**Original Article**

# Utility of Filter Paper for Preserving Insects, Bacteria, and Host Reservoir DNA for Molecular Testing

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## Abstract

**Background:** Appropriate methodology for storage biological materials, extraction of DNA, and proper DNA preservation is vital for studies involving genetic analysis of insects, bacteria, and reservoir hosts as well as for molecular diagnostics of pathogens carried by vectors and reservoirs. Here we tried to evaluate the utility of a simple filter paper-based for storage of insects, bacteria, rodent, and human DNAs using PCR assays.

**Methods:** Total body or haemolymph of individual mosquitoes, sand flies or cockroaches squashed or placed on the paper respectively. Extracted DNA of five different bacteria species as well as blood specimens of human and great gerbil *Rhombomys opimus* was pipetted directly onto filter paper. The papers were stored in room temperature up to 12 months during 2009 until 2011. At monthly intervals, PCR was conducted using a 1-mm disk from the DNA impregnated filter paper as target DNA. PCR amplification was performed against different target genes of the organisms including the ITS2-rDNA of mosquitoes, mtDNA-COI of the sand flies and cockroaches, 16SrRNA gene of the bacteria, and the mtDNA-CytB of the vertebrates.

**Results:** Successful PCR amplification was observed for all of the specimens regardless of the loci, taxon, or time of storage. The PCR amplification were ranged from 462 to 1500 bp and worked well for the specified target gene/s. Time of storage did not affect the amplification up to one year.

**Conclusion:** The filter paper method is a simple and economical way to store, to preserve, and to distribute DNA samples for PCR analysis.

**Keywords:** DNA, PCR, Insects, Bacteria, Vertebrate, Preservation, Filter paper

## Introduction

Vector-borne diseases (VBDs) such as malaria, dengue fever, leishmaniasis, filariasis, trypanosomiasis, yellow fever, onchocerciasis, plaque, and many other ones continue to threaten and to outbreak world health. Annually around 500 million people are affecting just from three VBDs: 300 M malaria cases, 120 million filariasis cases, and 50–100 M dengue cases (WHO 2000, 2009). The VBDs account for 17% of the global disease burden (Tabachnick 2010).

Understanding and predicting the VBDs epidemiology relies on clear knowledge about the basic biology of the organisms and the interactions among vectors-pathogens-hosts involved. Correct identification and population genetics of vectors, also detection and identification of pathogen species within insect or arthropod vectors, human, or animal host reservoirs are important for predictions of the risk and expansion of the disease in the endemic areas. Conventional methods such as dissection of individual insects under microscope, xenodiagnosis, culture media, and serological methods inherit a number of limitations including needs of fresh speci-

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mens, low sensitivity and specificity, requirement to a highly skilled person, high expenses, and time consuming. On the other hand, DNA based techniques are now available and becoming powerful tools for VBDs research.

Molecular diagnostic tests that require DNA or RNA from specimens may be used to detect and to identify the vectors, pathogens, blood meal origin, and reservoirs species (Oshaghi et al. 2003, 2006, 2008, 2010, 2011a, Nakazawa et al. 2009, Maleki-Ravasan et al. 2010, Mehravarana et al. 2011). The laboratories that are involved in VBDs in endemic areas now gradually accommodate to use DNA/RNA based methods and this need cheap, easy, and reliable protocols that allow for the preservation of nucleic acids specimens. The currently recommended method for long-term storage of specimens involves storing them in freezer at minus 20 to -70°C. Storage of the specimens in freezer takes a large amount of space particularly in the laboratory with high loads of samples. This might be less problematic for insect specimens but becomes extremely for tissues of animals.

In molecular studies, DNA is extracted from individual or pooled specimens using an expensive kit or by a complicated conventional protocol using proteinase K-containing DNA extraction buffer followed by phenol/chloroform extraction and ethanol precipitation. These methods are suitable for molecular testing of vector, reservoirs, human, and pathogens; however, less effort and cost are desirable to improve the methods (Weigle et al. 2002).

