FIND

Because diagnosis matters

Diagnostics for hepatitis C: Where do we stand and what lies ahead?

Sonjelle Shilton; Deputy Head HCV, Access

INHSU, 12 September 2019, Montreal, Canada

FIND is a global non-profit driving diagnostic innovation to combat major diseases affecting the world's poorest populations

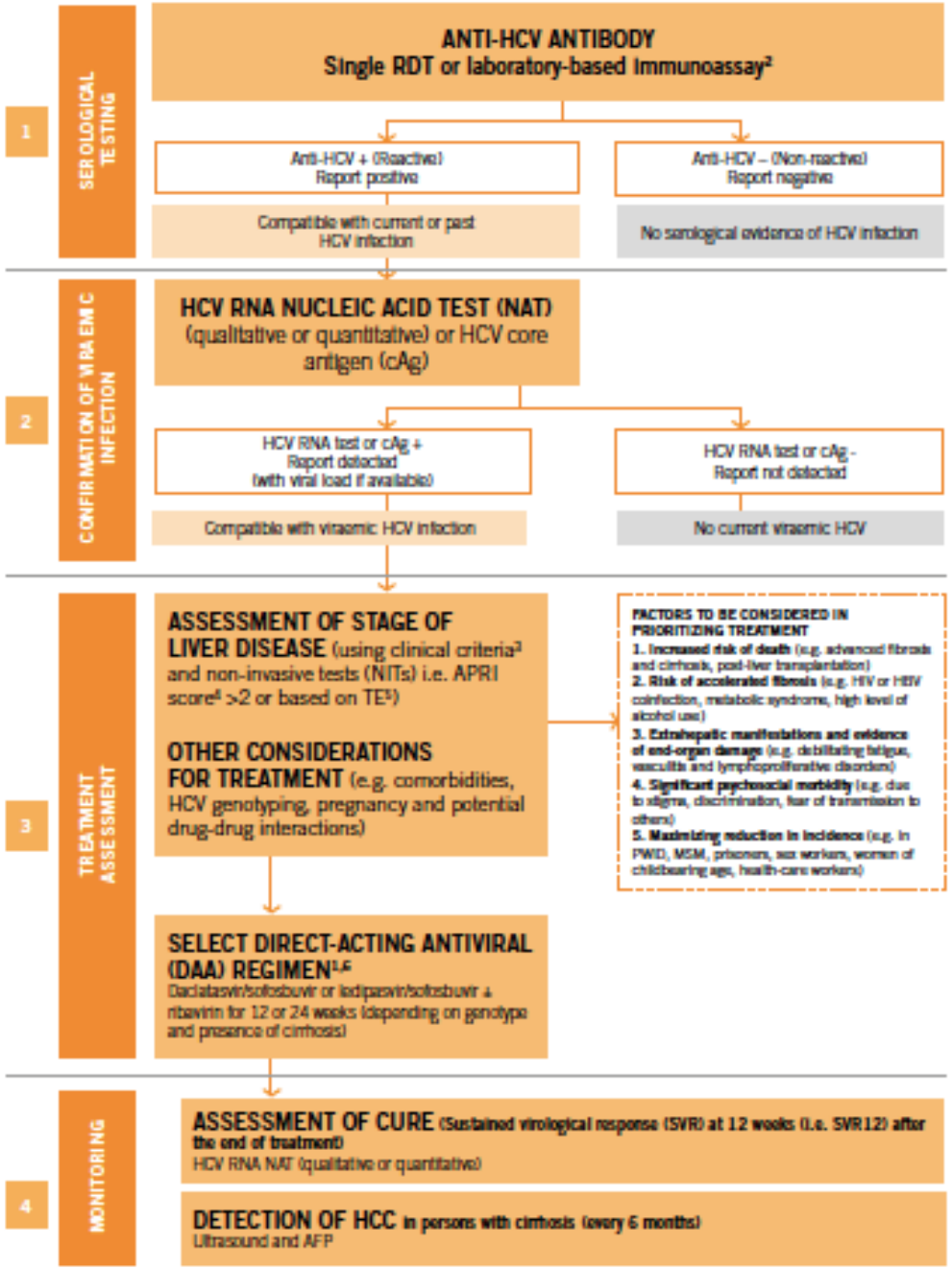
- WHO Collaborating Centre for Laboratory Strengthening & Diagnostic Technology Evaluation
- WHO SAGE-IVD member
- ISO-certified quality management system for IVD clinical trials

ANTIMICROBIAL RESISTANCE	HEPATITIS C & HIV	MALARIA & FEVER
NEGLECTED TROPICAL DISEASES	PANDEMIC PREPAREDNESS	TUBERCULOSIS

We address market failure by partnering to develop and deliver diagnostic solutions to LMICs

SUMMARY ALGORITHM FOR DIAGNOSIS, TREATMENT AND MONITORING[1] OF CHRONIC HCV INFECTION

WHO HCV Testing guidelines



Single assay: laboratory testing (EIA/CIA) or quality-assured RDT

Prompt or reflex HCV RNA or HCV core Ag testing

Assess and triage; Stage liver disease using NITs (APRI, FIB4, TE)

