JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2014. Vol. **8**(3), p. 2291-2297

Designation of Loop-Mediated Isothermal Amplification (LAMP) for Diagnosis of *Fusarium solani* in Corneal Samples from Suspected Herpetic Keratitis Cases

Atousa Ferdousi[1]*, Mohammad Hassan Shahhosseiny[2,3], Mansour Bayat[1], Seied Jamal Hashemi[4] and Mohammad Ghahri[5]

[1]Department of Pathobiology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]Department of Microbiology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran.
[3] Iranian Gene Fanavar Institute (IGF), Tehran, Iran.
[4]Department of Parasitology and Mycology, School of Public Health and Institue of Public HealthRresearches, Tehran University of Medical Science, Tehran, Iran.
[5]Department of Biology, Faculty of Science, Imam Hossein University, Tehran, Iran.

(Received: 19 December 2013; accepted: 06 March 2014)

***Fusarium solani,* as the most predominant agent of mycokeratitis, may also occur as a mixed infection with *HSV.* Furthermore due to similarities in their clinical features, sometimes it leads to misdiagnosis. The aim of this research ,was the rapid and accurate detection of *F. solani* as a main etiological agent or as a mixed infection in cases suspected to HSV. Accordingly, 65 negative, and 35 positive samples for *HSV* were collected. LAMP technique with (TEF-1α) as the target gene , was developed for detection of *F. solani.* The test was carried out in 1-h reaction at 65 °C in a heater block . Using this method for the total, 2 cases among the negative samples were found to be positive for *F. solani.* All were rechecked by PCR and the results were the same. The specificity of the test was 100% and its sensitivity was one copy of genome. It showed that, both methods were equal for this purpose. However, due to advantages of the LAMP technique, it can be a substitute for PCR, even in low technology laboratories. Besides this can be concluded, some cases suspected to herpeskeratitis ,are related to *F. solani*, which could be detected by molecular methods.**

Key words: *Fusarium solani*, LAMP, *HSV*, Keratitis.

Fusarium solani has been reported as the most common etiological agent of mycokeratitis in many surveys[1-10]. It may also occur as a mixed infection with *herpes simplex virus (HSV)*[10]. Furthermore, the clinical features of fungal keratitis are pleomorphic. The early stages of fungal ulcers appear like a dendritic ulcer of *HSV* origin. These features, sometimes, lead to misdiagnosis and prompt treatment with antiviral drugs or corticosteroids, ensuring treatment delays[11].

Early and specific diagnosis is very important to choose the best strategy for treatment. Traditional identification and classification methods primarily based on morphologic characteristics require relatively much time to reveal the final result, and sometimes it would be difficult to identify the fungi because of atypical cultural findings and a lack of sporulation. Therefore, further research needs to focus on the development of rapid, reliable and specific identification and diagnostic methods[1,3].

Molecular methods based on nucleic acid sequencing, especially gold standard polymerase chain reaction (PCR) method, are powerful tools for diagnosis of fungal infections and specific identification of etiological agent. However,

* To whom all correspondence should be addressed.
E-mail : Atousaferdousi@gmail.com
Tel.: [+9821] 44844946; Fax: [+9821] 44861889

because of the expensive equipments like thermocycler needed for gold standard PCR method, it cannot be used in low technology laboratories. Loop-mediated isothermal amplification (LAMP) is one of the molecular techniques that was initially designed by Notomi and his colleagues in 2000. This method employs a set of six primers that can recognize a total of eight distinct sequences on the target DNA. These primers are named FIP and BIP as inner primers, B3 and F3 as external primers, ,BLP and FLP as the loop primers. In this method, strand displacement DNA polymerase in isothermal conditions (approximately 65 °C) eliminates the need for a thermal cycler. The cycling reaction continues with accumulation of 10^9 copies of target in less than one hour [12, 13]. Currently, LAMP is mainly applied in the fields of medicine, virus detection, food safety testing, and so forth, with a relatively lesser application in Diagnosis of fungi, bacteria, nematode, insects and protozoa[14-19].

In the present study, LAMP method has been established for rapid and specific diagnosis of *F. solani* as a main etiological agent or as a mixed infection, in corneal samples which were suspected to herpetic keratitis. The results were compared with the results of PCR reference method by chi-square test.

