

Medical Science

The International Weekly Journal for Medicine

Cobas Amplicor Conventional PCR as a Routine Diagnostic tool for Quantification of HBV-DNA, HCV & HIV-RNA

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Publication History

Received: 17 November 2013

Accepted: 28 January 2014

Published: 19 February 2014

Citation

Ismail Mahmud Ali, Amirthalingam R. Cobas Amplicor Conventional PCR as a Routine Diagnostic Tool for Quantification of HBV-DNA, HCV & HIV-RNA. *Medical Science*, 2014, 5(17), 60-67

ABSTRACT

Liver cell carcinoma and liver failure are caused by both of viruses of hepatitis B and C. Acquired Immunodeficiency Syndrome (AIDS) is purposed by infection of human immunodeficiency virus (HIV). The aim of the study was to quantification of HBV DNA, HCV and HIV RNA titer in infected patients using Cobas Amplicor analyzer with monitor test methods for antiviral treatment and analyzed its disadvantages with current updated technology. Total of 130 for HBsAg positive, 72 for anti HCV positive, and 8 for anti HIV positive patients were preferred from serology division of laboratory. There are 130 HBV DNA, 72 HCV RNA, and 8 HIV RNA were extracted from positive specimens with test specific control and incorporated in this study. The viral load titer was analyzed individually by Cobas Amplicor analyzer. Out of 130 HbsAg positive cases, 121 cases of HBV DNA quantification were obtained with different ranges ($60-10^6$) and 9 cases were not detected because of lower limit of detection. All positive cases of anti HCV were confirmed as positive and HCV RNA measurement were obtained with different ranges ($600-10^6$). One case was not quantified out of eight anti HIV positive patients. This mean HIV RNA not detected or less than 50 copies/ml or it might be improper screening from serology division. The HIV RNA viral measurement was achieved with different ranges ($50-10^5$). The detection ranges might be compared with others updated and published technical data for future direction. Present updated molecular assays like Cobas Taqman and Abbott having greater sensitivity and accuracy than old version of Cobas Amplicor. Also, not possibility to get complete clearance of viral copy in antiviral treatment through Cobas Amplicor, because of this assay has minimum detection ranges 600 IU/ml for HCV, 62 IU/ml for HBV and 400 IU/ml for HIV (standard methods) viral load.

Key word: RT-PCR Viral load quantification, hepatitis B&C, HIV

Abbreviation: RT-PCR-Real Time Polymerase Chain Reaction, Human Immuno-deficiency virus

1. INTRODUCTION

Viral infectious diseases are common worldwide especially HBV, HCV and HIV. These viruses are causing million of death in every year. Among these viruses, the hepatitis B virus is a 42-nm element, double stranded DNA (3.2kb) virus of the Hepadnaviridae family. This virus is infecting merely in human liver organ and injuring the hepatocells foremost to liver cirrhosis and hepatocellular carcinoma (HCC) (MMWR, 2008; Fattovich et al. 2003; Perz et al. 2006). At present, 5-10% of cases of liver transplantation and more than 1 million people death yearly (Hadziyannis et al. 2006; Ganem et al. 2004). There are two billion people suffering with HBV infection globally and top 10 viral infectious diseases killer among viral disease. It is replicating with others viruses like hepatitis C, hepatitis delta virus (HDV) and human immune deficiency virus as a co infection with presence of other multi factors can change the normal path of HBV infection as well as effectiveness of antiviral advances (Fattovich et al. 2004). There are eight genotypes (A-H) have been identified up-to-date and distinct by genetics segregation and dispersed all over the world (Norder et al. 1993). The sera marker of antigens and antibodies associated with HBV infection including HBsAg, HBeAg, and HBeAg. The viral load analysis can be performed to assess the titer of circulating HBV DNA within 10-20 days before detection of hepatitis B surface antigen (Biswas et al. 2003).