Treat all with pangenotypic regimens

One test of cure SVR12: HCV RNA test

Serological testing for HCV

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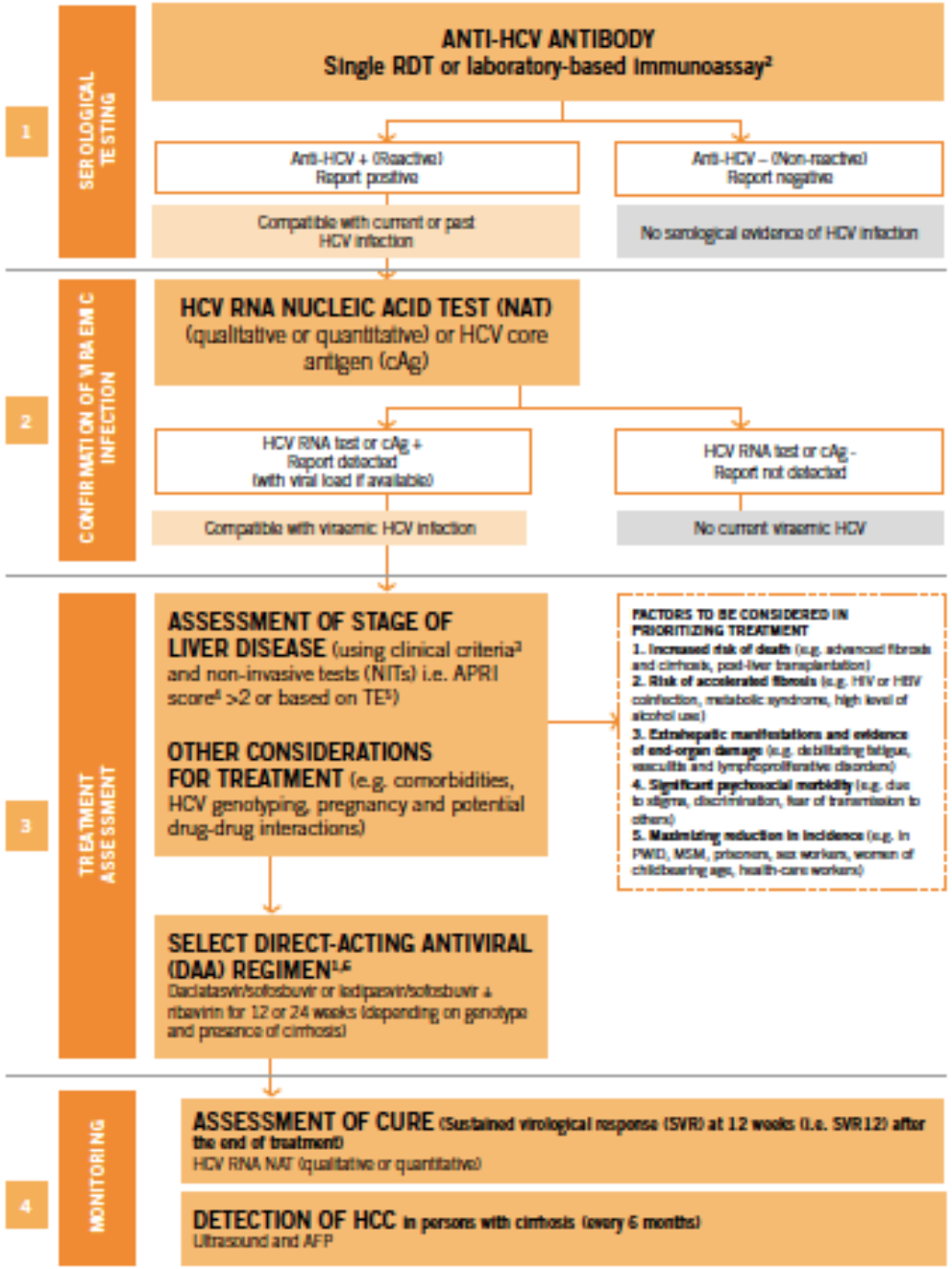
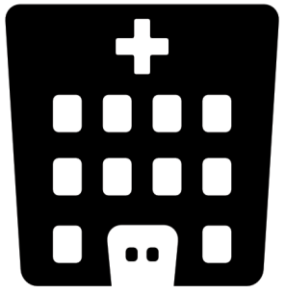
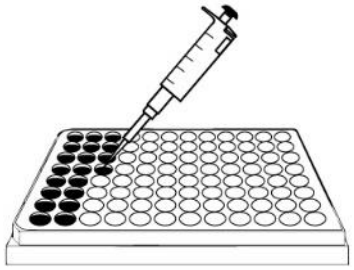


Single assay: laboratory testing (EIA/CIA) or quality-assured RDT

Screening for HCV

Centralized settings





EIA

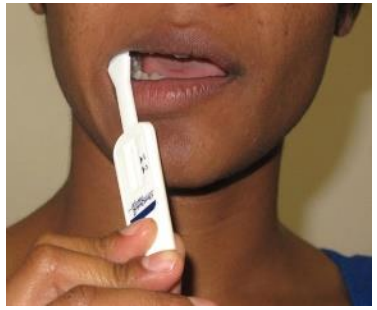
CIA

Dried Blood Spots

Currently off-label; needs field validation

Decentralized settings

RDT
(blood-based)



RDT
(oral fluid-based)

Settings: well-equipped lab

Operator: qualified lab technician

Specimen type: plasma, serum

Turnaround time: >2 hours

Settings: primary facility

Operator: trained healthcare worker

Specimen type: capillary blood, oral fluid

Turnaround time: 5-20 min

Hepatitis C antibody Rapid Diagnostic Test (RDT)

Product name	Manufacturer	Performance*	Sample type	WHO PQ?	Stringent Regulatory Authority	List (USD)
Rapid Anti-HCV Test	InTec PRODUCTS, INC	Sens: 99.2% Spec: 99.1% in mono-infected Sens: 91.7% Spec: 99.2% in HIV-co-infected	Serum/Plasma /Whole Blood	Yes	RoW	1.6 to 2.4
SD BIOLINE HCV	Standard Diagnostics, Inc.	Sens: 99.5% Spec: 99.6% in mono-infected Sens: 88.6% Spec: 99.7% in HIV-co-infected	Serum/Plasma /Whole Blood	Yes	RoW	1 to 2.4
OraQuick® HCV Rapid Antibody Test Kit	OraSure Technologies, Inc.	Sens: 99.5% Spec: 99.6% in mono-infected Sens: 89.4% Spec: 99.4% in HIV-co-infected	Serum/Plasma /Whole Blood/Body Fluids	Yes	CE mark	8 (MSF Access price) 14 (on US market)
First Response® HCV Card Test	Premier Medical Corporation Pvt. Ltd., Nani Daman, India	Sens: 99.5% Spec: 100% in mono-infected Sens: 90.5% Spec: 99.7% in HIV-co-infected	Serum/Plasma /Whole Blood	No-ERPD	CE mark	.60 to 1

