**Schistosoma real-time PCR as diagnostic tool for international travellers and migrants**

Lieselotte Cnops¹, Egbert Tannich², Katja Polman³, Jan Clerinx¹ and Marjan Van Esbroeck¹

¹ Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
² Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
³ Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

**Abstract**

OBJECTIVE To evaluate the use of a genus-specific PCR that combines high sensitivity with the detection of different *Schistosoma* species for diagnosis in international travellers and migrants in comparison to standard microscopy.

METHODS AND RESULTS The genus-specific real-time PCR was developed to target the 28S ribosomal RNA gene of the major human *Schistosoma* species. It was validated for analytical specificity and reproducibility and demonstrated an analytical sensitivity of 0.2 eggs per gram of faeces. Its diagnostic performance was further evaluated on 152 faecal, 32 urine and 38 serum samples from patients presenting at the outpatient clinic of the Institute of Tropical Medicine in Antwerp (Belgium). We detected *Schistosoma* DNA in 76 faecal (50.0%) and five urine (15.6%) samples of which, respectively, nine and one were not detected by standard microscopy. Only two of the 38 serum samples of patients with confirmed schistosomiasis were positive with the presently developed PCR. Sequence analysis on positive faecal samples allowed identification of the *Schistosoma* species complex.

CONCLUSION The real-time PCR is highly sensitive and may offer added value in diagnosing imported schistosomiasis. The genus-specific PCR can detect all schistosome species that are infectious to humans and performs very well with faeces and urine, but not in serum.

**keywords** real-time PCR, *Schistosoma*, diagnosis, travellers, imported schistosomiasis

**Introduction**

Schistosomiasis is a neglected parasitic infection that affects more than 200 million people worldwide (WHO 1985). It is a waterborne disease caused by trematode blood flukes of the genus *Schistosoma*. The three major schistosome species affecting man, *S. mansoni*, *S. haematobium* and *S. japonicum*, are distributed in various (sub) tropical areas (Doumenge & Mott 1984; Gryseels et al. 2006). *S. mansoni* is prevalent in many countries of Africa, parts of the Middle East, South-America and in the Caribbean. *S. haematobium* is distributed in parts of Africa and in the Middle East. *S. japonicum* is distributed in China, Indonesia and the Philippines. Other human species have a more restricted distribution in the Democratic Republic of Congo (*S. intercalatum*), in Central-Africa (*S. guineensis*) (Webster et al. 2006) and in Laos and Cambodia (*S. mekongi*).

Owing to growing (im)migration and to increasing travel to a variety of tropical destinations, people are at higher risk of becoming infected with one of the these *Schistosoma* species nowadays (Grobusch et al. 2003; Clerinx & Van Gompel 2011). In general, diagnosis by microscopy allows identification of all human *Schistosoma* species based on differences in morphology of the excreted eggs, but lacks precision in case of low egg load/excretion (Oliveira et al. 2010) or in case of mixed infections and hybrids (Taylor 1970; Huyse et al. 2009).

Owing to their high sensitivity, molecular techniques are of added value as diagnostic tools in non-endemic settings for several blood-borne parasitic infections such as malaria, leishmaniasis, trypanosomiasis and filariasis (Whitty et al. 2000; Cnops et al. 2010; Jiménez et al. 2011). For schistosomiasis, studies have so far focused on clinical samples from endemic regions (Table 1). Only few PCR studies investigated travellers to endemic regions (Sandoval et al. 2006; Wichmann et al. 2009) despite the reported increase in imported cases in Europe (Grobusch et al. 2003; Bierman et al. 2005). Over the past decade, several PCR formats have been successfully developed (Table 1), such as the first real-time PCRs in 2008 (Obeng et al. 2008; Ten Hove et al. 2008). Most formats targeted either *S. mansoni* in faeces (Pontes et al. 2003; Allam et al. 2009; Oliveira et al. 2010) or *S. haematobium* in urine.
### Table 1 Overview of molecular techniques described in the literature for diagnosis of schistosomiasis applied on human samples in endemic and non-endemic settings