Transporting the specimens to the laboratory without loss of DNA integrity under field conditions is important. Conventionally the insect specimens preserve dry or in alcohol or lactophenol, and transfer in box or in cold chain. Human or animal blood or tissues are preserved in citrated tubes and alcohol respectively. The specimens need additional labor costs for their storage if their DNA is not immediately extracted. Following DNA extraction, specimens should be kept again in an ultralow-temperature freezer which is expensive and inconvenient and occupies a considerable amount of laboratory space. This is more crucial for VBDs because the rate of infection in vectors usually is very low even in endemic areas (Oshaghi et al. 2009, 2010, 2011b) and one should extract or keep the extracted DNA of thousands insects individually. In addition, DNA infection and degradation of DNA by DNAase enzymes during the time of storage are another common issue raised in molecular laboratories.

Use of filter paper for storing DNA initially have been used for a wide range of biological sources such as whole blood, buccal scrapes, tissues, plasmids, plant material and microorganisms (Rogers and Burgoyne 1997, Devost and Choy 2000, Rogers and Burgoyne 2000, Natarajan et al. 2000, Lampel et al. 2000, Dobbs et al. 2002, Hide et al. 2003, Lampel et al. 2004) and later have been used for different goals in epidemiological studies of VBDs. The main advantage of filter papers is the long-term stability of genomic DNA within the cells of unfixed, dried whole body or blood cells stored at room temperature. The filter paper is provided in a small card size, which makes it possible to store several hundred samples at room temperature in a volume, the size of a small table driver. The filter paper matrix is impregnated with agents that denature infectious agents, and thus samples are no longer considered a biohazard. This allows for the storage and transport of samples without selective biohazard precautions.

Here, we investigated the use of filter paper cards as a means of storing either cell suspensions of different insect and vertebrate species including *Anopheles stephensi* Liston, *Culex quinqufaciatus* Say, *Phlebotomus papatasi* Scopoli, *Periplaneta americana*

Linnaeus, *Blattella germanica* Linnaeus, human, and rodent *Rhombomys opimus* Lichtenstein or extracted DNA of various bacteria comprising *Staphylococcus* spp., *Hafnia alvei, Cronobacter sakazaki, Escherichia coli,* and *Enterobacter* spp. The stability of the DNA within the suspension cells or the extracted DNA from the bacteria stored for as long as one year onto the filter papers were tested by polymerase chain reaction (PCR) of different loci of the vertebrates, invertebrates, and microorganism genomes.

## Materials and Methods

### Materials

Specimens of *An. stephensi, Cx. quinquefasciatus, P. americana, B. germanica* and *Ph. papatasi* were obtained from the insectariums of School of Public Health, Tehran University of Medical Sciences (SPH-TUMS). Human blood obtained from National Blood Center (Tehran), and *R. opimus* blood tissues provided from tails of the animal reared in animal house of the SPH-TUMS. The strains of *Staphylococcus* spp., *Hafnia alvei, Cronobacter sakazaki, Escherichia coli*, and *Enterobacter* spp bacteria obtained from individual colonies that were cultured in Brain Heart Infusion (BHI). These bacteria were isolated from the midguts of American cockroaches that were collected from a confectionary shop in Tehran.

Kawsar DNA bank card (DBC®) paper was obtained from Kawsar Biotech Company (KBC, IR Iran) consisting of filter paper impregnated with a proprietary mix of chemicals, which serve to lyse cells, to prevent the growth of bacteria, and to protect the DNA in the sample. The package also includes the reagents for the extraction of DNA from the cells trapped on the filter paper.

### DNA extraction

Fresh or alcohol preserved insect specimens were crushed individually using a glass pestle on DBC® card, allowed to air-dry and the cards were kept in sealed plastic bags at room temperature until subsequent experiments. Haemolymphs of the field or laboratory cockroaches were collected using yellow tips after dissection of the specimens and were pipetted directly on the filter paper. For bacteria, colonies of the species were used for DNA extraction using QIAGEN DNeasy Kit (Qiagen, Germany) according the manufacturer supplied procedure. Also fifty to hundred micro liter of blood tissue of human or rodent *R. opimus* was pipetted directly onto the filter paper. Based on the kind and volume of the specimen 3–10 square millimeter ($mm^2$) of the paper might be impregnated with DNA, which can provide 9–100 1-mm punches. The papers were stored in room temperature up to 12 months. At monthly intervals, the DNA was extracted from the dried cells on the filter paper card according to the manufacturer's instructions. A 1-mm hole was punched out from the center of the sample collection circle on the sample-impregnated filter paper and this punch was placed in a 1.5-mL microfuge tube for extraction of the DNA. The disks were washed with 200µl of DBC® buffer for 5 minute at room temperature. After removing the buffer, the punches were washed three times with 200µl ddH2O for five minutes. Then $ddH_2O$ completely emitted and the disks became air-dried at room temperature for 15 min. These washed filters were used directly in the PCR reactions.

