Screening of blood bank samples for the presence of malaria parasites by conventional methods and quantitative nucleic acid sequence-based amplification (QT-NASBA) assay

BAKRI Y. M. NOUR*, MD, PÈTRA F. MENS[†,‡], BSc, MSc, OSMAN K. SAEED*, MD, AHMED A. MOHAMADANI*, MD & HENK D. F. H. SCHALLIG[†], MSc, PhD

*Blue Nile Research and Training Institute, Faculty of Medicine, University of Gezira, Wad Medani, Sudan; [†]Koninklijk Instituut voor de Tropen (KIT)/Royal Tropical Institute, KIT Biomedical Research, [‡]Center for Infection and Immunity Amsterdam (CINEMA), Division of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Center, Amsterdam, the Netherlands

Correspondence to:
Dr H.D.F.H. Schallig, Koninklijk Instituut voor de Tropen/Royal Tropical Institute, KIT Biomedical Research, Meibergdreef 39, 1105 AZ Amsterdam, the Netherlands
E-mail: h.schallig@kit.nl

Publication data
Received: 9 May 2007
Revision received: 8 June 2007
Accepted: 11 June 2007

Keywords
- Blood safety
- Blood screening
- Malaria
- Nucleic acid sequence-based amplification
- Transfusion risks

SUMMARY

The potential of screening blood donations of apparently healthy donors for the presence of malaria parasites with quantitative nucleic acid sequence-based amplification (QT-NASBA) technology was assessed. One hundred samples were randomly collected from blood donations at the blood bank of Wad Medani Teaching Hospital (Central Sudan) and analyzed for *Plasmodium falciparum* contamination by standard microscopy, rapid diagnostic tests (RDTs) and QT-NASBA (lower detection limit for quantification is 0.1 parasite/μL of blood; absolute lower detection limit < 0.001 parasite/μL). Presence of *P. falciparum* could not be demonstrated in all samples analyzed by microscopy and RDTs. Eighty out of 100 samples were analyzed by QT-NASBA. Three samples were lost because of transportation difficulties and 17 gave an insufficient amount of RNA after extraction. Fifty-five donations were found to be negative for *P. falciparum*, i.e. < 0.01 parasite/μL of blood, four samples gave a result at the cutoff of the test, i.e. parasite count between > 0.001 and 0.1 parasite/μL. However, 21 of the 80 samples (26.3%) were found to be QT-NASBA-positive, i.e. parasites count > 0.1/μL of blood. Mean parasite count of the positive samples was 1.73 parasites/μL (ranging between 0.13 and 18.6 parasites/μL). The present study showed the sensitivity of QT-NASBA to detect low numbers of *P. falciparum* in apparently healthy donors.

INTRODUCTION

The safety of the blood supply is critical to many sectors of modern medicine and it is essential that transfusion services globally ensure the safety of the blood supply. Although most attention has been paid to viral infections as a complication of transfusion, a number of parasitic diseases, including malaria, are known or are

© 2007 The Authors
Journal Compilation © 2007 LMS Group
doi: 10.1111/j.1778-428X.2007.00065.x

suspected to be transmitted by blood transfusion.[1] The risk of transfusion-transmitted malaria (TTM) is not only present in highly endemic countries, but is also occurring in malaria-free countries.[2–7] Mortality due to TTM has been reported.[2,7]

Current measures to exclude potentially infected donors mainly rely on donor interviews, but the effectiveness of this is being debated.[6,8–10] Furthermore, immigration from and increased international travel to malaria-endemic countries by semi-immune people who still participate in blood donation may contribute to an increased risk of TTM.[2,10,11] On the other hand, many blood donations are discarded as a preventive measure, resulting in a significant loss of potentially noninfectious blood products, because the incidence of malaria in nonendemic counties is still low.[7,12]