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample type</th>
<th>Origin</th>
<th>Extraction method</th>
<th>Detection method</th>
<th>Target gene</th>
<th>Species</th>
<th>Detection limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pontes et al. 2002</td>
<td>Faeces (n = 7)</td>
<td>Brazil</td>
<td>ROSE method</td>
<td>Conventional PCR</td>
<td>121-bp tandem repeat</td>
<td>S. mansoni</td>
<td>1fg (2.16 EPG)</td>
</tr>
<tr>
<td></td>
<td>Serum (n = 2)</td>
<td></td>
<td>Glass-MAX system</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pontes et al. 2003</td>
<td>Faeces (n = 194)</td>
<td>Brazil</td>
<td>ROSE method</td>
<td>Conventional PCR</td>
<td>121-bp tandem repeat</td>
<td>S. mansoni</td>
<td></td>
</tr>
<tr>
<td>Sandoval et al. 2006</td>
<td>Urine (n = 18)</td>
<td>Spain</td>
<td>Nucleospin Trace kit</td>
<td>Conventional PCR</td>
<td>28S rDNA</td>
<td>Schistosoma spp</td>
<td>0.98 pg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>28S rDNA</td>
<td>S. mansoni</td>
<td>1.9 pg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ITS rDNA</td>
<td>S. japonicum</td>
<td>15 pg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ITS rDNA</td>
<td>S. haematobium</td>
<td>3.7 pg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. intercalatum/</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. bovis</td>
<td></td>
</tr>
<tr>
<td>Ten Hove et al. 2008</td>
<td>Faeces (n = 176)</td>
<td>Senegal</td>
<td>Adapted QIAamp Tissue mini kit</td>
<td>Triplex real-time PCR (probe)</td>
<td>Cytochrome c oxidase (cox1)</td>
<td>S. mansoni</td>
<td>100 fg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cytochrome c oxidase (cox1)</td>
<td>S. haematobium</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>gB DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obeng et al. 2008</td>
<td>Urine (n = 153)</td>
<td>Ghana</td>
<td>QIAamp DNA mini kit</td>
<td>Duplex real-time PCR (probe)</td>
<td>ITS2</td>
<td>PhHV-1</td>
<td>NA</td>
</tr>
<tr>
<td>Wichmann et al. 2009</td>
<td>Plasma (n = 52)</td>
<td>Germany</td>
<td>Large volume phenol/chloroform ROSE method</td>
<td>Real-time PCR (probe)</td>
<td>gB DNA</td>
<td>S. mansoni</td>
<td>68.8 copies/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. intercalatum/</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. haematobium</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PhHV-1</td>
<td></td>
</tr>
<tr>
<td>Lier et al. 2009</td>
<td>Faeces (n = 1727)</td>
<td>China</td>
<td>ROSE method</td>
<td>Real-time PCR (probe)</td>
<td>mitochondrial NADH</td>
<td>S. japonicum</td>
<td>1 EPG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>dehydrogenase I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allam et al. 2009</td>
<td>Faeces (n = 995)</td>
<td>Egypt</td>
<td>QIAamp Stool DNA mini kit</td>
<td>Conventional PCR</td>
<td>121-bp tandem repeat</td>
<td>S. mansoni</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gomes et al. 2009</td>
<td>Faeces (n = 67)</td>
<td>Brasil</td>
<td>QIAamp Stool DNA mini kit</td>
<td>Conventional PCR</td>
<td>121-bp tandem repeat</td>
<td>S. mansoni</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kjetland et al. 2009</td>
<td>Vaginal lavage (n = 83)</td>
<td>Zimbabwe</td>
<td>Wizard genomic DNA purification kit</td>
<td>Duplex real-time PCR (probe)</td>
<td>ITS2</td>
<td>S. mansoni</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Internal control</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. haematobium</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. mansoni</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PhHV-1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. bovis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. haematobium</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. mansoni</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. bovis</td>
<td></td>
</tr>
<tr>
<td>Huysse et al. 2009</td>
<td>Urine faeces (n = 575)</td>
<td>Senegal</td>
<td>Whatman FTA DNA</td>
<td>Duplex conventional PCR + sequencing</td>
<td>gB DNA</td>
<td>S. mansoni</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PhHV-1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. bovis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. haematobium</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. mansoni</td>
<td></td>
</tr>
</tbody>
</table>
Recently, the detection of schistosome DNA in serum was discovered to have diagnostic potential (Pontes et al. 2002; Wichmann et al. 2009; Xu et al. 2010). A molecular test that is applicable to different specimen types and able to detect all human schistosome species would be very useful for the diagnosis of intestinal and urogenital schistosomiasis in travellers and migrants. We therefore developed a real-time PCR that targets *Schistosoma* at the genus level and evaluated its performance in faeces, urine and serum.

