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| **STANDARD OPERATING PROCEDURE for Nigeria PreP Study** |
| **Study Site:**  | **SOPs Number** :LP-305 |
| **Title****HIV -1 VIRAL LOAD ASSAY** |
| **Version Number**:  | **Version Date:**  | **Effective date**:  |
| **Approval name Signature Date**  |

**Annual Review**

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| **Review date**  | **Revision Date**  | **Signature** |
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**Document History**

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| **Version number**  | **Reason for change**  | **Date**  |
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**Distribution List**

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1. **Introduction**

Human Immunodeficiency Virus (HIV) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS). Quantitative measurements of HIV viremia in the peripheral blood have shown that higher virus levels may be correlated with increased risk of clinical progression of HIV disease and transmission of infection. Conversely, the reductions in plasma virus levels may be associated with decreased risk of clinical progression and decrease in transmission of infection. HIV RNA plasma can be quantified by nucleic acid amplification technologies, such as Polymerase Chain Reaction (PCR). The COBAS Ampliprep/COBAS TaqMan HIV-1 Test, v2.0 use PCR technology to achieve maximum sensitivity and dynamic range for the quantitative detection of HIV-1 RNA in EDTA or ACD anti-coagulated plasma.

1. **Objectives**

This standard operating procedure (SOP) describes the protocol for the HIV-1 viral load estimation in the Nigeria PreP Study.

1. **Responsibility**

The procedure cited is and will be performed by;

* All trained laboratory personnel on the viral load assay
* All laboratory personnel certified okay after the training exercise by the trainer and head of the lab.
1. **Abreviations:**
* PCR – Polymerase Chain Reaction
* RNA- Ribonucleic acid
* DNA- De-oxyribonucleic acid
* HIV- Human Immunodeficiency Virus
1. **Maintenance**
2. Daily cleaning of the safety cabinets, **Ampliprep and Taqman instrument**, microcentrifuges and pipettes before and after use.
3. Daily UV sterilization of the safety cabinets after use for 15 minutes.
4. **Procedural notes**
5. Ensure that you use the specified lab coats for each of the viral load labs. No interchanging or transfer of lab coats between labs. The following are the specified lab coats for the various labs;
	1. Red label lab coats for specimen prep lab/ reagent lab
	2. Yellow label lab coats for PCR lab
6. Cross check separated plasma from the personnel on the separation bench with the separation register and photocopies of the sample register at the reception. Verify samples.
7. Duplicate samples for long term storage are also stored in storage boxes and the sample ID entered into the storage form as they are in the storage box. The storage form and storage box have same identification number.
8. Store samples to be run in the designated freezer (-20°C) and duplicate samples for long storage in the ultra-low freezer(s) (-80°C).
9. Samples for assaying are selected on a first come, first serve basis as arranged in the sample box.
10. Bench forms are always used for each run and the result print-outs (OD readings) duly signed by personnel performing the technical verification of the assay.
11. Kits are *always*handled with gloves and not with bare hands (because of RNase on the hand which could destroy the viral RNA to be extracted).
12. **The COBAS AmpliPrep/COBAS TaqMan HIV-1 Test v2.0**

**Reagents**

1. COBAS Ampliprep/COBAS TaqMan HIV-1 Test, v2.0

HIV-1 CS1 (HIV Magnetic Glass Particles Reagent Cassette), HIV-1 CS2 (HIV Lysis Reagent Cassette), HIV-1 CS3 (HIV Multi-Reagent Cassette), HIV-1 CS4 (HIV Test-Specific Reagent Cassette), HIV-1 H (+) C, v2.0 (HIV High Positive Control), HIV-1 L(+)C, v2.0 (HIV Low Positive Control), CTM (-) C (COBAS TaqMan Negative Control (Human Plasma)), HIV-1 H (+) C, v2.0 Clip (HIV High Positive Control Barcode Clip), HIV-1 L (+) C, v2.0 Clip (HIV Low Positive Control Barcode Clip), HIV-1 (-) C, v2.0 Clip (HIV Negative Control Barcode Clip).

