International Journal of Molecular Medicine and Advance Science 1 (2): 84-87, 2005

Application of Nucleic Acid Amplification Technology for Detection of Rna Viruses: Current Status and Future Prospects

[1] E. Mohamed Ahmed, [2] E. Imadeldin Aradaib
[1]Department of Surgery, Faculty of Medicine, ElNeelain University, Khartoum, Sudan, [2]Molecular Biology laboratory, Department of Medicine, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum, P.O. Box 32. Khartoum North, Sudan

Abstract: The application of bioengineering to study the molecular epidemiology of viral diseases should facilitate rapid detection of an active infection. The surge of new techniques in cellular immunology and molecular biology has made possible the development of rapid, sensitive, specific and inexpensive assays for detection of RNA viruses using nucleic acid amplification. Because of the Ribonucleic acid (RNA) nature of the virus genome, a Reverse Transcription (RT) step involving synthesis of complementary DNA (cDNA) from RNA template would be necessary, before cyclic amplification with PCR. In this study, we evaluated the diagnostic potential of the Reverse Transcriptase (RT) polymerase chain reaction (RT-PCR)-based assays, recently developed in our laboratory, for rapid detection of viral infections in cell culture and clinical samples.

Key words: Rna, viruses, molecular diagnoses, PCR

INTRODUCTION

Diagnostic methods currently applied for detection of RNA viruses include serology and virus isolation. Serology is useful in epidemiologic studies to identify previous infection[1]. The Agar Gel Immunodiffusion (AGID) test, currently used as standard serologic test, is complicated by cross reactions between closely related serogroup of viruses[2]. The problems associated with the use of AGID test have been solved by using Monoclonal Antibodies (MAb) in competitive ELISA (cELISA) technique. However, this technique is applicable only to blood (serum) and requires at least 1-2 weeks post infection for the production of specific antibodies by the susceptible host[3]. Conventional virus isolation is tedious, time consuming, labor intensive and expensive[4,5]. To address these problems, specific complementary RNA probes derived from different genes have been developed[6]. The use of RNA or cDNA probes in hybridization assay[7] was also proved useful for detection of RNA viruses. However, one of the disadvantages of hybridization assay is that the technique is incapable of direct detection of viral RNA in clinical samples from infected patients[8]. Hence, a prior amplification step by PCR technology deemed necessary. For the last twelve years, the major thrust of present research was directed towards the improvement of the existing techniques used for diagnosis of viral diseases. Recently, we have reported on application of Reverse Transcriptase (RT) Polymerase Chain Reaction (RT-PCR)-based assays for detection of viral RNA in cell cultures and clinical samples. These RT-PCR assays proved highly sensitive and specific method for detection of viral RNA. [6,7,9-18].

The development of a rapid, sensitive, specific and inexpensive method for diagnosis of the viral diseases would greatly facilitate clinical disease investigations epidemiological investigation and would enhance vaccination and control programs[8,16].

Polymerase Chain Reaction (PCR): Application of the Polymerase Chain Reaction (PCR) has proliferated because of its simplicity, rapidity, reliability,

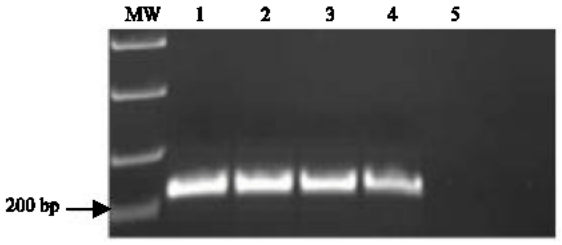

Fig. 1: The specific 240 bp-PCR product from 1.0 pg RNA of different vaccine strains of RVFV. Lane MW: molecular weight marker; Lane 1-4: 1.0 pg RNA extracted from RVFV Vaccine strains, Lane 5: Vero cell total nucleic acid extractdescribed different protocols for detection of RNA viruses in cell culture and a variety of clinical samples in previous studies [6,7,9-15,18].