The availability of a sensitive laboratory test to detect the presence of malaria parasites in potentially suspected blood donations would decrease the risk of TTM and increase the number of available donors. The screening of blood for the presence of *Plasmodium* species can be carried out with microscopy, antigen or antibody detection tests or molecular tests. Microscopy, the gold standard for the diagnosis of malaria, has been applied worldwide. However, even if expert microscopists perform the blood slide examination, the sensitivity of this technique is low (approximately 10–20 parasites/μL of blood) and time-consuming (20–30 minutes per slide at a low parasitemia),[13] rendering this methodology unsuitable for large-scale blood screening. Antigen detection tests, in the format of rapid diagnostic tests (RDTs), have become widely available. RDTs are based on the recognition of *Plasmodium* antigens in blood samples, in particular *Plasmodium* histidine-rich protein II (HRP-II) or lactate dehydrogenase (LDH), but at low parasitemia (< 100 parasites/μL) the sensitivity of these tests decreases significantly,[13] making currently available RDTs unsuitable for screening blood bank samples. Another drawback of RDTs may be the persistence of *Plasmodium* antigens, in particular HRP-II, in the blood circulation of the patient after parasite clearance, resulting in a false positive test.[13] Several antibody detection systems have been developed for screening purposes.[7,12] However, antibodies against *Plasmodium* may persist for a significant time after the patient has been cured, which would lead to some donors being excluded despite being parasite-negative. In contrast, it takes several days after infection before antibodies are produced against the parasite and thus a negative antibody test does not guarantee that the donor is free of malaria.[7,12,13]

Molecular tests have been proposed as cost-effective and highly sensitive tools for the detection of *Plasmodium* in blood donations.[9] Recently, our research group applied quantitative nucleic acid sequence-based amplification (QT-NASBA) technology for the detection and quantification of *Plasmodium* parasites in clinical samples.[14,15] This technology has several advantages over standard polymerase chain reaction (PCR); the NASBA amplification reaction is isothermal (at 41°C) not requiring a thermocycler, rapid (approximately 90 minutes for 48 samples), detects RNA in a background of DNA and is quantifiable. QT-NASBA is approximately 1000 times more sensitive than standard microscopy.[14]

In the present study, the utility of QT-NASBA to detect malaria parasites in blood bank donations was assessed. The study was performed in a malaria-endemic country, Sudan, to ensure that *Plasmodium*-contaminated samples would be available. In Sudanese blood banks, no active laboratory procedures are used to exclude potentially malaria individuals from donation. Only questionnaires including personal data and physical examination are being used to exclude blood donors who are at risk of malaria. However, these procedures do not exclude contamination of blood donations with *Plasmodium*.[16,17] The performance of the QT-NASBA was compared with routine microscopy ('gold standard') and with RDTs.

MATERIALS AND METHODS

Study site

The study was conducted during the peak transmission season of malaria (September to October) in Wad Medani (Gezira State), the principal town of an irrigation area in Central Sudan, where malaria is mesoendemic with an unstable transmission pattern. This town lies on the western bank of the Blue Nile River, at a junction of highways. The blood bank of Wad Medani Teaching Hospital (University of Gezira) serves around 1000 beds and supplies about 1000 donations per month for all hospitals in town.

Sample collection

One hundred blood donations were obtained from Wad Medani Teaching Hospital blood bank after being

© 2007 The Authors

screened for hepatitis B and C and HIV. Viral screening was performed before the actual donation was made. From each donation, a sample (50 μL) was put in 950 μL guanidinium isothiocyanate (GuSCN) L6 lysis buffer and subsequently stored at −70°C until further processing for QT-NASBA analysis. An additional sample from each donation was analyzed in the laboratory with RDTs (see below). Finally, from each donation a Giemsa-stained slide was prepared for microscopy (see below).

In addition, 20 blood samples were collected from healthy volunteers from the Netherlands. Appropriate malaria-positive control blood samples were obtained from the Royal Tropical Institute, Department of Biomedical Research, Amsterdam, the Netherlands.

