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Principles and Practice of Molecular Microbiology in Clinical Care and Public Health

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The International Diagnostics Centre

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Molecular Microbiology in Clinical Care and Public Health

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- **Recent advances in molecular technology for microbiology**
- **Molecular testing for common clinical syndromes**
- **Use of molecular testing for public health**
- **Summary**

Mention of company products does not imply endorsement

The Ideal Diagnostic Test

2003

- **A = Affordable**
- **S = Sensitive**
- **S = Specific**
- **U = User-friendly**
- **R = Rapid and robust**
- **E = Equipment-free**
- **D = Deliverable**

2018

- **R = Real time connectivity**
- **E = Ease of specimen collection**
- **A = Affordable**
- **S = Sensitive**
- **S = Specific**
- **U = User-friendly**
- **R = Rapid and robust**
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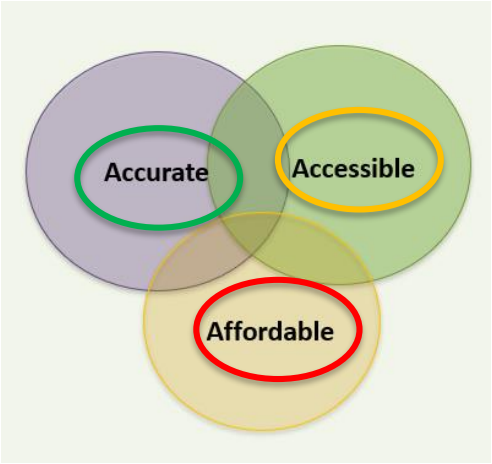
Molecular Tests



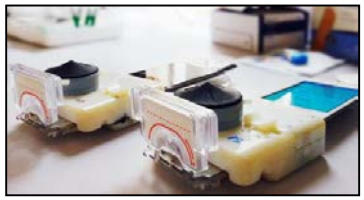
Mabey D, et al. Diagnostics for the developing world. Nature Rev Microbiol 2: 231-40, 2004.

Land KJ,et al. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. Nat Microbiol. 4(1):46-54.2019. e-pub Dec 2018.

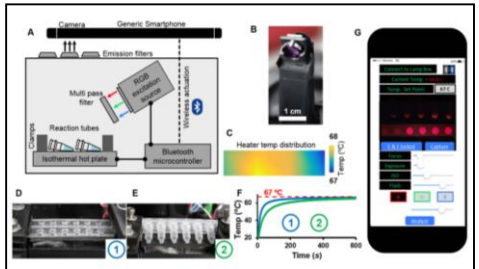
Explosion in Point-of-care (POC) Molecular Detection Technologies

Plug and play format:

- Minimal Hands on time
- Multiplex testing
- Rapid time to result
- Data transmission



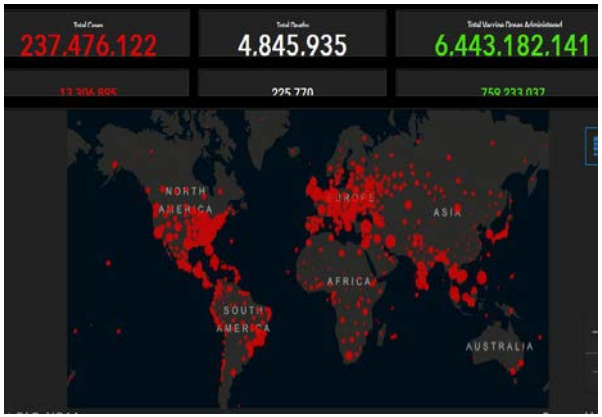
Laksanasopin et al. *Science Transl Med* 2015:7:273



Priye, A. et al. A smartphone-based diagnostic platform for rapid detection of Zika, chikungunya, and dengue viruses. Sci Rep 2017;7:44778

COVID-19 and Diagnostic Innovations

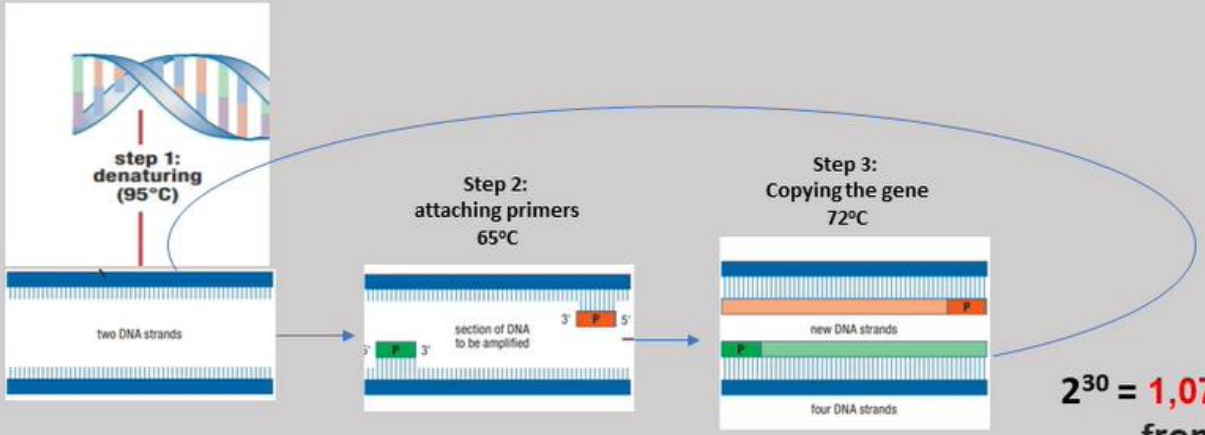
- More and better diagnostics
  - Unprecedented response from industry: > 1,000 tests
  - Faster molecular detection at point-of-care:
  - More sensitive rapid antigen tests
  - Home or self tests used in >100 countries
  - Diagnostics being used as public health tools in non-health care settings

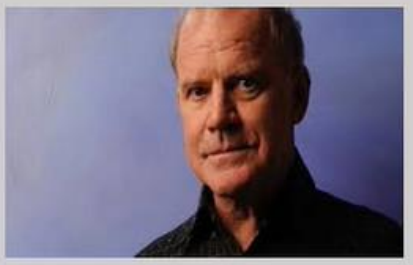


https://coronavirus.jhu.edu/map.html

- Increased testing capacity in countries, esp more molecular testing
- Increased data connectivity enabling the translation of data into real-time intelligence to provide timely alerts of outbreaks and to inform control strategies
- Data display (dashboards) allow transparency and engender trust among stakeholders including the public so that everyone can do their part in the pandemic response

Invention of the Polymerase Chain Reaction (PCR) Technology





Nobel Prize winner, Kary B. Mullis

2^{30} = 1,073,741,824 copies
from one copy of DNA

- PCR uses an enzyme called a **polymerase** to copy DNA
- The copying process works at 3 different temperatures
- After 30 cycles of this 3-step process (also known as a **chain reaction**), more than 1 billion copies of DNA can be obtained from a single copy in a few hours

Isothermal Amplification methods:

- Loop-Mediated Isothermal Amplification (LAMP) - 60-65°C, <1 hr
- Recombinase Polymerase Amplification (RPA) 37-42°C, 5-13 min
- Nucleic Acid Sequence-Based Amplification (NASBA) 41°C, 1-2 hrs

Cepheid: A Multi-disease, Random Access Real-time PCR Platform - 31 CE-IVD Tests

MTB/RIF (2hrs), MTB/RIF ultra (<80 min), **MTB/XDR** (<90 min)

SARS-CoV-2 (25 min), **Flu A & B, RSV, SARS-COV-2/Flu/RSV** (36 min), **Strep A** (18 min)

HIV early infant diagnosis, HIV Viral Load, HCV, CT/Ng (90 min); **Tv** (40-65 min); **HPV 16/18/45** (60 min); ***M. genitalium* + macrolide resistance** (2 hrs), ***Group B strep*** *(56 min)*

Healthcare Associated Infections: MRSA (60 min), **Carba-R** (50 min), ***C. difficile*** *(45 min),* **Norovirus, vanA/B** (48 min)

Ebola (Zaire – 94 min**)**

Multiplexing pipeline:
MTB/RIF/INH; Respiratory panel
GI panel; Tropical Fever panel;
Carba-R

Molecular tests with Faster time to Result: Abbott ID NOW Point-of-care Molecular Platform

Principle: Nucleic acid amplification system (iNAAT) that uses isothermal amplification and a fluorescence-based molecular signal for detection

Applications:

- Approved: COVID-19, Influenza virus A and B, RSV, Strep A
- In clinical trials: Ct/Ng
- In development: *C. difficile*

Operation:

- Adapted for use by non-laboratory staff
- 2 min of “hands on” time
- Time to result: 15 min

Connectivity:

- Cloud-based data storage
- Bi-directional connectivity

Multiplex Molecular PANELS

Principle: With integrated sample preparation, amplification, detection, and analysis, the BioFire System uses multiplex 2-staged nested PCR technology with dried reagents in a plastic pouch to simultaneously test for a comprehensive grouping of targets in about 1 hour.