B. COBAS AmpliPrep/COBAS TaqMan Wash Reagent

PG WR (COBAS AmpliPrep/COBAS TaqMan Wash Reagent).

**Stability of reagents**

1. Do not freeze reagents or controls
2. Store HIV-1 CS1, HIV-1 CS2, HIV-1 CS3 and HIV-1 CS4 at 2-8ºC. Unused, these reagents are stable until the expiration date indicated. Once used, these reagents are stable for 28 days at 2-8ºC or until the expiration date, whichever comes first. HIV-1 CS1, HIV-1 CS2, HIV-1 CS3 and HIV-1 CS4 can be used for up to a maximum of 4 instrument cycles64 hours cumulative on board the COBAS AmpliPrep Instrument. Reagents must be stored at 2-8ºC between instrument cycles.
3. Store HIV-1 H (+) C, v2.0; HIV-1 L (+) C, v2.0 and CTM (-) C at 2-8ºC. The controls are stable until the expiration date indicated. Once opened, any unused portion must be discarded.
4. Store Barcode clips [HIV-1 H (+) C, v2.0 Clip, HIV-1 L (+) C, v2.0 Clip and HIV-1 (-) C Clip] at 2-30ºC.
5. Store PG WR at 2-30ºC. PG WR is stable until the expiration date indicated. Once opened, this reagent is stable for 28 days at 2-30ºC or until the expiration date, whichever comes first.

**Instrumentation and Software**

COBAS AmpliPrep Instrument, COBAS TaqMan 48 Analyzer, AMPLILINK Software – revision 3.7 series, Pipettors (capacity 1000µL) with aerosol barrier tips, Computer system (Data Station) for the AMPLILINK Software, with printer, Vortex Mixer, Class 11 bio-safety cabinet, Inverter/UPS.

**Supplies**

Reagent Racks, Sample Racks, Rack of K-tips, SPU rack, K-carrier rack, K-tube capper, K-tube capper motorized, K-carrier Transporter, SPUs, Biohazard bags, discard jar, Disposable gloves (powder less), K-tube Box, Sample input tubes (S-tubes) with barcode clips

**Sample**

Sample Type: Plasma specimens only derived from blood collected using EDTA as anticoagulant is only used for this assay.

Amount Required: Minimum of 1050µL of plasma.

**Sample retention: Samples assayed are discarded and the long storage samples are stored at -20°C for 6 months and at -70°C for as long as possible.**

Transport, Storage and stability: Whole blood must be transported at 2-25°C and processed within 24 hours of collection. Plasma may be transported at 2-8°C or frozen -20°C to -8°C. Plasma may be stored at room temperature 25-30°C for up to 1 day, 2-8°C for up to 6 days or frozen at -20°C or -80°C for upto six weeks and thawed up to 3 times.

1. **Special safety precautions**
2. Specimens and controls should be handled as if infectious using safe laboratory procedures.
3. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.
4. HIV-1 QS CAP/CTM Mn2+ and HIV-1 MMX contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide buidup.
5. Wear laboratory coats and disposable gloves and work in the safety cabinet when handling any reagent. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills of these reagents occur, dilute with water before wiping dry.
6. Do not allow HIV-1 CS2 and liquid waste from the COBAS Ampliprep Instrument, which contain guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. These mixtures can produce a highly toxic gas.
7. When disposing of used COBAS Ampliprep SPUs, which contain guanidine thiocyanate, avoid contact with sodium hypochlorite (bleach) solution. These mixtures can produce a highly toxic gas.
8. **Quality control**

One COBAS TaqMan Negative Control, one HIV-1 Low Positive Control, v2.0 and one HIV-1 High Positive Control, v2.0 must be included in each test batch. The batch is valid if no flags appear for any of the controls [HIV-1 L (+) C, v2.0; HIV-1 H (+) C, v2.0 and CTM (-) C].