### Material and methods

#### Clinical samples

Clinical samples ($n = 222$) were collected between 2003 and 2011 from 140 patients who presented to the outpatient clinic of the Institute of Tropical Medicine (ITM) Antwerp, Belgium, or were sent by national laboratories for confirmation to the Central Laboratory of Clinical Biology (CLKB) of ITM (accredited according to ISO15189:2007) as national reference centre for parasitology. In total, 152 faecal, 32 urine and 38 serum samples were available. Patients either travelled individually ($n = 118$) or in groups ($n = 22$). One group of 13 travellers visited Rwanda (Clerinx et al. 2011), another group of nine patients travelled in Mali. PCR analysis was retrospectively performed between January and December 2011 on samples stored at less than $18\,\text{°C}$ until processing.

The faecal and urine samples came from either suspected or confirmed cases of schistosomiasis. Suspected patients presented with clinical symptoms or were selected based on laboratory data such as an increase in eosinophils ($>0.45 \times 10^9/l$), positive serology results (IHA titer $\geq 1/160$) or the presence of Charcot-leyden crystals in faeces. Confirmed cases were defined by the presence of eggs as determined by microscopy.

The serum samples were selected from patients with a confirmed *Schistosoma* infection. To prove the presence of schistosome DNA, the PCR targeting the 121-bp tandem repeat sequence (Wichmann et al. 2009) was established in all sera. The 121-bp PCR, designed for detection of cell-free parasite DNA in blood samples, was performed at the Bernhard Nocht Institute for Tropical Medicine (BNI, Hamburg, Germany) and at ITM.

#### Control samples

Positive control DNA of *S. mansoni* and *S. haematobium* and of three animal species (*S. bovis*, *S. rodhaini*, *S. curassoni*) was kindly provided by Dr. T. Huyse.
DNA extraction

DNA from faeces was extracted with the QIAamp DNA stool mini kit (Qiagen Benelux, Venlo, the Netherlands) according to the manufacturer’s guidelines with minor modifications. After dissolving 1 g of faeces in 5 ml ASL lysis buffer, samples were placed for 30 min on a vertical rotor to provide uniform homogenization. From the lysis buffer, samples were placed for 30 min on a vertical rotor to provide uniform homogenization. From the lysis buffer, samples were placed for 30 min on a vertical rotor to provide uniform homogenization. From the lysate, 1.6 ml was incubated for 10 min at 95 °C. Phocine Herpes Virus 1 (PhHV-1; 500 plaque forming units (PFU)/ml) was added to the AL lysis buffer as extraction and amplification control. DNA was eluted in a volume of 100 μl AE elution buffer.

DNA from urine was extracted as described by Haque et al. (2010). A volume of 10 ml urine was centrifuged for 5 min at 3000x g. The supernatant was discarded, and the remaining pellet was washed three times with 2 ml phosphate buffered saline (PBS). In case of absence of a visible pellet, at least 200 μl sediment was kept and washed with PBS. The 200 μl pellet solution was used for DNA extraction with the DNA mini kit (Qiagen) according to the manufacturer’s guidelines. DNA was eluted with 100 μl AE buffer.

For patients who travelled individually (n = 8), DNA was extracted from 1 to 2 ml serum with the QIAamp DNA MIDI kit (Qiagen) according to manufacturer’s guidelines at ITM. DNA was eluted with 200 μl AE buffer.

DNA from urine was extracted as described by Haque et al. (2010). A volume of 10 ml urine was centrifuged for 5 min at 3000x g. The supernatant was discarded, and the remaining pellet was washed three times with 2 ml phosphate buffered saline (PBS). In case of absence of a visible pellet, at least 200 μl sediment was kept and washed with PBS. The 200 μl pellet solution was used for DNA extraction with the DNA mini kit (Qiagen) according to the manufacturer’s guidelines. DNA was eluted with 100 μl AE buffer.

For patients who travelled individually (n = 8), DNA was extracted from 1 to 2 ml serum with the QIAamp DNA MIDI kit (Qiagen) according to manufacturer’s guidelines at ITM. DNA was eluted with 200 μl AE buffer.

Extraction of serum from patients that travelled in group (Clerinx et al. 2011; personal communication) was performed with the phenol/chloroform method at BNI (Germany). After extraction, part of the DNA was sent to ITM for PCR analysis.