Microscopic examination

Conventional thin and thick blood smears were made and stained with 10% Giemsa (at pH 7.2). The slides were subsequently examined for the presence of malaria parasites under oil immersion (×100 magnification). Two expert microscopists at the Malaria Diagnosis and Treatment Center in the Wad Medani Teaching Hospital independently read the blood smears.

RDTs

Paracheck test (Lot No. CB041A, Manuf. 05.2002, Exp. 04.2004, Orchid Biomedical Systems, India) to detect HRP-II and optiMAL test (Lot No. 13100.25.11, Exp. 2002.10, Diamed OptiMAL, Switzerland) to detect the parasite LDH were performed in Sudan on all blood donation samples strictly according to the manufacturer's instructions.

QT-NASBA

QT-NASBA analysis of the blood samples for the detection and quantification of *Plasmodium falciparum* was performed as described previously.[14] Immediately before RNA extraction, 1×10^7 molecules of *in vitro* Q-RNA, which serves as an internal standard, were added to the blood sample/L6 lysis buffer mixture as competitor RNA. Next, nucleic acids were isolated from the blood samples with the GuSCN-silica procedure as described.[18] The blood/lysis buffer mixture was mixed with activated silica. The nucleic acids bound to the silica were washed twice with wash buffer (10 M GuSCN, 100 mM TRIS-HCL, pH 6.4), twice with 70% ethanol and once with 100% acetone. Next, the nucleic acids were eluted from the silica with 100 μL of water. Finally, 2 μL of the nucleic acid isolate was used in the QT-NASBA analysis.

The NASBA reaction was performed and parasite counts were assessed as previously described.[14,15] Primers and probes for the NASBA reaction were selected on the basis of the published sequences of the 18S rRNA genes of *P. falciparum* primers Plas-1F (5′-TCAGATACCGTCGTAATCTTA-3′) and Plas-2R T7 (5′-AA TTCTAATACGACTCACTATAGGGAGAGAACTTTCTCGCT TGCGCGAA-3′). The NASBA reaction was performed at 41°C (isothermal amplification reaction using a final concentration of 70 mM KCl in the reaction mixture).

The NASBA amplification products were next diluted 25 times in water and were subsequently hybridized to a capture probe (5′-ACCATAAACTATGCCGACTAGG-3′) which was bound to streptavidin-coated magnetic beads. The samples were then separately hybridized to ruthenium-labeled WT (5′-CCTTATGAGAAATCA AAGTC-3′) and Q (5′-AATAACTGCACCAGTGTATA-3′) detection probes, followed by electrochemiluminescence (ECL) detection in a NucliSens ECL reader (Organon Teknika, Boxtel, the Netherlands). The light emitted is detected by a photoelectric cell, thus offering a precise measurement for quantification.

In order to quantify the number of parasites in the blood samples by QT-NASBA, the extracted *P. falciparum* RNA (WT-RNA) was coamplified with the internal standard RNA (Q-RNA) as a competitor by using the same amplification primers in a single-tube NASBA reaction. After hybridization of the NASBA product to the WT and Q detection probes, the ECL provided for each sample signals for WT RNA and Q-RNA. Because of the competition for the amplification primers, a low parasite concentration in the sample generates a low signal for WT RNA and a high signal for Q-RNA, whereas a high parasite concentration shows a high signal for WT RNA and a low signal for Q-RNA. The signals for WT RNA and Q-RNA are directly correlated to the number of parasites present in the samples.

In each experiment, a standard curve was made by using blood samples containing known numbers of parasites; i.e. 10^8, 10^6, 10^4, 10^3 and 0 *P. falciparum* parasites per mL of blood. Final ECL results were calculated as log [(WT RNA signal/Q-RNA signal) × 1000] and were plotted on a standard curve in which the *x*-axis represents the log parasite concentration and the *y*-axis

© 2007 The Authors

represents the final ECL result. Best-fit regression analysis was performed with the Excel software package.