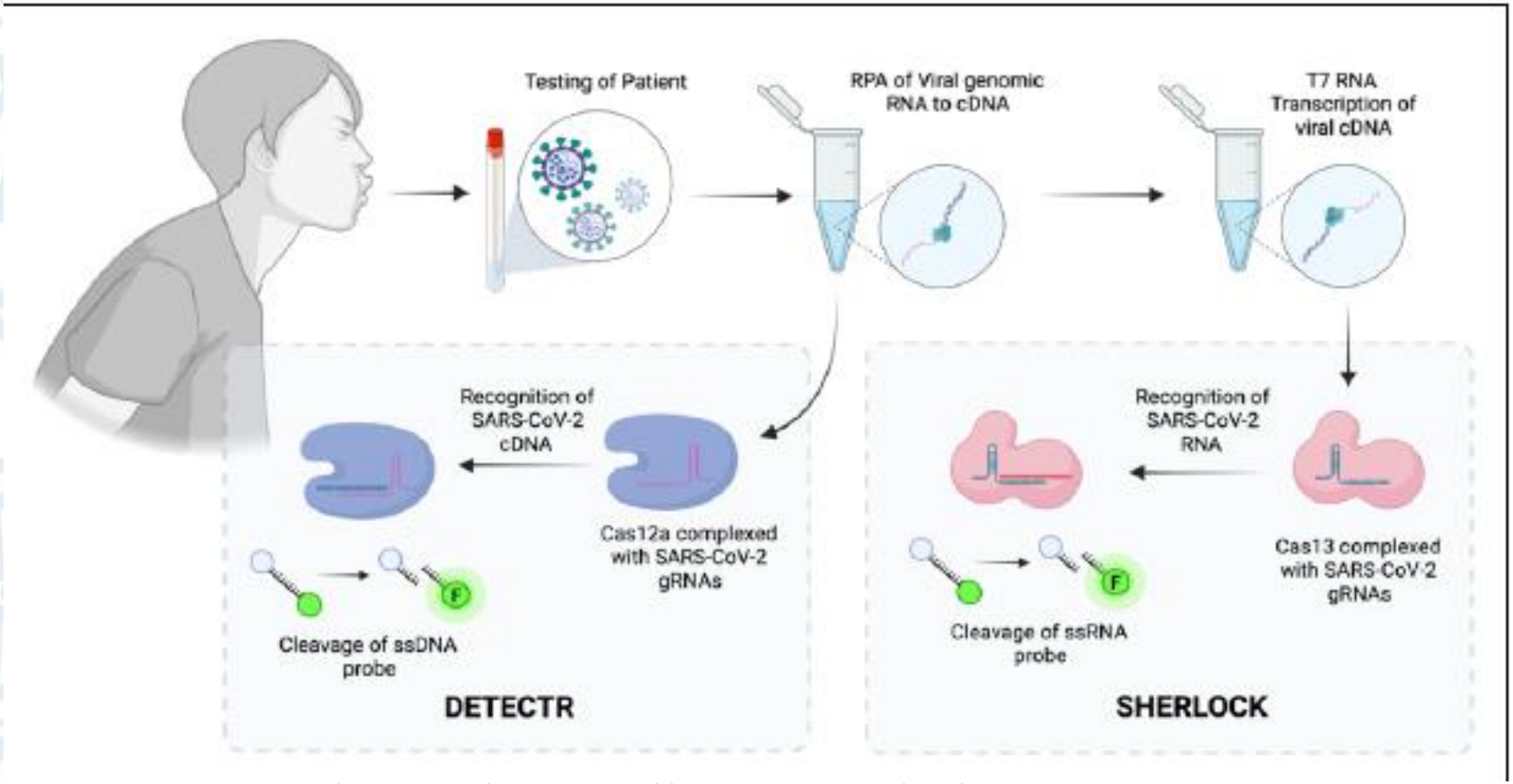
Applications :

- BIOFIRE Respiratory panel*: 23 Targets in One test. ~45 Minutes.
  - Sample Type: Nasopharyngeal swab
- BIOFIRE Blood Culture Identification: 43 Targets in One Test. ~1 Hour.
  - Sample Type: Positive blood culture
- BIOFIRE Gastrointestinal panel: 22 Targets in One Test. ~1 Hour.
  - Sample Type: Stool in Cary Blair
- BIOFIRE Meningitis/Encephalitis panel: 14 Targets in One Test. ~1 Hour.
  - Sample Type: Cerebrospinal fluid (CSF)
- BIOFIRE Pneumonia panel: 33 Targets in One Test ~1 Hour.
  - Sample Type: BAL: (including mini-BAL), Sputum: (including endotracheal aspirate)
- BIOFIRE Joint Infection: 39 Targets in One Test. ~1 Hour.
  - Sample Type: 0.2 mL of synovial fluid
  - Investigational use only.

*Respiratory panel: Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus OC43, Coronavirus NL63, Middle East Respiratory Syndrome CoronaVirus (Mers-CoV), Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Human Metapneumovirus, Human Rhinovirus/Enterovirus, Flu A, flu A/H1, flu A/H1-2009, flu A/H3, flu B, Parainfluenza 1-4, RSV, *Mycoplasma pneumoniae, Bordetalla pertussis, Bordetella parapertussis and Chlamydophila pneumoniae*

CRISPR Technology for Infectious Diseases

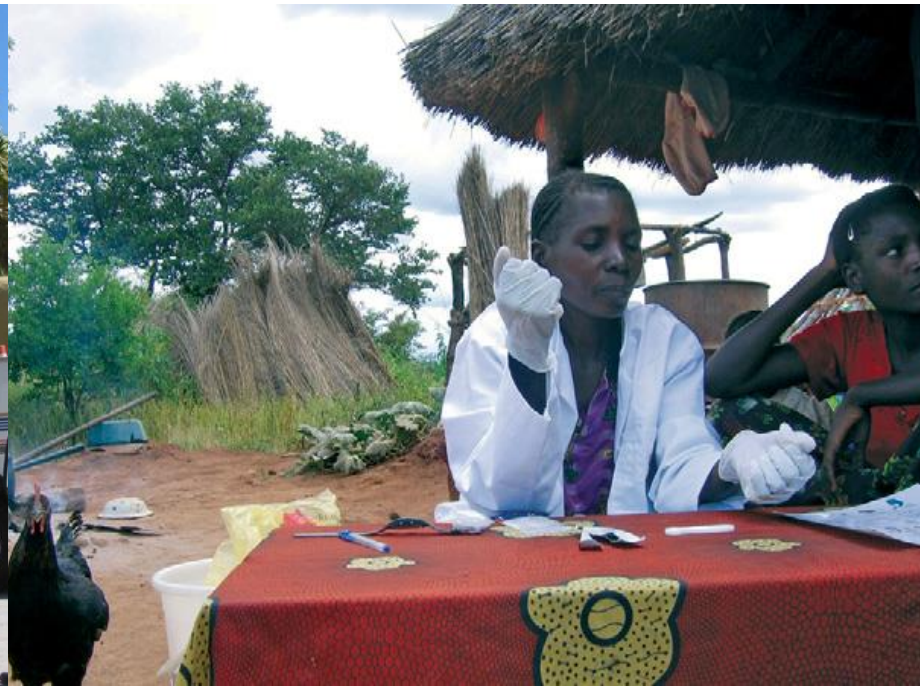
SHERLOCK and DETECTR have successfully been validated to detect SARS-CoV-2, Ebola Virus, flu A. Development of readout methodologies varies, spanning from fluorescence-based assays, that can be implemented in multiplex high-throughput screening platforms, or as inexpensive, field-deployable lateral flow strip assays.



