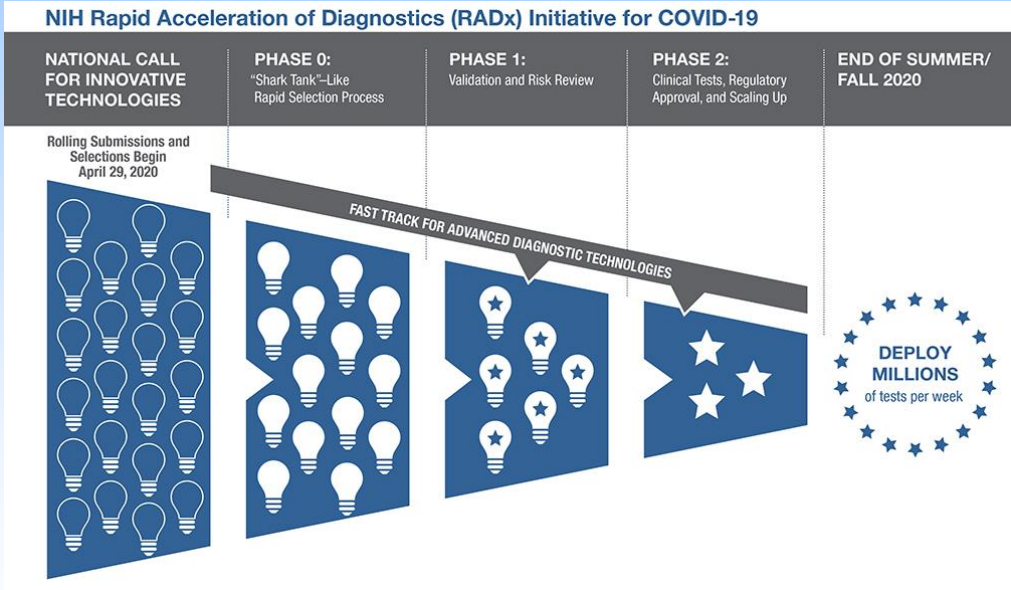




https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html; https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/covid-19-test-uses-faqs-testing-sars-cov-2; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7799021/; https://www.medrxiv.org/content/10.1101/2022.01.05.22268788v1; https://academic.oup.com/cid/article/73/9/e2861/6105729; https://www.nejm.org/doi/full/10.1056/NEJMp2025631; https://www.medrxiv.org/content/10.1101/2022.01.04.22268770v1.full.pdf; https://www.medrxiv.org/content/10.1101/2021.12.22.21268246v1.full; https://www.nejm.org/doi/full/10.1056/NEJMcp2117115?query=RP&cid=NEJM%20Recently%20Published,%20January%207,%202022%20DM609583_NEJM_Non_Subscriber&bid=763041445#section_key_clinical_points