MATERIALS AND METHODS

Extraction of DNA from standard strain

The standard lyophilized strain belonging to bacterial and fungal collection of Iranian Research Organization for Science and Technology (IROST), PTCC NO. 5284 (UMAH 7419), was cultured in *sabouraud dextrose broth (SDB)*. After one week incubation, 500 µl of the medium was taken and centrifuged at 10000rpm for 2 min. The supernatant was discarded and the sediment was suspended in 100 µl dabbled distilled water (ddw). Afterwards, DNA was extracted by DNG-plus method.

Designation of specific primers for LAMP technique

Primers were designed by Primer explorer V4 based on transcription elongation factor (TEF-1±) region of the organism genome (Table 1).

Reaction mixture for LAMP

LAMP reaction mixture was prepared as following: DDW: 5.2 µl, Betaine 5Mol: 4µl, dNTP (10 mM): 3.5 µl, 10X buffer: 2.5 µl, MgSo4 (100 mM): 1.8 µl, primer Mix(A): 1µl, primerMix(B): 1µl, Bst DNA polymerase enzyme (New England BioLabs;Lot:33/110806): 1 µl, target DNA (extracted DNA from standard strain): 5 µl, and total volume is 25 µl. primer Mix(A) contaning FIP, BIP primers concentration are 40, 10 µl DDW in 100 µl total volume respectively, and primer Mix (B) containing LF, LB concentration are 20 , 60 µl DDW in 100 µl total volume, respectively. The reaction followed by incubation at 65 °C for 1 h.

Analysis of LAMP product

At the end of the reaction, 1 µl of 0.1% SYBER Green was added to each tube, and then the tubes were visualized under UV light.

Identefication of LAMP sensitivity and specificity

To determine the sensitivity of the test, a serial dilution of fungal DNA from 1,000,000 copies of DNA (10^{-1} dilution) to 1 copy of DNA (10^{-6} dilution) was prepared.

DNA of *HSV, Streptococcus pneumoniae, Staphylococcus aureus* and some other species of *Fusarium* except for *solani* including *oxysporum, verticillioides, poea, graminearum* and *prolifratum* were used for specificity test.

Reaction mixture for PCR

The mt cytb gene was considered as a target gene for PCR, and ffuso1 (5'-CTC TGT TAA TAA TGC AAC TC-3') and rfuso2 (5'-TGG TAC TAT AGC TGG AGG A-3') were used as specific PCR primers (1). PCR was carried out in a total 25 µl reaction mixture containing 5µl DNA sample, 2.5 µl PCR 10x buffer, 1 µl of forward and reverse specific primer 10 mM, 0.75µl MgCl2 (50 mM),0.5 µl dNTP10 mM, 0.4 µl Taq DNA polymerase 5u/µl followed by primary denaturation at 94 °C for 2 min, cycles denaturation at 94 °C for 1 min, polymerization at 72 °C for 2 min, and final polymerization at 72 °C for 10 min. The total number of cycles was 35. The sensitivity and specificity of the test were evaluated by the same method described for LAMP technique.

Analysis of PCR product

The electrophoresis of the reaction product was carried on the agarose gel containing 1/5% syber green (sina colon Cat.No.: MR7730C) in TBE 0/5 x buffer.

Clinical sample collection and extraction of samples DNAs

100 samples of DNA which were extracted by DNG method from scrapping of ocular ulcer suspected to herpetic keratitis were collected. 65 of them were found to be negative for HSV, while 35 of them were observed to be positive for this virus using both LAMP and PCR methods. The samples belonged to Farabi hospital.

Application of optimized LAMP and PCR test for the clinical samples

Both of the optimized LAMP and PCR tests were carried out using the entire 100 DNAs, and the results were compared by chi-square test.

RESULTS

At the end of the LAMP reaction, after adding 5µl SYBR Green to each tube, the positive reaction tube was specified by a bright green flouresence under UV 366 nm light, while in negative control and negative reaction tubes no flouresence was observed under UV light (Fig. 1a). In parallel, the PCR products were confirmed by electrophoresis on 1.5% agarose jel; the size of PCR product was 330bp and the target gene was mt cyt b (Fig. 1b).