Kirby, E.N et al. CRISPR Tackles Emerging Viral Pathogens. Viruses **2021**, 13, 2157. https://doi.org/10.3390/v13112157

Rapid vs Point-of-Care (POC) Tests

Courtesy Dr. Ray Waters



Senior K. Lancet ID 9: 467 2009

Molecular Microbiology in Clinical Care and Public Health

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- Recent advances in molecular technology for microbiology
- **Molecular methods for common clinical syndromes**
- Use of molecular methods in public health
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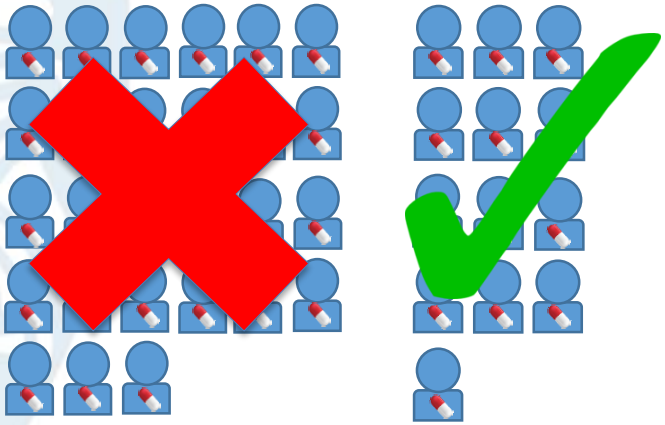
Respiratory Infections

Overuse of Antibiotics in Clinical Medicine

Fever, and respiratory infections are among the most common reasons why people seek care. Presumptive treatment with broad spectrum antibiotics has contributed and accelerated to the emergence of antibiotic resistance

In the US each year, approx. 40 million people are given antibiotics each year for respiratory issues but 27 million were given antibiotics unnecessarily.



Ref: Shapiro et al. Antibiotic prescribing for adults in ambulatory care in the USA, 2007-9. J Antimicrobial Chemother 2013.

Host biomarkers have been used to guide appropriate use of antibiotics:

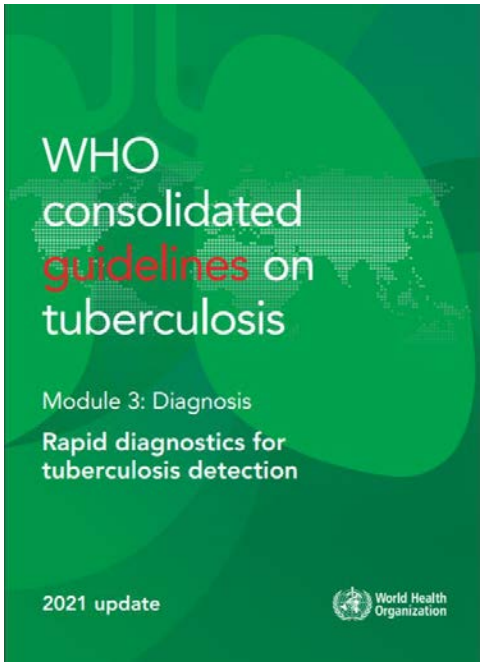
wbc, CRP, PCT, ESR

ELISA-based ImmunoExpert™ assay (MeMed Diagnostics, Israel) measures 3 different host proteins: CRP, TNF-related apoptosis-inducing ligand (TRAIL) and interferon gamma-induced protein 10 (IP-10)

FebriDx™ (RPS Diagnostics,USA), is a semi-quantitative test that combines CRP and the myxovirus resistance protein 1 (MxA), a marker for viral infection, and provides results within 15 min

Ross MH et al. Host Based Diagnostics for Acute Respiratory Infections. Clinical Therapeutics 2019; 41: 1923-1938

Updates to TB Diagnostic Guidelines



WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection

Guidelines included in the 2021 consolidated document:

- *Issued in 2021*
  - Moderate complexity automated NAATs for detection of TB and resistance to rifampicin and isoniazid
  - Low complexity automated NAATs for detection of resistance to isoniazid and second-line anti-TB agents
  - High complexity hybridization based NAATs for detection of resistance to pyrazinamide

- *Issued in 2020*
  - Xpert MTB/RIF and Xpert MTB/RIF (Ultra)
  - Truenat MTB, Truenat MTB Plus and Truenat MTB-RIF Dx

Source: WHO consolidated guidelines on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO

Updates to TB Diagnostic Guidelines

Technology class	Products included in the evaluation
	Xpert® MTB/RIF and Xpert® MTB/RIF Ultra (Cepheid)*
	Truenat™ (Molbio) *;
Moderate complexity automated NAATs for detection of TB and resistance to rifampicin and isoniazid	Abbott RealTime MTB and Abbott RealTime MTB RIF/INH (Abbott) BD MAX™ MDR-TB (Becton Dickinson) cobas® MTB and cobas MTB-RIF/INH (Roche) FluoroType® MTBDR and FluoroType® MTB (Hain Lifescience/Bruker)
	TB-LAMP (Eiken) *
Antigen detection in a lateral flow format (biomarker-based detection)	Alere Determine™ TB LAM Ag (Alere)
Low complexity automated NAATs for the detection of resistance to isoniazid and second-line anti-TB agents	Xpert® MTB/XDR (Cepheid)
Line probe assays (LPAs)	GenoType® MTBDR*plus* v1 and v2; GenoType® MTBDR*sl*, (Hain Lifescience/Bruker), Genoscholar™ NTM+MDRTB II; Genoscholar™ PZA-TB II (Nipro)

*These recommendations are currently product specific but will be changed to class-based to align with the other recommendations.

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Arboviruses Infections

Laboratory Diagnosis: Dengue

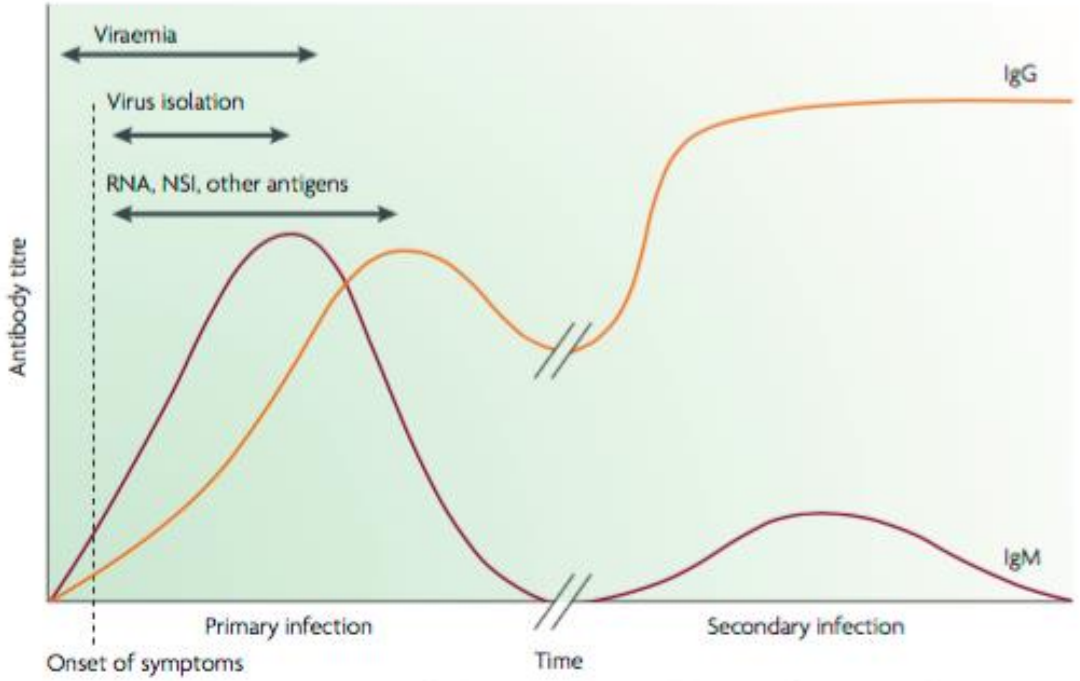


Figure 2 | **Major diagnostic markers for dengue infection.** The titre of the IgM and IgG response varies, depending on whether the infection is a primary or secondary infection.

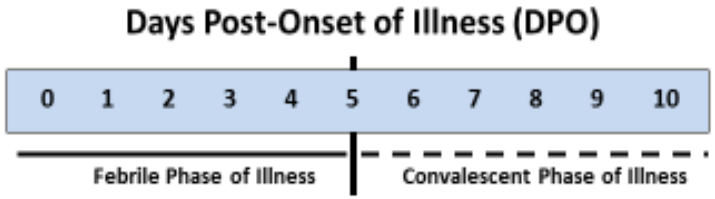
Patient Management:

- **Confirmed** :acute infection
  - Virus isolation
  - Nucleic acid detection
  - Antigen detection
  - Seroconversion for IgM
  - 4-fold rise in IgG titres
- **Highly suggestive**:
  - IgM positive

Reimagining the Future of the Diagnosis of Viral Infections

- 1,234 paired serum samples from laboratory confirmed dengue patients, archived between 2005-2011

- accurately identified >90% of primary and secondary dengue cases from a single serum specimen collected during the first 10 days of illness by using either:
  - DENV-1-4 real-time RT-PCR + IgM ELISA
  - NS1 antigen ELISA to detect DENV + IgM ELISA



Specimen from suspected dengue case by DPO	IgM anti-DENV	RT-PCR or NS1	Percent Positive	Decision
0-3	-	+	79-90%	One-Test
4-7	+	+	95-100%	Two-Test
>7	+	-	93-100%	One-Test