Hepatitis C virus has a single strand RNA virus in the family of Flaviviridae contains near 9400 nucleotide in its genomic sequence. There are six main genotypes have been isolated till now and each genotypes are further divided into subtypes (1a,1b,2a,2b,3a,3b,3c,4a,4b,4c,4d,5a,& 6a) based on mutation of nucleotide sequence (Halfon et al. 2005). Hepatitis C virus is occurring across the world with some countries having chronic infection rates more than 14.7% generally in Egypt (Khattab et al. 2011). Even though each year, 2.3–4.7 million people are chronically infected with the hepatitis C virus. There are 130 to 170 million people at possibility of emerging liver cirrhosis and liver cancer all over the world after outbreak of infection. Every year, more than 350 thousand people die from hepatitis C-related liver diseases (Laraba et al. 2010). The endurance of HCV infected healthy carriers showing patiently normal serum alanine transaminase (ALT) values and somewhat alteration in liver histology has been reported and also skillfully to distinguish the situation of hepatocellular carcinoma is significant therapeutic concern (Albadalejo et al. 1998). The HCV 3rd generation immune assay and quantification of RNA are used to confirm the diagnosis pathology condition and antiviral therapy management (Bimpson et al. 2006).

Another world top most human killer virus among infectious diseases is human immune deficiency virus (HIV) is a member of the genus lentivirus, branch of retroviridae family contains 9200 nucleotide bases in its genome (International Committee, 2006). It has been originated from the Pan troglodytes troglodytes' species of chimpanzees (Gao et al. 1999). It was acknowledged as an etiological agent of AIDS in 1983 (Montagnier et al. 2002). By 2012, there are more than 34 million people currently living with HIV and almost 30 million people have died of AIDS after outbreak (UNAIDS, 2012). There are four groups of HIV-1 have been identified and grouped according to genetics sequence of M, N, O, and P types. HIV-1 group M is more than 90% of infection worldwide and it's further subdivided into nine subtypes (A-I), based on the complete genome, which are geographically dissimilar (Dale et al. 1996). Group O is limited in West-Central Africa and N is very rare and is originated only in Cameroon (Plantier et al. 2009). The HIV-2 was isolated from AIDS patients in West African Country in the years 1986 and it was least pathogenic than HIV-1, derived from sooty mangabey the *Cercopithecus atys atys*. The replicative period of HIV viral pathogen (Piatak et al. 1993) and a gradual depletion of CD4+ T lymphocytes, foremost to rigorous immunodeficiency, more than a few possibility infections, tumor and end of life (Fauci et al. 1991). Screening procedure for HIV by detection of p24 antigen through EIA and western plot is poor and difficulty since the antigen is measurable in only 20% of carrier state of patients and 40-50% of threatening diseases of person (Hammer et al. 1993).

An advanced molecular technology like polymerase chain reaction is highly sensitive method for quantification of very low amount of nucleic acid than other methods even in window period. The measurement targeted DNA and RNA units are found in a milliliter of blood. High levels of DNA or RNA, which can range from minimum 10-50 to million units or copies/ml or even billion, indicate a high or low rate of viral replication. Practical guideline for the management of chronic hepatitis B, chronic hepatitis C and HIV through antiviral regimes have been published as clinical proof by a number of specialized scientific societies (Anna et al. 2009; European association, 2009; Hammers et al. 2008; Kanda et al. 2013). Viral load quantification plays a significant role during antiviral therapy with its specific genotypes, as nearly all current practical guideline projected that inhibition of viral replication. Present study was focused on Cobas Amplicor analyzer as a diagnostic tool for quantification of HBV DNA, HCV and HIV RNA for antiviral treatment and its methodology and precision are compared with recently updated published resource for viral diseases management and this methods has been in old versions and company stopped to supply kits because of new updated version was introduced recently for adequate treatment of viral diseases.

