World Health Organization
Myanmar

National Testing Guidelines for Viral Hepatitis Myanmar

National Health Laboratory
Department of Medical Services
Ministry of Health and Sports, Myanmar

September 2018 | Version 01

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Abbreviations

AFP	Alpha fetoprotein
ALT	Alanine aminotransferase
ANC	Antenatal care
Anti-HCV	HCV antibody
APRI	Aminotransferase/Platelet Ratio Index
ART	Antiretroviral Therapy
AST	Aspartate Aminotransferase
CBP	Complete Blood Picture
CDC	Centers for Disease Control and Prevention
CHAI	Clinton Health Access Initiative
CLIA	Chemiluminescence Immunoassay
CSO	Community Society Organization
DAA	Direct-acting antivirals
DHRH	Department of Human Resource for Health
DMR	Department of Medical Research
DMS	Department of Medical Services
DNA	Deoxyribonucleic Acid
DOPH	Department of Public Health
ECL	Electrochemiluminescence Immunoassays
EIA	Enzyme Immuno Assay
EPI	Expanded Program of Immunization
EQAS	External Quality Assessment Scheme
FSW	Female Sex Worker
HAV	Hepatitis A Virus
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C virus
HCVcAg	HCV core antigen
HEV	Hepatitis E virus
HRH	Human resource for Health
IBBS/PSE	Integrated Bio- Behavioural Surveillance/Population Size Estimation

ICT	Immuno Chromatographic Test
INGO	International non-governmental Organization
LFT	Liver Function Test
MCH	Maternal and Child Health
MLF	Myanmar Liver Foundation
MOHS	Ministry of Health and Sports
MSM	Men who have sex with men
NAP	National AIDS Program
NASBA	Nucleic Acid Sequence-based Amplification
NAT	Nucleic Acid Testing
NDACP	National Drug Abuse Control Program
NGO	Non-governmental organization
NHCP	National Hepatitis Control Program
NHL	National Health Laboratory
NS5B	Non-structural protein 5B (of HCV)
NTP	National TB Program
PCR	Polymerase Chain Reaction
PEG-IFN	Pegylated Interferon
PHL	Public Health Laboratory
PLHIV	People Living with HIV
PMTCT	Prevention of Mother to Child Transmission
PSE	Population Size Estimation
PWID	People Who Inject Drugs
RDT	Rapid Diagnostic Test
RMNCH	Reproductive, Maternal, Newborn and Child Health
RNA	Ribonucleic acid
SOP	Standard Operating Procedure
SVR	Sustained Virological Response
TB	Tuberculosis
TE	Transient Elastography
US FDA	United States of America Food and Drug Administration
VL	Viral Load
WHA	World Health Assembly
WHO	World Health Organization

Preface

Myanmar is taking a public health approach to respond of viral hepatitis infection. The National Hepatitis Control Program is established in 2014 and started to develop the National Strategic Plan for Hepatitis 2016-2020. The National Strategic Plan for Hepatitis is bounded by the following directions:

Strategic direction 1: Prevention of transmission of viral hepatitis
Strategic direction 2: Diagnosis, clinical care and treatment
Strategic direction 3: Workforce development
Strategic direction 4: Surveillance, research and strategic information

According to the strategic direction 2, the main goal is to develop and maintain services to provide the highest quality of viral hepatitis diagnosis, care and treatment. The NHCP has already adopted the service delivery model based on the approach of providing access to diagnosis, treatment, and care for viral hepatitis down to the township level. In the meanwhile, the NHCP has developed mechanisms and systems to facilitate the implementation such as procurement and access to diagnostics test kits and drugs, logistics and referral link to confirm the diagnosis of viral hepatitis infections with strong collaboration of the National Health Laboratory. The NHCP is currently expanding the sites of hepatitis C treatment clinics but also the private INGOs, local NGOs are also conducting the hepatitis B and C testing and the hepatitis C treatment implementation. To shed the light on this situation, the NHL considered producing the national hepatitis testing guidelines for those who are working on the viral hepatitis infections.

This Testing Guidelines for Viral Hepatitis Myanmar is adopted from World Health Organization "Guidelines on Hepatitis B and C testing February 2017". The purpose of this Myanmar Hepatitis Testing Guideline is to provide guidance mainly for the laboratory personnel and medical professionals working in the laboratory aspects. This guideline recommends who to test, how to test, monitoring of treatment scheme of hepatitis B and C and testing approaches of the other viral hepatitis infections.

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Glossary of terms

Markers for HBV infection

HbsAg
- First serological marker of HBV infection to appear
- Window period between HBV infection and detection of HBsAg estimated to be around 38 days, but depends on analytical sensitivity of assay used, immunocompetence of host and individual virus kinetics
- Occult HBV infection has been observed, i.e. HBsAg is undetectable but HBV DNA can be detected in individuals not in the window period
- Quantification of HBsAg is a potential alternative marker of viraemia

Anti-HBc IgM
- High levels present during acute infection but may remain detectable for up to 6 months
- Used to differentiate between acute and chronic HBV **infection,** but its reappearance during "flares" in chronic HBV infection make it an unreliable indicator of recent primary HBV infection

Anti-HBc (total)
- Develops around 3 months after infection and most constant marker of infection
- Together with anti-HBs, indicates resolved infection
- Anti-HBc, with or without anti-HBs, also indicates individuals who may reactivate in the context of immunosuppression

HbeAg
- Present when the virus is actively replicating in the liver
- Associated with high levels of HBV viraemia and is therefore a marker of "high infectivity"
- Associated with progressive liver disease

Anti-Hbe
- Represents host response to HBeAg and usually indicates decreasing HBV DNA and therefore infectivity
- Present in the immune-control and immune-escape phases
- May coexist with HBeAg during the period of seroconversion from e antigen to e antibody at the end of immune-tolerance phase

Anti-HBs
- Neutralizing antibody that confers protection from infection
- Present following spontaneous HBsAg clearance (with anti-HBc IgG)
- Generated by immunization and used to monitor post - immunization responses(anti-HBc absent)
- May coexist with HBsAg so presence cannot be used to exclude current infection

HBV DNA
- Used as a more direct and accurate measure of active HBV viral replication, which correlates with disease progression

- Serum HBV DNA is measured in international units (IU)/mL as the recognized international standard or copies/ml by nucleic acid testing (NAT) technologies
- Used to differentiate active from inactive HBeAg-negative, and to determine need for antiviral therapy in conjunction with ALT levels and degree of liver fibrosis
- Used to also monitor response to therapy (a rise may indicate inadequate adherence or the emergence of resistant variants) and as a marker of infectivity.
- May be detectable in early infection before HBsAg, and therefore useful in early diagnosis of at-risk individuals before HBsAg appears, but depends on sensitivity of the assay
- Also present at low levels in the absence of HBsAg in the context of occult infection

Markers for HCV infection

- Anti-HCV: Antibody to HCV, which can be detected in the blood usually within two or three months of HCV infection or exposure. The terms HCV antibody and anti-HCV are equivalent, but in these guidelines, anti- HCV is used throughout.
- HCV RNA: HCV viral genomes that can be detected and quantified in plasma by nucleic acid testing (NAT).
- HCV core antigen (HCVcAg): Nucleocapsid peptide 22 [p22] of HCV, which is released into plasma during viral assembly and can be detected from early on and throughout the course of active infection; not present in those with resolved infection

Natural history of viral hepatitis

- Chronic HBV infection: Persistence of HBsAg for at least six months. The persistence of HBsAg in two specimens six months apart is frequently used in clinical practice to confirm chronic hepatitis B infection.
- Chronic HCV infection: The presence of viraemic HCV RNA or HCVcAg in association with positive serology for HCV antibody.
- Viraemic infection: Hepatitis B or C infection associated with presence of virus in the blood (as measured by HBV DNA or HCV RNA), and often referred to as active, ongoing or current infection.
- Cirrhosis: An advanced stage of liver disease characterized by extensive hepatic fibrosis, nodularity of the liver, alteration of liver architecture and disrupted hepatic circulation.
- Hepatocellular carcinoma (HCC): Primary cancer of the liver arising from the hepatocytes and may be a complication of chronic hepatitis B or C infection.

Measures of treatment response

- HCV sustained virological response (SVR): Undetectable HCV RNA in the blood at defined time point after the end of treatment, usually at 12 or 24 weeks (SVR12 or 24)

Diagnostic testing for hepatitis B and hepatitis C

- Serological assays: Assays that detect the presence of either antigens or antibodies, typically in serum or plasma but also in capillary/venous whole blood and oral fluid. These include rapid diagnostic tests (RDTs) and laboratory-based immunoassays, e.g.

enzyme immunoassays (EIAs), chemiluminescence immunoassays (CLIAs), and electrochemiluminescence immunoassays (ECLs).

- Rapid diagnostic test (RDT): Immunoassays that detect antibodies or antigens and can give a result in less than 30 minutes. Most RDTs can be performed with capillary whole blood collected by finger-stick sampling. Serum or plasma can also be used after venepuncture.
- Enzyme immunoassay (EIA): Laboratory-based serological immunoassays that detect antibodies, antigens, or a combination of both
- Chemiluminescence immunoassay (CLIA): A variant of EIA which uses chemiluminescence as the detection signal. This technology may have increased sensitivity compared to enzymatic immunoassays. Usually used in automated platforms capable of high throughput.
- Nucleic acid testing (NAT): A molecular technology, for example, polymerase chain reaction (PCR) or nucleic acid sequence-based amplification (NASBA) that can detect very small quantities of viral nucleic acid (RNA or DNA), either qualitatively or quantitatively.