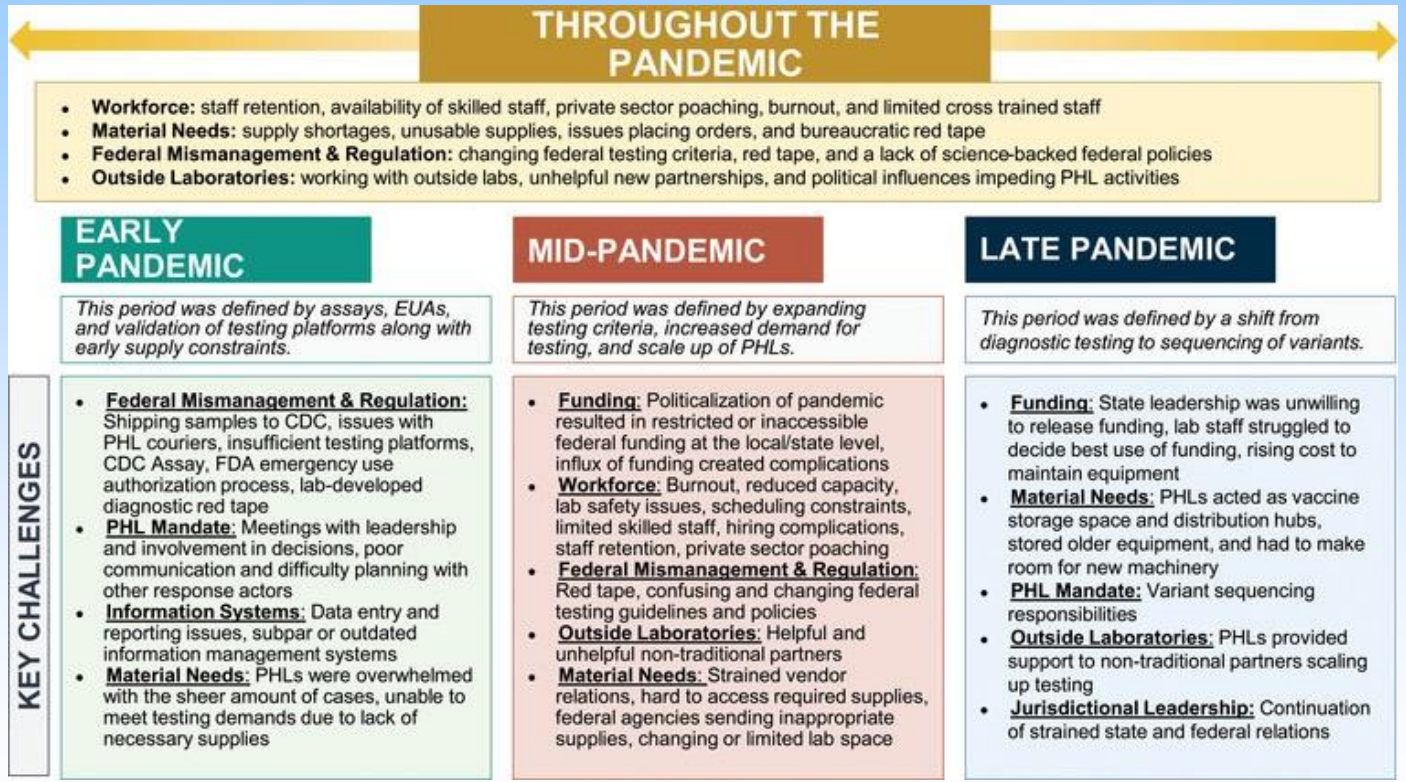
Performance evaluations of new diagnostics with emerging pathogens can be challenging

- No gold standard at the start of an outbreak with an emerging pathogen
  - Usually, molecular tests (questions around if nucleic acid correlates to infectious agent)
- Performance evaluations are dependent on clinical specimens which may be limiting and difficult to source
- Emergency Use Authorization/Emergency Use Listing often rely on performance evaluations with smaller numbers of specimens
  - Independent evaluations (e.g. FIND)
- Government-lead programs are aimed at accelerating development and evaluation of diagnostics (e.g. USG RADx)