A sample with a QT-NASBA count of > 0.001 parasite/μL was considered positive, and quantification of the number of parasites in a sample was achieved at a parasite density > 0.1/μL of blood.

Ethical considerations

The Ethical Committee of the Blue Nile Research and Training Institute/University of Gezira approved the study and permission was obtained from Gezira State Health Authorities.

RESULTS

Blood samples obtained from 100 blood donations were tested for the presence of malaria parasites by various techniques. As indicated in Table 1, no *Plasmodium* parasites were found by microscopy and all RDTs were negative within the advised reading time (10–15 minutes) described by the manufacturers.

The blood samples were further analyzed by QT-NASBA (lower detection limit for quantification 0.1 parasite/μL of blood) in the Netherlands. Three samples were lost because of transport conditions and 17 gave insufficient amounts of RNA after extraction. In these cases, the RNA may have been degraded during transportation or not been properly mixed with the L6 buffer immediately after collection of the blood sample, which may also result in insufficient preservation of RNA. The remaining 80 samples were analyzed by QT-NASBA and 55 were found negative for *P. falciparum*, i.e. < 0.001 parasite/μL of blood. Four samples gave a QT-NASBA result that was in the cutoff area of the test, i.e. parasite count between 0.001 and 0.1 parasite/μL of blood. Interestingly, 21 of the 80 samples (26.3%) were found QT-NASBA-positive, i.e. parasite count > 0.1/μL of blood. The mean parasite count of the positive samples was 1.73 parasites/μL of blood, ranging between 0.13 and 18.6 parasites/μL of blood. There were four samples that had a QT-NASBA count near the lower detection limit of expert microscopy (10–100 parasites/μL blood); these samples had counts of 1.9, 2.2, 2.7 or 18.6 parasites/μL of blood, respectively. It is unlikely that these parasite loads would be picked up by routine microscopy as employed in the present study. All other QT-NASBA positive samples had counts below 1 parasite/μL of blood.

The 20 samples obtained from the apparently healthy volunteers from the Netherlands were all found negative with QT-NASBA. Microscopy and RDT testing were not performed on these samples.

Table 1. Results of analysis of blood bank samples for the presence of *Plasmodium* parasites by microscopy, Paracheck test, optiMAL test and QT-NASBA

Test	Samples	Negative	Positive
Microscopy	100*	100	0
HRP-II	100*	100	0
OptiMAL (pLDH)	100*	100	0
QT-NASBA	80*	55	25‡
QT-NASBA	20†	20	0

*Samples from Sudan.
†Samples from the Netherlands.
‡Four samples were in the cutoff area of the QT-NASBA test, suggesting that parasite RNA was present in the sample, and were therefore included as being positive in the table.
QT-NASBA, quantitative nucleic acid sequence-based amplification; HRP-II, histidine-rich protein II; pLDH, parasite lactate dehydrogenase.

DISCUSSION

The presence of malaria parasites in blood donations from apparently healthy donors, who did not manifest clinical signs of malaria (no fever or recent history of fever and/or malaria), collected in a malaria-endemic region was assessed in the present study by microscopy, RDTs and a molecular test, i.e. QT-NASBA. In contrast to microscopy and RDT testing, QT-NASBA was able to detect *P. falciparum* in 26.3% of examined blood samples. This rather high incidence of malaria in the study population is most likely due to the fact that the samples were collected during the high transmission season for malaria in the study region. Furthermore, the study population comprised adult volunteers who during their life have acquired some degree of immunity against malaria. At low parasitemia, these individuals do not feel sick, nor have clinical signs of malaria. In Sudanese blood banks, no active laboratory procedures are in place to exclude potential malaria-infected individuals from donation; only a questionnaire including personal

© 2007 The Authors
Journal Compilation © 2007 LMS Group

data and a physical examination are used to exclude blood donors who are at risk of malaria. Malaria carriers with no clinical signs and a parasitemia below the detection level of routine microscopy can therefore easily be accepted as donors.[16,17]

Although in some cases ($n = 4$) QT-NASBA parasite counts were near the accepted detection limit of microscopy, most cases ($n = 21$) were below the detection limits of the applied technologies, i.e. RDTs (< 100 parasites/μL blood) or microscopy (in specialized centers, between 10 and 100 parasites/μL blood).[13] However, the mean number of parasites found in the blood samples is still high enough for the transmission of malaria via a blood donation.[7] This observation emphasizes the strong need for sensitive methods to exclude the presence of *Plasmodium* species in blood donations.