There are no requirements regarding the position of the controls on the sample rack. Check the batch printout for flags and comments to ensure that the batch is valid.

LJ charts are plotted using the low and high positive controls after every run on the PCR lab Computer, before results are entered and sent out.Charts are printed at the end of the month and pasted on the wall.

**Lot-t-lot Verification**

A recently known high and low positive samples are assayed with each new lot of kits.It is expected that the results are concordant and this is documented.

Negative Control

The CTM (-) C must yield a “Target Not detected” result. If the CTM (-) is flagged as invalid, then the entire batch is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If CTM (-) C is consistently invalid in multiple batches, contact your local Roche office for technical assistance.

Positive Controls

The assigned titer ranges for HIV-1 L (+) C, v2.0 and HIV-1 H (+) C, v2.0 is specific for each lot of reagents and are provided on the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test v2.0 reagent cassette barcodes.

The HIV-1 RNA IU/mL for HIV-1 L (+) C, v2.0 and HIV-1 H (+) C, v2.0 should fall within their assigned titer ranges. If one or both of the positive controls are flagged as invalid, then the entire batch is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If the HIV-1 RNA titer of one or both of the positive controls is consistently outside the assigned ranges in multiple batches, contact your local Roche office for technical assistance.

1. **Procedure**

Instructions for use

Note: For detailed operating instructions, a detailed description of the possible configurations, printing results and interpreting flags, comments and error messages, refer to the COBAS TaqMan 48 analyzer Instrument Manual for use the AMPLILINK software version 3.3 Series Application Manual, or the AMPLILINK software Version 3.3 Series Application Manual for use with COBAS AmpliPrep instrument, COBAS TaqMan analyzer, COBAS TaqMan 48 analyzer,

Batch Size: Each kit contains reagents sufficient for 48 tests, which may be performed in batches of 12 to 24 tests. At least one of each control (CTM (-) C, HIV-1 L (+)C and HIV-1 H(+)C, v2.0) must be included in each batch (see “Quality Control” section).

Workflow: The COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation.

Note: Do not freeze or store processed specimens and controls at 2-8ºC.

Specimen and Control Preparation

Note: If using frozen specimens, place the specimens at room temperature (15-30ºC) until completely thawed and vortex for10-15 seconds before use. Controls should be removed from 2-8ºC storage and equilibrated to room temperature before use.

COBAS AmpliPrep Instrument Set-up

Part A. Maintenance and Priming

A1. The COBAS AmpliPrep Instrument is ready for operation in stand-by mode.

A2. Turn the Data Station for the AMPLILINK software ON. Prepare the data Station as follows:

1. Log onto the Windows XP operating system
2. Double click the AMPLILINK software icon
3. Log onto AMPLILINK software by entering the assigned User ID and password

A3. Check the supply of PG WR using the Status Screen and replace if necessary.

A4. Perform all Maintenance that is listed in the Due Tab on the Maintenance section of the Status Screen. The COBAS AmpliPrep Instrument will automatically prime the system.

Part B. Loading of Reagent Cassettes

Note: All reagent cassettes should be removed from 2-8ºC storage, immediately loaded onto the COBAS AmpliPrep Instrument and allowed to equilibrate to ambient temperature on the instrument for at least 30 minutes before the first specimen is to be processed. Do not let reagent cassettes come to ambient temperature outside the instrument as condensation may form on the barcode labels. Do not wipe off condensation if it appears on the barcode label.

B1. Place HIV-1 CS1 onto a reagent rack. Place HIV-1 CS2, HIV-1 CS3 and HIV-1 CS4 onto a separate reagent rack

B2 Load the reagent rack containing HIV-1 CS1 onto rack position A of the COBAS AmpliPrep Instrument

B3. Load the reagent rack containing HIV-1 CS2, HIV-1 CS3 and HIV-1 CS4 onto rack position B, C, D or E of the COBAS AmpliPrep Instrument.