* FIND 2019, publication forthcoming

HCV EIA, WHO PQ'd

Product name	Manufacturer	Sample type	WHO PQ?	Stringent Regulatory Authority
ARCHITECT HCV Ag assay**	Denka Seiken Co., LTD, Kagamida Factory	Serum/Plasma	Yes	CE mark
INNOTEST HCV Ab IV	Fujirebio Europe NV	Serum/Plasma	Yes	CE mark
INNO-LIA HCV Score	Fujirebio Europe NV	Serum/Plasma	Yes	CE mark
Murex anti-HCV (version 4.0)	DiaSorin South Africa (Pty) Ltd.	Serum/Plasma	Yes	RoW
*Bioelisa HCV 4.0	Biokit S.A.	Serum/Plasma	Yes	CE mark

** ARCHITECT HCV Ag assay can be used for confirmation of viraemia, the other tests on this list can be used to determine presence of HCV antibodies

Self-testing (serology)

HCV self-testing

Currently available data on HCV self-testing is very limited

- One published report investigates acceptability of HCV self-testing among persons who inject drugs in the UK (Guise et al. 2018).
  - The study showed potential acceptability but also revealed multiple concerns associated with self-testing, primarily poor access to confirmatory testing and care.
- Another published study by Kimble and colleagues (2019) assessed the performance of OraQuick® HCV Rapid Antibody Test (Orasure Technologies, Inc., Bethlehem, PA) on oral fluid specimens when used by patients for self-testing.
  - The study included 95 participants and showed 88.4% sensitivity and 100% specificity of the test when used for self-testing compared to manufacturer-reported 98.1% and 99.6% when used by a professional healthcare provider (http://orc.orasure.com/default.aspx?pageid=1475).
  - Participants found testing procedure easy but reported some difficulties in interpreting test results. It is important to note that in this study graphical instructions for use were not provided by a test manufacturer but developed by the study team.

HCV self-testing: pilot feasibility study

- Objectives: determine acceptability and usability of HCV self-testing
- Several countries in different geographic regions:

Country	Settings	Population	Status
Egypt	District hospital (ALPC)	General population	Completed
China	CBO	MSM	Ongoing
Kenya	CBO	PWID	In preparation
Georgia	Harm reduction centers, PreP clinics	PWID, MSM	In preparation
Vietnam	CBO	PWID, MSM	In preparation

- 100-200 participants per site
- OraSure HCV Rapid diagnostic test adapted by the manufacturer for self-testing (research use only)

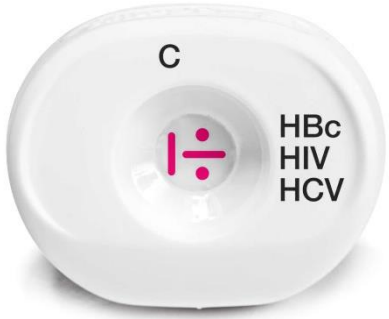
Combo testing (serology)

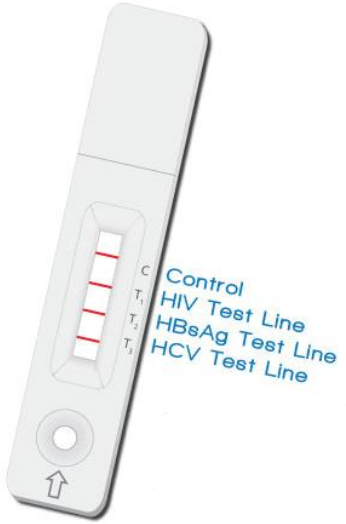
Multiplex serology testing (combo tests)

6.4.2 Integrating the diagnosis of hepatitis with diagnostic platforms and laboratory services used for other infections

Combination integrated multidisease serological tests

The use of combination integrated blood- or oral-based multidisease assays allow for integrated testing of HIV, HBV and HCV. Using a single specimen improves the efficiency of testing programmes, especially in populations with a high prevalence of HIV/HCV or HBV/HCV coinfection. While not yet fully validated, preliminary results of these combination assays appear promising *(160)*.





Multiplex serology testing (combo tests)

Test	Manufacturer	Detection			Regulatory status (SRA)
		HBV	HCV	HIV	
Detect 3 HIV/HCV/HBV combo kit	Artron Laboratories (Canada)	X	X	X	CE (plasma, serum)
Triplex HIV, HCV, HBsAg	Biosynex (France)	X	X	X	NA
Hep B, Hep C, HIV Combination Rapid Test	Maternova (US)	X	X	X	NA
Multiplo HBc/HIV/HCV	MedMira (Canada)	X	X	X	RUO
HBsAg/HCV Ab Rapid Test	Spectrum Diagnostics (Egypt)	X	X		NA
Rapid HBsAg/HCV/HIV/Syphlis Combo	Euro Genomas (Lithuania)	X	X	X	CE
OnSite HBsAg/HCV Ab Rapid Test	CTK Biotech (US)	X	X		NA
COMBIQUIC HIV/HCV	Qualpro Diagnostics (India)		X	X	NA
TriQuick HIV/HCV/HCV	Genlantis Diagnostics	X	X	X	NA