Antiviral Drugs:

There are different drugs are present in the drugs market to control viral infection like HIV, HBV and HCV. Drugs for HBV managements like L-nucleosides (lamivudine, telbivudine and emtricitabine); deoxyguanosine analogues (entecavir); and acyclic nucleoside phosphonates (adefovir and tenofovir) these are all approved by European Union. Drugs for HCV managements like Pegylated interferon (PEG-INF α 2a/b) and Ribavirin. For HIV, the drugs names are called as AZT, DDI, DDC, D4T, 3TC, Nevirapine, delavirdine, Saquinavir, Indinavir, Ritonavir and Nelfinavir. Working as protease and nuclease inhibitor has been approved by the department of Food and Drug Administration, USA.

Comparison:

The Cobas Amplicor assay is an un-updated methodology for antiviral treatment than updated methodology. The Taqman and Abbott have gaining major role in molecular diagnostic as well as in cancer genetics. New methodology is easy to handle and quick access in report when compare with Cobas Amplicor. In the detection of genotypes, Amplicor failure to cover complete genotype because of primer designing methods when compare with updated methodology. In antiviral management vise, Cobas has detected above 600IU/mL as minimum quantification and maximum 10^6 copies but in updated version it is 10 to 10^8 IU/mL, so updated technology are better than Amplicor for antiviral treatment.

Content:

All the information which I mentioned in this research papers were written or spoken about in a book, an article, a programme, a speech, etc.,

obtained from individual patients.

2.1.2. Specimen Collection

Approximately 10ml blood samples were collected from each patient. The patients serum and plasma sample were separated according to the test immediately by using centrifugation at 3000 rpm for 10 minutes and then each specimens was then aliquots into screw capped vials by labeling specific test name and stored at freezer (-80°C). These specimens were utilized for detection and quantification of HBV DNA, HCV and HIV RNA. Statistical analysis was performed using Microsoft office excel 2007.

2.2. Methods

2.2.1. HBV DNA Extraction

HBV DNA was isolated from serum samples using the polyethylene glycol (PEG) precipitation methods with alteration to the manufacturer's protocol (Roche Cobas Amplicor HBM v 2.0). Briefly, 50 μl of HBM QS copy (quantitative standard) was added to working lysis 2, mixed for 5 seconds; 50 μl of HBM working lysis 1 was added to each of the labeled tubes; 100 μl NHP (negative human plasma) to three control tubes was added. After that 100 μl serum samples were added; vortexes for 10 seconds then centrifuged at 15000 rpm for 5 minutes and supernatant were discarded. Further 25 μl controls (negative, low and high positive) was added to the labeled tubes; 100 μl working lysis 2 was added to all samples and controls and vortexes for 15 seconds; incubated at 60°C for 1 hour. Finally 100 μl working lysis 3 was added to all specimens and controls and further incubated at 100°C for 15 minutes, followed by centrifugation at 16000 rpm for 15 minutes. Supernatant discarded and diluted with 1 ml dilution buffer for PCR amplification.

2.2.2. HCV RNA Extraction

HCV RNA was extracted from serum by using chemotropic agent procedure with modification to the manufacture guideline (Roche Cobas Amplicor HCM v.2.0).

2.2.3. HIV RNA Extraction

HIV RNA was isolated from plasma by using standard specimen preparation procedures with adaptation to the maker principle (Roche Cobas Amplicor HIM-1 v.1.5). In brief, for each batch (12 specimens), added 100 μl of HIV-1 QS to the bottle of HIV-1LYS and mixed well. 600 μl of working lysis reagent was added to each of the labeled tubes. 50 μl of negative, low positive and high positive controls were added to control tubes with 200 μl of negative human plasma then mixed well. Added 200 μl of each patient's plasma to appropriately labeled tubes then mixed well and incubated at room temperature for 10 minutes. Afterward 800 μl isopropyl alcohol was added to each sample and control tubes mixed well; centrifuged at 16000 rpm for 15 minutes at room temperature and supernatant discarded. To each labeled tubes, 1.0ml of 70% freshly prepared ethanol was added, vortexes for 10 seconds and centrifuged at 16000 rpm for 5 minutes and supernatant discarded. Finally, 400 μl of dilution buffer was added to each tubes and vortexes for 10 seconds. The sample of isolated RNA is ready for amplification procedure.