Hunsperger et al JID Mar 2016

EUA-approved ZIKV Molecular Assays

- **RealStar® Zika Virus RT-PCR Kit U.S. (Altona Diagnostics GmbH, Germany)**
- ***Sentosa*® SA ZIKV RT-PCR Test (Vela Diagnostics U.S., Inc., U.S.)**
- **LightMix® Zika rRT-PCR Test (Roche Molecular Systems, Inc., U.S.)**
  - The claimed LoD of the test is 181 Copies/mL.
- **xMAP® MultiFLEX™ Zika RNA Assay (Luminex Corporation, U.S.)**
  - Claimed LoD is 687 copies/mL
- **VERSANT® Zika RNA 1.0 Assay (kPCR) Kit (Siemens Healthcare, Inc., U.S.)**
  - LoD of the test is 721 copies/mL
- **Zika Virus Real Time RT-PCR Kit (Liferiver™/Shanghai ZJ Bio-Tech Co, China)**
- **Aptima® Zika Virus Assay (Hologic, Inc.)**
- **Zika Virus Real-time RT-PCR (Viracor –IBT Laboratories)**

Not EUA- approved:

- **FTD Zika Virus (Fast Track Diagnostics Ltd., Luxembourg)**
- **Genesig® Kits for ZIKV (Primerdesign™ Ltd., UK)**
- **Zika Virus – Single Check FR325 (Genekam Biotechnology AG)**

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SEXUALLY TRANSMITTED AND BLOOD BORNE INFECTIONS

WHO STI POC Test Initiative

World Health Organization

human reproduction programme hrp research for impact
UNDP-UNFPA-UNICEF-WHO-WORLD BANK

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Sexual and reproductive health

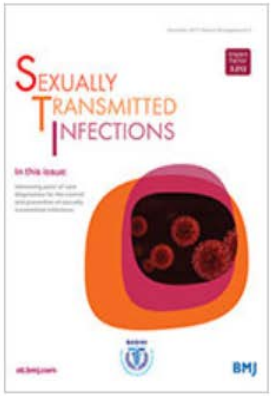
"The way forward": Quick, accurate tests to diagnose sexually transmitted infections

Greater investment needed worldwide in point-of-care tests

12 December 2017: A special supplement to the journal *Sexually Transmitted Infections* highlights the urgent importance of investing in the research, development and scaling up of the use of point-of-care tests.

– Download the supplement

Each year, there are an estimated 357 million new infections with 1 of the following 4 curable STIs: chlamydia, gonorrhoea, syphilis and trichomoniasis. An estimated 290 million women are infected with human papillomavirus – an STI which can cause cervical cancer. Herpes simplex virus and syphilis can increase the risk of



- Target Product Profiles for POCTs
- STI POCT Landscape
- Systematics reviews
- Protocols for POCT evaluations
- Multi-country evaluations

https://www.who.int/reproductivehealth/topics/rtis/pocts/en/

Toskin I et al. Advancing point of care diagnostics for the control and prevention of STIs: the way forward. Sex Transm Infect 2017;93:S81–S88.

Diagnosis of Genital Chlamydial and Gonococcal Infection

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CT:

- Culture and fluorescent microscopy require expensive equipment and technical expertise
- Molecular tests are highly sensitive and specific but not affordable

NG:

- Sensitivity of microscopy is >90% for men but only ~50% for women
- Culture is the gold standard but requires a laboratory

***Systematic review of C. trachomatis rapid POC antigen tests*:**

Pooled sensitivity (from 11 studies, 11,889 patients):

- **Vaginal swab**: **37%** (95% CI: 22.9 - 52.9%)
- **Endocervical swab**: **53%** (95% CI: 34.7 - 70.8%)

Systematic review of N. gonorrhoeae rapid POC antigen tests:

Mean sensitivity:

- **Vaginal swab: 54%** (95% CI: 37-71%)
- **Endocervical swab**: range: **12.5 -70%**

Kelly H, et al. STI 2017;93:S22–S30; Guy RJ, et al. STI 2017; 93:S16–S21.

Pooled Performance of POC Nucleic Acid Amplified Tests for *C. trachomatis*

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Nucleic acid amplification tests (NAATs) are gold standard and can be used with self-administered vaginal swabs, or urine samples in men, but are expensive

POC Test Xpert CT/NG	**N**	**Reference Standard**	**Sensitivity % (prevalence)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**
Gaydos et al[1]						
Vaginal:	581 S 1,132 A	BD ProbeTec and GenProbe	100 (5.2) 98 (4.3)	100 99	91 87	100 100
Cervical:	582 S 1,128 A	TMA	100 (5.1) 96 (4.3)	100 99	97 89	100 100
Urine (Women)	582 S 1,136 A		100 (5.3) 96 (4.5)	100 100	97 96	100 100
Urine (Men)	254 S 1,132 A		96 (21) 100 (2.6)	100 100	100 97	99 100
Goldenberg et al[2] Rectal (Men)	409	GenProbeTMA	86 (10.5)	99	93	98
Pooled values	**3,518**	**NAAT**	**95**	**100**	**94**	**100**

S = symptomatic; A = Asymptomatic

1 Gaydos et al JCM 2013; 2 Goldenberg et al JCM 2012

Pooled Performance of POC Nucleic Acid Amplified Tests for N. *gonorrhoeae*

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Nucleic acid amplification tests (NAATs) are gold standard and can be used with self-administered vaginal swabs, or urine samples in men, but are expensive

POC Test Xpert NG	**N**	**Reference Standard**	**Sensitivity % (prevalence)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**
Gaydos et al[1]						
Vaginal:	581 S	BD ProbeTec	100 (1.7)	100	91	100
	1,132 A	and	100 (1.1)	100	92	100
		GenProbe				
Cervical:	582 S	TMA	100 (1.7)	100	100	100
	1,128 A		100 (1.1)	100	100	100
Urine (Women)	582 S		100 (1.9)	100	100	100
	1,136 A		92 (1.1)	100	92	100
Urine (Men)	254 S		98 (17.7)	100	100	100
	1,132 A		100 (0.4)	100	83	100
Goldenberg et al[2]						
Rectal (Men)	409	GenProbeTMA	91 (14%)	100	100	99
Pooled values	**3,518**	**NAAT**	**98**	**100**	**95**	**100**

S = symptomatic
A = Asymptomatic

1 Gaydos et al JCM 2013
2 Goldenberg et al JCM 2012

binx *io*

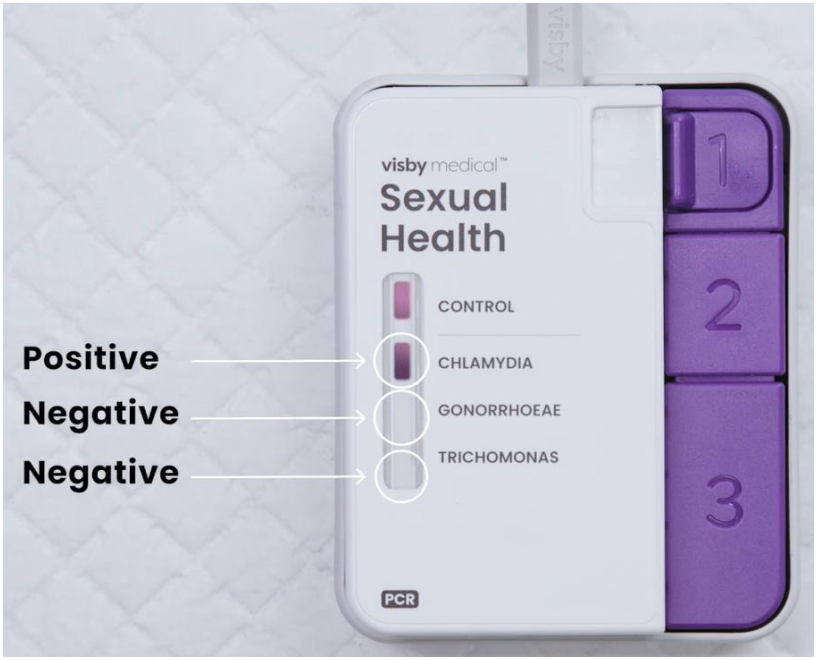
- FDA-cleared, CLIA-waived desktop instrument that processes a single-use cartridge
- no sample preparation, no calibration or preventive maintenance necessary
- fully automated, easy-to-use; time to result: 30-minutes

A noninterventional, cross-sectional clinical study at STI, HIV, family planning, and ob/gyn clinics:

	C. trachomatis		*N. gonorrhoeae*	
	Sensitivity	Specificity	Sensitivity	Specificity
Asymptomatic n=614	93.3% (56/60)	99.1% (549/554)	91.7% (11/12)	100% (602/602)
Symptomatic n=308	91.7% (55/60)	99.6% (247/248)	98.4% (61/62)	100% (246/246)
Total n=922	**92.5%** (111/120)	**99.3%** (796/802)	**97.3%** (72/74)	**100%** (848/848)

Van Der Pol, B. et al. (2020). **Evaluation of the Performance of a Point-of-Care Test for Chlamydia and Gonorrhea.** JAMA network open, 3(5), e204819

Visby Sexual Health Click Test for Women



First instrument-free, single use PCR test for STIs

Samples can be stored up to 4 hrs at room temp. or refrigerated if needed

Results available in 28 minutes

Company claim: >97% accuracy

	Positive Percent Agreement (PPA)	Negative Percent Agreement (NPA)
CT	97.4%	97.8%
NG	97.8%	99.1%
TV	99.3%	96.7%

https://www.visbymedical.com/news/visby-medical-receives-fda-clearance-and-clia-waiver-at-the-point-of-care-for-pcr-sexual-health-test

GARDP: Need for NG-AMR POCT:

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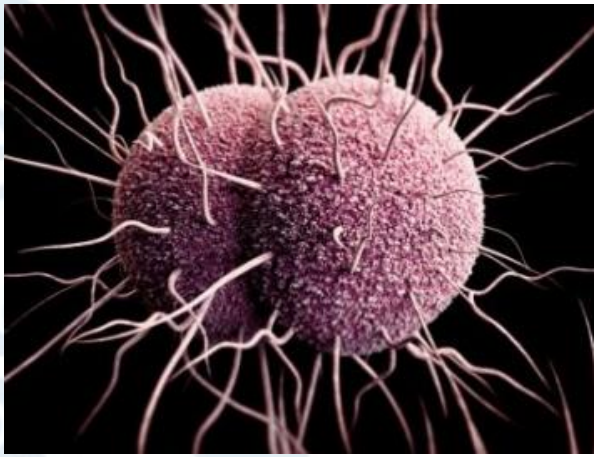


Source: US CDC Image Library

GARDP: the Global Antibiotic Research and Development Partnership (GARDP) is funding the development of new antibiotics and POC tests for NG

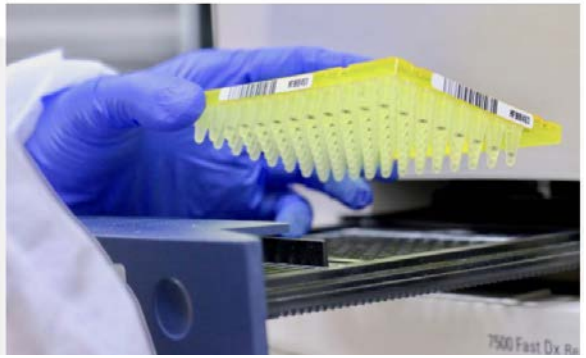
- The rapid emergence of antimicrobial resistance (AMR) by *Neisseria gonorrhoeae (*NG*)* has complicated the treatment
- In the UK antibiotic susceptibility testing is performed on NG isolates but results are not available to guide treatment
- An NG-AMR POCT can:
  √ reduce loss to follow up
  √ extend the life of current last-line treatment
  √ be cost-saving
- In 2014, 33, 431 ceftriaxone treatments were given for NG
- A modelling study showed that if a 30 min AMR POC test:
  - **for NG + penicillin resistance were available , 79% of current tx could be replaced by penicillin**
  - **for NG + ciprofloxacin resistance were available, 66% of current tx could be replaced by ciprofloxacin**

Ref: Turner KME, et al. BMJ Open 2017;7:e015447.

Laboratory-based assay for the Simultaneous Identification of *N. gonorrhoeae* and Resistance Gene Detection

Resistance*Plus*® GC simultaneously detects *Neisseria gonorrhoeae* and the gyrA S91 (wild type) or gyrA S91F (mutant) markers that are associated with ciprofloxacin resistance

- **Sample types**: Urine: Male and female; Swabs: anal, rectal, cervical, endocervical, vaginal, urethral, pharyngeal, and eye; pre-extracted samples
- **Equipment**: compatible with Roche LightCycler® 480 Instrument; Applied Biosystems® 7500 Fast and Fast Dx; Bio-Rad CFX96™ IVD and Touch
- **Reagent storage**: – 20°C
- **Regulatory approval**: CE-IVD; TGA



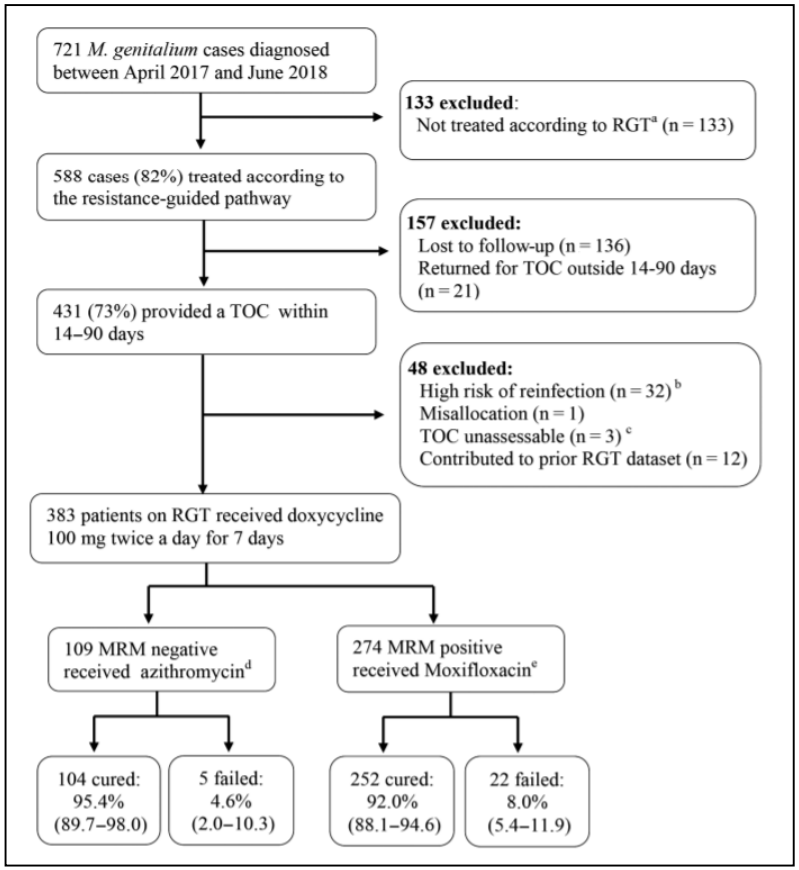
Gc detection: sensitivity 98.6%; specificity 100%; accuracy for GyrA S91 WT/S91F detection: 100%; accuracy in predicting phenotypic ciprofloxacin resistance: 99.8%.

Hadad R, and the European *collaborative group. Evaluation of the SpeeDx ResistancePlus® GC and SpeeDx GC 23S 2611 (beta)* molecular assays for prediction of antimicrobial resistance/susceptibility to ciprofloxacin and azithromycin in *Neisseria gonorrhoeae*. J Antimicrob Chemother. 2021 Jan 1;76(1):84-90.

Resistance Guided Therapy for *Mycoplasma genitalium*

Resistance*Plus*® MG simultaneously detects *Mycoplasma genitalium* and 5 mutations in the 23S rRNA gene associated with macrolide resistance

- **Sample types**: Urine: Male and female; Swabs: anal, rectal, cervical, endocervical, vaginal, urethral, pharyngeal, and eye; pre-extracted samples
- **Equipment**: compatible with Roche LightCycler® 480 Instrument; Applied Biosystems® 7500 Fast and Fast Dx; Bio-Rad CFX96™ IVD and Touch
- **Reagent storage**: – 20°C
- **Regulatory approval**: CE-IVD; TGA; Health Canada