Primer and probe design

From the different target genes described in the literature (Table 1), the 28S ribosomal (r)RNA was selected to detect the genus of Schistosoma in humans, regardless of the species or sample matrix. After alignment of GenBank accessions numbers AY157173.1, AY157262.1, AY157263.1, Z46504.4 and AY157253.1 (Figure 1), primers and a probe were selected in a homologous region that still contained enough inter-species differences to allow species identification to the level of the Schistosoma complex groups by sequencing. The S. mansoni complex included the human S. mansoni and rodent S. rodhaini species. The S. haematobium complex included the human parasites S. haematobium, S. intercalatum, S. guineensis and livestock parasites S. margrebowiei, S. bovis, S. leiperi, S. mattheei and S. curassoni (Webster et al. 2006). The S. japonicum complex included the human S. japonicum and S. mekongi species. One forward (FW) primer (GTGGAGTTGAATGCAAGGC), two reverse (RV) primers (RV1: CCATACGACACCGACGC; RV2: GCTCAACAWTAATAGTCAAACCTG) and one probe (ACTGACAAGCAGACCCTCACACC) were selected to detect Schistosoma at the genus level (Figure 1). The FW primer sequence was 100% identical for all five human species and was located in the same region as described for the conventional PCR described by Sandoval et al. (2006) but contained four additional nucleotides at the 5’end, and five nucleotides less at the 3’end. The RV1 primer was 100% homologous for S. mansoni, S. haematobium and S. intercalatum, and 86.7% homologous for S. mekongi and S. japonicum. To improve the detection of the latter two species, a second RV2 primer was designed that was specific for S. mekongi and S. japonicum. The genus-specific probe was located in a region that was 100% identical for the five species (Figure 1).

Real-time PCR

The 25 μl reaction mix contained 5 μl DNA, 1× Perfecta qPCR Supermix (Quanta Biosciences), 400 nM of FW, RV1 and RV2 primer, 600 nM of the FAM/BHQ1-labelled probe (Biolegio) and 0.1 mg/ml bovine serum albumin (BSA). The program consisted of an initial step of 2 min at 95 °C followed by 50 cycles of 15 s at 95 °C, 30 s at 60 °C and finally 30 s at 72 °C.
The reaction was run on the SmartCycler II (Cepheid Benelux, Belgium). DNA detection was expressed by cycle threshold (Ct)-values. In every run, the non-template control was negative (Ct = 0) and the positive control was positive.

For the detection of the extraction control (PhHV-1), an additional PCR was performed as described before (Cnops & Van Esbroeck 2010).

PCR validation

The primer and probe design were verified with Integrated DNA Technology (IDT) Oligo Analyzer software (v3.1) (http://eu.idtdna.com/analyzer/Applications/OligoAnalyzer/). Primer and probe specificity was checked in silico by BLAST analysis (http://blast.ncbi.nlm.gov/WriteBlast.cgi) and by 2% agarose gel electrophoresis at 100V for 35 min. PCR products were sequenced using a 3730XL-DNA Analyzer (Applied Biosystems) at the genetic service facility (VIB, Antwerp, Belgium). The analytical specificity of the PCR was tested on DNA of the 23 clinical control samples containing faecal or blood parasites (see above).

The detection limit was determined on a tenfold dilution series of a faecal sample containing 100 EPG of S. mansoni. It was diluted in a negative faecal sample, which was dissolved in ASL buffer (Qiagen). DNA was extracted from 10 faecal dilution. The highest dilution with a positive signal indicated the detection limit.

The variation in Ct-values was determined in a sample that was processed 8 times within the same run (reproducibility). The coefficient of variation (CV, in %) of the Ct-values was calculated.

Evaluation of PCR in comparison with microscopy

The diagnostic sensitivity (true positives/total positives) and specificity (true negatives/total of negatives) of PCR...
were determined in comparison with microscopy on faecal samples and expressed as percentages (%).

Standard microscopic analysis was carried out on a single faecal sample using the concentration method on 3 g of faeces that had been homogenised in 42 ml of 10% formaldehyde-saline solution (Laughlin and Spitz 1949). The infection intensity was expressed by the number of EPG. The limit of detection was 10 EPG.

The sediment of at least 20 ml end-stream urine was used for microscopic analysis of urine samples. Discrepant results between PCR and microscopy were assessed by serology data, clinical information, follow-up samples or external real-time PCR analysis at the laboratory of Leiden (LUMC, The Netherlands). The correlation between $C_t$-values and egg counts was determined and expressed by the Pearson correlation coefficient $R^2$.

Results

PCR validation

Integrated DNA Technology Oligo analysis approved no self- or heterodimerization between the three primers and the probe. BLAST analysis with probe and primers indicated 100% query coverage and maximum identity with the correlated species. Gel electrophoresis obtained a single band of the expected length (Figure 1) for DNA of *S. mansoni*, *S. haematobium* and *S. mekongi*, and no signal in the non-template control.