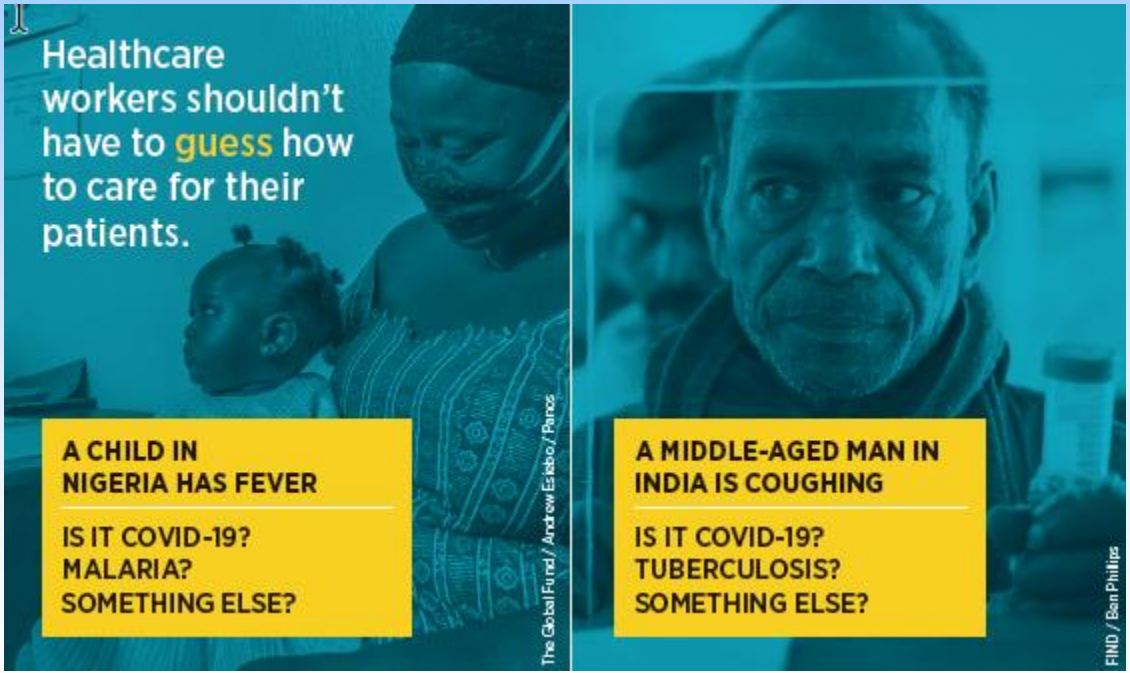
https://www.nih.gov/research-training/medical-research-initiatives/radx/radx-programs; Reitsma, Johannes B. et al. Journal of Clinical Epidemiology, Volume 62, Issue 8, 797 – 806; Escadafal C, et al. BMJ Glob Health. 2023 Jul;8(7):e012506; https://www.finddx.org/covid-19/find-evaluations-of-sars-cov-2-assays/

Summary of challenges



Potter, Christina et al. Journal of Public Health Management and Practice 28(6):p 607-614, 2022
Escadafal et al, BMJ Global Health, 2023

The diagnostic dilemma

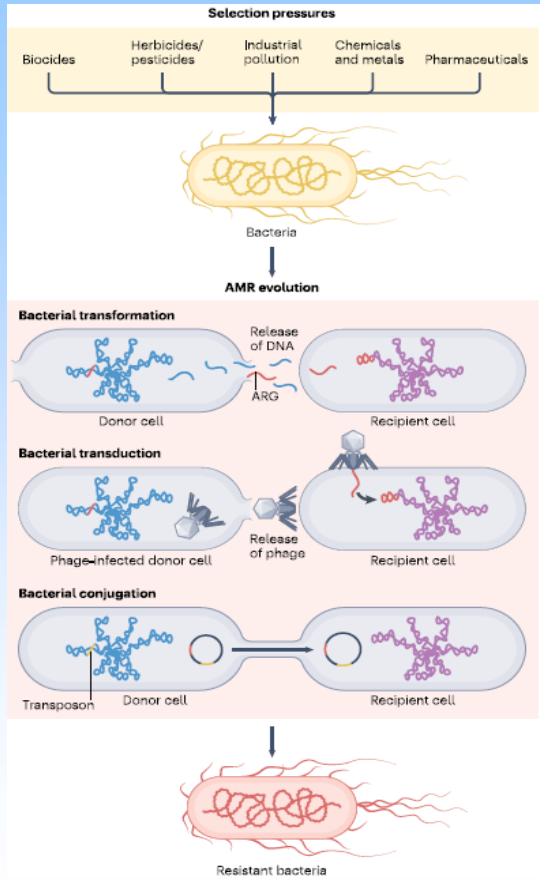


Pre-test probability for a given disease ?

How to deal with the dilemma ?