2. SCOPE OF STUDY

The HIV RNA quantification through Abbott assay is a major role in complete clearance of viral copy in antiviral treatment than others assay especially HIV-1 genotypes inclusivity. The hepatitis B and C virus quantification and genotypes through Cobas Tag man-48 assay is better than Cobas Amplicor assay and in feature need to replace according to the government policy in this region.

2.1. Materials

2.1.1. Patients

This potential research work was incorporated 130 HBsAg, 72 anti-HCV and 8 anti- HIV positive male and female patients. It has been randomly selected with clinical history of virus infection, who attended the medical outpatient department during the year of 2010 to 2011. The ethics panel and internal review board of the organization approved the procedure. Informed consent form was

RT-PCR:

Reverse Transcriptase
Polymerase Chain
Reaction for RNA
amplification and
quantification
technology.

2.2.4. Reverse Transcription

The extracted HIV and HCV RNA samples are added individually (50µl) to the amplify reaction mixture in amplification tube (A-tubes) in which both reverse transcription and PCR amplification occur with help of the thermo stable recombinant enzyme *thermus thermophilus* DNA polymerase. The antisense primer is biotinylated at the 5' end, the sense primer is not biotinylated. The reaction mixture is heated to allow the downstream primer to anneal particularly to the target RNA and the quantization standard RNA. In the presence of manganese²⁺ and excess deoxynucleotide triphosphates (dNTPs) and DNA polymerase enzymes extends the annealed primer forming a DNA strand (cDNA) to the RNA target.

2.2.5. Target Amplification

Following reverse transcription of the target and QS RNA, the reaction mixture is heated to denature the RNA: cDNA hybrid and release the primer target sequences. As the mixture cools, the primer anneals specifically to the cDNA stand, rTth DNA polymerase enzymes extend the primer to get second DNA strand. This phenomenon could continue up to performed cycle (40), each cycle effectively doubling the amount of amplicon DNA. But this procedure differs in HBV DNA target amplification that the processed DNA samples are added to the amplification mixture in amplification tube (A-tube) in which PCR amplification occurs. The analyzer thermal cycler heats the reaction mixture to denature the double stranded DNA and expose the specific primer target sequence on covalently circular DNA of HBV genome. The biotinylated HBV primer ((HBV-104UB) and non-biotinylated primer (HBV-104D) anneal to the target DNA and it produce a 104 base pair as an amplicon as like standard control. Amp Erase has been used to avoid contamination in PCR amplification. For all tests, the required number of cycle is pre programmed in the Cobas Amplicor analyzer and reports were released automatically.

2.2.6. Detection

After amplification reaction, Cobas Amplicor analyzer was automatically added 100µl de-naturation solution to the amplification tubes to chemically denature test target and quantitative standard amplicons to form single-stranded DNA. To achieve quantitative reports over a broad dynamic range, the system performs dilution in D cups orderly. An oligonucleotide probe with suspension of coated magnetic particle for target as well as quality control is added to total of four dilutions including target and controls. The biotin-labeled PCR amplicon is hybridized to the target probe which is bound to the magnetic particle. After hybridization reaction, the analyzer removed unbound beads and conjugate by washing procedure, then added substrate in orderly (TMB-3, 3, 5, 5-tetramethylbenzidine), to form color complex, the absorbance of which was measured at 660 nm, was produced as results.

2.2.7. Quantification

The absorbance at 660nm for each target is depending to the total of target or control amplicon in the detection cup. The system automatically calculates a total absorbance by multiplying the absorbance of the detection cup by the amplicon dilution factor of that cup. The amount of target amplicon in each test was quantitating from the ratio of the total target absorbance to the total control absorbance and the input number of copy by using the following equation: Total test target/Total QS target x test specific QS IU/ml x sample volume=DNA/RNA IU/ml, Where total DNA/RNA A660 is calculated total DNA/RNA absorbance, total QS A660 is calculated total QS absorbance, input QS copies is the number of QS copies in each PCR mixture (lot specific), and 45 (200 for HCV) is a factor to convert copies or IU/ PCR.

