Primer Design Ltd

R00117

Human Immunodeficiency Virus Types 1 & 2 and Hepatitis Viruses B and C

Kit version: 3

Gag gene (HIV1)

5’UTR (HIV2)

S gene (HBV)

POLY gene (HCV)

genesig®PLEX kit

100 tests

For general laboratory and research use only

GENESIG

Kits by Primerdesign

Introduction

HIV1 & HIV2

Human immunodeficiency virus (commonly known as HIV, and formerly known as HTLV-III and lymphadenopathy-associated virus) is a retrovirus that is the cause of the disease known as AIDS (acquired immunodeficiency syndrome), a syndrome where the immune system begins to fail, leading to many life-threatening opportunistic infections.

HIV is transmitted through direct contact of a mucous membrane with a bodily fluid containing HIV, such as blood, semen, vaginal fluid, pre-seminal fluid or breast milk. This transmission can come in the form of penetrative (anal or vaginal) sex, oral sex, blood transfusion, contaminated needles and exchange between mother and infant during pregnancy, childbirth, or breastfeeding.

Since the start of the epidemic in 1981, AIDS has been responsible for the deaths of over 40 million people, making it one of the most devastating pandemics in recorded history. According to the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO), an estimated 1.3 million people were newly infected with HIV in 2022. Of the estimated 624,000 people who died of AIDS-related illnesses in 2022, approximately 16 per cent of them were under 20 years of age. An estimated 130,000 children aged 0-9 contracted HIV in 2022, bringing the total number of children in this age group living with HIV to 930,000. Almost 85% of these children reside in sub-Saharan Africa. Every week, 4,000 adolescent girls and young women aged 15–24 years became infected with HIV globally in 2022. Almost 80% of these infections occurred in sub-Saharan Africa. To reduce HIV-related mortality and morbidity among these highly vulnerable populations, early testing and treatment is essential.

Two species of HIV infect humans: HIV-1 and HIV-2. HIV-1 is hypothesized to have originated in southern Cameroon after jumping from wild chimpanzees (Pan troglodytes troglodytes) to humans during the twentieth century. HIV-2 is hypothesized to have originated from the Sooty Mangabey (Cercocebus atys), an Old World monkey of Guinea-Bissau, Gabon, and Cameroon. HIV-1 is more virulent, more easily transmitted and is the cause of the majority of HIV infections globally, while HIV-2 is less easily transmitted and is largely confined to West Africa.

HBV

The hepatitis B virus (HBV) is a member of the Hepadnavirus family. HBV infects the liver and can cause both acute and chronic disease including a high risk of death from cirrhosis or liver cancer. Acute symptoms can include jaundice, dark urine, fatigue, nausea, vomiting and stomach pain. The virus can be spread through close contact with infected bodily fluids such as saliva, blood, semen, and vaginal fluids, and can also be passed from a mother to a child during birth.

The HBV consists of a proteinaceous core particle and an outer lipid-based envelope with embedded proteins. The inner core contains the viral genome in the form of double

stranded DNA with single stranded regions, The envelope proteins are involved in viral binding and entry into susceptible cells. HBV is one of a few known non-retroviral viruses which employs reverse transcription as part of its replication process.

HBV has caused epidemics in parts of Asia and Africa, with around 70% of all cases occurring in Africa, and is recognised as endemic in China and other parts of Asia. Over one-third of the world's population has been or is actively infected with HBV.

HCV

Hepatitis C Virus (HCV) is a bloodborne virus primarily affecting the liver, causing both acute and chronic infections; transmission occurs through exposure to infected blood.

Symptoms of acute HCV infection can vary from mild to severe, including fever, fatigue, nausea, abdominal pain, jaundice (yellowing of the skin and eyes), dark urine, and clay-coloured stools. However, many infected individuals might not exhibit symptoms during the acute phase. If left untreated, most cases progress to chronic infection, often remaining asymptomatic for years or even decades. Over time, chronic HCV can lead to severe liver damage, cirrhosis, liver cancer, and liver failure.

Diagnosis of HCV involves blood tests to detect the virus and determine its genotype. These tests aid in planning appropriate treatment strategies. Fortunately, advancements in medicine have led to highly effective antiviral medications for Hepatitis C, taken to cure the infection, prevent further liver damage, and complications. The treatment duration and type typically depend on the virus genotype and the individual's overall health.