The results of specificity test indicated that there was no cross reactivity in LAMP technique and its specificity for detection of *F. solani* was 100% (Fig. 2a).The same results were obtained by PCR method (Fig. 2b). The detection DNA limit of LAMP technique is one copy of genome(Fig. 3b). The same results were obtained by PCR test(Fig. 3a).

Using the LAMP method for 100 clinical samples, 2 out of 65 negative samples for *HSV* were found to be positive for *F. solani* (Figs. 3a). However, among the 35 positive samples for HSV, no positive case for *F. solani* was detected (data not shown). The same results were obtained by the PCR test (Fig 3b). Comparison of the results of the two methods by chi-squared test revealed that both of them are equal for diagnosis of *F. solani* in corneal samples.

Table 1. Features of primers

Name of primer	Sequence of primer
F3fso	5'-GCTTCTCCCGAGTCCCAA-3'
B3fso	5'-AGGAACCCTTACCGAGCT-3'
FIPfso	5'-CTTTGTCCAACGTCGCCCGAGTTTTGCGGTTCGACCGTAAT-3'
BIPfso	5'-AACACCAAACCCTCTTGGCGCAGCGGCTTCCTATTGTTGAA-3'
Lbfso	5'-GCATCACGTGGTTCATAACAGACA-3'
Lffso	5'-GGGGTAAATGCCCCACCAAAAA-3'

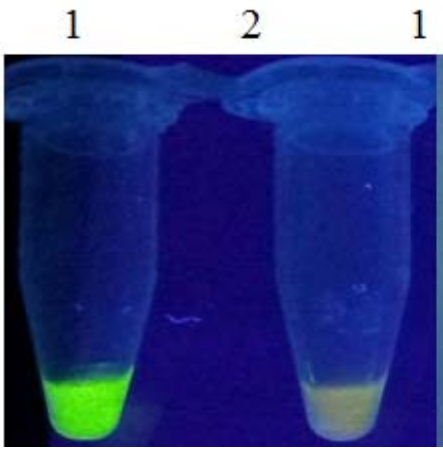

Fig. 1(a). LAMP optimization
1: Positive reaction
2: Negative reaction

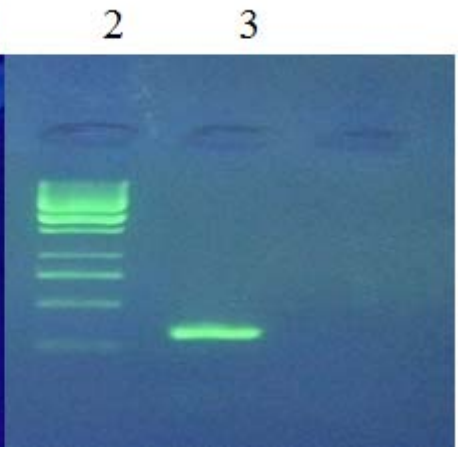

Fig. 1 (b). PCR optimization
1: Size marker 1kb termoscientific
2: Positive control (330bp)
3: Negative control

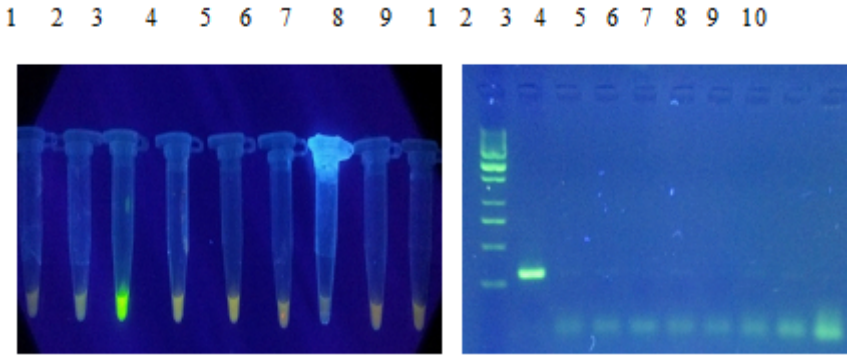

Fig. 2a: 1: *Streptococcus pneumoniae*
2: *HSV*
3: *F. solani*
4: *Staphylococcus aureus*
5: *F. oxysporum*
6: *F. verticillioides*
7: *F. poea*
8: *F. graminearum*
9: *F. prolifratum*