Testing terminology

- Testing algorithm: The combination and sequence of specific assays used within hepatitis B and C testing strategies
- Testing approach: In the context of these guidelines, the testing approach describes both "who to test" i.e. different populations and "where to test" i.e. different settings. Testing approaches include general population testing, focused testing of high-risk groups, or of antenatal clinics. These can be delivered through either health-facility or community-based testing.

Testing approaches terminology

- Key populations: Groups of people who due to specific high-risk behaviours, are at increased risk for HBV, HCV and HIV infection irrespective of the epidemic type or local context. Key populations often have legal and social issues related to their behaviours that increase their vulnerability to HIV, HBV and HCV infection. These guidelines refer to the following groups as key populations: men who have sex with men (MSM); people who inject drugs (PWID); people in prisons and other closed settings; sex workers; and transgender people.
- Vulnerable populations: Groups of people are particularly vulnerable to HBV/HCV infection in certain situations or contexts. These guidelines refer to the following groups as vulnerable populations: migrant and mobile workers, and indigenous populations.
- General population testing: This approach refers to routine testing throughout the entire population without attempting to identify high-risk behaviours or characteristics. It means that all members of the population should have potential access to the testing programme.
- Antenatal clinic testing: This approach means routine testing of pregnant women especially in settings where there is an intermediate or high seroprevalence, to identify women in need of antiviral treatment for their own health and additional interventions to reduce mother-to-child transmission (MTCT).

- Facility-based testing: Includes testing in primary care clinics, inpatient wards and outpatient clinics, including specialist dedicated clinics such as HIV, STI and TB clinics, in state, regional and district hospitals and their laboratories, and in private clinical services.

Service delivery terminology

- Integration: The co-location and sharing of services and resources across different disease areas. In the context of hepatitis B or C infection, this may include the provision of testing, prevention, care and treatment services alongside other health services, such as HIV, tuberculosis (TB), sexually transmitted infections (STI), antenatal clinic (ANC), contraceptive and other family planning services.
- Decentralization: The process of delegating significant authority and resources to lower levels of the health system (state, regional, district, township, station hospitals, primary health care and community).
- Linkage to care: A process of actions and activities that support people testing for HBV/HCV to engage with prevention, treatment and care services as appropriate for their hepatitis B and C status.

Executive summary

In Myanmar, the parenterally (blood borne) transmitted hepatitis viruses (HBV and HCV) and the fecal-orally transmitted hepatitis viruses (HAV and HEV) are widely prevalent. Given the differences in the geographic distribution, the populations affected, the routes of transmission and treatment, tailored strategies are required.

To effectively manage the prevention and control viral hepatitis infections, the National Hepatitis Control Program (NHCP) was established in 2014. Under the guidance of the Department of Public Health, the NHCP has developed the National Strategic Plan (2017-2020) and the simplified treatment guidelines of Hepatitis C infection. The treatment program on hepatitis C infection had started as "Quick start" program since June 2016. The NHCP is currently providing care and treatment of hepatitis C infection in collaboration with the medical services at the government general hospitals. In the light of this, diagnosis of hepatitis B and C infection is one important component of response to hepatitis as outlined in second strategic direction of the National Action Plan for Hepatitis Response.

Above all, the National Health Laboratory and partners adopted the viral hepatitis testing guidelines in line with WHO guidelines on hepatitis B and C 2017. In this national hepatitis testing guidelines, the two tests algorithm will be used for both viral hepatitis B and C infections.

For viral hepatitis C infection, many rapid diagnostic tests (RDT) are available in Myanmar though, the WHO prequalified test kits must be used for the first test as screening of hepatitis C antibody. If RDT screening test is reactive, that reactive sera should be confirmed by second test – nucleic acid test is preferable to GeneXpert testing.

For viral hepatitis B infection, hepatitis surface antigen (HBsAg) should be tested by single RDT test or laboratory based immunoassay and if reactive, the HBV DNA nucleic acid testing should be followed for confirmation.

Early diagnosis of persons with chronic HBV and HCV infections enables us to identify the status of their infection and they are able to access treatment and care to prevent or delay progression of liver disease. The testing and diagnosis is a crucial component of epidemic response to hepatitis.

This guideline outlines the public health approach to describe who to test, how to test and where to test in the country.

1. Introduction

Viral hepatitis is a global public health problem. Globally, approximately 71 million persons were living with HCV infection and 257 million persons were living with chronic HBV infection. There were estimated that 1.34 million deaths each year. Mortality rate has been increasing unless people with HBV and HCV infections are diagnosed and treated[1] . The goal of the WHO Global Health Sector Strategy on viral hepatitis (2016-2021), is to eliminate viral hepatitis as a major public health threat by 2030[2] .

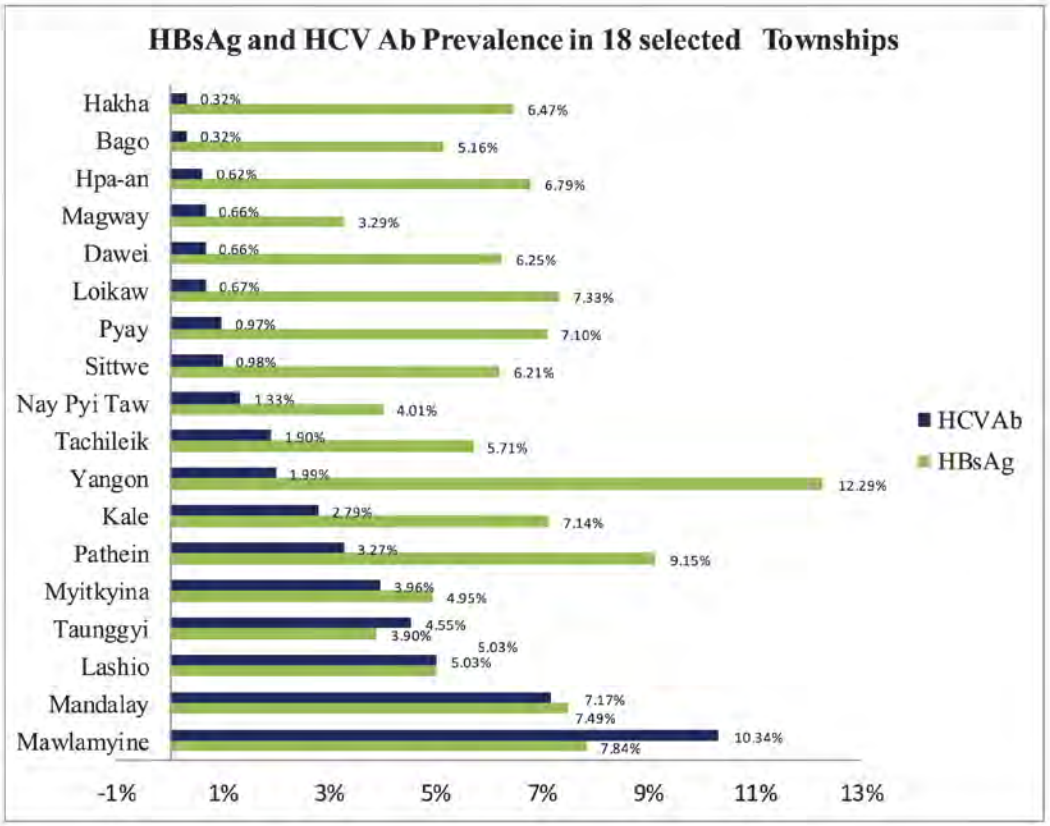
The low and middle-income countries in Asia and Africa carry a significant burden of HBV and HCV. The majorities of people in these populations are unaware of their infection and therefore often present only when they have advanced disease, in addition to being a source of ongoing transmission of infection. Factors that contribute to the low rates of hepatitis testing in these regions include: limited facilities and services for testing, lack of policies and guidelines for hepatitis testing, costly diagnostic assays, poor laboratory capacity and quality systems and ignorance about these infections.

1.1. Viral hepatitis epidemiology in Myanmar

In Myanmar, the parenterally (blood borne) transmitted hepatitis viruses (HBV and HCV) in addition to the fecal-orally transmitted hepatitis viruses (HAV and HEV) are widely prevalent, and are of major public health concern. The Department of Medical Research conducted a national prevalence survey for HBV and HCV between May to November 2015 across 18 study sites covering all States and Regions[3]. The HBV and HCV prevalence in the general population was found to be 6.51% and 2.65% respectively. The highest occurrence of HBsAg positivity was found in Yangon (12.29%), Pathein (9.15%), and Mawlamyine (7.84%). The highest occurrence of anti-HCV positivity was found in Mawlamyine (10.34%), Mandalay (7.17%) and Lashio (5.03%) respectively[3]. Prevalence of HBV and HCV in multi-transfused was 6.1% and 3.1%; in HIV-infected was 4.9% and 12.8%; and in PWID was 8.2% and 47.7%. Figure 1 and Table 1 show the prevalence across the various sites and the prevalence in the general population and some high risk groups.

