

## Original article

# Enhancing Domestic Molecular Biology for Viral Hepatitis in Cameroon

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## ABSTRACT

**Background:** Molecular biology (MB) is an essential tool for the diagnosis and evaluation of treatment efficacy against viral hepatitis. This tool was not available in Cameroon for viral hepatitis B and C, until recently. Blood samples to be examined were systematically sent abroad via the Centre Pasteur du Cameroun (CPC).

**Purpose:** to assess the accuracy of the results from a domestic new laboratory of MB compared to those from a laboratory abroad.

**Methods:** We included in the study, 15 subjects randomly selected from the queue of patients followed at viral hepatitis clinic of the Yaoundé University Hospital Center. They were all carriers of chronic hepatitis C. Two blood samples of the same volume were sent the CPC and to a new MB laboratory at the Chantal Biya International Research Center (CIRCB). Neither the CPC nor the CIRCB were aware of the study. One of the two tests was free for all patients. To be acceptable to the result from the CIRCB should be the same from the outside or with than 12 IU / ml above.

**Results:** We did not observe any significant differences from the results issued by the laboratories.

**Conclusion:** These first results from a domestic MB laboratory are encouraging. One should promote Molecular biology should hence be promoted.

**Key words:** Molecular biology; viral hepatitis; Chantal Biya international reference center.

## INTRODUCTION

Viral hepatitis C is a worldwide public health problem. It touches up to 3% of the general population (1). There is meanwhile differences in the magnitude of the problem in between countries. As a matter of fact, WHO classifies the world from very high, to low endemic zones, according to the average prevalence of each viral hepatitis. Cameroon is among the high and very high endemic zones for viral hepatitis B (VHB) and viral hepatitis C (VHC) respectively. Hepatitis B or C can lead to chronic liver diseases namely, cirrhosis and hepato cellular carcinoma. (2) An essential tool for the diagnosis, treatment and follow up of chronic hepatitis, is molecular biology (M B). An accurate and reliable quantitative assay for hepatitis C virus (HCV) and hepatitis B virus (HBV) is therefore essential for the

## RESUME

**Introduction :** La biologie moléculaire (BM) est un outil essentiel pour le diagnostic et l'évaluation de l'efficacité des traitements contre les hépatites virales. Cet outil n'était pas disponible au Cameroun pour les hépatites virales B et C, jusqu'à un passé récent. Les échantillons de sang à examiner étaient systématiquement envoyés à l'étranger via le Centre Pasteur du Cameroun.

**Objectif :** Juger la véracité des résultats obtenus localement dans un nouveau laboratoire de BM en le comparant à ceux qui proviennent d'un laboratoire à l'étranger.

**Patients et méthodes :** Nous avons inclus dans l'étude, 15 patients suivis au Centre Hospitalier et Universitaire de Yaoundé pour hépatite chronique. Deux prélèvements de sang de même volume ont été envoyés Au Centre Pasteur du Cameroun (CPC) et au tout nouveau laboratoire de BM du Centre international de recherche Chantal Biya (CIRCB). Pour être jugé acceptable le résultat issu du CIRCB devait être identique à celui provenant de l'étranger ou supérieurs de 12 u i/ml.

**Résultats :** Des résultats obtenus des deux laboratoires, nous n'avons pas observé de différence significative.

**Conclusion :** Ces premiers résultats issus d'un laboratoire local de BM sont encourageants. L'on devrait promouvoir la biologie moléculaire pour ce qui concerne hépatites virales.

**Mots clés :** Biologie moléculaire ; hépatites virale ; Centre International de référence Chantal Biya.

diagnosis and the assessment of antiviral therapeutic efficacy. Up to a recent time, M B was not available for hepatitis B and C in Cameroon, and the blood samples of the patients were systematically sent to a specialized laboratory in France. This situation, made the time too long to have the results, and the exams, too expensive, for most patients. We conducted this study with the aim of assessing the veracity of results from a domestic M B laboratory, namely the Chantal Biya International Research Center (CIRCB) which has recently acquired an M B set for VHC.

## PATIENTS AND METHODS

### A. Patients selection:

We randomly selected 15 patients from the viral hepatitis clinic of the University Hospital Center in Yaoundé. They were 9 females and 6 males divided

into 8 naïve patients and 7 others under treatment. The patients were then sent the same day, for blood sampling at a reference biology center, namely the "Centre Pasteur du Cameroun" (CPC), which subcontracts for another reference laboratory in France, and at the CIRCB. The same volume of blood sample was taken for CPC and for CBIRC. Neither the CPC, nor the CBIRC knew about the study, but patients were aware, and one of the two exams, was free of charge for each patient.

#### **B. Biological sampling procedure at the CIRCB**

At the CIRCB, Abbott Real Time HCV assay was used. The Real time HCV assay (Abbott Molecular) was performed according to the manufacturer's specifications.