Part C. Loading of Disposables

Note: Determine the number of COBAS AmpliPrep reagent cassettes, Sample Processing Units (SPUs), Input Sample tubes (s-tubes), K-tips and K-tubes needed. One SPU, one Input S-tube, one K-tip and one K-tube are needed for each specimen or control.

Multiple workflows for use of the COBAS AmpliPrep Instrument with the COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer are possible. Depending on the workflow used, load the appropriate number of reagent cassette racks, sample racks with Input S-tubes, SPU racks, K-tip racks, K-tube racks and K-carriers on K-carrier racks onto the respective rack positions of the COBAS AmpliPrep Instrument.

C1. Place the SPUs in the SPU rack(s) and load the rack(s) onto rack position J, K, or L of the COBAS AmpliPrep Instrument.

C2. Depending on the workflow used, load full K-tube rack(s) onto rack position M, N, O or P of the COBAS AmpliPrep Instrument.

C3. Load full K-tip rack(s) onto rack position M, N, O or P of the COBAS AmpliPrep Instrument.

C4. For workflow 3 using the COBAS TaqMan 48 Analyzer, load K-carriers on K-carrier rack(s) onto rack position M & N, or O & P of the COBAS AmpliPrep Instrument.

Part D. Ordering and Loading of Specimens

D1. Prepare sample racks as follows: Attach a barcode label clip to each sample rack position where a specimen (S-tube) is to be placed. Attach one of the specific barcode label clips for the controls [CTM(-) C, HIV-1 L(+)C, v2.0 and HIV-1 H(+)C, v2.0] to each sample rack position where the controls (S-tube) are to be placed. The barcode label clips for controls should have the same control lot number as the lot number on the control vials in the kit. Take care in assigning the right control to the position with the appropriate control barcode clip. Place one Input S-tube into each position containing a barcode label clip.

D2. Write the sample rack order on the HIV-1 viral load bench form.

D3. Using the AMPLILINK software, create specimen orders for each specimen and control in the orders window sample folder. Select the appropriate test file and complete by saving.

D4. Assign specimen and control orders to sample rack positions in the Orders window

Sample Rack folder. The sample rack number must be for the rack prepared in step D1.

D5. Print the Sample Rack Order report to use as a worksheet.

D6. Prepare specimen and control racks in the designated area for specimen and control

addition as follows: Vortex each specimen and control [CTM (-) C, HIV-1 L (+) C, v2.0

and HIV-1 H (+) C, v2.0] for 10-15 seconds. Avoid contaminating gloves when

manipulating the specimens and controls.

D7. Transfer 1000µL of each specimen and control [CTM (-) C, HIV-1 L(+)C, v2.0 and

HIV-1 H(+)C, v2.0 to the appropriate barcode labeled Input S-tube using a micropipettor

with an aerosol barrier or R Nase-free tip. Avoid transferring particulates and/or fibrin clots from the original specimen to the Input S-tube. Specimens and controls should be transferred to tube positions as assigned and recorded on the worksheet in step D2. The barcode label clips for controls should have the same control lot number as the lot number on the control vials in the kit. Assign the right control to the position with the appropriate control barcode clip. Avoid contaminating the upper part of the S-tubes with specimens or controls.

D8. For workflow 3 using the COBAS TaqMan 48 Analyzer, load sample rack(s) with

Input S-tubes and K-tubes (one for each Input S-tube, loaded in the right position adjacent to Input S-tubes) onto rack position F, G or H of the COBAS AmpliPrep Instrument.

Part E. Start of COBAS AmpliPrep Instrument Run

E1. Start the COBAS AmpliPrep Instrument using the AMPLILINK software.

Part F. End of COBAS AmpliPrep Instrument Run and Transfer to COBAS TaqMan

48 Analyzer

F1. Check for flags or error messages.

F2. Remove processed specimens and controls from the COBAS AmpliPrep Instrument on K-carrier racks (for COBAS TaqMan 48 Analyzer), depending on the workflow.