[1] World Health Organization. Global hepatitis report 2017.
[2] World Health Organization. Global health sector strategy on viral hepatitis 2016-2021, towards ending viral hepatitis. June 2016.
[3] Department of Medical Research, Ministry of Health and Sports, National Prevalence survey report 2015

Figure 1: The prevalence of hepatitis B surface antigen and hepatitis C antibodies among general population in 18 selected townships in 17 States and Regions of Myanmar (2015)[3]



[3] Department of Medical Research, Ministry of Health and Sports, National Prevalence survey report 2015

Table 1: Burden of HBV and HCV in the population

Prevalence	Population	Prevalence rate (%)
Mono-infection of HBV		
	General population (2015)[4]	6.51
	Adult Males	8.95
	Adult Females	5.47
	Males under 10 years	7.25
	Females under 10 years	10.81
	PWID[5]	7.3
	Among blood donors (2015)	2.3
Mono-infection of HCV		
	General population (2015)	2.65
	Adult males	3.51
	Adult females	2.29
	Among blood donors (2015)	0.5
	PWID (2014)[5]	47.7
	PWID (Yangon) (2014)[5]	84.4
	PWID (Lashio) (2014)[5]	83.9
	PWID (Bahmo) (2014)[5]	73.9
Co-infection of HBV & HCV		
	PWID (2007)[6]	6.7
	PWID (Myitkyina) (2007)	9.5
	PWID (Moegaung) (2007)	8.5

Viral hepatitis, particularly HBV and HCV prevalence is higher among some groups such as PWID, MSM, sex workers, prisoners and also those with low socioeconomic status, where there is typically poor access to health care. Co-infection rates with HIV and TB are higher in these groups as well.

Rates of HBV seroprevalence in the general population and blood donors are higher than HCV seroprevalence. In some townships of Yangon, Pathein and Mawlamyine, HBsAg positivity rates range from 7.84% to 12.29%. Additionally, HBsAg positivity is more prevalent in younger adults (20-39%) and is more efficiently sexually transmitted. The risk factors associated with transmission of Hepatitis B were male gender, history of liver disease or hepatitis and history of household contacts.

The risk factors associated with the transmission of hepatitis C were male gender, age > 50 years, history of blood transfusion, dental treatment, surgery and history of liver disease or hepatitis.

[4] Department of Medical Research. Ministry of Health and Sports. *National Prevalence survey report* .2015
[5] Department of Health. *Integrated Bio-Behavioural Survey among people who inject drugs*. 2014
[6] Aung-Thu, et al., 2008.Myanmar Health Sciences Research Journal, 20 (3).

An Integrated Bio-Behavioral Survey/Population Size Estimation (IBBS/PSE)[6] study in 2014, among persons who inject drugs (PWID), the highest anti-HCV positivity rates were seen in Yangon (84.4%), Lashio (Shan North State) (83.9%), and Bhamo (Kachin State) (73.9%) . Among PWID, HIV/HBV co-infection was 2.2%, HIV & HCV was 20.1% and HIV & HBV/HCV was 20.7% respectively. Co-infection with HIV significantly increases rate of progression to cirrhosis and liver cancer, thereby the risk of liver- related mortality.

1.2. Estimated number of infected chronic hepatitis B and C

HCV: Estimated total of 1 .4 million HCV infected in Myanmar according to the HCV prevalence in 2015. This is based on prevalence of viraemia of 1.88%[7], in a population of 51.4 million[8]. Hepatitis C genotype 6 was most prevalent genotype (49%), followed by HCV genotypes 3 (39%), 1 (11%), and 2 (0.7%). HCV genotype 6 was the most common in northern cities and genotype 3 in the southern and western cities[9].

It is estimated that around 50% of HCV infected population are those with higher risk behaviours and exposures. This includes HIV infected (For HIV infection, there are an estimated 220,000 HIV infected, of which 30% are PWID), PWID, MSM, CSW, prisoners, repeated transfusion recipients, health care workers, haemodialysis patients and those who have been tattooed. It also includes patients in health care facilities with signs, symptoms and laboratory features of liver pathology. Table 2 summarizes estimated number infected in some of these key populations[10].

Table 2: Estimation of number of HCV and HBV infections National Strategic Plan for HIV and AIDS (2016-2020) which includes size estimation and prevalence of HIV and HIV coinfections in selected key populations[11]

Key population	PSE	HIV prevalence	New infection	Co-infected with HIV and HBV	Estimated No. HIV and HBV	Co-infected with HIV and HCV	Estimated No. HIV and HCV
PWID	83,000	28.5%	28%	14%	11,620	59.7%	49,551
MSM	253,210	11.6%	13%	15.2%	38,488	5.7%	14,433
FSW	66,056	8%	8%	10.3%	6,804	9.2%	6,077

[7] Nakai K, Win KM, Oo SS, Arakanwa Y and Abe K. Molecular characteristic-based epidemiology of hepatitis B, C and E viruses and GB virus C/hepatitis G virus in Myanmar. J Clin Microbiol. 2001. Apr, 30(4): 1536-9.
[8] Ministry of Immigration and Population. The Republic of Union of Myanmar. The 2014 Myanmar population and housing census. 2014.
[9] Lwin A, Shinji T, Khin M, Win N, Obika M, Okada S, Koide N. Hepatitis C virus genotype distribution in Myanmar: Predominance of genotype 6 and existence of new genotype 6 subtype. Hepatol Res 2001; 37:337-345.
[10] National AIDS Program. Ministry of Health and Sports. National Strategic Plan on HIV and AIDS (2016-2020) 2016.
[11] Ministry of Health and Sports, National AIDS Program, National Strategic Plan for HIV and AIDS (2016-2020). 2016.

HBV: Roughly estimated 3.3 million persons were living with the Hepatitis B infection according to the HBV prevalence of 6.5%.

1.3. National viral hepatitis response

In November 2014, Myanmar established the National Hepatitis Control Program (NHCP). After several consultative meetings with the government and civil society partners and deliberations of several Technical Working Groups, the Myanmar Action Plan for Viral Hepatitis Response was adopted in October 2016 encompassing four strategic directions of prevention of transmission of viral hepatitis; diagnosis, clinical care and treatment; workforce development; and surveillance and research/strategic information. There is now a costed operational and M&E plan, and training for advanced trainers planned.

A treatment strategy with initially seven Government Hospitals provided HCV treatment in Yangon, Nay Pyi Taw, Mandalay for 1,200 mono HCV infected and 800 HIV-HCV co-infected. The initial treatment targets for 2017 are 2,000 patients, with aim to scale up to 10,000 in 2018 and 25,000 in 2019, with expansion to state and district level hospitals/township clinics in future. National programme has purchased 6,500 RNA tests. Training has been provided by CHAI based on treatment guidelines, and CDC has also provided laboratory training.

1.4. Hepatitis C treatment facilities in Myanmar

The NHCP is being implemented hepatitis C treatment and care in the general hospitals in Yangon and Mandalay since June 2017. At first, the Hepatitis treatment is focused at facility based government hospitals where the consultant hepatologists and physicians are available however, NHCP will expand to the community level in the coming years. In 2018, as shown in the map in Figure 2, the seven general hospitals and fortunately in 2018 three additional general hospitals are being provided hepatitis C drugs: Sofosbuvir and Daclasivir in an account of government budget. In 2017, approximately 2,000 hepatitis C infected persons are being treated including 800 HIV and Hepatitis C co-infected persons at the ART providing hospitals. In 2018, Mawlamyine General Hospital and Myitkyina General Hospital are selected to treat both HCV monoinfection and HIV and HCV co-infection.

Figure 2: Hepatitis C treatment facilities in Myanmar (2018)

Myitkyina

1. Myitkyina General Hospital (monoinfection)
2. Myitkyina General Hospital (coinfection)

Mandalay

3. Mandalay General Hospital (monoinfection)
4. Mandalay General Hospital (coinfection)

Nay Pyi Taw

5. 1,000 Bedded Nay Pyi Taw General Hospital (monoinfection)
6. 1,000 Bedded Nay Pyi Taw General Hospital (coinfection)

Yangon

7. 500 Bedded Yangon Specialty Hospital (monoinfection)
8. North Okkalapa General Hospital (moninfection)
9. Waibagi Infectious Diseases Hospital (coinfection)
10. Mingalardon Specialist Hospital (coinfection)
11. Thaketa Specialist Hospital (coinfection)

Mawlamyine

12. Mawlamyine General Hospital (monoinfection)
13. Mawlamyine General Hospital (coinfection)

To treat HIV-HCV co-infection

To treat mono HCV infection

Testing and diagnosis: Viral hepatitis diagnosis is a distinct component of one of its Strategic Directions. Testing and diagnosis of these viral infections are key to ensuring access to highly effective curative short course treatment for HCV and for long term treatment for HBV, as well as prevention and care. Testing also provides an opportunity to introduce specific interventions to reduce further transmission such as counselling for high risk behaviour, provision of prevention commodities (such as sterile needles and syringes), safe health care injection safety and HBV vaccination. Given the differences in the geographic distribution, the populations affected, the routes of transmission and treatment, tailored strategies are required.

For both HCV and HBV infection, the majority of the population is unaware of their infection status. In accordance with the National targets for viral hepatitis response in Myanmar for 2030, 50% of the population should be diagnosed for HBV and HCV infection and also receive treatment. While efforts are being made to ensure birth dose of HBV vaccine for every newborn and also ensuring completion of the 3rd dose in the vaccination schedule, diagnosis of HBV and HCV infection is an important intervention to reduce burden of infection and transmission.

In a country with limited resources, integration of National Hepatitis Control Program with other already established services/ programs (e.g. National AIDS Program (NAP), National TB Program (NTP), National Drug Abuse Control Program (NDACP), Expanded Programme of Immunization (EPI), Reproductive, Maternal, Newborn and Child Health(RMNCH) is crucial for optimal delivery of services. The National Hepatitis Control program also needs the collaborative involvement of relevant departments within the Ministry of Health and Sports; Department of Public Health, Department of Medical Services, Department of Medical Research and Department of Human Resource for Health.