Field validation is needed to assess diagnostic accuracy of these tests

Diagnosis of hepatitis C virus: confirmation of active infection

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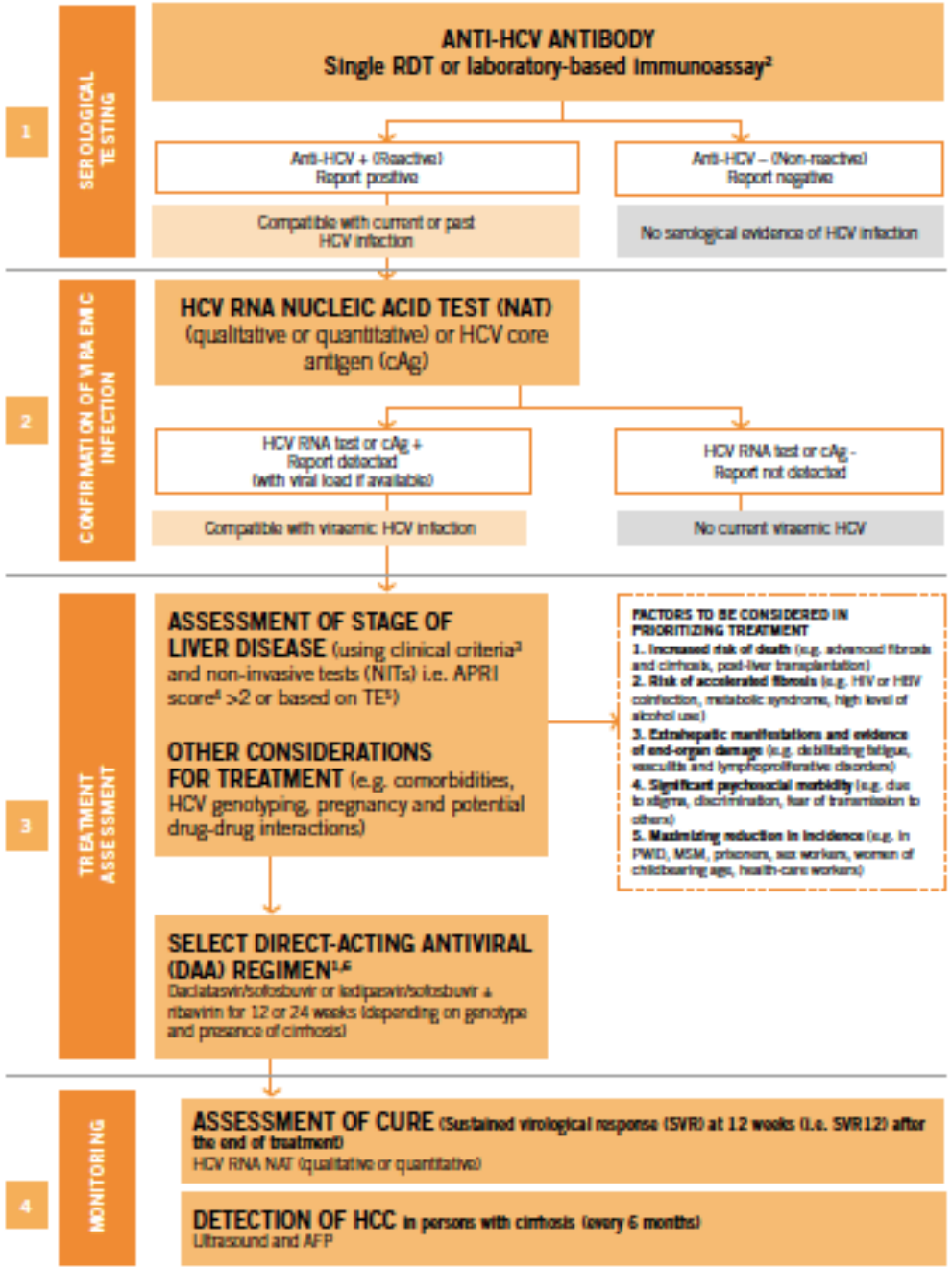


Prompt or reflex HCV RNA or HCV core Ag testing

Confirmation of viremia for HCV

Centralized settings

Dried Blood Spots

Decentralized settings

Currently off-label;

needs field validation

In development:

HCV RNA

HCV core antigen test

HCV RNA

HCV core antigen RDT

Settings: well-equipped lab

Operator: qualified lab technician

Specimen type: plasma, serum

Turnaround time: >5 hours

Settings: district hospitals

Operator: trained healthcare worker

Specimen type: capillary blood/plasma

Turnaround time: 60-90 min

Centralized molecular platforms

Legend: Commercially available

Manufacturer	Platform	HIV	HCV	HBV	TB	HPV
Abbott	M2000					
Abbott	Alinity					
Beckman Coulter	Veris					
Biomerieux	NucliSENS easyQ					
Bioneer	ExiStation					
Cepheid	Infinity					
Hologic	Panther					
Qiagen	QIAsymphony					
Roche	Cobas CAP/CTM					
Roche	Cobas 4800					
Roche	Cobas 6800/8800					
Sacace Biotech	SA Cycler					

Each conventional molecular instrument possesses unique features, which need to be considered when defining the optimal device mix within a Lab Network

Instrument	Cobas 4800-6800-8800	CAP/CTM 96	m2000sp	Panther
Supplier	Roche	Roche	Abbott	Hologic
				
Assays	HIV (EID;VL);HPV;HCV; HBV; TB	HIV (EID;VL);HCV; HBV; TB	HIV (EID;VL);TB; HPV; HCV; HBV	HIV (EID; VL); HPV; HCV; HBV
HIV sample types	DBS; Plasma; PSC (VL)	DBS; Plasma	DBS; Plasma	DBS (VL) ; Plasma
HCV sample types	Plasma (PSC and DBS in the pipeline)	Plasma	Serum; Plasma (DBS in the pipeline)	Serum; Plasma (DBS in the pipeline)
Infrastructure Requirements				
Space requirements	1.8 m^2- 5.5 m^2, Fixed	3 m^2, Fixed	3.15. m^2, Fixed	1 m^2, Moveable
Human resources	1-2 FTEs/machine	1-2 FTEs/machine	1-2 FTEs/machine	1 FTE/<4 machines
Workflow Requirements				
Workflow	Batch	Batch	Batch	Random access
Throughput (8hr)	192-960	168	96	320
Time to first result (hr)	< 3.5	~5.5	~5.5	< 3.5

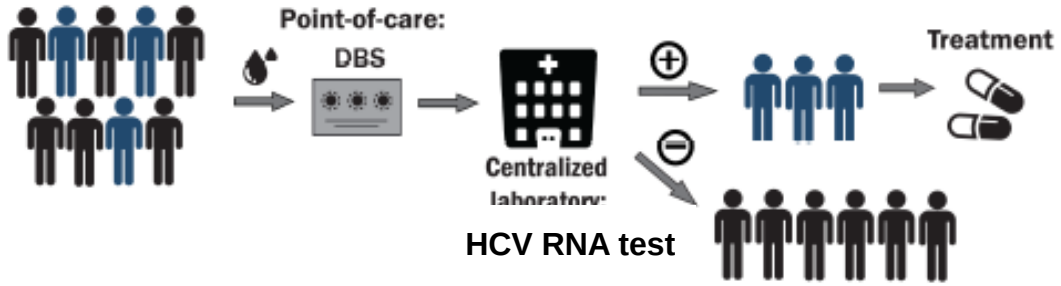
Dried blood spot (DBS)