F3. Remove waste from the COBAS AmpliPrep Instrument

Note: All processed specimens and controls should not be exposed to light after completion of specimen and control preparation.

Amplication and Detection

COBAS TaqMan 48 Analyzer Set-up

The COBAS TaqMan 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation.

Note: Do not freeze or store processed specimens and controls at 2-80C

Part G. Loading Processed Specimens

G1. Depending on the workflow, perform the appropriate steps to transfer the K-tubes to

the COBAS TaqMan 48 Analyzer.

Workflow 3: Manual transfer of K-carrier on K-carrier rack(s) to the COBAS TaqMan 48 Analyzer. Manual transfer of K-carriers into COBAS TaqMan 48 Analyzer using the K-carrier Transporter.

Part H. Start of COBAS TaqMan 48 Analyzer Run

H1. Start the COBAS TaqMan 48 Analyzer by one of the options below depending on

the workflow used.

Workflow 3: Fill K-carrier with empty K-tubes if there are fewer than 6 K-tubes on the K-carrier. Filling is guided by the AMPLILINK software. Open thermal cycler cover, load K carrier into thermal cycler and close lid. Start the COBAS TaqMan 48 Analyzer run.

Part I. End of COBAS TaqMan 48 Analyzer Run

I1. At the completion of the COBAS TaqMan 48 Analyzer run, print Results Report.

Check for flags or error messages in the Result report. Specimens with flags and

comments are interpreted as described in the Results section. After acceptance, store

data in archive.

I2. Remove used K-tubes from the COBAS Taqman 48 Analyzer.

1. **Results**

The COBAS TaqMan 48 Analyzer automatically determines the HIV-1 RNA concentration for the specimens and controls. The HIV-1 RNA concentration is expressed in copies/mL. The conversion factor between HIV-1 RNA copies/mL and HIV-1 RNA IU/mL is 0.6 copies/IU, using the WHO 1st International Standard for HIV-1 RNA for Nucleic Acid Technology (NAT) Assays Testing (NIBSC 97/746).

 AMPLILINK Software

* Determines the Cycle Threshold value (Ct) for the HIV-1 RNA and the HIV QS RNA
* Determines the HIV-1 RNA concentration based upon the Ct values for the HIV-1 RNA and the HIV-IQS RNA and the lot-specific calibration coefficients provided on the cassette barcodes.
* Determines that the calculated CP/mL for HIV-1 L(+)C, v2.0 and HIV-1 H(+)C, v2.0 fall within he fixed ranges

 Batch Validation – AMPLILINK Version 3.3 Series

Check AMPLILINK software results window or printout for flags and comments to ensure that the batch is valid. For control orders, a check is made to determine the CP/mL value for the control is within its specified range. If the CP/mL value for the control lies outside of its range, a FLAG is generated to show the control has failed.

The batch is valid if no flags appear for any of the controls [HIV-1 L (+) C, v2.0 and CTM (-) C].

The batch is not valid if any of the following flags appear for the HIV-1 Controls:

**Negative Control:**

|  |  |  |
| --- | --- | --- |
| **Flag** | **Result** | **Interpretation** |
| NC\_INVALID | Invalid | An invalid result or a “valid” result that was not negative for HBV target |

**HIV-1 Low Positive Control, v2.0:**

|  |  |  |
| --- | --- | --- |
| **Flag** | **Result** | **Interpretation** |
| LPCINVALID | Invalid | An invalid result or a control out of range |

**HIV-1 High Positive Control, v2.0:**

|  |  |  |
| --- | --- | --- |
| **Flag** | **Result** | **Interpretation** |
| HPCINVALID | Invalid | An invalid result or a control out of range |

If the batch is invalid, repeat the entire batch including specimen and control preparation, amplification and detection.

**Interpretation of Results**

For a valid batch, check each individual specimen for flags or comments on the results printout. Interpret the results as follows:

* A valid batch may include both valid and invalid specimen results depending on whether flags and/or comments are obtained for the individual specimens.