3. RESULTS

A total of 130 HBV, 72 HCV and 8 HIV patients were enrolled in this study. Highly risk group of 20-40 years aged HBV infected patients were more among male (47%) then female (32%) population and 9% of infected persons were not quantified because of lower limit of detection or window period. The HBV DNA viral quantification was between 2-5 log₁₀ for male and 2-4 logs₁₀ for female. Similarly for HCV infected patients were higher in male (35%) then female (18%) population with different age groups. The HCV RNA viral load was between 6 log₁₀ for male and 5-6 log₁₀ for female and all of them quantified. Likewise, HIV infected most of patients were male with different age groups (12-50) and medium HIV RNA viral load (4 log₁₀) except 1 patient because of lower limit of detection less than 50c/ml or might be screening error from serology section. The limit of detection of Cobas Amplicor monitor test was determined by analysis of standard that was associated with WHO international standard (96/790). The limit of detection for HIV RNA 50-400copies/ml; 60 IU/ml for HBV DNA and 600IU/ml for HCV RNA with more than 95% positivity rate. The maximum linear ranges of viral quantification are 6 log₁₀ for all versions (Figure 1, 2, 3). The performance of the analyzer on genotypes inclusivity for HIV-1 M groups A to G subtypes; for HCV genotypes 1 to 6 and for HBV A-G subtypes only. The optimal density of analyzer was 0.15 to 2.5 logs₁₀ in detection cup dilution. This

Table 1

Comparison of commercially available new assays with Cobas Amplicor assay

Diagnosis Assay-FDA/CE	Methodology	Genotypes	Lower Limit	Upper Limit	Target genes	Specificity Processing Time/s.volumes	
Cobas Amp HBM V2.0	Target Amp	A to G	60 IU/ml	Log 10 ⁶	Precore Core region	97%	10h 100µl
HCM V2.0	Target Amp	1a,1b,2a,2b,3a,4a,5,6	600 IU/ml	Log 10 ⁶	5-UTR	97%	10h 100µl
HIM V 1.5	Target Amp	Group M-A-H subtypes	50-400 C/ml	Log 10 ⁶	Gag gene	95%	10h 200l
Cobas TaqMan48 HBV V2.0	RT-PCR	Genotypes A to G	6-10 IU/ml	Log 10 ⁷	Surface, Core regions	98.2%	8h 500ul
HCV V2.0	RT-PCR	1a,1b,2a,2b,3a,4a,5,6	10 IU/ml	100 million	5-UTR	99%	8h 650ul
HIV V2.0	RT-PCR	Group M (A-H) subtypes	20 C/ml	Log 10 ⁷	Gag gene	98%	8h 650ul
Abbott m2000rtHBV	RT-PCR	Genotypes A to H	10 IU/ml	1 Billion Log 10 ¹⁰	N-S region gene	99%	9h 500ul
HCV	RT-PCR	1to6 genotypes	12 IU/ml	100-million	5-UTR	99.5%	9h 500ul
HIV	RT-PCR	A-H+O+N subtypes	12-40C/ml	10 million Copies/ml	Pol/IN region	99.2%	9h 200ul
Bayer bDNA HBV V3.0	Signal amp	A-F subtypes	357 IU/ml	Log 10 ⁷	Entire genome	99%	18h 50ul
HCV V3.0	Signal amp	1-6genotypes	615 IU/ml	Log 10 ⁷	5NCR	98.2%	18h 650ul
HIV V 3.0	Signal amp	A-G subtypes	50C/ml	Log 10 ⁷	Pol region	99%	18h 650ul
Digene HBV V II	Hybrid Capture	A-G subtypes	100 IU/ml	Log 10 ⁹	Precore/ Core region	95%	6h 30ul
HCV V II	Hybrid Capture	1-6genotypes	-	Log 10 ⁷	5NCR	96%	6h
HIV V II	Hybrid Capture	A-G subtypes	-	Log 10 ⁷	Pol/IN region	96%	6h
ArtusQiagen HBV	Rotor PCR	A to H subtypes	10.2	32 to 10 ⁸	Precore Core region	95%	6h 140ul
ArtusQiagen HIV	Rotor PCR	A to H subtypes	66.9IU	120 to 10 ⁸ IU	Gag gene	95%	6h 140ul