### PCR

PCR amplifications were performed using the sets of the specific primers previously used to amplify the ITS2-rDNA of mosquitoes (Litvaitis et al. 1994), mtDNA-COI of insects (Folmer et al. 1994), mtDNA-CytB of vertebrates (Boakye et al. 1999), and 16SrRNA of bacteria (Favia et al. 2007). The sequences of the primers are given in table 1. Amplification reaction was performed in

volume of 20 µl containing 1-mm punch paper as target template, 10 pmol of each primer, and PCR Master Mix (iNtRON®, South Korea) includes 1.0 mM Mgcl2, 100 µM dNTPs, 2 µl 10X PCR buffer, and 1unit of Taq polymerase.

For ITS2, PCR was optimized for mosquito templates. An initial denaturation of 5 min at 94 °C was followed by 32 cycles at 94 °C for 1 min, 57 °C for 1 min and 72 °C for 1 min. The final extension step was 72 °C for 10 min. For COI, PCR was optimized for sand flies and cockroaches templates. An initial denaturation of 2 min at 94 °C was followed by 5 cycles at 94 °C for 40 sec, 45 °C for 40 sec, 72 °C for 1 min and 35 cycles at 94 °C for 40 sec, 51 °C for 40 sec, 72 °C for 1 min. The final extension step was 72 °C for 10 min. For CytB, PCR was optimized for vertebrate templates. An initial denaturation of 5 min at 94 °C was followed by 35 cycles at 95°C for 1 min, 58 °C for 1 min and 72 °C for 1 min. The final extension step was 72 °C for 7 min. The universal primers of 16suF and 16suR (Favia et al. 2007) were used to amplify about 1.5 kilo base (kb) partial sequence of the 16S rRNA gene of the bacteria. The PCR conditions were set as an initial denaturation at 94°C for 10 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 56.5°C for 40 seconds, and extension at 72°C for 30 seconds, and final extension at 72°C for 8 minutes. Fifteen microliters of PCR products were run along with a 100 bp ladder on a 1.2% agarose gel containing ethidium bromide for 1 h at 70V and observed on a UV transilluminator.

## Results

Insects, bacteria, or blood samples were obtained for at least 20 specimens and their suspension cells or their extracted DNA was stored on DNA cards. These paper cards were kept at room temperature until use, with a variable storage period ranging from 30 days to one year. The suspension cells of sand flies, mosquitoes, and cockroaches as well as the bacterial DNA were pale or did not stain the paper whereas the dried bloods stained the specimen cards. DNA of the punched discs was tested in 1% agarose gel and quality and quantity were examined by comparison of the sharpness of DNA bands. The yields and quality of DNA per disc were variable based on the organisms and tissue stored on the paper. Generally blood tissues made fewer smears and more sharpness DNA than other tissues.

The quality of purified DNA was assessed by PCR amplifications of different loci of the organisms. Except a few cases, results of PCR showed 100% success rate according to the presence/absence of the length specific PCR products (Fig. 1–2). There were no differences in the robustness of the PCR reactions between the various species as well as their DNA extracted at different times. The cells were stored on the filter paper cards up to 12 months preserved the DNA healthy before DNA extraction. Following DNA extraction, no PCR failures were seen in the specimens stored for about one year. The PCR products of different loci against the DNA extracted from the filter paper are shown in Fig. 1 and 2. For cytB gene of the vertebrate specimens, the amplified products were a single band of the expected size (624 bp) on the agarose gel. For ITS2-rDNA the PCR products were varied that was 462 bp for *Cx. quinqufaciatus* and 640 bp for *An. stephensi*. The amplicon for the COI gene of *Ph. papatasi* was 711 bp. The biggest PCR product with 1500 bp was for the bacteria. *Hafnia alvei*, *Enterobacter sakazakii*, *Escherichia coli*, and *Enterobacter* bacteria are gram negative and species of *Staphylococcus* spp. are gram positive.