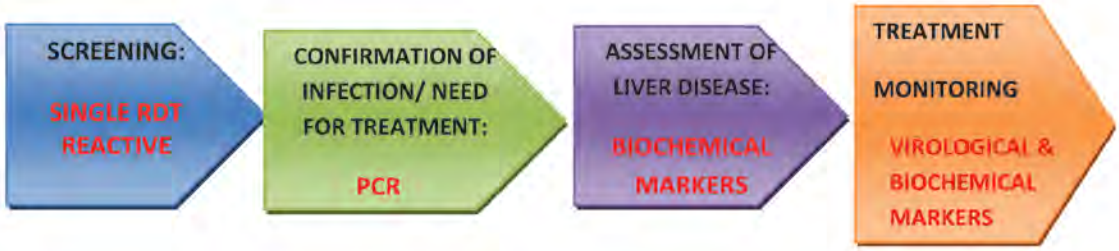
1.5. Aim of testing guidelines

These guidelines aim to establish hepatitis testing services and expand access to these testing services, especially for key populations.

- To Provide guidance on who to test and how to test and monitor (assays and testing algorithms) for chronic hepatitis B and C virus infection using the 2017 WHO guidelines of HBV and HCV testing as the framework, but adapted to the Myanmar context
- To propose a mix of testing strategies appropriate to the Myanmar context
- To Provide a framework for implementation of testing for viral hepatitis, with a mapping of existing lab infrastructure, a proposed sample referral network that could be employed in other Control programs, quality assurance, training and capacity building and laboratory information system.

- To Support effective prevention, treatment and care of chronic HBV and HCV infections.

Figure 3: Broad framework of testing and monitoring for chronic HBV and HCV infections



1.6. Target Audience

These Myanmar Hepatitis Testing Guidelines are intended to assist national program managers and service providers in planning for and implementing testing, treatment, care and as much as possible integrate with already existing national programs such as the NAP and NDACP.

1.7. Scope of guidelines

These guidelines are based on the WHO 2017 Guidelines of Hepatitis B and C testing and WHO 2016 Technical Considerations and Case Definitions to Improve Surveillance for Viral Hepatitis, but adapted to the Myanmar context. They cover the following topics:

- Introduction
- Existing infrastructure to support hepatitis testing
- Proposed arrangements for hepatitis laboratory testing strategies in the NHCP
- Recommended approaches on who to test and what laboratory assays to use for chronic hepatitis C and B infections
- HIV and HBV co-infections
- Clinical evaluation and laboratory work up prior to starting treatment
- Monitoring and follow-up after treatment
- Hepatitis B virus
- Monitoring HBV patients on treatment
- HIV and HCV co-infections

- Counseling messages for those identified as anti-HCV or HBsAg positive and negative
- Viruses causing acute hepatitis
- Training and capacity building
- External quality assessment scheme
- Future laboratory initiatives

1.8. Guiding principles of Hepatitis testing in Myanmar

Equity of access: People with HCV infection and (HBV infection) frequently come from vulnerable groups because of low socioeconomic status, poor access to appropriate health care, or belong to marginalized or stigmatized populations such as PWID and MSM. Thus, screening for HCV must not be used as a means to discriminate against those testing positive, for example, by denying them employment or education.

Adoption of five "C"s: It is advised that the WHO "5Cs"of consent, confidentiality, counseling, correct test results and connection that apply to all HIV testing services (HTS) in Myanmar be also implemented in the hepatitis testing program in Myanmar.

Quality assured testing: All testing devices [Rapid Diagnostic Tests (RDT)] or assays used for this program should be WHO pre-qualified or be approved by a stringent regulatory authority [eg. US FDA, CE marked (European approval)]. Only in the event of lack of availability of either and due to cost constraints, the choice of RDT or assays for use may be guided by availability of published (peer reviewed) performance of testing tools.

2. Existing infrastructure to support Hepatitis Testing

2.1. Existing testing services for HIV to support hepatitis testing

Testing facilities are available for

HIV infected persons: HIV/ART clinics: Test at 94 HIV clinic sites, and 42 STI clinic sites
PWID: Test at 51 Drug treatment centers/Methadone Maintenance Therapy sites
Prisoners: 2 Prisons of Yangon and Mandalay
Blood bank centers: Blood donor screening done at hospital blood bank
INGO and NGO sites: Those working on the harm reduction activities for HIV and AIDS project

2.2. Existing services and laboratory assays to support hepatitis testing

- **Serological diagnosis**: In the public sector, routine HBV and HCV screening is done using RDTs or for screening of blood donors, a laboratory-based immunoassay (ELIA or CLIA). However, diagnostic testing for HCV, HBV, HAV and HEV are not uniformly available in all hospital laboratories of the public sector. ELISA is available but not used routinely for HBV and HCV diagnosis. The National Health Laboratory (NHL) in Yangon (reference laboratory) has capacity to perform ELISA or Chemiluminescence based immunoassays (CLIA) for HCV, HBV, HAV and HEV. ELISA/ CLIA testing is currently not feasible in the State, Region and Township laboratories due to the requirement of 24 hours electricity for test kits storage.

- **Confirmatory viral load testing for HBV and HCV** (and HIV for viral load estimations) is conducted routinely at National Health laboratory (NHL) and the Mandalay Public Health Laboratory (PHL).

- **Liver function testing and serum creatinine estimation** is available in Tertiary Hospitals, States and Regional Hospitals and some District Hospitals.

- Facilities for **assessment of stage of liver disease** (degree of fibrosis) using Fibroscan are very limited in the country. However, basic biochemistry and platelet count to obtain APRI scores for staging of fibrosis can be done at the township level.

- **Ultrasonography and alpha fetoprotein** (AFP) are available in district hospitals in Myanmar.

Table 3: Summarizing existing access to serological and virological assays

Facility	Serology	Confirmatory Virological testing	Staging of liver disease
National Health laboratory (NHL)	ELISA or Chemiluminescence based immunoassays (CLIA) for HCV, HBV, HAV and HEV	- Three GeneXpert machine (4 modules) are used for HCV VL (RNA PCR) - One Rotor gene Real Time PCR is used for HBV VL (DNA PCR) - Two Abbott m2000rt amplification machine and one Abbott m2000sp automatic extractor is used for HIV viral load (RNA PCR) and EID (DNA PCR) - Abbott m2000 is 96 well high-throughput viral load instrument	
Mandalay Public Health Laboratory (PHL)	ELISA/RDT for HCV, HBV, HAV and HEV	- One GeneXpert machine (4 modules) is used for HCV VL (RNA PCR) - One Abbott m2000rt amplification machine and one Abbott m2000sp automatic extractor is used for HIV viral load (RNA PCR) and EID (DNA PCR)	
Tertiary hospital, states and regional hospital, and some district hospitals	RDT for screening tests	**Two other Abbot m2000 instruments:** ▪ Magwe AIDS/STD Team ▪ Mingaladon specialist hospital	Liver function and serum creatinine Ultrasound and AFP
Township level	RDT for screening tests		Basic biochemistry and platelet count for calculation of APRI score

3. Logistics of screening and confirmatory testing for HCV and HBV in Myanmar

3.1. Sample collection

Blood collection: 5ml of venous blood aseptically in an EDTA tube. Plasma is separated using centrifuge (800-1600g for 20 minutes).

Testing: Plasma tested by RDT (SD Bioline HCV) for anti-HCV, strictly as per manufacturer's instructions:

- If screening test is reactive (positive for anti-HCV), then plasma (minimum 2.5 ml per patient) has to be sent to the designated Referral hospital as shown in Table 3, based on site of collection. Until time of dispatch sample may be stored in refrigerator (2°C- 8°C) for a maximum of 2-3 days.

Packing of samples for referral: For dispatch to referral laboratory, the plasma (minimum 2.5 ml per patient) sample tube must be tightly capped, clearly labelled with name, hospital registration number and site of collection and placed along with other samples in a leak proof zip-lock bag/plastic bag along with referral slips, then packed with cold packs and then placed in an outer insulated box/container. These boxes/containers must be delivered to referral hospitals (laboratories) within 24 hours.

3.2. Confirmatory sites

- Receipt of samples at Referral laboratory: The box/container must be opened using universal precautions. Individual plasma samples are taken from the box and stored at 2-8° C if HCV VL test run within 3 days. If HCV VL test cannot run within 3 days, samples must be stored at _15∘C to _70∘ C, until time of testing.
- Repeated freezing & thaw of samples must be avoided. Freezing and thawing should not be more than three times.
- Samples must be completely thawed and then vortexed using a vortex mixer and then centrifuged before performing the confirmatory HCV RNA test (GeneXpert HCV VL).
- Turnaround time for confirmatory testing at the Referral laboratory:
  In the interest of clinical care and maximizing follow-up of patients (particularly those belonging to key populations), a maximum turnaround time of 3 days is recommended.
- Reporting of results from Referral Laboratory:
  Reports are sent to the appropriate screening site (Table 4). The development of a laboratory information system is strongly recommended. All access to patient's

details must be by only registered users with password protection. Registers and patient records must be given restricted access.

4. Proposed arrangements for hepatitis laboratory testing strategies in the NHCP

The initial strategy of HCV and HBV testing for this programme will be facility-based testing in General / Specialist hospitals with confirmation is done at the NHL and PHL. Confirmatory testing using GeneXpert could be expanded to other district hospitals in future. In that regard, table 4 shows the sample referral plan from the screening sites to referral laboratories for confirmation for the hepatitis C testing.