Antimicrobial resistance

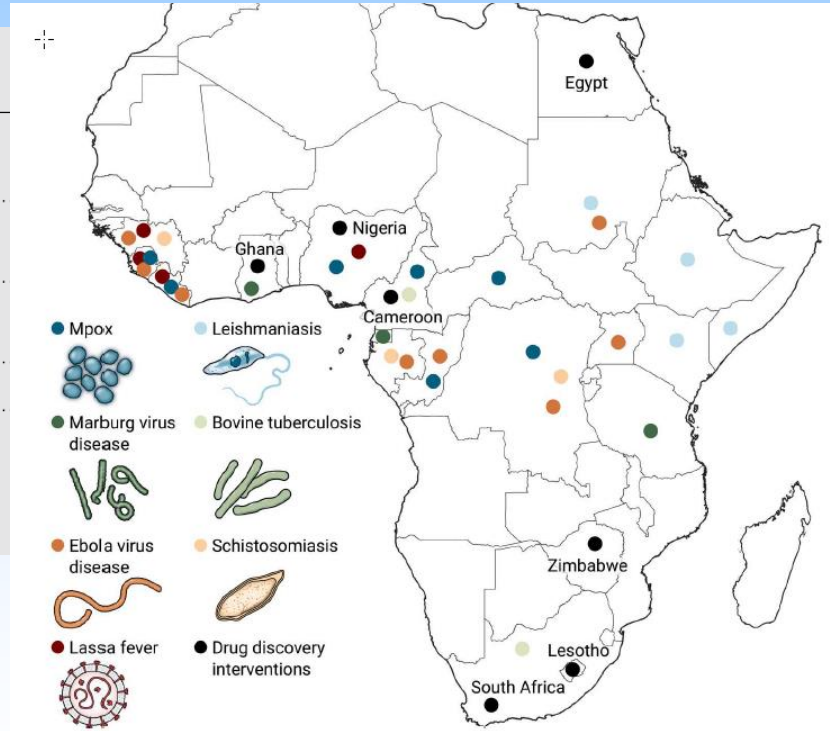
- Caused by interaction between
  - Humans, food-producing animals, wildlife, insects and the environment
- Genomic technologies can be used to monitor
  - “One-health” genomic surveillance



Djordjevic et al, Nature Reviews Genetics, 2023

Zoonotic outbreak in last decade in Africa*

Disease area	Number of people affected	Animal reservoir	Year
Leishmaniasis	≍1 million (*29*)	Dogs, rock hyraxes, rodents, weasels, and hedgehogs	Annually
Schistosomiasis	≍68 million (*5*)	Rodents, cattle, goats, sheep, horses, and camels	2021
Ebola	≍28,600 (*30*)	Bats	2014–2016
Marburg	25 (*31*)	Bats	2023
Mpox	≍1400 (*32*)	Unknown (*33*)	2022



*63% increase in number of zoonotic outbreaks in last decade compared with former decade

https://www.afro.who.int/news/africa-63-jump-diseases-spread-animals-people-seen-last-decade