The present study confirmed that currently available malaria parasite detection methods, i.e. microscopy and RDTs, are not sensitive enough to detect low parasitemia that may be present in apparently healthy donors. Improved screening of blood banks for malaria parasites to reduce the risk of TTM is therefore necessary. Screening blood donations with molecular techniques can improve the quality of the blood banks, as demonstrated in the present study and by others.[9] The present study demonstrated that QT-NASBA is a very sensitive tool for analyzing blood samples for the presence of *Plasmodium* parasites, even at submicroscopic levels. It has also been established that this technology has superior properties in terms of sensitivity and efficiency above other amplification methods.[19] Furthermore, microscopic reading of blood slides at low parasitemia is extremely laborious – it can take 30 minutes to read one slide – and may be erroneous. In contrast, 48 samples can be analyzed with QT-NASBA in 90 minutes.[14,15,19] Moreover, the availability of fully automated RNA extraction methods allows the development of a high throughput system, enabling the number of samples that can be analyzed on a working day to be increased.[15,20,21] Furthermore, the recent development of a real-time QT-NASBA system with a closed-tube format of the assay will greatly reduce the risk of contamination and thus false positive results.[19]

The relatively high costs of applying QT-NASBA (approximately €5/sample for consumables, excluding labor costs) compared with microscopy (< €1/sample for consumables) may limit the implementation of this technology in routine screening of blood bank donations. However, Shehata *et al.* performed a cost-effectiveness study of four donor screening strategies for malaria, including testing blood donors with risk factors for malaria with PCR, and concluded that such a strategy is economically attractive in countries where malaria is not endemic.[9]

In resource-poor disease-endemic countries, however, the implementation of such a strategy is possibly unfeasible and investments should be made toward improved microscopic screening of risk donors, which will ultimately lead also to improved quality of the blood banks in malaria-endemic countries.[16]

Finally, we would like to note that it will be possible to develop NASBA technology for the detection and quantification of other protozoan diseases that may contaminate blood donations and thus bear the inherent risk of transfusion-transmitted diseases. Notably, Chagas disease and leishmaniasis are diseases that jeopardize the safety of blood products in Europe and America.[22,23] Carriers can be chronically infected with parasites causing these diseases for a very long period and still qualify according to European guidelines as donors.[24]

ACKNOWLEDGEMENTS

This work was supported by a grant from the Netherlands Organization for Scientific Research–Netherlands Foundation for the Advancement of Tropical Research (NWO-WOTRO WB 93-388) and by a grant from the Knowledge and Innovation Fund of the Royal Tropical Institute (Amsterdam, the Netherlands).

REFERENCES

1 Snyder EL, Dodd RY. Reducing the risk of blood transfusion. *Hematology Am Soc Hematol Educ Program* 2001; 433–42.

2 Frey-Wettstein M, Maier A, Markwalder K, Munch U. A case of transfusion transmitted malaria in Switzerland. *Swiss Med Wkly* 2001; **131**: 320.

3 Mungai M, Tegtmeier G, Chamberlard M, Parise M. Transfusion-transmitted malaria in the United States from 1963 through 1999. *N Engl J Med* 2001; **344**: 1973–8.

© 2007 The Authors

4 Slinger R, Giulivi A, Bodie-Collins M, *et al*. Transfusion-transmitted malaria in Canada. *CMAJ* 2001; **164**: 377–9.