Table 4: Sample referral plan from testing sites to Referral Laboratories for confirmatory testing using high throughput lab platforms or GeneXpert

Hospitals performing anti-HCV antibody screening and other serological investigations i.e., AST, Platelets	Hospitals/ Referral Laboratories performing Confirmatory HCV RNA testing
Specialist hospital Thaketa	National Health Laboratory, Yangon
North Okkalapa General Hospital	
Infectious Disease Hospital, Waibagi	
Specialist Hospital, Mingaladon	
500 Bedded Yangon Specialty Hospital	
Mandalay General hospital	Public Health Laboratory, Mandalay (for HCV)
Nay Pyi Taw 1,000 Bedded Hospital	
Mawlamyine General Hospital	Laboratory at Mawlamyine General Hospital
Myitkyina General Hospital	Laboratory at Myitkyina General Hospital

Table 5 shows the sample referral plan from the screening sites to referral laboratories for confirmation for the hepatitis C testing. The Sample referral plan for HBV from testing sites to the Referral Laboratory (NHL) is the same as for HCV.

Table 5: Sample referral plan for HBV from screening sites to the Referral Laboratory (NHL)

General Hospitals performing HBsAg screening and other serological investigations i.e., AST, Platelets	Hospitals / Referral Laboratories performing Confirmatory HBV DNA quantification
Specialist hospital Thaketa	National Health Laboratory, Yangon
North Okkalapa General Hospital	
Infectious Disease Hospital, Waibagi	
Mandalay General hospital	
Nay Pyi Taw 1,000 Bedded Hospital	
Specialist Hospital, Mingaladon	
500 Bedded Yangon Specialty Hospital	
Mawlamyine General Hospital	
Myitkyina General Hospital	

- Counselling of patients at time of providing confirmatory test results follow broadly the framework of that following the screening test (see above).

4.1. Requirements for testing sites facilities

Testing sites:

1. The site where specimens are taken and tests performed must be clean and comfortable for patients and the testing provider.
2. Adequate lighting must be available for visually read assays.
3. A safe working environment must be maintained with necessary procedures in place: universal precautions and prevention of and/or response to needle stick injuries and other occupational exposures, chemical biological safety, spill containment, waste disposal and appropriate use of personal protective equipment.
4. Where possible, testing should be done in climate- controlled settings.
5. There must be proper waste disposal for biological (infectious and non-infectious), chemical and paper waste and most importantly for sharps.

Confirmatory site:

1. The NHL and the PHL must have all of the above facilities and practices and additionally will have appropriate power back-up facilities to support the use of equipment for nucleic acid testing and various laboratory assays such as enzyme immunoassays and chemiluminescence immunoassays.

2. The testing will be supervised by a senior laboratory specialist or consultant.
3. All preventive and corrective equipment maintenance must be in place and on installation of new equipment, appropriate validation and calibration must be done.

5. Recommended approaches on who to test and what lab assays to use for chronic hepatitis C and B infection

Testing for HCV is a priority due to the availability of directly acting anti-viral agents in Myanmar. Duration of treatment with these drugs is for a shorter duration (12/24 weeks), associated with fewer side effects and yield significantly superior sustained virological response as compared to the earlier standard of care i.e., pegylated interferon and ribavirin.

5.1. Who to test for chronic hepatitis C and B

The initial testing policy will be to focus on priority or most affected populations as summarized in table 6 below.

Table 6: Testing approaches and populations to get tested

S No.	Testing approach	Population	Comments on implementation at sites
1	Focused testing in most affected and at-risk populations in Myanmar	HIV infected persons, People who Inject drugs (PWIDs), Men who have Sex with Men (MSM), Female Sex Workers (FSW), Multi-transfused recipients, Health care workers, Hemodialysis patients, Prisoners	**HIV infected persons:** HIV/ART clinic. Test at 94 HIV clinic sites, and 42 STI clinic sites **PWID**: Test at 51 Drug treatment / OST sites or outreach sites **Prisoners**: 2 Prisons of Yangon and Mandalay **Multi-transfused recipients and thalassemia**: Patient registers in specialist clinics **Haemodialysis**: Dialysis units (public and private) **Healthcare workers:** Pre-employment, medical and nursing students
2.	Persons with signs and symptoms of disease	Patients admitted in hospitals with signs and symptom of liver pathology (i.e. jaundice, abdominal pain, fatigue, nausea, vomiting, or abnormal liver function tests or ultrasound). This will be done at the discretion of the treating physicians.	Adults, adolescents and children in Hospital Inpatients and outpatients in State, Regional and District Hospitals
3.	Antenatal clinic	Pregnant women Offspring of HCV positive	- Routine ANC testing alongside HIV/HBV testing - Screen all children of positive mothers at 18 months

4.	Blood donors	The anti-HCV testing of blood and organ donors is mainly for screening but those identified positive should be referred for further evaluation to treatment sites.	Blood banks need to refer all positive cases to local sites.

6. HCV testing algorithm

6.1 Screening for anti-HCV antibody

Initial screening for HCV infection is done by testing for anti-HCV antibodies (anti-HCV Ab) using a single WHO pre-qualified RDT or Laboratory-based immunoassay-(ELISA/CLIA). The single initial screening test is recommended before confirmatory testing regardless of the prevalence level within the population. RDTs should be prioritized over immunoassays in settings where they will increase access to testing.

Currently, SD Bioline (Standard Diagnostics), which is a WHO prequalified RDT, have been already procured for the NHCP. Anti-HCV Ab positivity (reactive) implies exposure to HCV and cannot differentiate between current (viraemic) infection and past infection. Therefore, anti-HCV Ab positivity needs to be confirmed by tests that can detect current infection. Anti-HCV Ab negative (non-reactive) result suggests absence of serological evidence of HCV and no further testing is required (Figure 4).

Re-testing: Patients with ongoing risks should be re-tested.

Patients with both positive and negative serology result should be appropriately counseled as per earlier published recommendations.

6.2 Confirmatory test for HCV

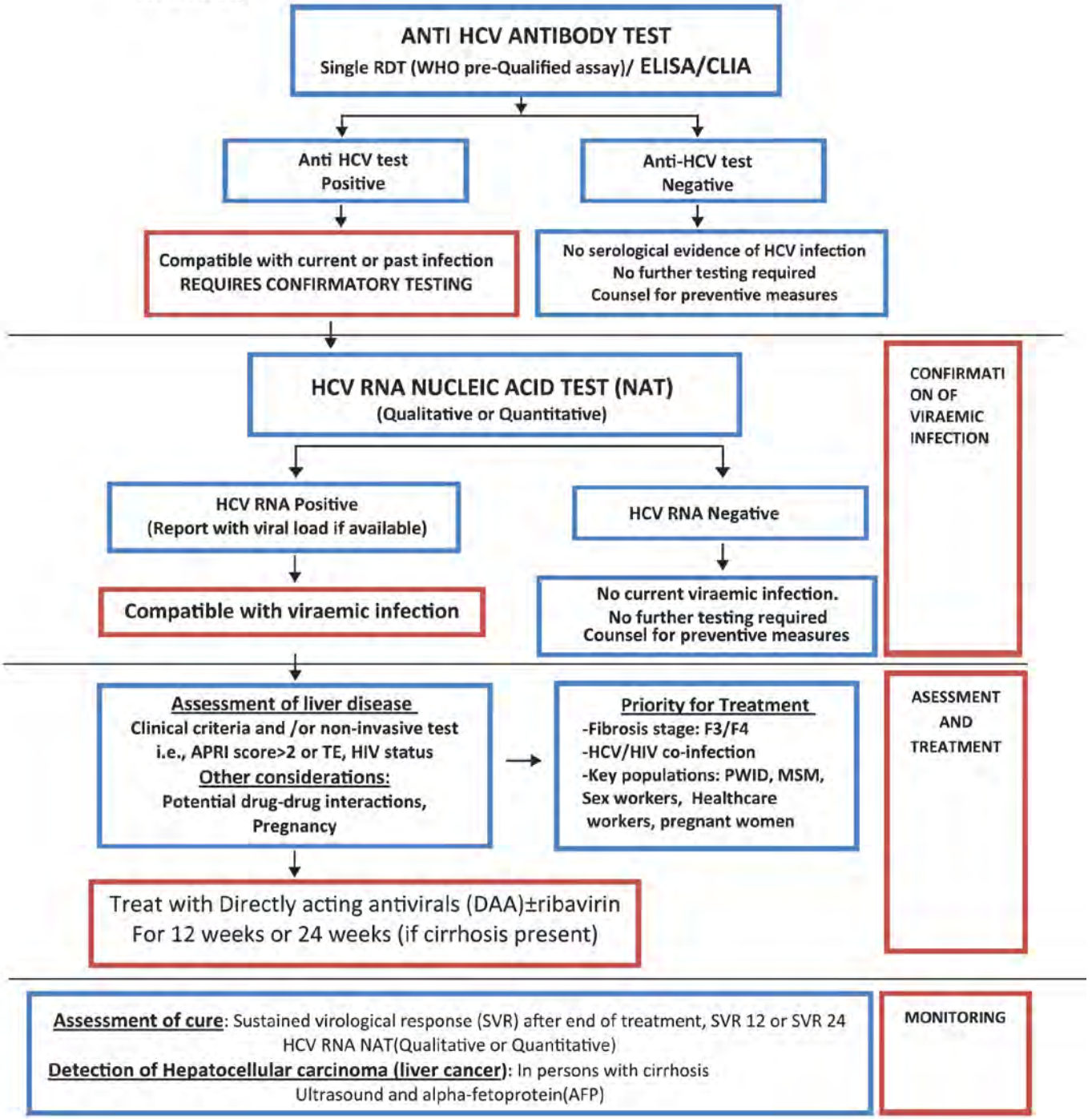
It is recommended that all HCV antibody positive samples be confirmed using nucleic acid testing (NAT) for HCV RNA (either qualitative or quantitative) or the HCV core antigen (HCVc Ag). The latter is an alternative test to confirm current viraemic (active) HCV infection, detecting directly the HCV core antigen (HCVcAg) on an automated immunoanalyzer platforms. Only patients who are confirmatory test positive (either NAT or core antigen test) should be assessed for treatment eligibility and placed on treatment (Section 2 of Figure 6). HCV RNA testing can be done on several NAT platforms i.e, VERSANT HCV RNA 1.0 Assay kPCR (Siemens), CAP/CTM Quant Test v2.0 High Pure (Roche), GeneXpert HCV VL(Cepheid) and Real Time HCV (Abbott). On completion of

treatment, a sustained viral response to anti-viral treatment is tested by performing a HCV RNA test (Figure 4).