**Table 1.** The primer sequences, target genes and species, and length of PCR products used in this study

| Primer | Sequence 5'-3' | Locus | Target genome | PCR size (bp) |
|---|---|---|---|---|
| **5.8S** | TGTGAACTGCAGGACACATGAA | ITS2-rDNA | *Culex quinqufaciatus* | 462 |
| **28S** | ATGCTTAAATTAGGGGGTAGTC | | *Anopheles stephensi* | 640 |
| **COIF** | TTAAACTTCAGGGTGACCAAAAAATCA | mtDNA-COI | *Phlebotomus papatasi* | 709 |
| **COIR** | GGTCAACAAATCATAAAGATATTGG | | *Periplaneta americana* *Blattella germanica* | |
| **Un1025F** | GGTTGTCCTCCAATTCATGTTA | mtDNA-CytB | *Homo sapiens* | 624 |
| **UN403R** | TGAGGACAAATATCATTCTGAGG | | *Rhombomys opimus* | |
| **16SUF** | TCGACATCGTTTACGGCGTG | 16S rRNA | *Staphylococcus* spp., *Hafnia alvei*, *Cronobacter sakazaki*, *Escherichia coli*, *Enterobacter* spp | 1500 |
| **16SUR** | AGAGTTTGATCCTGGCTCAG | | | |

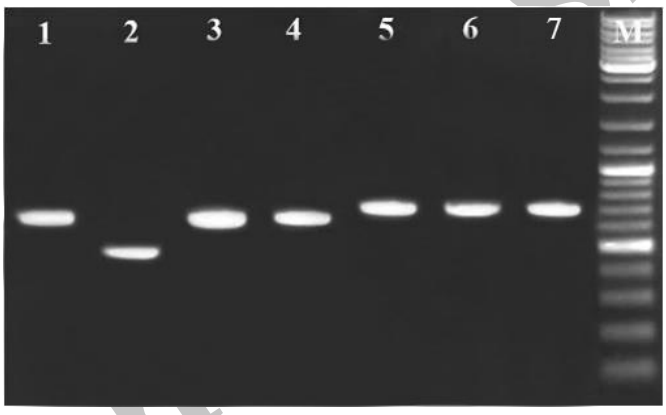


**Fig. 1.** Agarose gel showing the PCR products amplified from filter paper extractions: lane 1, ITS2-rDNA of *An. stephensi* (640 bp), lane 2, ITS2-rDNA of *Cx. quinquefasciatus* (462 bp), lanes 3–4, host-specific cytochrome *b* of human and *R. opimus* (624 bp) respectively; lanes 5–7, mtDNA-COI of *Ph. papatasi*, *Periplaneta americana,* and *Blattella germanica* (709 bp) respecyively, and M, a 100 bp ladder (Fermentas)

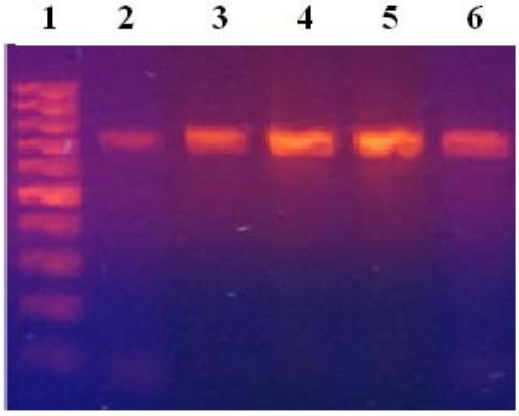


**Fig. 2.** 16SrRNA gene PCR products (1500 bp) of bacteria amplified from their DNA stored on filter paper: lane 1, a 200 bp ladder, Lane 2, *Staphylococcus* sp, lane 3, *Hafnia alvei*, lanes 4, *Cronobacter sakazaki*, lane 5, *Escherichia coli*, lane 6, *Enterobacter* sp.