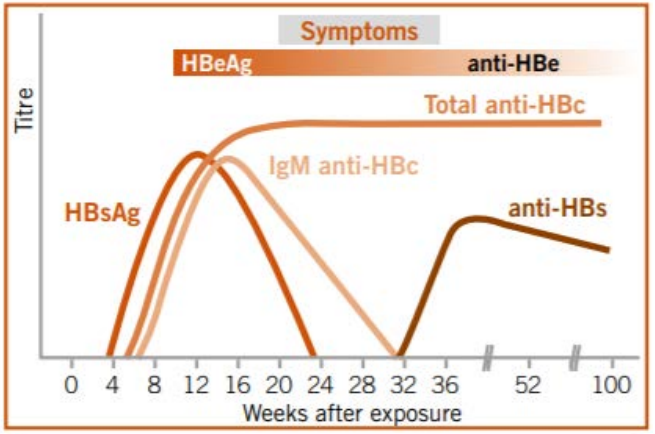


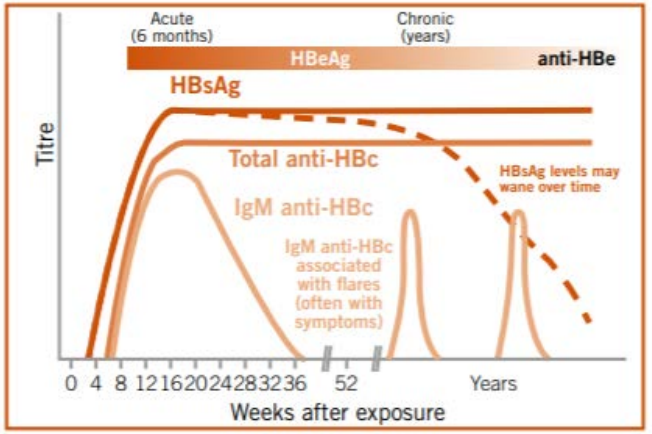
Durukan D, et al. Resistance-Guided Antimicrobial Therapy Using Doxycycline-Moxifloxacin and Doxycycline-2.5 g Azithromycin for the Treatment of *Mycoplasma genitalium* Infection: Efficacy and Tolerability. Clin Infect Dis. 2020 Sep 12;71(6):1461-1468

Hepatitis B Virus (HBV): biomarkers of infection

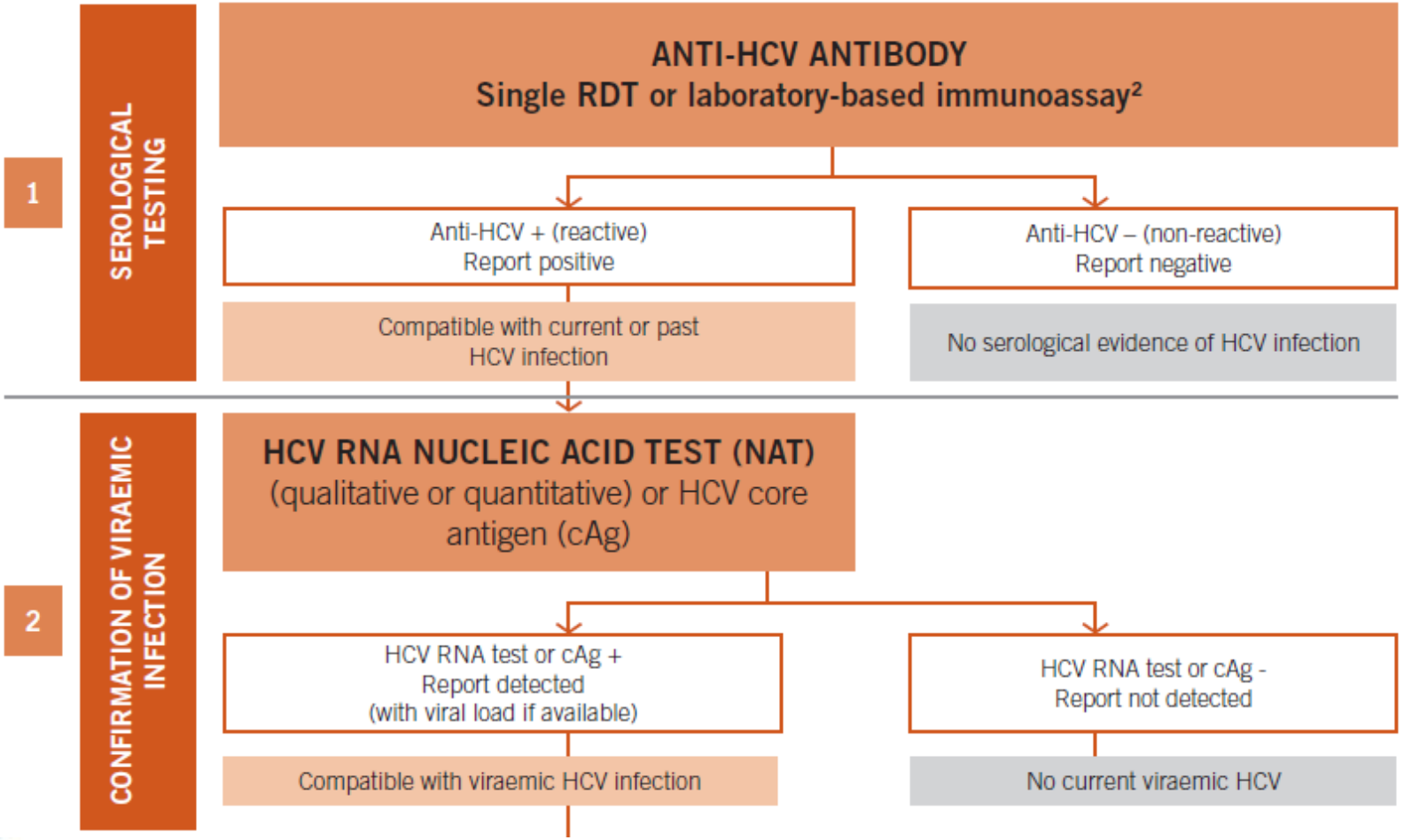
Markers for HBV infection

HB surface antigen (HBsAg)	HBV envelope protein often produced in excess and detectable in the blood in acute and chronic HBV infection
HB core antigen (HBcAg)	HBV core protein. The core protein is coated with HBsAg and therefore not found free in serum
HB e antigen (HBeAg)	Viral protein found in the high replicative phase of HBV. HBeAg is usually a marker of high levels of replication with wild-type virus but is not essential for viral replication
HB surface antibody (anti-HBs)	Antibody to HBsAg. Develops in response to hepatitis B vaccination and during recovery from hepatitis B, denoting past infection and immunity
HB core antibody (anti-HBc)	Antibody to HBV core (capsid) protein. Anti-HBc antibodies are non neutralizing antibodies and are detected in both acute and chronic infection
anti-HBc IgM	Subclass of anti-HBc. Detected in recent HBV infection but can be detected by sensitive assays in chronic HBV infection
HBV e antibody (anti-HBe)	Antibody to HBeAg. Detected in persons with lower levels of HBV replication but also in HBeAg-negative disease (i.e. HBV that does not express HBeAg)
HBV DNA	HBV viral genomes that can be detected and quantified in serum by nucleic acid testing (NAT)





Diagnostic Algorithm for HCV

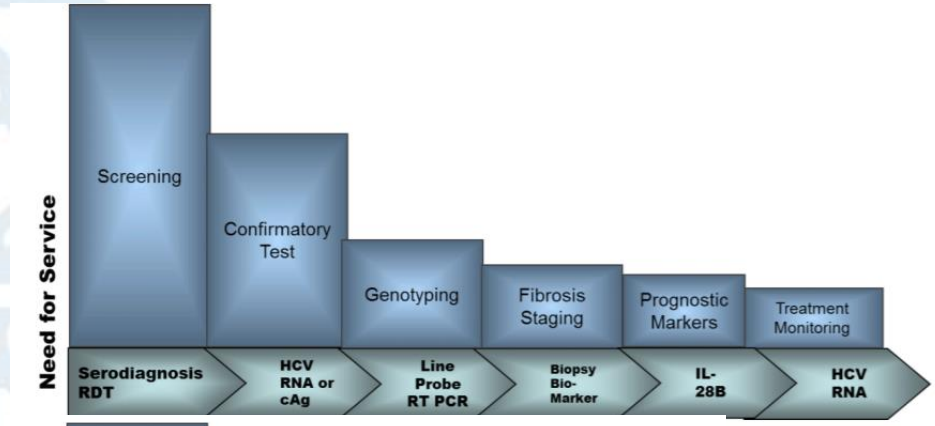


WHO Guidelines on Hepatitis B and C Testing (accessed July 2018). Available at: http://apps.who.int/iris/bitstream/10665/254621/1/9789241549981-eng.pdf?ua=1.