Confirmatory HCV RNA testing should also be considered in severely immunocompromised patients from HIV infection or other reasons because of the potential for false-negative HCV serological test results. This may occur in up to 6% of persons with HIV who undergo testing using a second-generation anti-HCV enzyme immunoassay (EIA), but may occur more often among persons with advanced immunosuppression due to HIV and during early HCV infection.

Viral load confirmatory testing is particularly important for patients with advanced HIV infection and/or unexplained active liver disease (high transaminases) with initial anti-HCV negative test.

Figure 4: Summary Algorithm for HCV Diagnosis, Assessment, Treatment and Monitoring



Several GeneXpert (Cepheid) systems are already installed in Myanmar as part of the NTP and the NAP. Additionally, the PHL in Mandalay and the NHL have their own GeneXpert machines to perform Hepatitis testing. At the Stakeholders meeting held in March, 2017 at NHL, it was proposed to consider GeneXpert HCV VL (Cepheid) for Hepatitis HCV viral load testing as a convenient platform for decentralized HCV viral load testing at treatment sites, but as temporary approach at NHL until demand for VL increases.

6.3 Proposed approach

The NHL will be the main Reference Laboratory for performing confirmatory viral load testing and will use a high throughput platform such as the *Real Time* HCV (Abbott), which are already available at the NHL.

Rationale for adoption of high throughput Abbott is primary foundation for VL confirmatory testing rather than GeneXpert. Abbott platforms are already available in reference labs and GeneXpert machines will not serve anticipated future high demand for VL testing in NHL and PHL reference labs. Therefore, placement there would only be there on a temporary basis.

Key caveat are the requirement for high volume to make this approach cost effective, the need to establish a formal arrangement for sharing of instrumentation with HIV programme, a robust sample transport system and for timely transmission of results.

7. HIV and HBV co-infections

Clinical impression and/or distinct risk factors for co-infection should be investigated. HIV testing and anti-retroviral therapy are be supported by the NAP. HBV testing will be done as part of the NHCP in the future. Prior to starting treatment for HCV, it is advisable to test for HBsAg since there is a potential but uncertain risk of HBV reactivation and worsening of liver disease during after HCV clearance.

8. Clinical evaluation and laboratory work up prior to starting treatment

- To assess fibrosis in the liver, for the calculation of APRI score second blood draw is required **(Figure 4)**
- Platelet count: blood to be collected in K3 EDTA tube
- Aspartate aminotransferase (AST): Blood to be collected in plain tube to obtain serum

9. Monitoring and Follow-up after treatment

Patients with cirrhosis or risk for liver cancer may require an ultrasound and alpha-fetoprotein (AFP) determination from blood.
A repeat HCV RNA test is to be done 3 months (12 weeks) after completion of treatment **(Figure 4)** to assess sustained virus response (SVR12).
When first line treatment fails, genotyping can be considered to guide the appropriate second line treatment.

10. Hepatitis B virus (HBV)

Rates of HBV seroprevalence in the general population and blood donors are higher than HCV seroprevalence. In some townships of Yangon, Pathein and Mawlamyine, HBsAg positivity rates range from 7.84% to 12.29%. Additionally, HBsAg positivity is more prevalent in younger adults (20-39%) and is more efficiently sexually transmitted.

Like HCV infection, the majority of the population is unaware of their infection status. As per the National targets for viral hepatitis response in Myanmar for 2030, 50% of the population should be diagnosed for HBV and HCV infection and also receive treatment[12]. While efforts are being made to ensure birth dose of HBV vaccine for every newborn and also ensuring completion of the 3rd dose in the vaccination schedule, diagnosis of HBV infection is an important intervention to reduce burden of infection and transmission.

10.1. Screening tests for HBV

Initial screening for HBV infection is done by testing for HBsAg using an RDT or laboratory based immunoassay such as ELISA. In Myanmar, due to constraints of resources and infrastructure, RDTs may be prioritized over immunoassays to increase access to testing. Assays should meet minimum acceptance criteria of either WHO pre-qualified or a stringent regulatory review for in-vitro diagnostics.

A reactive HBsAg result in a screening test (RDT) is considered positive for HBsAg and is compatible with current HBV infection. A non-reactive HBsAg result in the RDT is considered negative for HBsAg, suggesting no serological evidence of HBV infection **(Figure 5)**.

A testing strategy defines the sequence of tests to be followed for a specific testing objective (i.e. to identify infected and non-infected individuals). The choice between a one-assay versus two-assay serological testing strategy will depend on the seroprevalence in the population to be tested and diagnostic accuracy (sensitivity and specificity) of the assays used [13].

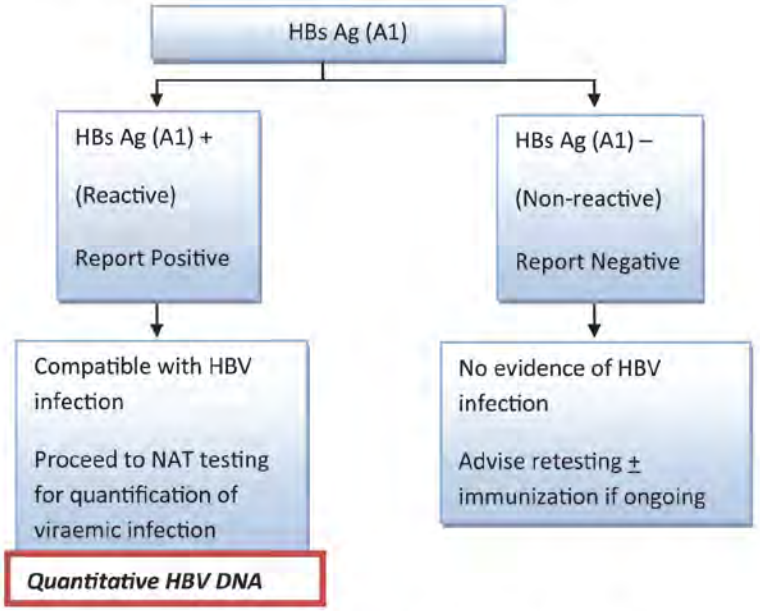
[12] National Hepatitis Control Program. Ministry of Health and Sports. Operational Plan for Viral Hepatitis C (2017-2020). 2017.

10.1.1. One-assay serological testing strategy

A one-assay serological testing strategy (Figure 5) is when a single serological test is performed. If the test result is reactive, a "compatible with positive infection" status is reported. If the initial test result is non-reactive, a "negative infection" status is reported. This testing strategy efficiently rules out most uninfected individuals correctly, and rules in those who are likely to be infected and therefore in need of further HBV DNA and HCV RNA NAT testing and staging of liver disease using NITs and clinical evaluation. This testing strategy is particularly suitable for high prevalence settings due to the relatively higher positive predictive values (PPVs), but needs a highly sensitive and specific assay to maintain acceptable predictive values[13].

Figure 5: Hepatitis B virus serological testing (Single assay)

(A) Single assay (HBs seroprevalence ≥0.4%)



10.1.2. Two-assay serological testing strategy

Two-assay serological testing strategy (Figure 6) differs in that two different assays are used sequentially, to improve the Positive Predictive values (PPV) of the testing strategy, and reduce the number of false positive results and therefore the number of individuals inappropriately referred on to more specialist services. In low prevalence settings, there

[13] World Health Organization. Guidelines on Hepatitis B and C Testing, February, 2017.

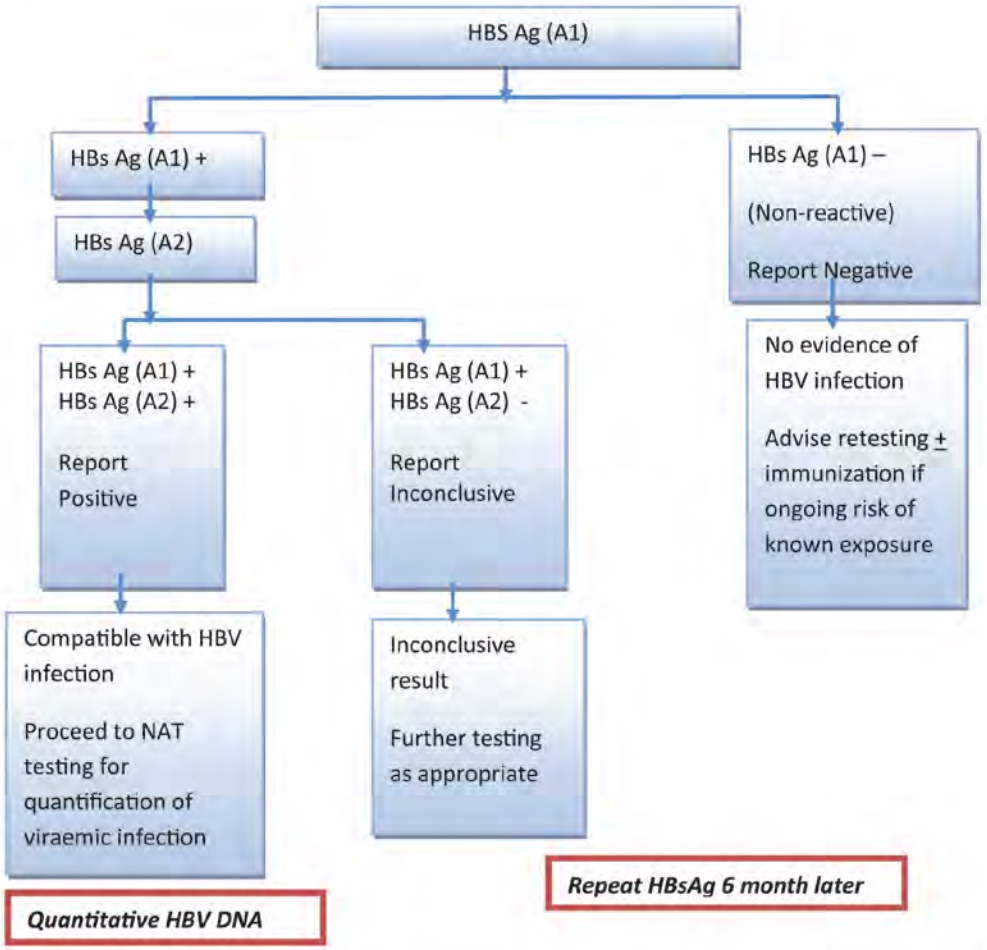
will be more false positive results, even with a test of 99% specificity. Employing two assays with a specificity around 99% increases the ratio of true-positive to false-positive diagnoses from 0.2 to 32-40.