Globally, Hepatitis C represents a significant public health challenge, affecting millions of people. Chronic HCV infection is a leading cause of liver transplantation due to severe liver disease. However, with improved awareness, access to healthcare, and effective treatments, efforts aim to reduce the burden of this disease. If there's a suspicion of exposure or experiencing symptoms related to hepatitis, seeking prompt medical advice is crucial for proper evaluation, diagnosis, and treatment.

Specificity

The genesig®PLEX kit is designed for the in vitro detection of human immunodeficiency viruses 1 & 2 and human hepatitis viruses B and C.

Due to the complexity of HIV1 subtype variation, this assay is designed to detect subtypes A1, A2, A3, A6, B, C and AE. When reviewing this HIV1 kit, sequences from the previous three-year period were analysed from the Los Alamos National Laboratory database. Due to the sequence evolution observed in the HIV1 genome over time, this is considered to be an adequate time period for analysis.

The HIV2 assay within this kit is predicted to detect over 95% of sequences available on the Los Alamos National Laboratory database at the time of design.

The HBV and HCV assays within this kit are predicted to detect over 95% of sequences available on the NCBI database at the time of design.

The dynamics of genetic variation means that new sequence information may become available after the initial design. Primerdesign periodically reviews the detection profiles of our kits and when required releases new versions.

The HIV1/HIV2 assay is predicted to cross react with simian-human immunodeficiency virus and simian immunodeficiency virus (SIV) which would give signal in the FAM channel. The HBV assay is predicted to detect hepatitis B viruses that infect species other than humans which would give signal in the VIC channel.

If you require further information or have a specific question about the detection profile of this kit, then please send an email to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

- **Multiplex primer/probe mix (100 reactions BROWN)**
  FAM, VIC, ROX and Cy5 labelled (see table below)

Target	Fluorophore
HIV1/HIV2	FAM
HBV	VIC
HCV	ROX
Endogenous control	Cy5

- **Multiplex positive control template (RED)**
- **2x Lyophilised OneStep Master Mix (GOLD)**
- **2x oasig® resuspension buffer (BLUE)**
- **Template preparation buffer (YELLOW)**
  for resuspension of positive control template
- **RNase/DNase free water (WHITE)**
  for resuspension of primer/probe mix

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Extraction kit

This kit is recommended for use with genesig® EASY DNA/RNA Extraction kit. However, it is designed to work well with all processes that yield high quality DNA and RNA with minimal PCR inhibitors.

Pipettors and tips

Vortex and centrifuge

Thin walled 1.5ml tubes

qPCR plates

Kit storage and stability

This kit is stable at room temperature but should be stored at -20ºC on arrival. Once the lyophilised components have been resuspended they should not be exposed to temperatures above -20ºC for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances. Primerdesign does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and DNA/RNA integrity.

Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template.

Principles of the test

Real-time PCR
Individual primer and probes designed for each virus have been combined into a single reaction and these can be detected through the different fluorescent channels as described in the kit contents.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target cDNA. Fluorogenic probes are included in the same reaction mixture which consists of a DNA probe labelled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control
The kit positive control contains templates for the 3 target channels: HIV1 will give a signal in FAM, HBV will give a signal in VIC and HCV will give a signal in ROX. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting each virus are working properly in that particular run. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false positive results. This can be achieved by handling these components in a post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control
To confirm the absence of contamination, a negative control, or No Template Control (NTC) reaction should be included every time the kit is used. For this reaction, the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run.

Endogenous control
To confirm extraction of a valid biological template, the multiplex primer/probe mix supplied also contains primers and probe designed to detect an endogenous gene. Detection of the endogenous control is through the Cy5 channel. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

Resuspension protocol

To minimise the risk of contamination with foreign DNA/RNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the primer/probe mix in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component – resuspend in water	Volume
Pre-PCR pack	
Multiplex primer/probe mix (**BROWN**)	110µl

3. **Resuspend the positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component – resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
Positive control template (**RED**)*	500µl

*** This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.**

4. **Resuspend the lyophilised OneStep Master Mix in oasig resuspension buffer, according to the table below:**

Component – resuspend in oasig resuspension buffer	Volume
Lyophilised OneStep Master Mix (GOLD)	525µl

OneStep RT-qPCR detection protocol

For optimum performance and sensitivity
All pipetting steps and experimental plate set up should be performed on ice. After the plate is prepared, proceed immediately to the OneStep amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. **For each RNA/DNA sample prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

Component	Volume
Lyophilised OneStep Master Mix (GOLD)	10µl
Multiplex primer/probe mix (**BROWN**)	1µl
RNase/DNase free water (**WHITE**)	4µl
Final volume	**15µl**

2. **Pipette 15µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Pipette 5µl of RNA/DNA sample into each well according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

4. **Pipette 5µl of positive control template into each well according to your plate set up.**
   The positive control contains templates for HIV1, HBV and HCV. The final volume in each well is 20µl.