Corresponding Author: E. Mohamed Ahmed, Department of Surgery, Faculty of Medicine, ElNeelain University, Khartoum, Sudan, P.O. Box 32. Khartoum North, Sudan

reproducibility, sensitivity and specificity for monitoring of microorganisms (Fig. 1). PCR proved satisfactory to replace the immunological and DNA hybridization methods using DNA probes. In our Laboratory, we have

Reverse Transcriptase (RT- PCR) : RT-PCR has been successfully applied for detection of RNA viruses by addition of a complementary DNA (cDNA) synthesis step using Reverse Transcriptase (RT) enzyme, before cyclic amplification by PCR. RT- PCR-based assay for detection of viral RNA in cell culture and clinical samples, using primers derived from conserved genes sequence analysis of EHDV-2 Alberta strain, was described[7].

RT-PCR was compared with different Virus Isolation (VI) procedures from a variety of tissue samples. The results indicated that the sensitivity of RT-PCR assay is comparable or even more sensitive than VI method in cell cultures or Embryonated Chicken Egg (ECE). In addition, the RT-PCR assay could provide a superior diagnostic alternative to replace the current cumbersome and time-consuming virus isolation procedures[9,11].

Detection of serogroup-specific PCR-based assay could be done using well-characterized serogroup-specific primers derived from a conserve gene among cognates of different serotypes[3,7,19]. The serogroup-specific PCR products are usually visualized on ethidium bromide-stained agarose gel or detected with chemilumiescent hybridization. The RT-PCR assay provides an attractive diagnostic alternative to the lengthy and cumbersome conventional virus isolation procedures. Probe derived from conserve genes hybridized with cognates of different serotypes of the same serogroup [12].

Sserotype-specific identification, in cell culture or tissue samples, was described using RT-PCR-based assay [10]. Similar study was conducted to demonstrate specific identification of in cell culture and clinical samples[20]. These RT-PCR assays were based on nucleotide sequences of genes, which are associated with serotype specificity or neutralizing antibodies. The nucleotide sequences of these genes were found to be variable among cognate genes of different serotypes of serogroup. Widespread application of the molecular biological techniques described in this study should facilitate rapid detection and epidemiological investigation of outbreaks of viral diseases among susceptible humans or animal populations.

Nested Polymerase Chain Reaction : In the nested PCR, two pairs of oligonucleotides primers are required. The PCR reaction is carried out in 2 amplification steps. The first pair of primers (outer primers) is used to amplify specific PCR product. The second pair of

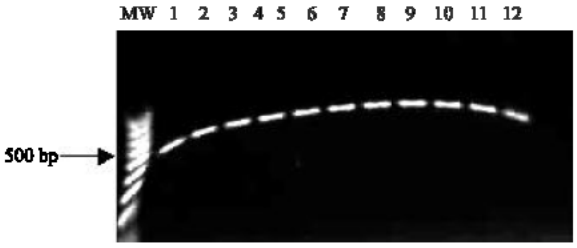

Fig. 2: Nested Amplifcation of the nested 520- bp specific-BTV PCR product on ethidium-bromide-stained agarose gel from 1.0 pg of RNA of eight different BTV field isolates. Lane MW: molecular weight marker (100 bp ladder); Lane 1-5: North American BTV serotypes 2, 10, 11, 13 and 17; Lane 6-9: Sudanese BTV serotype 1, 2, 4 and 16 field isolate; Lanes 10: BTV-4 from Senegal; Lane 11: BTV-4 from South Africa; Lane 12: non-infected Vero cells total nucleic acid extract