Fig. 2b: 1: Middle Range DNA Ladder 1113 Termoscientific
2: *F.solani*
3: *HSV*
4: *Streptococcus pneumoniae*
5: *Staphylococcus aureus*
6: *F. oxysporum*
7: *F. verticillioides*
8: *F. poea*
9: *F. graminearum*
10: *F. prolifratum*

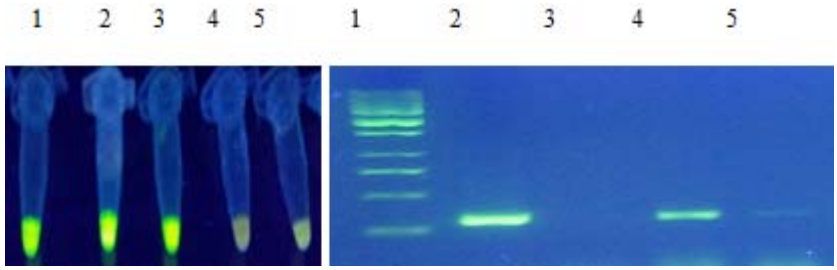

Fig.3a: 1: Positive control
2&3: Positive sample
4: Negative sample
5: Negative control

Fig. 3b: 1: Size marker DNA Ladder 1Kb Termoscientific
2: Positive control
3: Negative control
4: Positive sample
5: Negative sample

DISCUSSION

F. solani is the most virulent *Fusarium* species, which has been reported as an species most frequently isolated from mycokeratitis cases, over the last two decays[1-10]. However, the epidemiological pattern of *Fusarium keratitis* varies from country to country[10, 11]. As it mentioned before, it may also occur as a mixed infection with herpes simplex virus. Moreover, similarities in its clinical features sometimes lead to misdiagnosis[11]. Early identification of *Fusarium* at the species level is very important. It has a high level of resistance to several antifungal drugs, on the other hand, the different species have different patterns of sensitivity to antifungal drugs. Thereby, sometimes it may require combination therapy[3, 20, 21].

Identification of the pathogen at genus level is not difficult, while identification of it at the species level requires a greater degree of expertise. Traditional identifications are very time-consuming, and they are based on morphological methods which are cumbersome and requiring adequate training. Consequently, the identification of 33 to 55% of *Fusarium* isolates is either erroneous or missing [(3, 22)]. To solve these problems, new molecular diagnostic tests have been developed. PCR-based methods have been developed to detect *F. solani* in corneal samples[1, 2]. Although these assays are effective for *F. solani* detection, the necessity of utilizing relatively expensive laboratory equipment limits their

usefulness. On the other hand, LAMP can be performed with minimal laboratory facilities such as heating block or water bath. Furthermore, the use of a fluorescent dye or visual assessment of turbidity reduces the need for time-consuming post-PCR procedures such as agarose gel electrophoresis.

LAMP method is a very powerful tool for identifying species, particularly, in a mix infection. Because of using six primers for target gene in this technique, it is highly specific to identify species. Some important factors, including efficacy of DNA extraction and quality of designed primers, can affect the sensitivity and specificity of the assay[23-25]. In order to use molecular methods like RAPD, RFLP and PCR for isolation of Fusarium, the DNAs were previously developed[26-28]. The major objective of the current study was to develop LAMP method and further optimize it for specific, sensitive and rapid detection of organisms directly in a sample, even in a low technology laboratory. In this study, LAMP test was carried out in 1 h. However for the same analysis by PCR method, the time consumed was 3 h. In addition to rapidity, we observed an equal level of sensitivity and specificity using the LAMP method compared to gold standard PCR test. In some other studies, a higher level of sensitivity has been reported for LAMP method as compared with PCR[29-32]. In general, LAMP offers a better alternative, with its major advantages being possibility of visual judgment by color, being time saving, and being independent of costly PCR apparatus and gel scanner.