OneStep RT-qPCR amplification protocol

Amplification conditions using lyophilised OneStep Master Mix

	Step	Time	Temp
	Reverse transcription	10 mins	55°C
	Enzyme activation	2 mins	95°C
Cycling x 50	Denaturation	10 secs	95°C
	DATA COLLECTION*	60 secs	60°C

* Fluorogenic data should be collected during this step through the FAM, VIC, ROX and Cy5 channels.

Interpretation of results

Positive control
The positive control well should give an amplification plot through the FAM channel (HIV1/HIV2), the VIC channel (HBV) and the ROX channel (HCV). There is no endogenous control template within the positive control so the Cy5 channel should give no signal (flat amplification plot). The positive control signals indicate that the kit is working correctly to detect each virus.

No template control (NTC)
The NTC should give a flat line (flat amplification plots) through all channels. Signals in the NTC indicate cross contamination during plate set up.

Endogenous control
The signal obtained from the endogenous control reaction will vary according to the amount of biological material present in each sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.

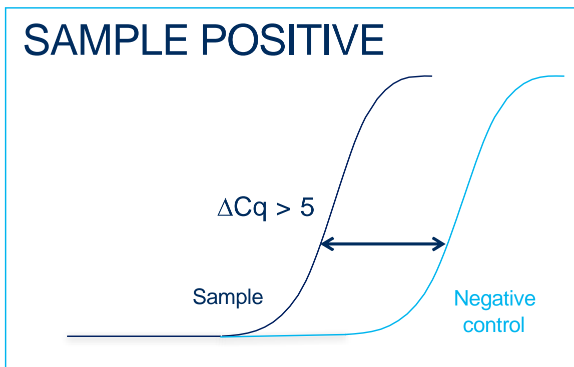
Sample data
Presence of the viruses are detected in the channels indicated in the kit contents section. Positive signals indicate positive tests for those viruses. It may be possible for samples to contain multiple viruses, therefore positive results in the FAM, VIC and ROX channels may be present.

Summary of data interpretation

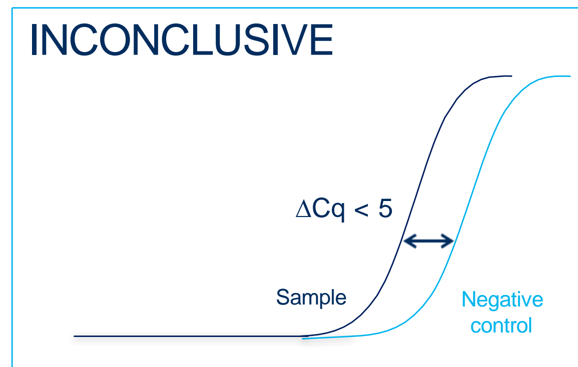
Target (FAM/VIC/ROX)	Endogenous control (Cy5)	Positive Control	Negative Control	Interpretation
FAM +	**+ / -**	**+**	**-**	**HIV1/HIV2 POSITIVE RESULT**
VIC +	**+ / -**	**+**	**-**	**HBV POSITIVE RESULT**
ROX +	**+ / -**	**+**	**-**	**HCV POSITIVE RESULT**
-	**+**	**+**	**-**	**NEGATIVE RESULT**
+ / -	**+ / -**	**+**	**≤35**	**EXPERIMENT FAILED** Due to test contamination
+ / -	**+ / -**	**+**	**>35**	*
-	**-**	**+**	**-**	**SAMPLE PREPARATION FAILED**
+ / -	**+ / -**	**-**	**+ / -**	**EXPERIMENT FAILED**

Positive control template (RED) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

* Where the test sample is positive, and the negative control is positive with a Cq >35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control then the positive sample result is invalidated and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated

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