5 Busch MP, Kleinman SH, Nemo GJ. Current and emerging infectious risks of blood transfusions. *JAMA* 2003; **289**: 959–62.

6 Kitchen AD, Barbara JA, Hewitt PE. Documented cases of post-transfusion malaria occurring in England: a review in relation to current and proposed donor-selection guidelines. *Vox Sang* 2005; **89**: 77–80.

7 Kitchen AD, Chiodini PL. Malaria and blood transfusion. *Vox Sang* 2006; **90**: 77–84.

8 Kitchen A, Mijovic A, Hewitt P. Transfusion-transmitted malaria: current donor selection guidelines are not sufficient. *Vox Sang* 2005; **88**: 200–1.

9 Shehata N, Kohli M, Detsky A. The cost-effectiveness of screening blood donors for malaria by PCR. *Transfusion* 2004; **44**: 217–28.

10 Purdy E, Perry E, Gorlin J, Jensen K. Transfusion-transmitted malaria: unpreventable by current donor exclusion guidelines? *Transfusion* 2004; **44**: 464.

11 Baas MC, Wetsteyn JC, van Gool T. Patterns of imported malaria at the academic medical center, Amsterdam, the Netherlands. *J Travel Med* 2006; **13**: 2–7.

12 Seed CR, Cheng A, Davis TM, *et al*. The efficacy of a malarial antibody enzyme immunoassay for establishing the reinstatement status of blood donors potentially exposed to malaria. *Vox Sang* 2005; **88**: 98–106.

13 Moody A. Rapid diagnostic tests for malaria parasites. *Clin Microbiol Rev* 2002; **15**: 66–78.

14 Schoone GJ, Oskam L, Kroon NC, Schallig HD, Omar SA. Detection and quantification of *Plasmodium falciparum* in blood samples using quantitative nucleic acid sequence-based amplification. *J Clin Microbiol* 2000; **38**: 4072–5.

15 Schallig HD, Schoone GJ, Lommerse EJ, *et al*. Usefulness of quantitative nucleic acid sequence-based amplification for diagnosis of malaria in an academic hospital setting. *Eur J Clin Microbiol Infect Dis* 2003; **22**: 555–7.

16 Ali MS, Gader AA, Kadaru AA, Mustafa MS. Screening blood donors for malaria parasite in Sudan. *Ethiop J Health Dev* 2004; **18**: 70–4.

17 Elfaki EA, Ghali RG, El Fadil S. Malaria among the causes of postoperative fever in Wad Medani Hospital, central state, Sudan. *Sudan Med J* 1993; 77–85.

18 Boom R, Sol CJ, Salimans MM, *et al*. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 1990; **28**: 495–503.

19 Schneider P, Wolters L, Schoone G, *et al*. Real-time nucleic acid sequence-based amplification is more convenient than real-time PCR for quantification of *Plasmodium falciparum*. *J Clin Microbiol* 2005; **43**: 402–5.

20 Cook L, Ng KW, Bagabag A, Corey L, Jerome KR. Use of MagNA pure LC automated nucleic acid extraction system followed by real-time reverse transcription-PCR for ultrasensitive quantitation of hepatitis C virus RNA. *J Clin Microbiol* 2004; **42**: 4130–6.

21 Hourfar MK, Michelsen U, Schmidt M, *et al*. High-throughput purification of viral RNA based on novel aqueous chemistry for nucleic acid isolation. *Clin Chem* 2005; **51**: 1217–22.

22 Young C, Losikoff P, Chawla A, Glasser L, Forman E. Transfusion-acquired *Trypanosoma cruzi* infection. *Transfusion* 2007; **47**: 540–4.

23 Cardo LJ. Leishmania: risk to the blood supply. *Transfusion* 2006; **46**: 1641–5.

24 Reesink HW. European strategies against the parasite transfusion risk. *Transfus Clin Biol* 2005; **12**: 1–4.

© 2007 The Authors
Journal Compilation © 2007 LMS Group