## Discussion

In this study, we demonstrated the utility of using the filter paper cards to preserve the extracted DNA or DNA in cells respectively from bacteria or from fresh or alcohol preserved mosquitoes, sand flies, cockroaches or blood tissues of human and rodent specimens. We found the filter paper as a convenient and relatively inexpensive method for the storage and transportation of the specimens that may require DNA testing. Successful PCR amplification of four different loci (ITS2-rDNA, 16SrRNA, mtDNA-COI, and mtDNA-CytB genes) proved that DNA extracted from the cells stored on the filter paper functioned very well with no significant differences in the ability of the templates to produce PCR products. Use of filter papers previously have reported for malaria parasite species identification in human blood (Syafruddin et al. 2007, Lekweiry et al. 2009, Ataei et al. 2011), for extraction and storage of insect DNA in forensic entomology (Harvey 2005), for detection and identification of the filarial parasite *Brugia timori* in human blood (Fischer et al. 2002), for source of mosquito blood meals (Ansell et al. 2000), and for detection of *Wuchereria bancrofti* DNA in human blood (Rao et al. 2006). Also, filter papers have been used for blood meal identification and *Leishmania* parasite detection in sand fly *Lutzomyia longipalpis* (Schaefer et al. 1995, Sant'Anna et al. 2008), identification of trypanosome in wild tsetse populations (Adams et al. 2006), diagnosis and surveillance of *Trypanosoma vivax* in ruminants (Gonzales et al. 2006), detection of *Cyclospora cayetanensis* and *Cryptosporidium parvum* from food samples and human fecal specimens (Orlandi and Lampel 2000), and archiving and processing DNA from fresh water protozoans (Hide et al. 2003). The application of the filter paper for molecular testing using RNA such as microarrays or RT-PCR assays, which need a stable source of RNA, was not addressed in this study. However, others have shown that RNA is stable on the filter paper (Nakazawa et al. 2009).

In this study, the preservation is only tested on DNA regions that have high copy number on the genome which are easy to amplify. Therefore to make a better assessment of the filter paper, it is recommended to test them against the genes/loci with low or single copy number.

The archival storage of specimens for future DNA analysis is greatly facilitated by using the filter paper because the cards are small and are stored at room temperature in a compact file until molecular studies are needed. In this study, the DNA was shown to be stable for up to one year, and DNA has been reported by the manufacturer to be stable on the cards for at least 30 years. This stability diminishes the need for an ultralow-temperature freezer dedicated to specimen storage, which both is expensive and requires laboratory space. In addition, specimens stored at -20 °C to -70 °C in an ultralow-temperature freezer are at risk should the freezer suffer a mechanical or electrical failure. Once a frozen specimen thaws, severe degradation of the DNA can result unless it is immediately extracted. The diameter of punched disc from filter papers was as low as 1-mm hence allows using the rest of specimens for many other molecular analyses. In addition, it is shown that the reagents on the filter paper are designed to kill pathogens upon contact and the papers protect DNA within the samples for several years at ambient conditions (Smith and Burgoyne 2004).

In conclusion, the filter paper is an inexpensive and user-friendly method for storing cell suspensions from different specimens. The card size index allows the convenient storage and transportation of speci-

men DNA and makes the system ideal for small laboratories that rely on sending specimens to larger facilities for molecular analysis. Further studies are needed to validate this method of storage using a wide range of molecular methods including PCR, DNA microarrays, and expression analysis with RNA.

It seems that filter paper is a suitable, fast, safe and easy to use tool for storage and transportation of the samples directed from the field to the laboratory for DNA extraction or storage of extracted DNA.

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## References

Adams ER, Malele II, Msangi AR, Gibson WC (2006) Trypanosome identification in wild tsetse populations in Tanzania using generic primers to amplify the ribosomal RNA ITS-1 region. Acta Trop. 100: 103–109.

Ansell J, Hu JT , Gilbert SC, Hamilton KA, Hill AV, Lindsay SW (2000) Improved method for distinguishing the human source of mosquito blood meals between close family members. Trans Royal Soc Trop Med Hyg. 94: 572–574.