As it mentioned above,quality of designed primers and the sequences of elected target genes can affect the sensitivity and specificity of the molecular assays[3, 29, 33]. Different molecular studies employ richly varied sequences of multiple loci such as elongation factor 1α (EF-1α)), β-tubulin (β-TUB), calmodulin (CAM), RNA polymerase II second largest subunit (RPB2), the nuclear ribosomal ITS region, domains D1 and D2 of the 28S ribosomal DNA (rDNA) large subunit for *Fusarium* sp. identification. However, the available data clearly demonstrate that sequences from the nuclear ribosomal Internal Transcribed Spacer (ITS) region and domains D1 and D2 of the 28S ribosomal DNA (rDNA) large subunit are too conserved to resolve most of the clinically important fusaria at the species level. Moreover, use of (ITS) and ²-tubulin within *F. solani* species complex should be avoided due to paralogous or duplicated divergent alleles[3, 34-36]. In this study, TEF-1α was considered as the target gene. According to the reported results by Arif et. al. (2012), this sequence was highly specific for *F. solani* and did not show cross reaction with any other similar species of *Fusarium*[36]. This agreed with the results obtained in the current study. As shown in Fig. 2a, the specificity of the test is 100%. Therefore, the established test can precisely detect *F. solani* in the sample and differentiate the organism even in a mixed infection. Moreover, as it can detect even one copy of genome in the sample, this technique can be very effective for rapid and accurate diagnosis of *F. solani* in ocular clinical samples.

In the current study, no cases of *F. solani* as a mixed infection were observed among the 35 positive samples.Perhaps, some more cases needed to be examined to investigate the probability of mixed infection. In the other group of samples, among the 65 negative samples for *HSV*, two cases were found to be positive for *F. solani*. Supposedly, because of similarities in clinical features they had been initially misdiagnosed as herpetic keratitis.

Early diagnosis is important to manage the consequences of infections. The results of different researches have shown combination therapy to be effective in curing fusarium infections[37-40]. LAMP, as a more recent molecular technique for diagnosis, enjoys some advantages like rapidity, sensitivity, cost effectiveness as allowing for easy visual judgment of the result. To the best of our knowledge, this is the first attempt to establish this technique for detection *F. solani* in corneal samples. The obtained results were compared with the results of gold standard PCR method by chi-squared test. Both tests were equal for diagnosis of *F. solani* in corneal samples. Considering the specificity and sensitivity of the optimized test, it can be useful for precise identification of pathogen at early stage of infection and it can be used in every clinical laboratories instead of PCR. This method can be suggested to be applied for groups which also bear a high risk for disseminated fusariosis.

CONCLUSION

The results of this study indicate that some cases of corneal infection suspected to herpetic keratitis are due to *F. solani* which can be detected by molecular methods. Some advantages like rapidity, accuracy and simple equipments required for LAMP assay have made a better alternative for gold standard PCR even in low technology laboratories.

ACKNOWLEDGEMENTS

The authors would like to appreciate the science based Iraian gene fanavar (IGF) institute and its respectful personnel (Sam Masahi, Atefeh Shahhosseiny, Elham Moslemi, Mohammad Amin Mahmudi), for great help and generosity and also wish to extend their gratitude to Dr. M. J. Najafzedeh, Dr. S. Bayanolhagh and Dr. A. R. Khosravi for providing the samples.