Taq polymerase enzymes:

A Thermus aquatics polymerase enzyme is derived from heat resistant bacteria at 120C°. It is performing gene amplification of any nucleic acid molecules.

result compared with existing published data source like Cobas TaqMan and Abbott assays are minimum detection ranges for 6-10IU/ml for HBV;10 IU/ml for HCV; and 20copies/ml for HIV. In Abbott, 10 IU/ml for HBV; 12IU/ml for HCV and 12-40 copies/ml for HIV. The maximum detection ranges in TaqMan were log10⁸ for HBV and HIV; for HCV 100million IU/ml as well as in Abbott were 1 Billion for HBV; 100 million for HCV and 10 million for HIV. The genotypes inclusivity in Taqman and Abbott was more advanced than Cobas Amplicor assay.

4. DISCUSSION

Cobas Amplicor monitor assay has somehow better accurateness in quantification of viral load with target amplification methodology. Its permits identical detection and quantification of DNA/RNA with least of subtypes of viruses' genotypes in human serum or plasma specimens. Cobas Amplicor uses sequential dilution of amplicon, followed by hybridization, washing, and enzymes related color development, and need at least 4 h after amplification as long time to gain results when compare with new methodology . It has minimum detection ranges (600 IU/ml for

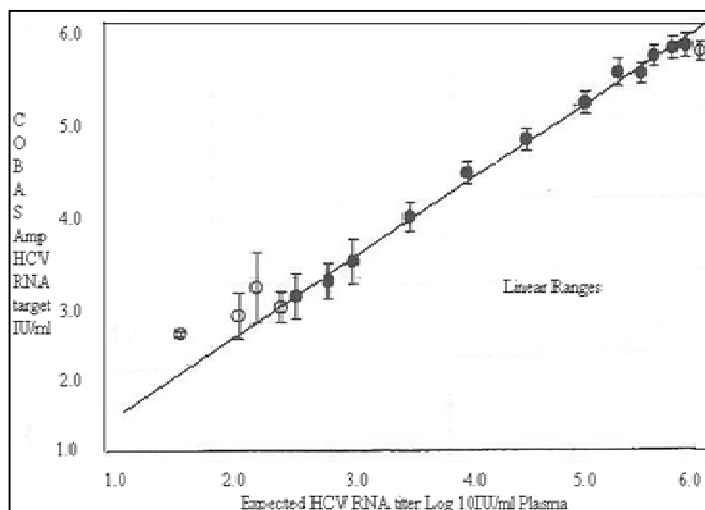


Figure 1

HBV DNA Linearity

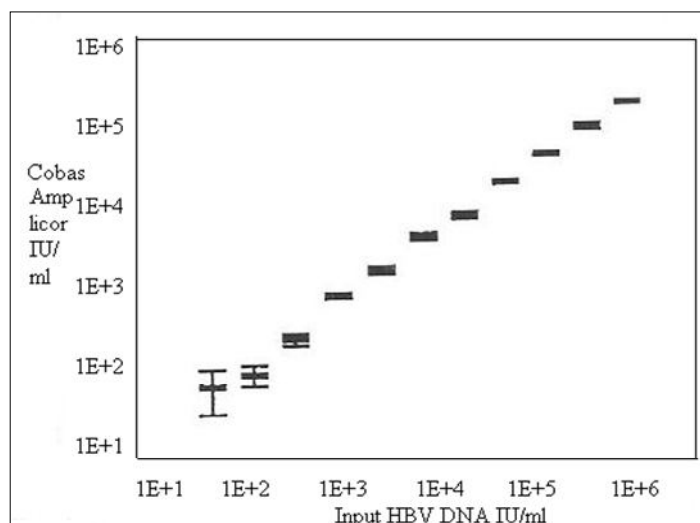


Figure 2

HCV RNA Linearity

HCV v-2.0, 61 IU/ml for HBV v-2.0 and 50copies/ml for HIV v-1.5) using low specimen volume of only 100µl (HBV, HCV) to 200µl (HIV), at least 6 log₁₀ maximum detection ranges need for specimen dilution, as well as good clinical specificity. Food and Drug Administration (FDA) and Europeans Counseling approved the best assays with device are available in the Molecular diagnostic market for detection and quantification of DNA/RNA in human serum or plasma integrated the Cobas Taqman48 (v2.0) assay depend on target specific dual labeled oligonucleotide probes with RT-PCR; the Abbott m2000^{rt} assay relied on nucleic acid based target amplification with RT-PCR; the Bayer versant assay (v.3.0) based on branched DNA signal amplification; the Digene hybrid capture II assay utilize chemiluminescent signal amplification; the NucleiSens assay depend on isothermal nucleic acid amplification; the Artus assays based on real time quantification and finally the