Ataei S, Nateghpour M, Hajjaran H, Edrissian G, Foroushani AR (2011) High specificity of semi-nested multiplex PCR using dried blood spots on DNA Banking Card in comparison with frozen liquid blood for detection of *Plasmodium falciparum* and *Plasmodium vivax*. J Clin Lab Anal. 25: 185–190.

Boakye DA, Tang J, Truc P, Merriweather A, Unnasch TR (1999) Identification of blood meals in haematophagous Diptera by Cytochrome b heteroduplex analysis. Med Vet Entomol. 13: 282–287.

Dobbs LJ, Madigan MN, Carter AB, Earls L (2002) Use of FTA gene guard filter paper for the storage and transportation of tumor cells for molecular testing. Arch Path Lab Med. 126: 56–63.

Devost NC, Choy FY (2000) Mutation analysis of Gaucher disease using dot-blood samples on FTA filter paper. Am J Med Genet. 94: 417–420.

Favia G, Ricci I, Damiani C, Raddadi N, Crotti E, Marzorati M, Rizzi A, Urso R, Brusetti L, Borin S, Mora D, Scuppa P, Pasqualini L, Clementi E, Genchi M, Corona S, Negri I, Grandi G, Alma A, Kramer L, Esposito F, Bandi C, Sacchi L, Daffonchio D (2007) Bacteria of the genus Asaia stably associate with Anopheles stephensi, an Asian malarial mosquito vector. Proc Natl Acad Sci USA. 104: 9047–9051.

Fischer P, Wibowo H, Pischke S, Rückert P, Liebau E, Ismid IS, Supali T (2002) PCR-based detection and identification of the filarial parasite Brugia timori from Alor Island, Indonesia. Ann Trop Med Parasit. 96: 809–821.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Marine Biol Biotech. 3: 294–299.

Gonzales JL, Loza A, Chacon E (2006) Sensitivity of different *Trypanosoma vivax* specific primers for the diagnosis of livestock trypanosomosis using different DNA extraction methods. Vet Parasitol. 136: 119–126.

Harvey ML (2005) An alternative for the extraction and storage of DNA from

insects in forensic entomology, J Forensic Sci. 50: 627–629.

Hide G, Hughes JM, McNuff R (2003) A rapid and simple method of detection of *Blepharisma japonicum* using PCR and immobilisation on FTA paper. BMC Ecol. 3: 7.

Lampel A, Orlandi PA, Kornegay L (2000) Improved template preparation for PCR based assays for detection of food bourne bacterial pathogens. App Environ Microb. 66: 4539– 4542.

Lekweiry KM, Abdallahi MO, Ba H, Arnathau C, Durand P, Trape JF, Salem AO (2009) Preliminary study of malaria incidence in Nouakchott, Mauritania. Malaria J. 8: 92.

Litvaitis MK, Nunn G, Thomas WK, Kocher TD (1994) A molecular approach for the identification of meiofaunal turbellarians (Piatyhelminthes, Turbellaria). Marine Biol. 120: 437–442.

Maleki-Ravasan M, Oshaghi MA, Javadian E, Rassi Y, Sadraei J, Mohtarami F (2009) Blood Meal Identification in Field-Captured Sand flies: Comparison of PCR-RFLP and ELISA Assays. Iran J Arthropod-Borne Dis. 3: 8–18.

Mehravaran A, Oshaghi MA, Vatandoost H, Abai MR, Ebrahimzadeh A, Moazeni Roodi, A, Grouhi A (2011) First report on *Anopheles fluviatilis* U in southeastern Iran. Acta Trop. 117: 76–81.

Natarajan P, Trinh T, Mertz L, Goldsborough M, Fox DK (2000) Paper-based archiving of mammalian and plant samples for RNA analysis. Biotechniques. 29: 1328–1333.

Nakazawa S, Marchand RP, Quang NT, Culleton R, Manh ND, Maeno Y (2009) *Anopheles dirus* co-infection with human and monkey malaria parasites in Vietnam. Int J Parasitol. 39: 1533–1537.

Orlandi PA, Lampel KA (2000) Extraction-free, filter-based template preparation for rapid and sensitive PCR detection of pathogenic parasitic protozoa. J Clin Microbiol. 38: 2271–2277.