This can be achieved by either (1) repeating the serological test using a different assay of similar sensitivity , or (2) in case of HBsAg , performing a neutralization test using a specific anti-HBs containing reagent in the same first line assay after appropriate dilution of the specimen under test.

If the first test result is non-reactive, a **"negative infection"** status is reported. If both test results are reactive, the status is report as **"presumptive positive status infection for further diagnostic testing".** If the second test result is non- reactive, the status is reported as **"infection inconclusive; requires additional testing".**
Both categories of patients should be appropriately counseled in accordance with published recommendations (see below).

Figure 6: Hepatitis B virus serological testing (Double assay)

(B) Two assays (HBs seroprevalence <0.4%)



10.2. Additional tests in decision making to start treatment

10.2.1. Assessment of stage of liver disease

This is done using clinical criteria and assessment of fibrosis using the APRI score (AST/platelet ratio). Additionally repeated ALT measurements may be required to further assess the level of inflammatory activity in the liver. These tests can be done in the General Hospitals where anti-HCV antibody screening is done.

10.2.2. Confirmatory HBV DNA (quantitative) test

- This is done for guidance to treat/not treat in patients who do not have cirrhosis. In patients with cirrhosis, antiviral therapy should be given irrespective of HBV DNA viral load. The HBV DNA quantitative assay is currently done in the NHL. The same steps of blood collection, sample processing, packing for dispatch to the Referral Laboratory and storage, described earlier for HCV apply to HBV as well.
- Turnaround time for confirmatory testing at the referral laboratory:
  In the interest of clinical care and maximizing follow-up of patients (particularly those belonging to key populations), a maximum turnaround time of 3 days is recommended.

  Reporting of results from Referral Laboratory:
- Reports are sent to the appropriate Screening site (Table 4). The development of a laboratory information system is strongly recommended. All access to patient's details must be by only registered users with password protection. Registers and patients records should be restricted access
- After clinical evaluation and review of the biochemical test, APRI score and HBV DNA viral loads, the decision to treat/no treat is made by the attending clinician (Figure 7)

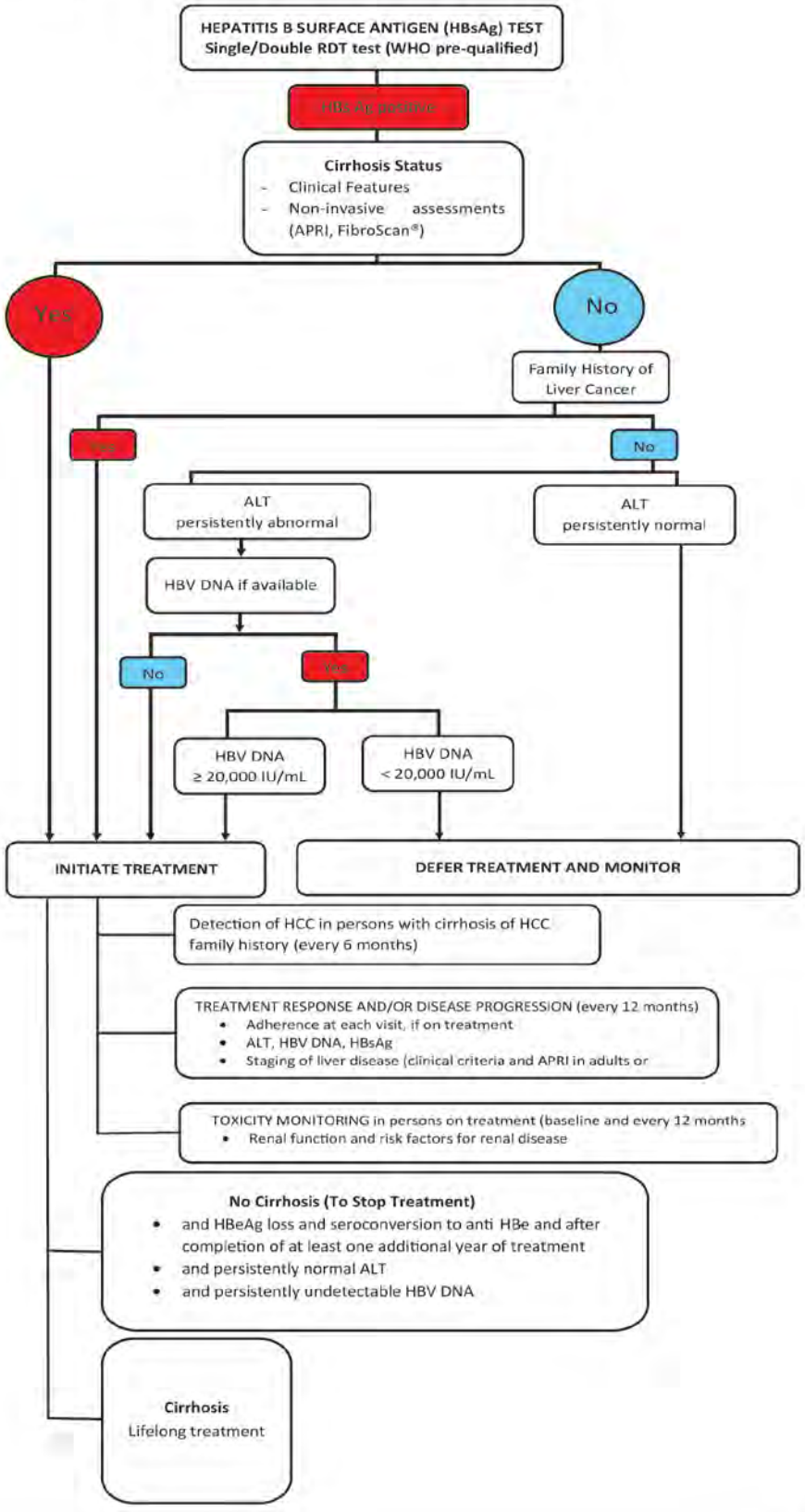
11. Monitoring HBV patients on treatment

- Ultrasound and serum alpha-fetoprotein levels: these tests may be required in patients with cirrhosis and/or family history of liver cancer. This is to be done every 6 months
- Quantitative HBV DNA test, hepatitis B e antigen (HBeAg) and ALT levels need to be monitored every year to assess response to therapy.
- Adherence to therapy must be checked by asking discreet questions
- Renal function test (serum creatinine) will need to be done annually for patients on tenofovir therapy (Figure 7).

12. HIV and HCV co-infections

Clinical impression and/or distinct risk factors for co-infection should be investigated. HIV testing can be supported by the NAP. HCV testing will be done as part of the NHCP.

Figure 7: Summary Algorithm for HBV Diagnosis, Assessment, Treatment and Monitoring



13. Counselling messages for those identified as Anti-HCV antibody or HBsAg positive and negative

13.1. People Testing Anti-HCV and HBsAg Positive

Explanation of the results and implications: Patient has to be told that he/she has been infected with the hepatitis C virus. All people who receive a positive anti-HCV Ab. test result should receive education and counselling about their HCV infection, care and treatment. The aim of the counselling should be to encourage confirmatory testing and to prevent transmission before confirmatory testing. The counselling session should include the following:

- That patient may or may not currently have hepatitis C as some people are able to clear the virus, although many do not. Patient will need to have a further blood test (confirmatory test) to find out if he/she currently has hepatitis C. Patient needs to be told that his/her sample will be now be sent to a referral laboratory.
- For HBV, Patient needs to be told that his/her sample will be now sent to a referral laboratory as part of further assessment prior to treatment.
- Emphasis on the need for confirmatory testing and assistance with determining next steps.
- Patients need to be informed that if the confirmatory test is positive, he/she will be contacted on the mobile or through contacts to come back to the hospital for further clinical evaluation with the doctor and a second blood test (AST, platelets) for planning for anti-viral therapy.
- Acknowledgement of concerns about HCV and HBV transmission, barriers to returning for additional testing, and addressing questions regarding potential illness must be done.
- For HBsAg positive, Advice about getting partner(s) and children HBV vaccinated.
- General health promotion and education, with emphasis on prevention and modes of transmission.