REFERENCES

1. Dan H., Jilong H., Zhang B., Yang Y., Song W., Zhang Y., Yokoyama K. and Wang L.,Pathogenic Spectrum of Fungal Keratitis and Specific Identification of *Fusarium solani.* iov.2011; **52** (5); 2804-2808.
2. Godoy P., Cano J., Gené J., Guarro J., Ana Luisa Höfling-Lima, and Arnaldo Lopes Colombo. Genotyping of 44 Isolates of *Fusarium solani* the Main Agent of Fungal Keratitis in Brazil. *J Clin Microbiol*. 2004; **42**(10): 4494–4497.
3. Jain P.K. , Gupta V.K. , Misra A.K. , Gaur R. , Bajpai V. and Issar S. Current Status of Fusarium Infection in Human and Animal. *Asian Journal of Animal and Veterinary Advances*.; 2011; **6**: 201-227.
4. Srinivasan M. , Gonzales C.A., George C. *et al.* Epidemiology and etiological diagnosis of corneal ulceration in maduria, south India. *Br. J. Ophthalmol.* 1997); **81**: 965-971.
5. Thomas P. A., Abraham D.J. , Kalavathy C.M., Rajasekaran J. Oral itraconazole thrapy for mycotic keratitis. *Mycoses*. 1998; **31**: 271-279.
6. Gugnani H.C., Gupta S., Talwar R.S. Role of opportunistic fungi in ocular infections in Nigeria. *Mycopathologia*, 1978; **65**: 155-166.
7. Mselle J. Fungal keratitis as an indicator of HIV infection in Africa. *Trop. Doct.;* 1999; **29**: 133-135.
8. Rosa R.H., Miller D., Alfonso E.C. The changing spectrum of fungal keratitis in sauth of florida. *Ophthalmology*. 1994;**101**: 1005- 1013.
9. Gupta V., Dada T., Vajpayee RB., Sharma N., Dada VK. Polymicrobial keratitis after laser in situ keratomileusis. *J. Refract surg* . 2001;**1**, NO17:147-148.
10. Dóczi I., Gyetvai T., Kredics L., Nagy E. Involvement of *Fusarium* spp. in fungal keratitis.*Clinical Microbiology and Infection*.2004 ;**10**(9), 773–776.
11. Akshaya.R, Amrutha.P.A Study on the Epidemiological and Microbiological Aspects of Keratomycosis, Hyderabad, South India.*The journal of young investigators.* 2010; **20**(1) :2-7
12. Mori Y., Hirano T., Notomi T. Sequence specific visual detection of LAMP reactions by addition of cationic polymers . *BMC Biotechnology.* 2006; 3-6.
13. Nagamine K., Kuzuhara Y., Notomi T. *Isolation of Single-Stranded DNA from Loop-Mediated Isothermal Amplification Products*. *Biochemical and Biophysical Research Communications*. 2002; **290**. 1195–1198.
14. Fukuta S., Ohishi K., Yoshida K., Mizukami Y., Ishida A., and Kanbe M. Development of immunocapture reverse transcription loop-mediated isothermal amplification for the detection of tomato spotted wilt virus from chrysanthemum," *Journal ofVirological Methods*.2004:**121**(1), 49–55.
15. Guan X. Y., Guo J. C., Shen P., Yang L. T., and Zhang D. B. Visual and rapid detection of two geneticallymodified soybeanevents using loop-mediated isothermal amplification method . *Food Analytical Methods*. 2010; **3**(4), 313–320.
16. Nemoto J., Sugawara C., Akahane K.et al.Rapid and specific detection of the thermostable direct hemolysin gene in *Vibrio parahaemolyticus* by loop-mediated isothermal amplification. *Journal of Food Protection.* 2009; **72**(4), 748–754.
17. Tsujimoto M., Nakabayashi K., Yoshidome K., et al. One-step nucleic acid amplification for intraoperative detection of lymph node metastasis in breast cancer patients," *Clinical Cancer Research.* 2007; **13**(16), 4807–4816.
18. Nie X. Z. Reverse transcription loop-mediated isothermal amplification of DNA for detection of *Potato virus Y. Plant Disease.* 2005; **89**(6), 605–610.
19. Yang H., Lee Y., Inoue N., Kumar Jha B., Sylvatrie D.,et al. Loop-Mediated Isothermal Amplification Targeting 18S Ribosomal DNA for Rapid Detection of *Acanthamoeba*. Korean J Parasitol., 2013; **51**(3):269-277.
20. Jing L., Liping Xu., Jinlong G., Rukai C., Paul

M., and Youxiong Q. Development of Loop-Mediated Isothermal Amplification for Detection of *Leifsonia xyli* subsp. *xyli* in Sugarcane. Hindawi Publishing Corporation BioMed Research International. 2013; Article ID 357692, 8 pages