Cobas Amplicor assay depend on target amplification with biotinylated primers followed by amplicon hybridization within detection cup containing attached detection probe (Weiss et al. 2004). These types of commercial assays are routinely used in clinical laboratories; various assays have margins in sensitivity, edge of detection, linear range, genotypes inclusivity, time of process and specimen quantity. The comparison of these marketable assays with the Cobas Amplicor PCR assay is shown in Table 1. For instance, the minimum detection ranges of the Bayer bDNA, artus RT-PCR and Digene assays might be not sufficient to detecting low levels of viral load in patients undergoing antiviral therapy. In same time, Bayer bDNA; NucleiSens, artus RT-PCR; Cobas Amplicor and the Digene were not detected all genotypes inclusivity normally HIV-1 O and N types. Hence, the recently introduced advance molecular assay like the Abbott m2000^{rt} is real time target based amplification and equally amplify all major genotypes and subtypes of HIV-1. The minimum detection ranges of at least 8 log₁₀ and good accuracy are comparable to the Cobas Amplicor and RT-PCR assay. Although, in Cobas TaqMan48 and Abbott m2000^{rt} assays uses more sample volume than Cobas Amplicor and others RT-PCR assays giving results in greater clinical sensitivity. However, the clinical sensitivity of assay might be improved by similar sample volume of above assays but the dilution procedure totally different from it. The primer probe sets of the Cobas Amplicor and Cobas

Taqman48 HBV assays target the precore/ core region, while the others assays like Bayer b DNA target the entire genome, Abbott2000^{rt} target N-S region gene and Digene assays target precore/core regions. But some in house RT-PCR assay they developed the own targets region. Cobas TaqMan 48 HCV RNA analytic-specific reagents (ASR) have a least amount border of sensitivity between 10 and 100 million IU/ml, and are ranges to maximum 100million IU/ml (Barbeau et al. 2004).The Cobas Amplicor competitive HIV RNA PCR 1.5 v assay has a limit of detection of 50 copies/ml (Sun et al. 1998), while b DNA 3.0 assay has a detection limit is 50 to 100 copies/ml (Collins et al. 1997). The Cobas Amplicor assay (HBV V2.0) was more accessible than Digene assay v2.0 for linear detection and quantification of HBV viremia (He-Jun Yuan et al. 2004).