In the first step, total nucleic acid was extracted from 0.5 ml serum or plasma by magnetic micro beads technology, using the Abbott Sample Preparation System, followed by manual reaction assembly. The internal control (IC), derived from the hydroxyl pyruvate reductase gene of the pumpkin plant, *Cucurbita pepo*, was introduced as armored RNA into the sample lysis buffer. The IC was processed simultaneously with each sample. RNA was captured by magnetic micro beads; the micro beads were washed to remove unbound sample components. Bound RNA was eluted.

The second step consisted of adding 50µl of master mix (HCV oligonucleotide reagent including primers and probes, thermostable rTth polymerase enzyme, and activation reagent) into a 96-well optical reaction plate, to which 50µl of the extracted nucleic acid sample were added. The Plate was sealed and placed on an Abbott m2000rt instrument (m2000rt; Abbott Molecular Inc) for reverse transcription, PCR amplification, and detection/quantification.

The HCV probe is a short linear oligonucleotide labeled with a fluorophore at the 5' end and a quencher at the 3' end. The PCR primers and probe target conserved regions of the 5' untranslated region of the HCV genome. In the absence of an HCV target, the fluorescence is quenched. In the presence of the HCV target sequence, the HCV probe preferentially hybridizes to the target sequence, allowing fluorescence detection. A different set of primers and probe labeled with a different fluorophore are used for amplification and detection of the internal control. The IC probe is a single-stranded DNA oligonucleotide with a fluorophore at the 5' end and a quencher at the 3' end. IC probe fluorescence is quenched in the absence of IC target sequences. In the presence of IC target sequences, IC probe hybridization to complementary sequences, separate the fluorophore and quencher and allow fluorescence emission and detection. The noncompetitive internal control is detected at all HCV levels.

The HCV-and IC- specific probes are each labeled with a different fluorophore, thus allowing for

simultaneous detection of both amplified products at each cycle. The amplification cycle at which a reactive level of fluorescent signal is detected by the m2000rt instrument is proportional to the log of the HCV RNA concentration present in the original sample. The homogeneous format and sealed PCR tray eliminate the risk of contamination by amplified products.

#### **C. Results consideration**

As we were comparing the CIRCB to an already established M B laboratory, and taking into account the threshold of detection of 12 IU/mL, we set that an acceptable result from CIRCB should be similar to CPC result or + 12 IU/mL. We compared the results issued by the two M B laboratories on the rapid virologic response (RVR) or early virologic response (EVR) depending on the case. The predictive value of RVR was partial (pRVR) if there was a decline  $\geq 2 \log_{10}$ . It was considered complete (cRVR) if HCV-RNA was undetectable. Early virologic response, partial or complete (pEVR/cEVR) was to be evaluated.

## **RESULTS**

We selected 15 patients (6 males and 9 females) aged 59 to 65 years. Out of the 15 patients, 8 were naïve of any treatment, whereas 7 were treated. Patients were bearers of genotype 1, 2 and 4 (Table I).

**Table I:** patient's characteristics.

Characteristics	Value
Patients age (years)	
Range	49-65
Mean $\pm$ SD	54.6 $\pm$ 5.2
Gender	
Male	6
Female	9
HCV genotypes	
1	4
2	5
4	6
Treatment status	
Naïve	8
Treated	7
Fibrosis evaluation (Metavir)	
F2	11
F3	4

Mean viral load in the 8 naïves patients was 5113355 IU/mL from CIRCB and 5113354 IU/mL from CPC.

Amongst the 7 treated patients, 4 had no viral RNA detected, meaning a cRVR. This result was identical in the two laboratories settings.

Three 3 other patients had positive results, with a mean of 1 442 321 IU/mL against 1442317 from CPC. We registered a pRVR for patient N°10; 4 cRVR (patients N°11; 13; 14; 15).

Patient N°9 was a non responder, and patient N°12 was a relapse.

## DISCUSSION

We have presented results from a domestic M B laboratory, comparing them to those of an established one. The purpose of this study was to assess the veracity of a newly established HCV diagnostic assay in a country where it is really in needed. We found no significant difference in the results from the two laboratories.

CIRCB has used The Abbott Real Time HCV assay. This assay meets the second WHO International Standard for HCV RNA .The lower limit to detection is 12 IU/ml, with more than 95% probability. It detects and quantifies genotypes 1 to 6. The efficacy of Abbott has been reported as suitable for use in routine diagnostic with a good time to result (3). It has a high sensitivity and a wide dynamic range. The

worry in a newly set laboratory might be the strict respect of usage recommendation from the provider. It is by the way, known that in VHC, the viremia can be better quantified by assessment of HCV core Ag level. (4, 5, 6)

Compared to other assays Abbott real time HCV has proven its reliability (7) and even in multy center evaluation including specimen from Europe, Asia and United States of America (8). But the main problem might be the availability of reagents and the affordability for the patients, with limited resources in sub-Saharan Africa. There is, by the way, a need for capacity building in the field of molecular biology, as it appears to be the corner stone for diagnosis and follow up of many viral diseases.

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