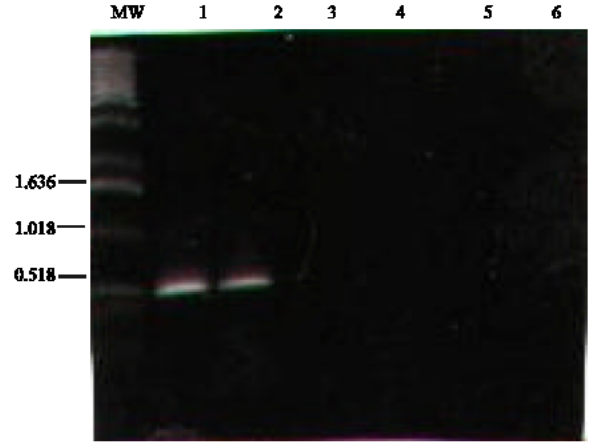

Fig. 3: A: Chemiluminescence hybridization of the specific 518 bp PCR product from epizootic hemorrhagic disease virus A: Lane MW: molecular weight marker; Lane 1-6: 1 ng; 100 pg; 1.0; 100 fg; 10 fg; 1 fg RNA extracted from EHDV

nested primers (internal primers) is designed internal to the annealing sites of the outer primers to amplify specific PCR product, which is shorter than that produced by the first pair of primers (Fig. 2).

The second amplification step using the nested primers is necessary to confirm the specificity of the first amplified product and to increase the sensitivity of the PCR-based assay.

The use of nested PCR removes the hazardous and cumbersome radioactive laboratory procedures of working with ^{32}P or ^{33}P[21] (Fig. 3b).

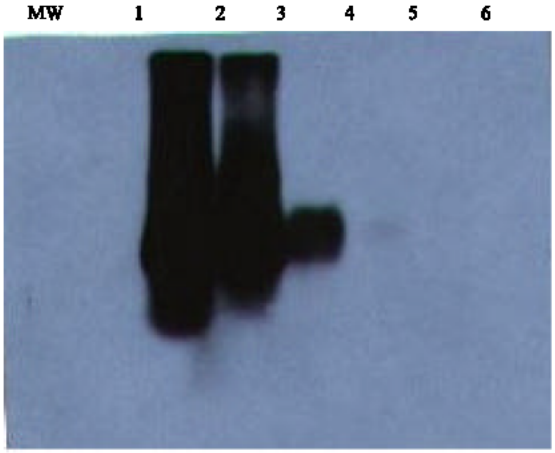

Fig.3:B: Chemiluminescence hybridization of the above gel

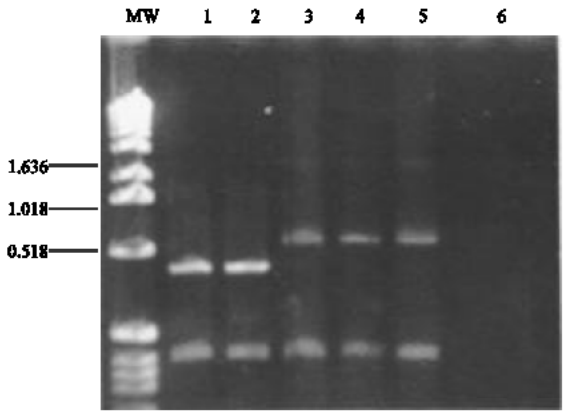

Fig.4: A Multiplex RT-PCR for simultameous detection and differentiation of orbiviruses

Multiplex Polymerase Chain Reaction: In this type of PCR, different primer pairs derived from different serotypes of the same serogroup, are used in a single-tube PCR amplification. A multiplex RT-PCR for simultaneous serogroup-specific detection and serotype-specific identification could be developed. A multiplex RT-PCR-based assay, for simultaneous detection and differentiation of closely related serotypes of the same serogroup, in cell culture was also described[13]. The multiplex RT-PCR assay is a simple procedure that utilized a single-tube PCR amplification in which different primers for 2 or more viruses were used simultaneously[8].

Simultaneous detection and differentiation of RNA viruses will simplify the assay, save time and above all save on cost, because each clinical sample will be tested once instead of testing the suspected samples individually. Because of its rapidity, sensitivity and specificity, the multiplex RT-PCR assay would be advantageous in a variety of circumstances including epidemiological investigations. In addition, multiplex RT-PCR could also be used to determine the prevalence and frequency of viral infections in susceptible human or animal populations. More over multiplex RT-PCR could also be used for evidence of viral incursion in a particular geographical region.