The PCR was validated for analytical specificity. DNA controls of the nine *Schistosoma* species gave a positive PCR signal while no amplification was seen in the 23 clinical control samples with faecal and blood parasites other than *Schistosoma*. The analytical sensitivity was tested on serial faecal dilutions and demonstrated a detection limit of 0.2 EPG. Repeatability and reproducibility testing revealed a CV of 0.5% and 1.2%, respectively.

Sequence analysis of the amplified positive controls for *S. mansoni* and *S. haematobium* demonstrated 100% sequence identity to the target sequence of the corresponding *Schistosoma* complex. DNA of *S. mekongi* could be identified to the species level as the amplicon contains two specific nucleotides that differ from the *S. japonicum* target sequence.

PCR analysis on faeces

Seventy faecal samples that were positive by microscopy for *S. mansoni* ($n = 60$), *S. haematobium* ($n = 9$) and *S. mekongi* ($n = 1$) and 82 faecal samples that were microscopically negative were analysed by PCR. Egg loads varied between 10 and 580 EPG with a median of 33 EPG. DNA extraction and PCR were ineffective in three faecal samples with only few eggs. This was attributable to the insufficient amount of stool (<0.15 g) in two samples and dehydration of the faeces after 4 years of storage in the third sample. Repeated PCR analysis on those three samples in our institute and in the LUMC (The Netherlands) gave the same negative results. These three samples were excluded from analysis.

PCR detected *Schistosoma* DNA in 76 of the 149 faecal samples (Table 2) with $C_t$-values ranging between 23.44 and 47.97 and a median $C_t$-value of 30.36. There was no correlation between the $C_t$-value and the egg count ($R^2 < 0.1$). The diagnostic sensitivity for PCR was 100%.

In the 73 faecal samples without detectable *Schistosoma* DNA, no eggs were seen. Nine faecal samples were positive by PCR (with $C_t$-values varying between 28.09 and 33.78) but negative during parasitological examination (Table 2), indicating a diagnostic specificity of 89% (C.I. 82, 3–95.8) for PCR. With PCR being more sensitive than microscopy, the comparison to the ‘gold standard’ resulted in an apparent reduced specificity. Clinical symptoms and laboratory data (positive serology, eosinophilia) were supportive of the positive PCR results. Sequence analysis was performed on three of the nine samples and indicated the presence of parasite DNA from the *S. mansoni* complex.

PCR analysis on urine

PCR analysis on urine revealed five positive results. Eggs of *S. haematobium* were seen in four of these five samples (Table 3) and the fifth in which no eggs were found was
taken from a patient who travelled to Mali in the group of patients with confirmed schistosomiasis. No Schistosoma DNA was detected by PCR in the urine of 27 patients. For five of those, intestinal schistosomiasis was diagnosed by microscopy and PCR on faeces.

**PCR analysis on serum**

The PCR was tested on 38 serum samples of patients with confirmed schistosomiasis. Only two of 38 samples were positive with $C_T$-values of, respectively, 38.29 and 47.05.

**Discussion**

The presently developed PCR was designed as a highly sensitive diagnostic tool for schistosomiasis in travellers and migrants. We opted for the use of a real-time format, which has a short turnover time and is preferred to conventional PCR methods as it avoids post-PCR handling and contamination (Mackay 2004).

In view of our international patient population, a genus-specific format was chosen with the 28S rRNA as target gene (Sandoval et al. 2006) to enable the detection of all infectious Schistosoma species. Most of the PCR methods described so far have been developed to detect only one or two of the human Schistosoma species. Most of the PCR methods described so far have been developed to detect only one or two of the human Schistosoma species (Table 1). Pontes et al. (2002) were the first to report the use of PCR for diagnosing Schistosoma, that is, detection of S. mansoni in human faecal and serum samples, targeting the highly repetitive 121-bp tandem repeat sequence that was described 10 years earlier by Hamburger et al. (1991). In 2010, Oliveira et al. demonstrated that the 28S PCR was more sensitive than the 121-bp tandem repeat PCR for faecal samples. For S. haematobium (Hamburger et al. 2001) and S. japonicum (Lier et al. 2009; Xu et al. 2010), species-specific target genes were described. Ten Hove et al. (2008) used the Cox I gene to differentiate between S. mansoni and S. haematobium, but the sensitivity appeared to be lower than the ITS-2 PCR described by Obeng et al. (2008), which detect both species without differentiation.