Validation of DBS sampling

DBS sampling for HCV RNA test

Aim: provide HCV diagnostics in the settings with no access to laboratory infrastructure

Concept:



FIND study: multicenter diagnostics accuracy study to obtain evidence of the performance of HCV RNA tests from DBS/PSC (with the intention of data to be included to companies' regulatory dossiers)

- real-life conditions: RT transport and storage of DBS and PSC samples
- DBS and PSC processing using manufacturers' protocols
  - Abbott M2000
  - Roche cobas® 4800 and 6800 (PSC and DBS)
  - Hologic Panther

Study sites:

- Georgia
- Greece
- Cameroon
- Rwanda
- Australia (NRL – central testing)

Sample size: 415 HCV RNA positives, 415 HCV RNA negatives

Timeline: Q1 2019 – Q3 2019

Point of Care (POC) and near POC

Near-POC HCV RNA assays available on the market

PLATFORM	Xpert HCV VL assay	Xpert HCV Fingerstick VL assay	GeneDrive HCV ID assay
			
SAMPLE TYPE	Plasma	Capillary blood	Plasma
SENSITIVITY	99%	98%	98%
SPECIFICITY	100%	100%	100%
SAMPLE PREPARATION	Integrated	Integrated	Off-board (several pipetting steps)
TIME TO RESULT	110 min	60 min	~120 min
REGULATORY STATUS	CE-IVD, WHO PQ	CE-IVD	CE-IVD
POWER SUPPLY	Need electricity supply		Need electricity supply
DATA ANALYSIS	PC		Integrated
TEST MENU	TB, HIV, HBV and many others		TB in development
TEST COST	US$ 14.95 ex works		Not disclosed
INSTRUMENT COST	US$ 17,500		Not disclosed

Molbio Trueprep™/Truelab™

The platform:

- Commercially available and used for TB testing in India
- Internal, rechargeable batteries allow implementation in facilities with no electricity support
- Instrument costs: US$ 12,000

Prototype Truenat™ HCV assay:

Parameter	TPP target	Molbio HCV assay prototype
Analytical sensitivity	<1000 IU/mL	250 IU/mL EDTA plasma 1'500 IU/mL whole blood
Diagnostic sensitivity	>95%	97.9% (94.1-99.6%)
Specificity	>98%	98.8% (95.8-99.9%)
Genotype inclusivity	All 6 genotypes detected	All genotypes detected
Analytical specificity	No cross reactivity with endogenous substance and exogenous factors (e.g. HIV, HSV, HTLV, EBV)	No cross reactivity with HBV, HIV, HSV, HTLV, EBV, and others
Specimen type	Capillary blood	500 µL EDTA plasma 200 µL capillary blood
Sample preparation	Maximum 2 steps	Separate sample preparation
Time to result	<60 min	60 min

FIND is preparing a multicentre prospective diagnostic accuracy study (Q4 2019)

Alere/Abbott: mPIMA

The platform:

- Commercially available
- External batteries allow implementation in facilities with no electricity support
- Instrument costs: US$ 25,000
- Test costs: not disclosed

Prototype HCV assay:

Parameter	TPP target	M-Pima HCV assay prototype
Analytical sensitivity	<1000 IU/mL	250 IU/mL
Diagnostic sensitivity	>95%	99.2% (97.5-100%)
Specificity	>98%	99.5% (98.5-100%)
Genotype inclusivity	All 6 genotypes detected	All genotypes detected
Analytical specificity	No cross reactivity with endogenous substance and exogenous factors (e.g. HIV, HSV, HTLV, EBV)	No cross reactivity with No cross reactivity with HBV, HIV-1/2, HSV, HTLV-I/II
Specimen type	Capillary blood	50 µL capillary blood
Sample preparation	Maximum 2 steps	Integrated: sample to answer
Time to result	<60 min	65 min

Diagnostic for the Real World: SAMBA II

The platform:

- Commercially available
- Assay control by tablet
- Needs electricity supply
- Instrument cost: US$18,000 to 24,000 depending on volume
- Test costs (HIV EID&VL): TBD

HCV assay:

Parameter	TPP target	SAMBA II HCV assay prototype
Analytical sensitivity	<1000 IU/mL	500 IU/mL
Diagnostic sensitivity	>95%	97% (92-99%): *WB spiked with HCV+ plasma*
Specificity	>98%	100% (92-100%) : *WB spiked with HCV+ plasma*
Genotype inclusivity	All 6 genotypes detected	All genotypes detected
Analytical specificity	No cross reactivity	No cross reactivity with HBV, HIV-1/2, EBV, HTLV-I/II
Specimen type	Capillary blood	100 µL capillary blood
Sample preparation	Maximum 1 steps	Sample in – Result out
Time to result	<60 min	90 min

Blink

The platform:

- Under development
- Multipurpose platform: can detect DNA, RNA, protein, cells
- Integrated battery, compatible with external batteries
- Instrument costs: US $5,000
- Test costs: projected launch ex works price US $13;
- Continue development of HCV assay: complete product development and validation, target launch in Q1 2021

Prototype HCV assay:

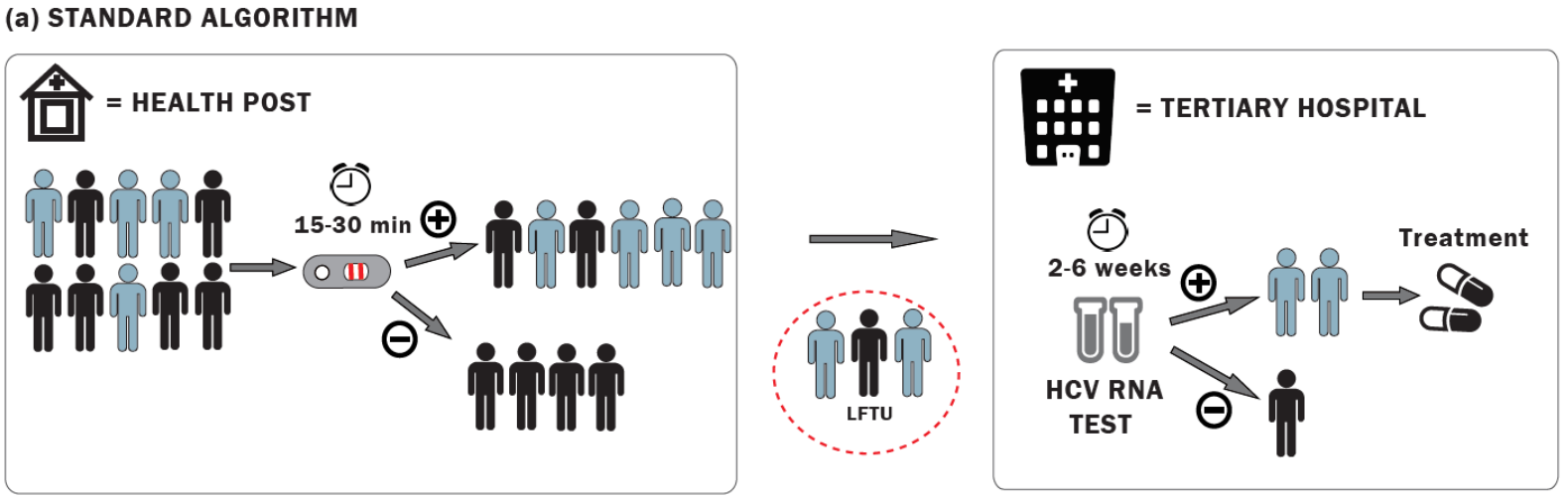
Parameter	TPP target	BLINK HCV assay prototype
Analytical sensitivity	<1000 IU/mL	58 IU/mL: WB
Diagnostic sensitivity	>95%	100% (97 -100%): plasma
Specificity	>98%	100% (95-100%): plasma
Genotype inclusivity	All 6 genotypes detected	All genotypes detected
Analytical specificity	No cross-reactivity with endogenous substance and exogenous factors (e.g. HIV, HSV, HTLV, EBV)	No cross-reactivity with HBV, HIV, HSV
Specimen type	Capillary blood	100 µL capillary blood
Sample preparation	Maximum 2 steps	Integrated: sample to answer
Time to result	<60 min	25 min

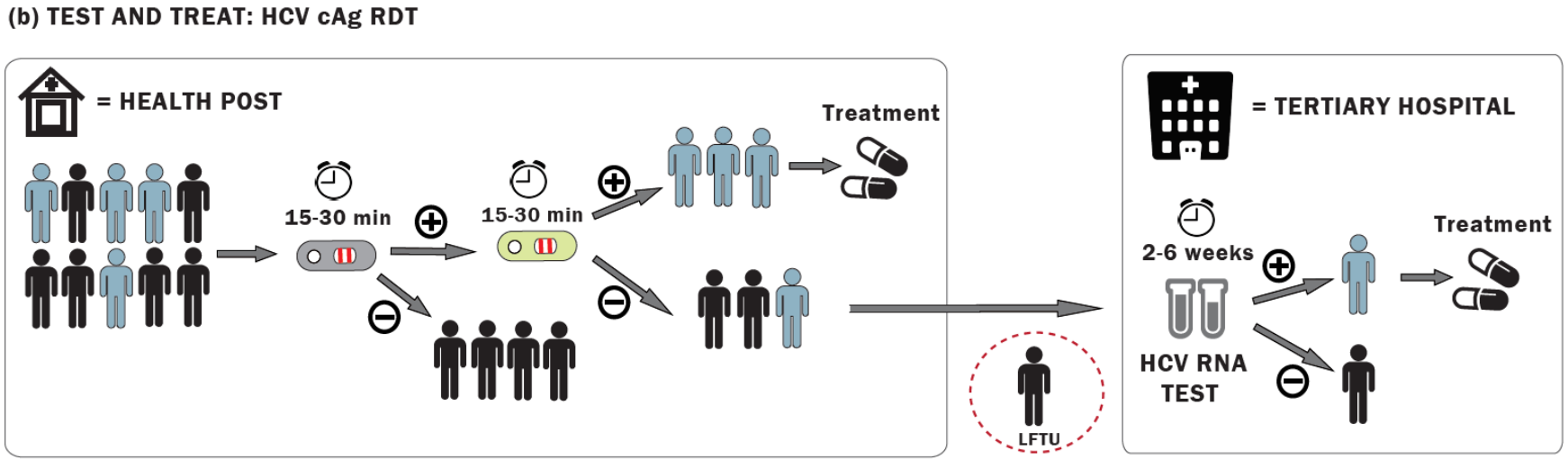
cAg RDT

HCV core Ag RDT concept

Main technical challenge: high analytical sensitivity requirements unlikely to be met in RDT format

➡ HCV core Ag RDT will have 75-85% clinical sensitivity, but impact will likely outweigh suboptimal sensitivity





HCV core antigen RDT: current status

- FIND has ongoing partnerships with DCN Diagnostics (USA) and Mologic (UK) to develop HCV core antigen test
- Early prototype reached 70% sensitivity target (measured on a panel of frozen plasma specimens)
- Further development is ongoing. Another 2 years will be needed for development and clinical validation
- FIND has published a call to identify a commercialization partner: IVD company that will take the developed test to LMIC markets. The call is closed, applications are under review.