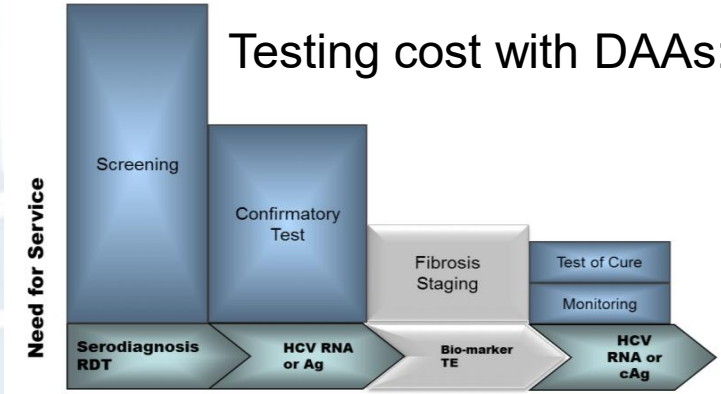
Innovations and Future of HCV Testing

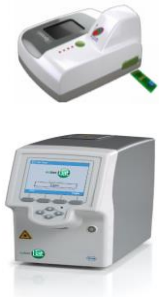
LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Testing cost with current regimens: 220-1,100 USD



Testing cost with DAAs: 30-120 USD





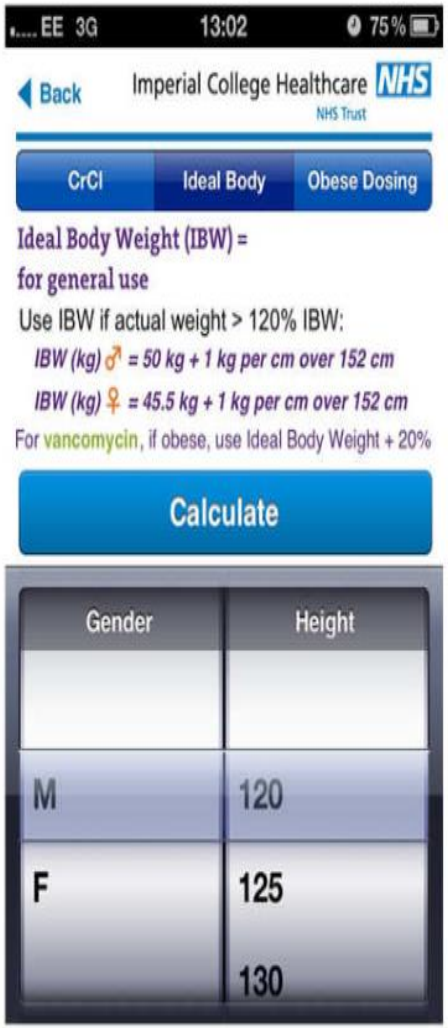
UNITAID "Hepatitis C Medicines and Diagnostics in the Context of HIV/HCV Co-Infection: A Scoping Report" 2013. Available at: https://unitaid.eu/assets/Hepatitis-C_October-2013.pdf.

- Simplifying testing as a result of improved drug regimens
- Patient centred testing using multiplex tests - HIV, HCV, syphilis, HBV testing using a single specimen at a single visit
- Increasing access to:
  - screening using oral or blood based RDTs
  - confirmatory testing – Dried Blood Spots for RNA testing or detection of HCV core antigen
- Leveraging investment in POC molecular testing capacity for COVID-19, TB and HIV
- Data connectivity to monitor quality of individual and cascade of care

Improving Patient Management through Electronic Decision Support

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

The Imperial Antibiotic Prescribing Policy (IAPP) smart phone app provides clinical decision support at the point of care to improve antimicrobial stewardship and appropriate prescribing:



Molecular Microbiology in Clinical Care and Public Health

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- Recent advances in molecular technology for microbiology
- Molecular methods for common clinical syndromes
- **Use of molecular methods in public health**
- Summary

Mention of company products does not imply endorsement

The Director-General of the World Health Organization urged countries to "**test, test, test**."

He said,"**testing, isolation, and contact tracing should be the backbone of the global pandemic response."**

Evolving Role of Diagnostics: from Pandemic Response to Control

Dec 2019: China identified first human cases
Jan 30 2020: WHO declared Public Health Emergency of International Concern
Mar 11 2020: WHO declared COVID-19 a pandemic

Pathogen identified and genome sequence known

- **Confirm clinical diagnosis of symptomatic individuals**
- Refine **COVID19 case definition**
- Testing of **contacts** of confirmed cases
- **Enable research** to determine the mode and speed of transmission & monitor impact of interventions

Mar-Apr 2020: asymptomatic and pre-symptomatic transmission confirmed

Community transmission confirmed

- Testing of **symptomatic individuals** and **contacts** continues
- Screening of **populations at high risk of acquisition and transmission** e.g. health and elder home care workers
- Testing in non-health care settings - **schools, workplaces, mass gatherings**, and **travel**

Sept 2020 onwards: rapid spread of Variants of Concern (VOCs) confirmed; Vaccinations start to provide immunity, esp for vulnerable

Vaccination and VOCs)

- Testing of symptomatics and those requiring hospitalisation
- Testing now important for **surveillance,** esp to track VOCs
- Testing for **travel** to include pre-boarding and on-arrival testing
- **Testing in schools, work and mass gatherings to save livelihoods**

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Test to enable: re-opening of schools, workplaces and mass gatherings

Lockdowns and border closures impose mental, social and financial hardships in many societies

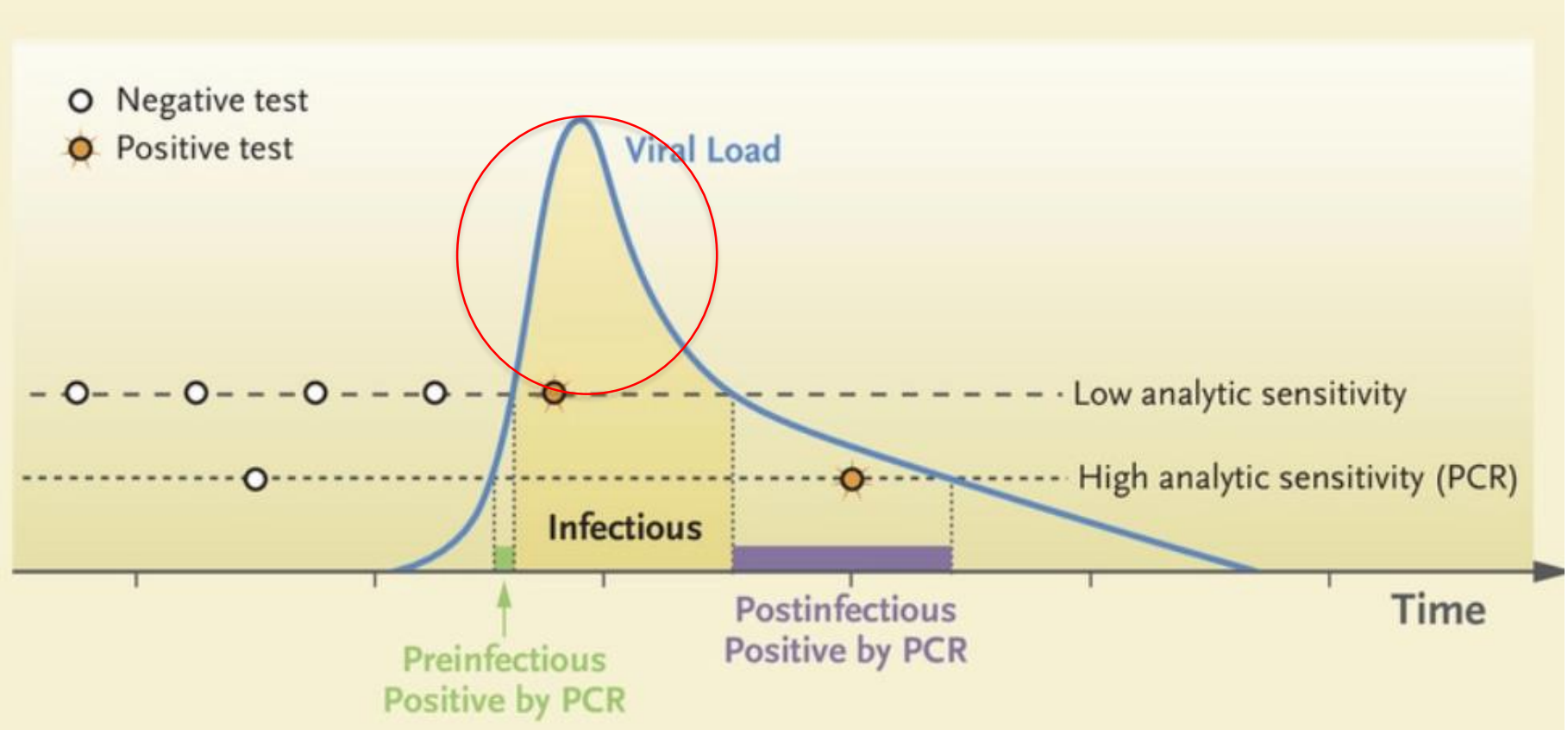
https://www.who.int/publications/i/item/considerations-for-school-related-public-health-measures-in-the-context-of-covid-19

Courtesy of Dr. Yap Boum, Cameroon

Layered interventions to reduce risk of transmission: face masks, distancing, hand hygiene and ventilation

An Evidence Co-op for Sharing Antigen Testing Strategies and Shaping Best Practices
https://courses.globalhealthcpd.com/courses/evidence-coop-for-antigen-testing-strategies