What do we detect by PCR? In general, all PCRs that detect genomic DNA of Schistosoma should be able to detect DNA of the parasite in all stages of its life cycle: eggs containing the miracidium, cercariae, schistosomulae and adult worms. The PCR presented here was able to detect DNA from adult worms, as demonstrated in the control samples. In faecal samples, it is most likely that it detected DNA from eggs (devitalised or containing a living miracidium), including cell-free DNA released from damaged eggs. We cannot rule out that any juvenile or adult worm DNA breakdown products would be present in urine or even in faeces although the latter is rather unlikely. Until now, only circulating cathodic antigens (CCA) secreted by the adult worms were demonstrated in urine (Midzi et al. 2008; Obeng et al. 2008). In summary, a positive PCR signal indicates the presence of parasite DNA without providing information on the life cycle stage or viability of the parasite. This possibly explains why we did not observe a correlation between the $C_T$-value and the number of eggs.

The PCR was applied on three different sample matrices in which human schistosome DNA could be present: faeces for species causing intestinal schistosomiasis, urine for S. haematobium and serum. The latter sample type appeared to be useful for the diagnosis of acute infections by S. mansoni using a PCR that targeted the highly repetitive 121-bp target sequence (Wichmann et al. 2009; Clerinx et al. 2011). The currently presented PCR using the 28S rRNA as target gene was highly sensitive for the detection of DNA in faeces and urine, but not in serum. Almost all sera tested were negative, regardless the extraction method that was used. The highly repetitive 121-bp target sequence, which represents 11% of the genome, seems to be a more suitable target to pick up cell-free Schistosoma DNA in serum (Wichmann et al. 2009).

PCR on faeces and urine were successful in spite of the difficult nature of these matrices. A sample-specific extraction method was used to remove the inhibitory factors, and BSA was added to the PCR mix to lower the effect of inhibition (Cnops & Van Esbroeck 2010). We also found that a sufficient amount of faeces (approximately 1 g) should be processed for DNA extraction to obtain optimal PCR results.

The presently described PCR performed well for specificity, sensitivity and reproducibility and did not show cross-reactivity with other parasites. The analytical sensitivity of 0.2 EPG is excellent, keeping in mind that the detection limit was determined on a clinical sample starting from extraction while other studies (Table 1) mainly used dilutions of DNA controls.

In this study, the analytical specificity tests demonstrated that in addition to the six human Schistosoma species, animal species were also recognised. Some authors consider it a drawback that for example S. bovis is detected in human faecal samples (Webster et al. 2010). In our non-endemic setting, mainly patients with clinical symptoms suspected for schistosomiasis are investigated. Occasional infection with non-human schistosomes may be of clinical significance. As hybridization events between human and animal species are more frequently reported nowadays (Vercruysse et al. 1994; Huyse et al. 2009), a tool that detects both human and animal species has additional value.

The most important reason for our approach using a genus-specific PCR is that anti-schistosome therapy is
independent of the parasite species causing the infection. Thus, sensitive detection of all Schistosoma species is preferred to identification of the species. In this study, 10 (12.5%) additional schistosomiasis cases were demonstrated with PCR. There are a number of situations in which PCR is of added value. Its high sensitivity allows diagnosis of schistosomiasis when no eggs are detected by microscopy. This is especially the case during the acute phase of the infection when worms have not yet produced large numbers of eggs, and during active and chronic infections with low egg loads, as frequently seen in travellers and migrants, respectively (Nicolls et al. 2008; Grobusch et al. 2003; Clerinx & Van Gompel 2011) and in cases with low egg excretions as seen in immunocompromised persons (Mwinzi et al. 2004). PCR followed by sequence analysis could be also beneficial to confirm the diagnosis in cases where species identification by microscopy is difficult because of unclear egg morphology or the presence of mixed infections or hybrids.

In conclusion, we demonstrated that the genus-specific Schistosoma real-time PCR is a sensitive diagnostic tool, with added value to microscopy in non-endemic settings for diagnosis of schistosomiasis on faeces and urine, especially in patients in whom no eggs were found.

Acknowledgements

We would like to thank Hilde Cox, Kathy Demeulemeester, Idzi Potters, Lien Van Puyenbroeck and Henk Vereecken for their excellent technical support.

References


**Corresponding Author** Lieselotte Cnops, Department of Clinical Sciences, Institute of Tropical Medicine (ITM), Kronenburgstraat 43/3 2000 Antwerp, Belgium. Tel.: +32 3 2476436; Fax: +32 3 2476440; E-mail: lcnops@itg.be