The NASBA(Nucleic Acid Sequence Based Amplification) NucleiSens assay, using the long terminal repeat region as a target, was revealed to detection and quantification of HIV-1 group M subtypes A to M and group O virus (De Baar et al. 1999), while the Cobas Amplicor assay representing suboptimal quantification of subtypes (HIV) A- G (Emery et al. 2000). Most of commercial assay were unable to detect and quantitative HIV group O isolate and HIV-2 but only Abbott assay has a great specificity to detect and measurement of viral load of HIV-1 group M subtypes A-H, Group N and O not HIV-2.The Abbott Real Time PCR assay was a great sensitive and accuracy instrument for an excellent quantification of HCV viral load genotypes, generally for genotype 1b than Cobas TaqMan 48 and Amplicor assay (Heidi LaRue et al. 2012). Measurement levels gained by Digene capture II assay were lower than Cobas Amplicor

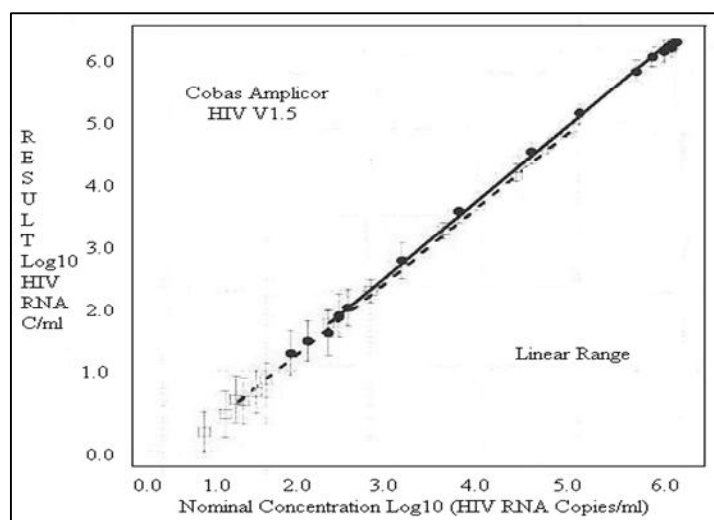


Figure 3

HIV RNA Linearity

assay and in same time there was no polyethylene glycol precipitation or proteinase K digestion in Digene capture assay. The attendance of too many proteins could be disturbing the performance of the test (Niesters et al. 2000).

5. CONCLUSION

The Cobas Taqman and Abbott assays are recent updated version especially for antiviral treatment in end stage clearance of viral copy. Both assays have an excellent detection ranges when compare with old version of Cobas Amplicor and others conventional methods. This information collected through different research source which was published early by scientific groups and it was clearly briefed in discussion. The clinical sensitivity of TaqMan assays were $\log 10^8$ for HIV & HBV; 100 million IU/ml for HCV; as well as in Abbott assays were for HCV 100million IU/ml, for HBV 1 billion IU/ml and for HIV 10 million copies/ml. Those parameters are important role to find the pathogenity, treatment decision making, complete clearance viral load and diseases management. Disadvantages of Cobas Amplicor assays included the following: Length procedure (10-12h) for reporting; limit of detection (600IU for HCV, 60 IU for HBV, 50-400copies/ml for HIV; washing and hybridization procedure with serial dilution; limited quantification of genotypes and cost of assay more than commercial assay and it need to update according to current treatment strategy.

SUMMARY OF RESEARCH

1. Cobas Amplicor assay has been used for quantification of HBV DNA, HIV-1 RNA and HCV RNA from different ward for treatment purposes since 2002.
2. Quantification were received and compared with existing data for accuracy and maximum and minimum detection ranges with recent updated technology which was mentioned in discussion in details.
3. The advantages and disadvantages have been mentioned in details about the Cobas Amplicor conventional polymerase chain reaction with updated RT-PCR technology.

FUTURE ISSUE

Quantifications of viral load could be in least and highest ranges in the cases of antiviral treatment as well as viral detection to know about the pathological condition and completely clearance of viral copy from blood. These types of clinical service may helpful to get free of virus from the blood and saving of individual as well as country.

DISCLOSURE STATEMENT

There is no financial support for this research work from the finding agency.

ACKNOWLEDGEMENT

We are grateful to serology laboratory staffs for assistance of this study.

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