The multiplex RT-PCR assay could be used for export regulation to certify animals free of infectious diseases. (Fig. 4). A definitive diagnosis of infected clinical sample using the RT- PCR-based detection assay with chemiluminescent hybridization could be obtained within 2-3 days[7] and within the same working day using a nested PCR-based assay[5,8,22].

In general, the RT-PCR-based detection assay is an extremely sensitive procedure and hence PCR-positive and virus isolation-negative results from the same clinical sample are not surprising. This is because PCR positive results could be obtained from clinical samples containing incomplete virions or viral nucleic acid as well as infectious virus. Therefore, the biological significance of a PCR- positive result has to be interpreted with caution in light of presence of viral nucleic acid and absence of infectious virus. PCR-positive, but virus isolation-negative blood samples, may not be infectious either to the invertebrate vector or susceptible humans or animal populations. It is probably that individuals whose blood samples test PCR-positive, but virus isolation-negative, are unimportant in the epidemiology of insect-transmitted RNA viruses including viral hemorrhagic fevers. The RT-PCR-based detection assays, will continue to be important diagnostic tools for rapid detection and differentiation of viral infections during epizootics of the disease, where at least 2-4 weeks are required for conventional isolation and identification of the virus in a susceptible cell line (Fig. 3a).

It is worth mentioning that conventional virus isolation procedures will remain important for recovery of an infectious virion and detection of genetic diversity of different strains of the same serogroup of viruses.

REFERENCES

1. Meegan, J.m., R.J., Yedloutschnig, B.A. Peleg, C.J. Peters, J.S. Walker and R.F. Shope, 1987. Enzyme-linked immunosorbent assay for detection of antibodies to Rift Valley Fever in ovine and bovine sera. American . J. of Veterinary Research, 48: 1138-41.
2. Borden, E.C., R.E. Shope and F.A. Murphy, 1971. Physicochemical and morphological relationships of some arthropod-borne viruses to bluetongue virus-a new taxonomic group. Physicochemical and serological studies. J. Gen. Virol., 3: 261-271.