**Specimen results are interpreted as follows:**

|  |  |
| --- | --- |
| **Titer Result** | **Interpretation** |
| Target Not Detected | Ct value for HIV-1 above the limit for the assay or no Ct value for HIV-1 obtained. Report results as “HIV-1 RNA not detected”. |
| <2.00E+01 cp/mL | Calculated CP/mL are below the Limit of Detection of the assay. Report results as “HIV-1 RNA detected, less than 20 HIV-1 RNA cp/mL’’. |
| ≥2.00E+01 cp/mLand≤1.00E+07 cp/mL | Calculated results greater than or equal to 20 CP/mL and less than or equal to 1.00E+07CP/mL are within the Linear Range of the assay. |
| >1.00E+07 cp/mL | Calculated CP/mL are above the range of the assay. Report results as “greater than 1.00E+07 HIV-1 RNA cp/mL”. If quantitative results are desired, the original specimen should be diluted 1:100 with HIV-1-negative human EDTA plasma and the test repeated. Multiply the reported result by the dilution factor. |

* Note: The analytical measurement range of analyte values that can be directly measured on a specimen without any dilution using the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 is 20 to 1.0E+07 cp/mL.
* Note: Specimens above the range of the assay that produce an Invalid result with a flag “QS\_INVALID” should not be reported as >1.00E+07 cp/mL. The original specimen should be diluted with HIV-1-negative human EDTA-plasma and the test repeated. Multiply the reported result by the dilution factor.
* Note: Titer Result “Failed”. Interpretation: Specimen is not correctly processed during specimen preparation on the COBAS AmpliPrep Instrument.
* Note: Titer Result “Invalid”. Interpretation: An Invalid Result.
1. **Procedural precautions**

As with any test procedure, good laboratory technique is essential to the proper performance of this assay.

1. **Procedural limitations**
2. This test has been validated for use with only human plasma collected in EDTA anticoagulant. Testing of other specimen types may result in inaccurate results.
3. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.
4. The presence of AmpErase enzyme in the COBAS AmpliPrep/COBAS TaqMan HIV-1 Master Mix, v2.0 reduces the risk of amplicon contamination. However, contamination from HIV-1 positive controls and clinical specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in this package insert.
5. Use of this product should be limited to personnel trained in the techniques of PCR
6. This product can only be used with the COBAS AmpliPrep Instrument and the COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer.
7. Though rare, mutations with the highly conserved region of the viral genome covered by the COBAS AmpliPrep /COBAS TaqMan HIV-1 Test, v2.0 primers and/or probe may result in the under-quantitation of or failure to detect the virus.
8. Detection of HIV-1 RNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods and patient factors (i.e. age, presence of symptoms, and/or stage of the infection.
9. **Interfering substances**

Elevated levels of triglycerides(up to 3500mg/dL), bilirubin(up to 28mg/dL), albumin(up to 8900mg/dL), hemoglobin(up to 900mg/dL) and human DNA(up to 0.4mg/dL) in specimens as well as the presence of autoimmune diseases or respective markers such as Systemic Lupus Erythematous(SLE), Rheumatoid Arthritis (RA) and Antinuclear Antibody (ANA) were shown not to interfere with the quantitation of HIV-1 RNA or impact the specificity of the COBAS AmpliPrep /COBAS TaqMan HIV-1 Test v2.0.The evaluation was performed according to CLSI guideline EP7-A2 using one lot COBAS AmpliPrep /COBAS TaqMan HIV-1 Test v2.0. reagents.

The following drug compounds tested at 3 times of the Peak Plasma Level(Cmax) have been shown not to interfere with the quantitation of HIV-1 RNA or impact the specificity of the COBAS AmpliPrep /COBAS TaqMan HIV-1 Test v2.0.

**References**

Refer to kit insert in COBAS AmpliPrep/COBAS TaqMan HIV-1 Test version 2.0 manuals.

**This SOP has been read and understood by:**

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