13.2. People Testing Anti-HCV antibody or HBsAg negative

All patients who are confirmed anti-HCV Ab or HBsAg negative should receive post-test counselling with the aim of reducing or eliminating risky behaviour which can lead to future acquisition of infection. The counselling session should include the followings:

- Explanation of the results and implications: HCV antibody or HBsAg test negative or nonreactive implies “no antibodies”
- Absence of anti-HCV or HBsAg in the blood should not be confused with future immunity to HCV infection

- If the patient has recent or ongoing risk, an explanation of the "window" or "lag" period must be given along with the recommendation of retesting in 6 months.
- General disease education with emphasis on prevention and modes of transmission.
- Discussion of benefits of retesting in the future must be also done in high risk populations.
- For HBsAg negative, patients should be specifically told where to get vaccinated or offered vaccination on site.
- Until the confirmatory test results are known, counseling on standard prevention practices to avoid transmission, in case chronic infection does exist.

14. Viruses causing acute hepatitis

Hepatitis A and E viruses can be transmitted through the fecal-oral route. Poor sanitary and hygienic practices, unsafe water supplies and poor food hygiene are prevalent in Myanmar and are common modes of transmission, contributing to occurrence of outbreaks. Hepatitis A and E infections are associated with poor outcomes in alcoholics and patients with chronic liver disease.

HAV and HEV are not associated with cirrhosis and hepatocellular carcinoma. However, in pregnant women, especially in the last trimester of gestation, HEV can lead to development of acute fulminant hepatitis followed by liver failure and eventually death. Less frequently but not rarely, HBV infection can present as acute hepatitis. HCV should be also considered especially in patients with high parenteral risk or risky lifestyle.

14.1. Clinical features of acute hepatitis

Fever, malaise, fatigue and liver damage [e.g. anorexia, nausea, jaundice, dark urine, right upper quadrant tenderness, and/or raised alanine aminotransferase (ALT) levels more than ten times the upper limit of normal.

14.2. Testing approaches to investigation of acute viral hepatitis

Since there are no WHO pre-qualified RDTs available for use in Myanmar, as a priority, the National Reference Laboratory such as NHL must seek to evaluate the available RDTs against ELISA/CLIA that are WHO pre-qualified or approved by a stringent regulatory authorities [(US FDA/CE marked(European approval)]. Until such assays are available for use in the general and district hospitals, patients' samples need to be sent to NHL for diagnosis with ELISA. The laboratory approach of investigation of acute viral hepatitis is shown in Table 7.

Table 7: Laboratory tests for investigation of acute viral hepatitis in Myanmar (done at NHL and PHL)

Virus	Virus marker	Type of test
HAV	Anti-HAV IgM	ELISA/RDT
HEV	Anti-HEV IgM	ELISA/RDT
HBV	HBs Ag +Anti-HBc IgM	ELISA/RDT
HCV	Anti-HCV	RDT or Lab-Based Immunoassay (CLIA/ELISA)

HCV is usually investigated for only if the above HAV, HEV and HBV markers are negative.

15. Training and capacity building

The National Health laboratory will take the initiative to provide an orientation and training to all Laboratory In-charge: Microbiologists, Pathologists and Medical Technologists on the laboratory testing algorithms. Inputs from Hepatologists and Clinicians would provide a comprehension picture of the whole NHCP.

Training should be in the form of:

- Sample collection and processing of samples
- Hands-on training in the use of the RDTs and observation and interpretation of the molecular tests (confirmatory assays)
- Packing of samples for referral and transport
- Appropriate storage of samples in the refrigerator or freezer
- Staff from the Referral Hospitals must be trained in the use of GeneXpert HCV VL since NHL is already routinely using this platform for diagnostic tests
- All trainees must be provided with an outline of the testing algorithm as well as the appropriate SOPs for display
- A mapping of the referral network should be also provided for streamlining the movement of samples and reports between the screening sites and the Referral Laboratories.

16. External Quality Assessment Scheme (EQAS)

Quality of tests and timely reporting of results are very central to the management, care and treatment of the patients.

- An EQAS network must be established between all participating laboratories. Inter-laboratory comparisons between the Referral laboratories and between the General hospital (screening sites) may be a simple, pragmatic initiative.

- Staff should be trained in maintenance of Inventory and Records and forecasting of supplies for smooth functioning of the program.

- Training and re-training programs should be held twice a year.

- A Quality manual and Safety manual on use of Personal Protective Equipment, Injection Safety and Biohazard waste Management must available at each site.

- Contact information of all Laboratory staff participating in the Hepatitis testing must be available in a dedicated directory.

- On-site visits by Laboratory consultants from the Referral laboratory must be made bi-annually.

- The Program should ensure that all equipment, refrigerators and freezers are annually serviced and in good working condition.

- Myanmar National Policy on Health Laboratory was developed in 2016.

- NHL established evaluation of HIV testing and syphilis testing by using a proficiency panel and hepatitis proficiency panel will be built up in 2019

17. Future laboratory initiatives

The role of the Public Health laboratory services is pivotal for the second strategic direction of the NHCP i.e., diagnosis of infection. This laboratory serves as the key Referral laboratory. Additionally the mandate of the NHL is to undertake or support:

- **Validation of different RDTs in country**: Country evaluation of different RDTs to inform procurement Identify quality assured (preferably WHO pre-qualified), affordable testing systems to be used to execute and sustain this program. This may be done by conducting evaluations of new kits in the NHL

- **Forecasting and procurement**: Conduct of diagnostics forecasting for hepatitis testing needs and consideration of pooled procurement with HIV programme to ensure optimal pricing

- **HBV vaccination of all laboratory staff**: Take initiative to ensure that all laboratory staff have been immunized against HBV

- **Dissemination of testing algorithms and best practice**: Engage with counterparts in the private sector so that uniform testing algorithms can be used nationally

- **Accreditation**: The NHL must take every effort to gain accreditation so that they can subsequently empower smaller laboratories to also do the same

- **GeneXpert**: A complete mapping of all GeneXpert machines must be made within Myanmar to inform the potential for decentralized confirmatory testing

- **DBS protocols:** Support in country validation of testing protocols to evaluate the use of Dried Blood Spots (DBS) using commercial assays for serology and virology, and also using the GeneXpert platform if protocol becomes available. This has the potential to simplify the transport of samples from the periphery and provides scope for more laboratories to join this network. DBS versus plasma performance must be compared on the GeneXpert platform. The RealTime HCV (Abbott) platform, at the NHL may be used for comparison.

- **Training:** The long term goal of the NHCP is to decentralize testing as more laboratory staff get trained. With progressive sharing of resources across other national programs such as the NAP, the GeneXpert could be used in a point-of-care setting

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Annex 1 Staging of liver disease for treatment of Hepatitis C infection[14]

After confirmation of HCV viraemia, further assessment of liver disease [clinical evaluation and/or estimation of AST/Platelet Ratio Index (APRI) score (to assess fibrosis)] is needed before treatment can be started.

APRI = [AST(IU/L)/ AST_ULN(IU/L)) x 100] / platelet count (10^9/L)
ULN = upper limit of normal for AST in the laboratory where it was tested

FIB-4 = Age(yr) x AST(IU/L)/platelet count(109/L) x [ALT(IU/L)1/2]

	Fibrosis stages assessed	Cut-off values for detection of fibrosis	
		Cirrhosis (METAVIR F4)	Significant fibrosis (METAVIR ≧F2)
APRI	≥F2, F4	High cut-off 2.0	High cut-off 1.5
FIB-4	≥F3	High cut-off 3.25	
FibroTest	≥F2, F3, F4	0.32-0.48	0.58-0.75
FibroscanR	≥F2, F3, F4	>11-14 kPa	>7-8.5 kPa

Annex 2. Biomarkers for HCV serology

Anti HCV	HCVcAg	HCV RNA	Interpretation
-	-	-	Never infected
-	+	+	Recent infection
+	+	+	Chronic infection
+	-	-	Previously infected Infection resolved or cured

[14] National Hepatitis Control Program. Ministry of Health and Sports. *Simplified Treatment Guidelines for hepatitis C infection.* Feb 2017.

Annex 3. Biomarkers for HBV serology

HBsAg	Anti-HBs	IgM Anti-HBc	(Total) Anti-HBc	HBeAg	Anti-HBe	HBV-DNA	Interpretation
-							**Uninfected**
-	-	**N/A**	-	**N/A**	**N/A**	**N/A**	**Never infected**
-	+	**N/A**	-	**N/A**	**N/A**	**N/A**	**Immunity due to vaccination**
-	+	**N/A**	+	**N/A**	**N/A**	**N/A**	**Immunity due to natural infection and recovery**
+	-	+	+				**Recent infection**
+	-	+	+	**N/A**	**N/A**	**N/A**	**Acutely infection**
+	-	-	+				**Chronic infection**
+	-	-	+	+	-	**High**	**Chronic infected, high level of viral replication**
+	-	-	+	-	+	**Low**	**Chronic infected, low level of viral replication**

Annex 4. Timeline and targets for hepatitis testing

Sr. No	Activity	2018	2019	2020	2030
1.	Identify suitable screening RDTs for HBsAg and HCV Ab testing	√			
2.	Training Microbiologists and Technologists	√	√	√	
3.	Vaccination of all healthcare workers	√	√	√	
4	Dissemination of SOPs and Algorithm	√			
5.	Initiate EQAS network		√		
6.	Establish Laboratory Information Management system		√		
7.	Produce biosafety guidelines, waste management & infection control manual	√			
8.	Accreditation of NHL		√		
9.	Liaise with Department of Medical Research on research and surveillance		√		
10.	Accreditation of all participating laboratories			√	
11	Expansion of network of laboratories				√

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