3. Aradaib, I.E., M.M. Sawyer and B.I. Osburn, 1994b. Experimental epizootic hemorrhagic disease virus infection in calves: Virologic and Serologic studies. J. Vet. Diagn. Invest., 6: 489-492.
4. Fosgerg, S.A., E.H. Stuber and H.W. Renshaw, 1977. Isolation and characterization of epizootic hemorrhagic disease virus from White-tailed deer in eastern Washington. Am. J. Vet. Res., 38: 361-65.
5. Ibrahim, M.S., J.M. Turell, K.F. Knauer and R.S. Lofts, 1997. Detection of Rift valley fever virus in mosquitoes by RT-PCR. Molecular and cellular Probes., 11: 49-53.
6. Mohammed, M.E.H., I.E. Aradaib, M.M. Mukhtar, H.W. Ghalib, H.P. Riemann, A.Oyejide and B.I. Osburn, 1996. Application of molecular biological techniques for detection of epizootic hemorrhagic disease virus (EHDV-318) recovered from a sentinel calf in Central Sudan. Veterinary Microbiology., 52: 201-208.
7. Aradaib, I.E., G.Y. Akita and B.I. Osburn, 1994a. Detection of epizootic hemorrhagic disease virus serotype 1 and 2 in cell culture and clinical samples using Polymerase Chain Reaction. J. Vet. Diagn. Invest., 6: 143-147.
8. Sall, A.A., J. Thomon, O.K. Sene, A. Fall, M. Ndiaya, B. Baudez, C. Mathio and M. Bouloy, 2001. Single-tube and nested Reverse Transcriptase-Polymerase Chain Reaction Valley fever virus in human and animal sera. J.Virological Methods, 91: 85-92.
9. Aradaib, I.E., G.Y. Akita, J.E. Pearson and B.I. Osburn, 1995a. Comparison of Polymerase Chain Reaction and virus isolation for detection of epizootic hemorrhagic disease virus in clinical samples from naturally infected deer. J. Vet. Diagn. Invest., 7: 196-200.
10. Aradaib, I.E., W.C. Wilson, C.W. Cheney, J.E. Pearson and B.I. Osburn, 1995b. Application of the polymerase chain reaction for specific identification of epizootic hemorrhagic disease virus serotype 2. J. Vet. Diagn. Invest., 7: 388-392.
11. Aradaib, I.E., M.E.H. Mohammed, M.M. Mukhtar, H.W. Ghalib and B.I. Osburn, 1997a. Serogrouping and topotyping of Sudanese strains of epizootic hemorrhagic disease virus using Polymerase Chain Reaction. Comp. Immunol. Microbiol. Infec. Dis., 20:211-218.
13. Aradaib, I.E., 1999. Detection and differentiation of Bluetongue and Epizootic hemorrhagic disease viruses. Sudan J. Vet. Sci. Anim. Husb., 38: 39-43.
14. Aradaib, I.E., A. E. Karrar and M. A. Abdalla, 2002. PCR detection of Sudanese serotypes of epizootic hemorrhagic dsease virus serogroup. The 27th International Congress of the World Veterinary Association, Ghartage, Tunisia.
15. Aradaib, I. E., A. E. Karrar, E.H.M. Mohammed, S.M. Elamin and M.M. Salih, 2003a. Simultaneous Detection and differentiation of Epizootic hemorrhagic disease virus serotype 1 and 2 using RT-PCR. J. Anim. and Vet. Adv., 10: 584-588.
16. Aradaib, I.E., W.S. Smith, J.S. Cullor and B. I. Osburn, 2003b. A multiplex PCR for simultaneous detection and differentiation of North American serotypes of bluetongue and epizootic hemorrhagic disease viruses. Comparative Immunology, Microbiology and Infectious Diseases, 26:77-87.
17. Aradaib, I. E. and N.O.M. Ali, 2004. A review on epizootic hemorrhagic disease virus infection: Current status and future prospects. Veterinariski Arhiv 74: 63-83.
18. Aradaib, I. E., M. E.H. Mohammed, N. O. M. Ali, A. A. Majid, S, H. Idris and A. E. Karrar, 2005. Asimple and Rapid method for Detection of African horse sickness virus serogroup using RT-PCR. Veterinary Research Communication (In press).
12. Aradaib, I.E., M.E. Mohammed, C.E. Schore, W.C. Willson and J.S. Cullor and B.I. Osburn, 1998a. PCR detection of North American and Central African Isolates of epizootic hemorrhagic disease virus based on genome segment 10 sequence analysis of EHDV-1. J. Clinical. Microbiol., 36: 2602-2608.
19. Aradaib, I.E. and B..I. Osburn, 1994c. Application of PCR for detection of epizootic emorrhagic disease virus. Sudan J. Vet. Sci. Anim. Husb., 33: 79-85.
20. Aradaib, I.E., C.E. Schore, J.S. Cullor, and B.I. Osburn, 1998b. A nested PCR for detection of North American isolates of Bluetongue virus based on NS1 genome sequence analysis of BTV-17. Vet. Microbiol.,.59: 99-108.
21. Aradaib, I.E., J.W. Mc Bride, W.C. Wilson and B.I. Osburn, 1995c. Development of Polymerase Chain Reaction for specific identification of epizootic hemorrhagic disease virus serotype 1. Archives of Virology, 140: 2273-2281.
22. Sall, A.A., P.M. Zanotto, O.K. Sene, H.G. Zeller, J.P. Digoutte, Y. Thiongane and M. Bouloy, 1999. Genetic reassortment of Rift Valley Fever virus in nature. J. Virology., 73: 8196-200.