Oshaghi MA, Sedaghat MM, Vatandoost H (2003) Molecular characterization of the *Anopheles maculipennis* complex in the Islamic Republic of Iran. East Mediterr Health J. 9: 659–666.

Oshaghi MA, Chavshin AR, Vatandoost H (2006) Analysis of mosquito blood meals using RFLP Markers. Exp Parasitol. 114: 259–264.

Oshaghi MA, Yaghobi-Ershadi MR, Shemshad K, Pedram M, Amani H (2008) The *Anopheles superpictus* complex: introduction of a new malaria vector complex in Iran. Bull Soc Path Exo. 101: 429–434.

Oshaghi MA, Maleki Ravasan N, Javadian EZ, Mohebali M, Hajjaran H, Zare Z, Mohtarami F, Rassi Y (2009) Vector incrimination of sand flies in the most important visceral Leishmaniasis focus in Iran**.** Am J Trop Med Hyg. 81: 572–577.

Oshaghi MA, Rasolian M, Shirzadi MR, Mohtarami F, Doosti S (2010) First report on isolation of *Leishmania tropica* from sandflies of a classical urban Cutaneous leishmaniasis focus in southern Iran. Exp Parasitol. 126: 445–450.

Oshaghi MA, Rassi Y, Tajedin L, Abai MR, Akhavan AA, Mohtarami F (2011a) Mitochondrial DNA diversity in the populations of great gerbils, *Rhombomys opimus*, main reservoir of cutaneous leishmaniasis. Acta Trop. 119(2–3): 165-17.

Oshaghi MA, Vatandoost H, Gorouhi A, Abai MR, Madjidpour A, Arshi S, Sadeghi H, Nazari M, Mehravaran A (2011b) Anopheline species composition in border line of Iran-Azerbaijan. Acta Trop. 119(1): 44–49.

Rao RU, Atkinson LJ, Ramzy RM, Helmy H, Farid HA, Bockarie MJ, Susapu M,

Laney SJ, Williams SA, Weil GJ (2006) A real-time PCR-based assay for detection of Wuchereria bancrofti DNA in blood and mosquitoes. Am J Trop Med Hyg. 74: 826–832.

Rogers CD, Burgoyne LA (1997) Bacterial typing: storing and processing of stabilized reference bacteria for polymerase chain reaction without preparing DNA-an example of an automatible procedure. Anal Biochem. 247: 223–227.

Rogers CD, Burgoyne LA (2000) Reverse transcription of an RNA genome from databasing paper (FTA). Biotechnol Appl Bioc. 31: 219–224.

Syafruddin D, Asih PB, Wahid I, Dewi RM, Tut,i S, Laowo I, Hulu W, Zendrato P, Laihad F, Shankar AH (2007) Malaria prevalence in Nias District, North Sumatra Province, Indonesia. Malaria J. 6: 116.

Sant'Anna MR, Jones NG, Hindley JA, Mendes-Sousa AF, Dillon RJ, Cavalcante RR, Alexander B, Bates PA (2008) Blood meal identification and parasite detection in laboratory-fed and field-captured *Lutzomyia longipalpis* by PCR using FTA databasing paper. Acta Trop. 107: 230–237.

Schaefer KU, Schoone GJ, Gachihi GS, Muller AS, Kager PA, Meredith SE (1995) Visceral leishmaniasis: use of the polymerase chain reaction in an epidemiological study in Baringo District, Kenya. T Roy Soc Trop Med H. 89: 492–495.

Smith LM, Burgoyne LA (2004) Collecting, archiving and processing DNA from wildlife samples using FTA databasing paper. BMC Ecol. 84: 4.

Tabachnick WJ (2010) Challenges in predicting climate and environmental effects on vector-borne disease episystems in a changing world. J Exp Biol. 213: 946–954.

WHO (2009) World malaria report, WHO Press, World Health Organization, Geneva.

WHO (2000) The World Health Report, WHO Press, World Health Organization, WHO Health Report- Prelims i-ixx/E.

Weigle KA, Labrada LA, Lozano K, Santrich C, Barker DC (2002) PCR Based Diagnosis of Acute and Chronic Cutaneous Leishmaniasis Caused by *Leishmania* (Viannia). J Clin Microbiol. 40: 601–606.