Test Sensitivity is Secondary to Frequency of Testing and Turnaround Time



O denotes testing point

Mina MJ et al. Rethinking strategy for containment. N Engl J Med 2020; 383:e120

The right test for the right patient at the right time in the right setting

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Diagnostic Tests	Target	Use Case	Optimal time for use*	Accuracy		Access ibility	Afford ability	Time to result
				Sens	Spec			
Molecular:	Viral RNA	confirm infection	day 0-7	****	****			
Lab						√	$$$	1-2 hrs
POC**						√√		15-45 min
Antigens:	Viral Proteins	confirm infection	day 0-7	**	***			
Lab						√√	**$$**	~3 hrs
POC						**√√√**		15-20 min
Serology:	Host Antibodies	exposure Surveill-ance	day 7-40	***	***			
Lab						√√	$	~3 hrs
POC						√√√		15-20 min

*Days post onset of symptoms

**supply may be limited by speed of manufacture

Ref: Peeling RW, Heymann DL, Teo YY, Garcia PJ. Diagnostics for COVID-19: moving from pandemic response to control. Lancet. 2022 Feb 19;399(10326):757-768. doi: 10.1016/S0140-6736(21)02346-1. Epub 2021 Dec 20. PMID: 34942102; PMCID: PMC8687671.

COVID-19 Pandemic:
Policy decisions on scaling up testing

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Access options:

Health Facilities

Community Venues

Diagnostic options:

Lab based Molecular Tests

Rapid Molecular/Antigen Tests

- Testing of symptomatic individuals
- Testing contacts of cases
- Screening of health care workers, care home workers, first responders

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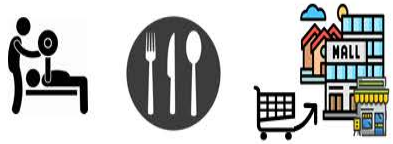
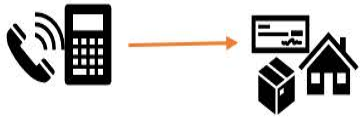
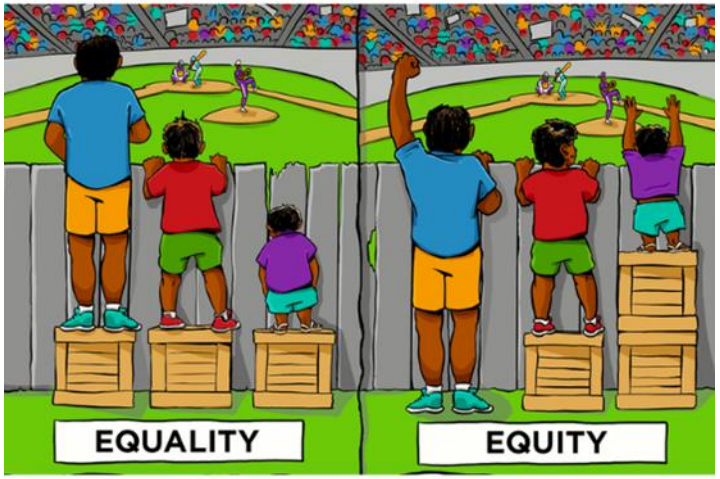
Mass Gatherings



Online



Border Crossings

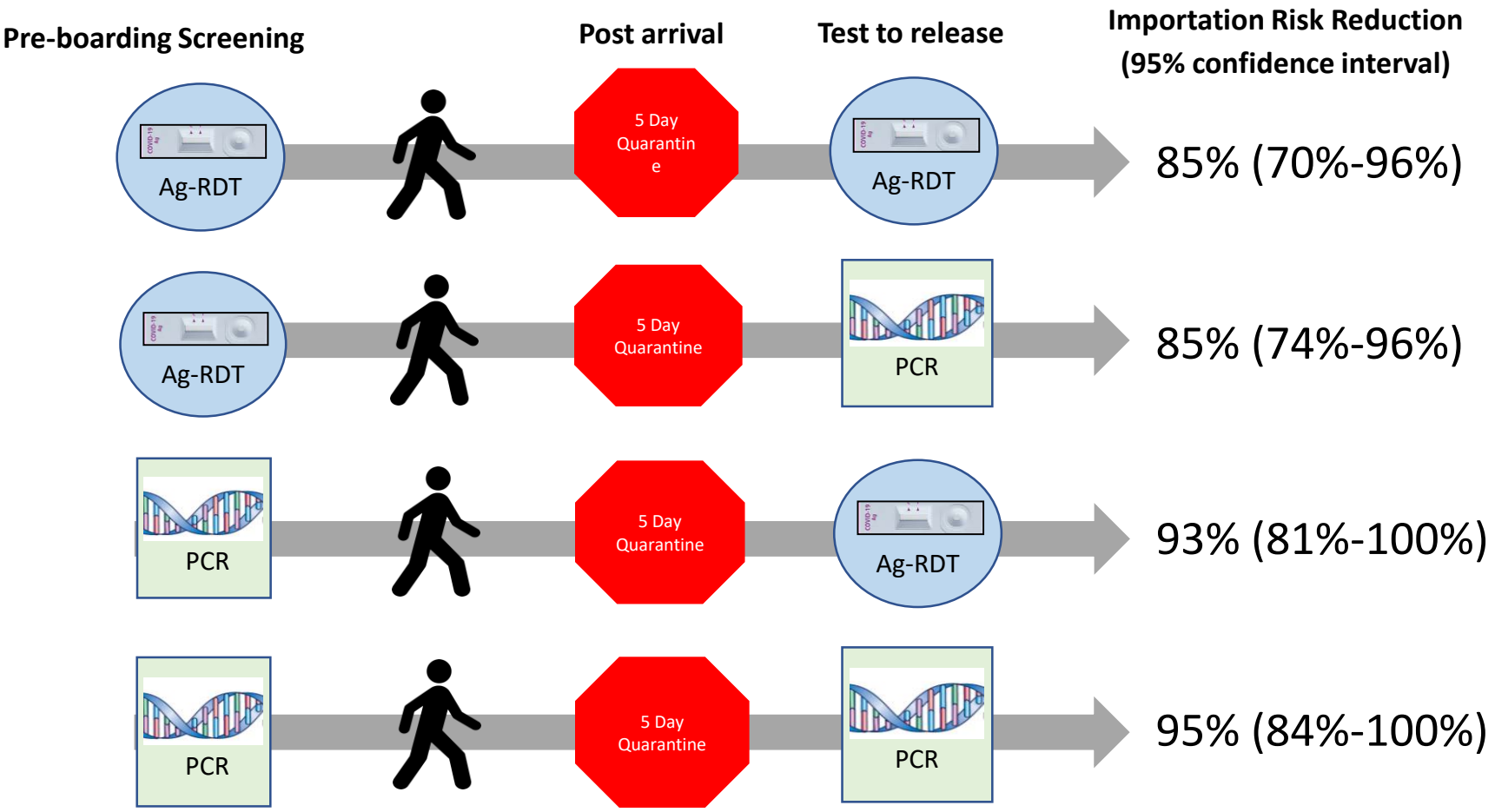


No one is safe until we are all safe

Modelling Strategies for Reducing Importation Risk of COVID-19 Cases

Dickens BL et al. J Travel Med Aug 2020

	Reduction in case rates relative to S1
No controls	
Testing on arrival;**+ve** **quarantine 7 days**	**90.2%**
Testing on arrival; **+ve** **quarantine 14 days**	**91.7%**
Quarantine 7 days	**55.4%**
Quarantine 14 days	**91.2%**
Testing on arrival; **denied entry if positive**	**77.2%**



Quilty BJ, et al. Quarantine and testing strategies to reduce transmission risk from imported SARS-CoV-2 infections: a global modelling study. medRxiv preprint doi: https://doi.org/10.1101/2021.06.11.21258735; posted June 14, 2021.

Summary

- Molecular testing gives highly accurate results and are increasingly more rapid, accessible and affordable, enabling its use to address inequity of diagnostic access in remote settings and for marginalised populations

- Molecular testing at the point-of-care has the potential to improve patient management through more timely diagnosis and reduce the risk of antimicrobial resistance although the presence of resistance genes may not imply their expression

- For public health, molecular testing is critical for confirmation of clinical diagnosis and for screening high risk populations. It is also used for waste water surveillance to monitor pathogens in communities.

Thank you

LSHTM/International Diagnostics Centre: David Mabey, Joe Tucker, Dan Wu, Debi Boeras, Noah Fongwen, Susan Nkengasong, Eneyi Kpokiri, Tuesday Doherty, Jennifer Ishiodu

Funding: Wellcome Trust, WHO, Bill & Melinda Gates Foundation, US NIH, Newton Fund