21. Ashwini K. Esnakula, Irorere Summers, and Tammey J. Naab. Fatal Disseminated *Fusarium* Infection in a Human Immunodeficiency Virus Positive Patient .Case Reports in Infectious Diseases . (2013);Volume 2013, Article ID 379320, 5 pages.
22. Nucci M. and Anaissie E. Fusarium infections in immunocompromised patients. *Clinical Microbiology Reviews;* 2007; **20**(4), 695–704.
23. Lo¨effler J., Schimdt K., Herbart H., Schumacher U., Einsele H. Automated extraction of genomic DNA from medically important yeast species and filamentous fungi by using the MagNA Pure LC system. *J Clin.Microbiol* . 2002 ; **40**: 2240–3.
24. Lo¨effler J., Herbart H., Schumacher U., Reitze H., Einsele H. Comparison of different methods for extraction of DNA of fungal pathogens from cultures and blood. *J Clin Microbiol* . 1997 ;**35**:3311–2.
25. Einsele H., Herbart H., Roller G., *et al*. Detection and identification of fungal pathogens in blood by using molecular probes. *J Clin Microbiol*.1997; **35**:1353–60.
26. Francois-Xavier H., Huerre M., Ange Rouffault M. and de Bievre C.Specific Detection of Fusarium Species in Blood and Tissues by a PCR Technique. *J. Clin. Microbiol*. 1999; **37** (8); 2434-2438.
27. Godoy P., Cano J., Gene J., Guarro J., Hofling-Lima AL., Lopes Colombo A.Genotyping of 44 isolates of *Fusarium solani*, the main agent of fungal keratitis in Brazil. *J Clin Microbiol.* 2004; **42**:4494-4497.
28. Thomas PA. Fungal infections of the cornea. *Eye (Lond).* 2003; **17**: 852-862.
29. Oriel M., Thekisoe M., *et al.* (LAMP), PCR and parasitological tests for detection of Trypanosoma evansi in experimentally infected pigs. Veterinary Parasitological. 2005; **130**: 327–330.
30. Kuboki, N. *et al*. Loop-mediated isothermal amplification for detection of African trypanosomes. *J. Clin.Microbiol* 2003; **41**: 5517–5524.
31. Bakheit MA., Torra D., Palomino LA., Thekisoe OM., Mbati PA., Ongerth J., Karanis P. Sensitive and specific detection of Cryptosporidium species in PCR-negative samples by loop-mediated isothermal DNA amplification and confirmation of generated LAMP products by sequencing. *Vet Parasitol*. 2008; **25**;158(1-2):11-22.
32. O'Donnell K., Sarver B.A.J., Brandt M., Chang D.C. , Noble-Wang J. , *et al*. Phylogenetic diversity and microsphere array-based genotyping of human pathogenic fusaria, including isolates from the multistate contact lens-associated U.S. keratitis outbreaks of 2005 and 2006. *J. Clin. Microbiol.* 2007; **45**: 2235-2248.
33. Lewis White P., Christopher J. Linton, Michael D. Perry, Johnson M., and Rosemary A. Barnes. The Evolution and Evaluation of a Whole Blood Polymerase Chain Reaction Assay for the Detection of Invasive Aspergillosis in Hematology Patients in a Routine Clinical Setting. *Clinical Infectious Diseases.* 2006; **42**: 479-86.
34. Helm T.N., Longworth D.L., Hall G.S., Bolwell B.J., Fernandez B. and Tomecki K.J. Case report and review of resolved fusariosis. *J. Am. Acad. Dermatol.* 1990; **23**: 393-398.
35. Guarro J., Rubio C., Gene J., Cano J.and Gil J. *et al*. Case of keratitis caused by an uncommon *Fusarium* species. *J. Clin. Microbiol.* 2003; **41**: 5823-5826.
36. Arif M., Chawla S., Zaidi N. W., Rayar J. K. Variar , M. , Singh U. S. Development of specific primers for genus Fusarium and F. solani using rDNA sub-unit and transcription elongation factor (TEF-1α) gene. *African Journal of Biotechnology* 2012; **11**(2): 444-447.
37. Eljaschewitsch J., Sandfort J., Tintelnot K., Horbach I., and Ruf B. Port-a-cath-related Fusarium oxysporum infection in an HIV-infected patient: treatment with liposomal amphotericinB, *Mycoses*. 1996; **39**(3-4): 115–119.
38. Muhammed M., Coleman J. J., Carneiro H. A., and Mylonakis E. The challenge of managing fusariosis. *Virulence*. 2011; **2**(2): 91–96.
39. Perfect J. R.The impact of the host on fungal infections. *American Journal of Medicine*. . 2012; **125**(1): S39–S51.
40. Ho D. Y., Lee J. D., Rosso F., and Montoya J. G. Treating disseminated fusariosis: amphotericin B, voriconazole or both?.*Mycoses*. 2007; **50**(3): 227–231.