Hikaambo et al, *Sci. Transl. Med.* 15, 2023

Global inequity

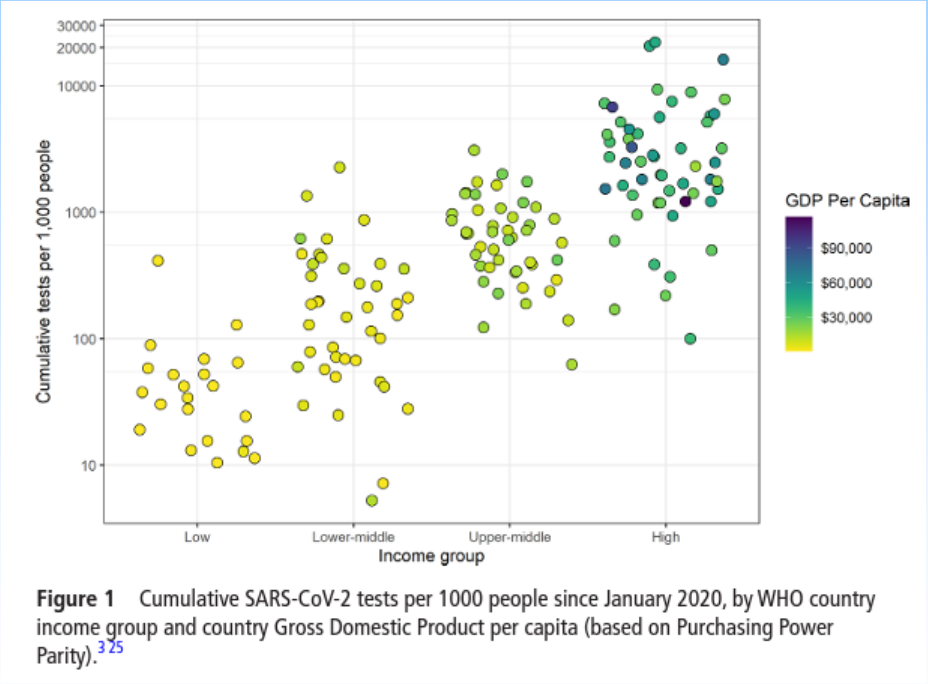


Figure 1 Cumulative SARS-CoV-2 tests per 1000 people since January 2020, by WHO country income group and country Gross Domestic Product per capita (based on Purchasing Power Parity).[3 25]

- Global inequality: resource limited/Global South often not able to purchase and support testing capacity
  - Leveraging of resources in West Africa grown out of the Ebola outbreak in 2014
- Importance of lower cost diagnostics with the ability to test near the point-of-care (without elaborate laboratory setup)

Narayanasamy S, Okware B, Muttamba W, et al. J Epidemiol Community Health 2022;76:972–975; Diarria, B, et al. Front. Trop. Dis, 31 January 2022 Sec. Disease Prevention and Control Policy Volume 2 - 2021

Key areas to build from COVID-19 that can boost access to testing for all diseases

- Political focus and collaboration
- Sequencing capacity for disease surveillance and rapid response
- Diagnostic prioritization within health systems
- Technologies to improve diagnostics use, including mobile and digital tools
- Health worker training and diagnostic literacy
- Innovation and manufacturing capacity

ACT-Accelerator Diagnostics Pillar working group leads

Table 1 Challenges and opportunities in conducting evaluation studies of diagnostic tests during outbreak situations and identified opportunities for improvement

Challenge	Opportunities
Access to well-characterised clinical samples and reference materials	► Identify mechanisms that can ensure timely ethical approval for collection, study and storage of specimens ► Implement virtual biobanks and biobanking networks at regional and international levels ► Create online catalogues of all available reference and control materials for specific diseases
Delays in importation and customs clearance processes	► Ensure logistics teams are sufficiently staffed, well organised and experienced both at sponsor and study sites ► Form relationships with national regulatory authorities ► Develop regulatory/import processes that allow for rapid approval of permits and customs clearance during outbreak situations
Delays in drafting, approving and implementing study materials (eg, protocols, tools, contracts)	► Develop 'emergency mode' procedures that can scale-up human resources and fast-track internal processes during health emergencies ► Prepositioning of generic templates and adaptable systems that can be adopted rapidly during emergencies ► Develop generic and adaptive protocols and study documents (eg, case report and informed consent forms) that have already been reviewed by key actors and require minimal input to be implemented in a timely manner
Limited or stretched resources at study sites during an outbreak affecting conduct of evaluation studies	► Conduct clinical and laboratory assessments prior to any evaluation ► Establish contingency funding to be triggered once an outbreak and need for test clinical evaluations is identified, to support human resource needs and purchasing of supplies, equipment and personal protective equipment
Hesitation to participate in a study due to stigma linked to the disease or fear of negative impact on employment	► Integrate social sciences and engage local communities in the early stages of clinical study design
Constantly changing disease incidence and evolving testing strategies affecting ability to achieve study objectives	► Establish network of partners with agreements already in place so evaluation plans can shift from one site to another ► Ensure that import permits are already approved for all tests across all potential partner sites ► Integrate adaptive clinical study designs during protocol development and study implementation ► Ensure that tests are compatible with stored samples and universal media
Travel restrictions preventing in-person training and monitoring visits at study sites	► Use of teleconferencing tools ► Develop detailed assessment, training and monitoring tools (including online/remote options) and electronic data capture systems with audit trail
Variable quality of clinical trial data across sites	► Implement network of study sites to ensure common practices are performed to a high standard
Difficulties ensuring independence of study conduct and data analysis	► Apply well-defined and transparent scoring processes ► Do not accept tests free of charge ► Submit all data to open-access repositories, independently of the manufacturer's opinion of the results