Liver staging

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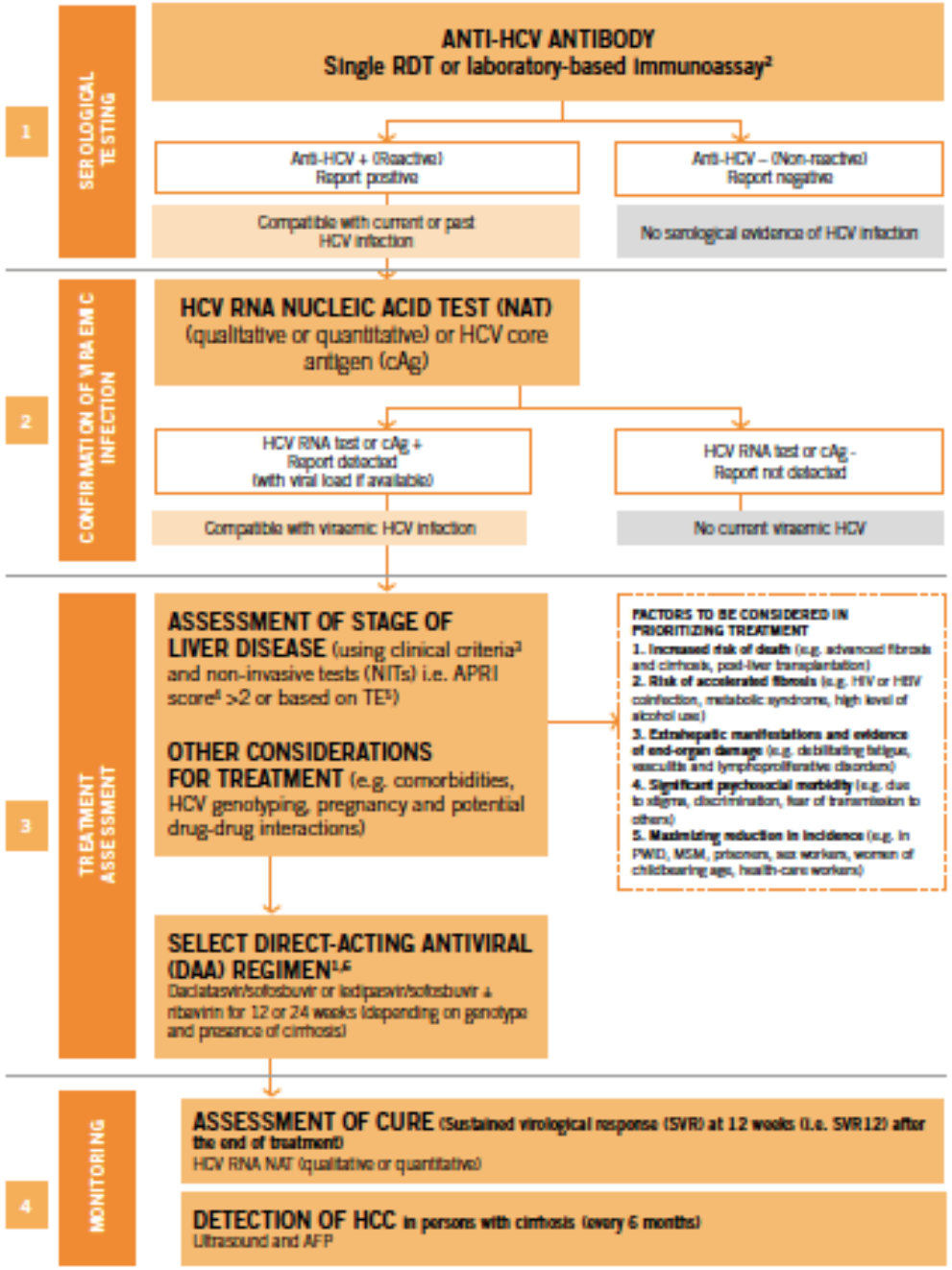
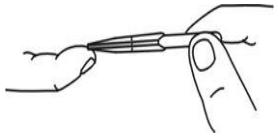


Assess and triage; Stage liver disease using NITs (APRI, FIB4, TE)

Existing liver staging options

Biochem	aspartate aminotransferase to platelet ratio index (APRI)	Uses blood test for a blood test to measure your aspartate aminotransferase (AST) and a platelet count	Machines needed to conduct the blood tests can usually be found at level 1 health centers. Is relatively inexpensive
	FIB4	Is a formula based on several laboratory tests: (Age x AST) / (Plts x (sqr (ALT))	
Fibroscan	Machine which can provide liver staging results		Machine is expensive Requires trained technician Is not widely available in all countries/contexts
Ultra sound	Can be used to provide liver staging results		While often machines are already in place for other services requires trained technician Wait times for ultrasound appointment can be long as other patient types may be prioritized (pregnant women)

Burnet Institute / Nanjing BioPoint – ALT1 test



Add 40 μL of whole blood to the well

Add 3 drops of buffer to the well

Wait for **20 minutes** then read result

南京澳百生物技术有限公司
Nanjing BioPoint Diagnostics

Burnet Institute
Medical Research. Practical Action.

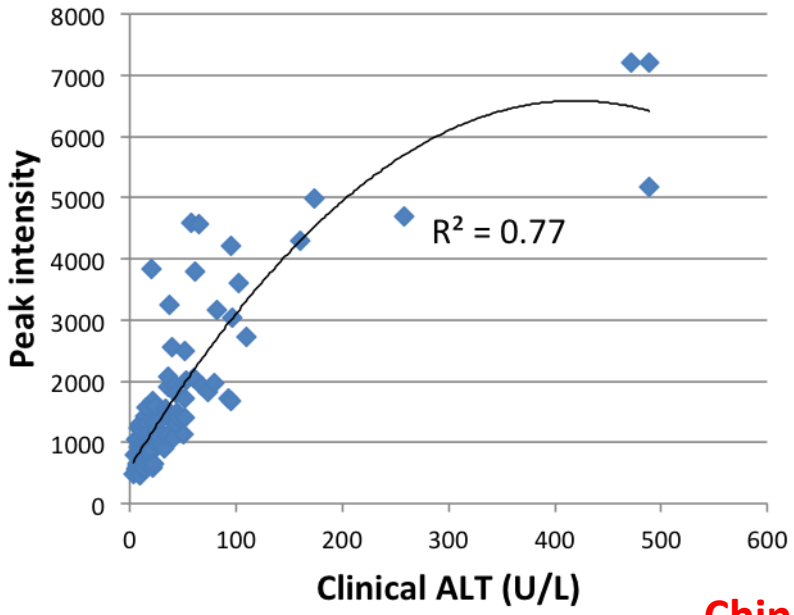
Correlation with standard (enzymatic) ALT

40 µl whole blood

	Clinical ALT		
ALT1 rapid	≥40U/L	<40U/L	Total
≥40U/L	28	7	35
<40U/L	5	66	71
Total	33	73	106

Visual read:

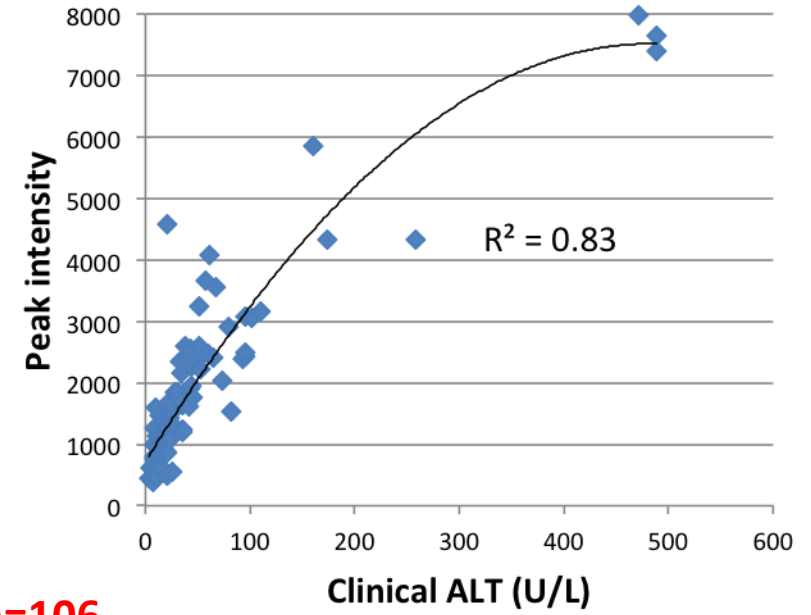
Sensitivity	85 %
Specificity	90 %
Accuracy	89 %



15 µl plasma

	Clinical ALT		
ALT1 rapid	≥40U/L	<40U/L	Total
≥40U/L	31	11	42
<40U/L	2	62	64
Total	33	73	106

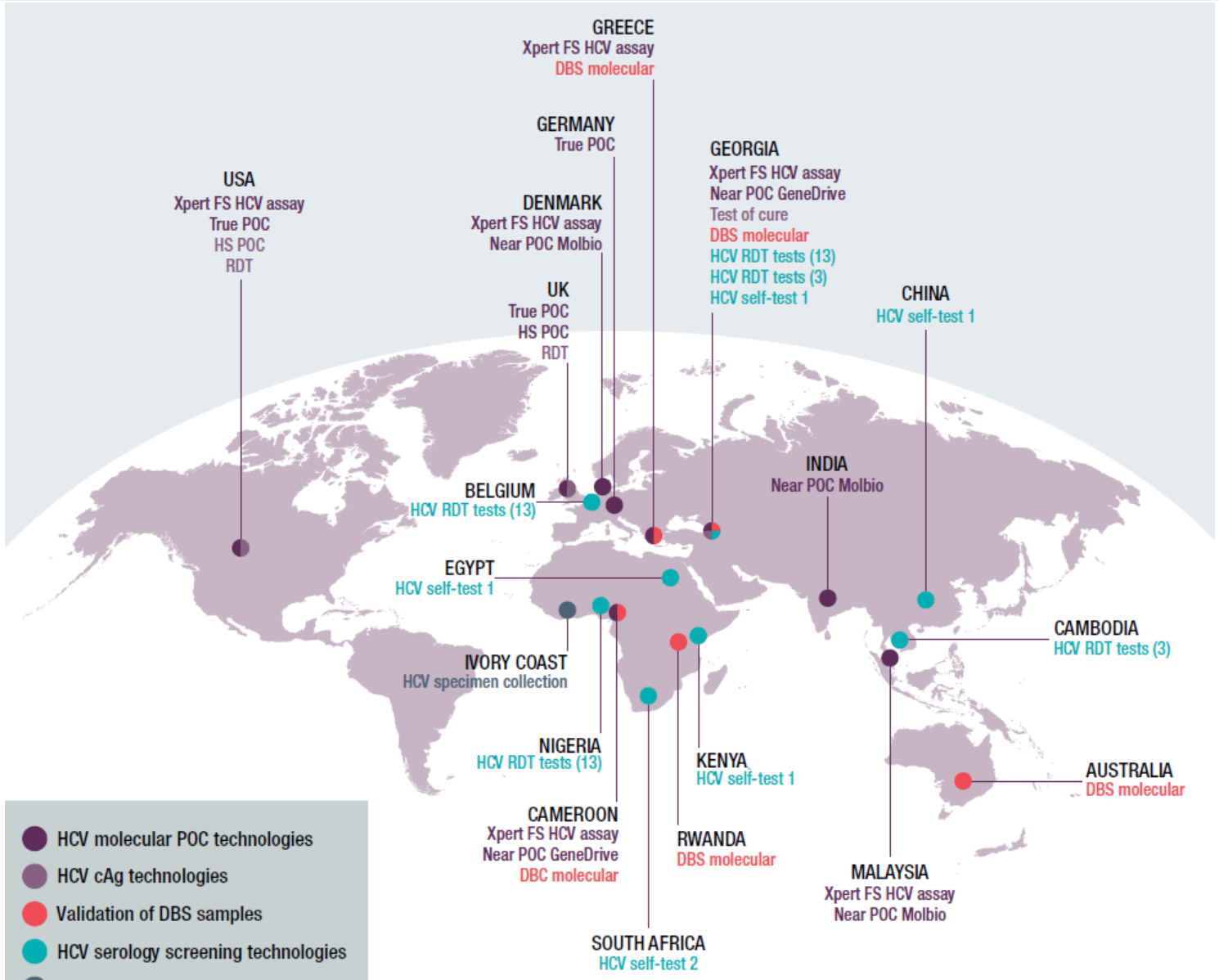
Sensitivity	94 %
Specificity	85 %
Accuracy	88 %



China, n=106

Burnet Institute
Medical Research. Practical Action.

HEAD-Start R&D activities, trials, studies



HCV product pipeline

GeneDrive HCV test

2018

Q1 Q2 Q3 Q4

Xpert HCV FS (Cepheid)

2019

Q1 Q2 Q3 Q4

DBS HCV RNA
on Roche CAP/CTM
Abbott m2000
Hologic Panther

TrueNAT HCV (Molbio Dx)

HCV self-test

2020

Q1 Q2 Q3 Q4

SAMBA II HCV assay

2021

Q1 Q2 Q3 Q4

HCV cAg RDT

HCV test on BlinkOne (Blink Dx)