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An Onyx Quencher™ Dual-Labeled Probe for the Detection of Schistosomiasis via qPCR

Parasitic infection resulting from human acquisition of trematodes has been a significant health issue for centuries, and it continues to be an issue to this day. Trematodes are also commonly referred to as "flukes," and include intestinal, liver, lung and blood flukes. There are three species of schistosome blood flukes that have particularly strong impact on society, Schistosoma mansoni, S. japonicum and S. haematobium. This study is focused on the detection of S. mansoni using the quantitative real-time polymerase chain reaction (qPCR).

S. mansoni originated in the African continent and Arabian peninsula areas, but was brought to various parts of the western hemisphere including Brazil, Surinam, Venezuela and the Caribbean, as a result of forced human migration during the African slave trade period (Markell and Voge, 2005). Recent estimates suggest that approximately 200 million people in seventy four different nations have varying degrees of schistosomiasis infection (Bergquist, R., et. al. 2002). The infection itself may present in different ways and is dependent upon the nature of the schistosome life cycle. Schistosome larvae enter and develop inside snails found in fresh water ponds and rivers, etc. They leave the snails as cercaria, which may then enter humans through their skin. Once inside the body, the parasites develop into adults within liver veins. They then reproduce and eggs are released into the environment in human feces. If in water, those eggs then hatch, develop into larvae, and the cycle continues. Symptoms may occur during any of these phases, including initial hemorrhages, fever, intestinal complaints, cough and hepatitis. The major issue for most patients is the development of chronic stages of the disease impacting the liver, spleen, intestinal, lung and neurological organ systems. A rapid and sensitive method of detecting schistosome presence is therefore a priority for these types of parasites.

The source of the targets for detection are typically human serum and fecal samples. Over the past two decades, a number of studies have been published describing the use of standard PCR to detect the presence of infectious agents (see Heath et. al. 2013). The assay developed and demonstrated in this note uses for detection a repetitive DNA sequence originally discovered by Hamburger et. al. (1991). Hamburger and colleagues then went on to develop a traditional PCR primer set using that target (Hamburger et. al. 1998).

Most qPCR reactions are developed with amplicons that are less than 200 base pairs in length. For the development of assays used to detect parasites, and also other targets, this has a very useful effect. As it is now possible to manufacture synthetic DNA oligonucleotides of up to 200 bases on a routine basis, a synthetic assay target may be made and used to optimize the reaction protocols, etc. before any patient samples are required. In the case of the S. mansoni assay, the synthetic amplicon has the following sequence of 110 bases, taken from the Hamburger finding:

5′-GATCTGAATCCGACCAACCGTTCTATGAAAATCGTTGTATCTCCGAAACCACTGGAC-GGATTTTTA

TGATGTTTGTTTTAGATTATTTGCGAGAGCGTGGGCGTTAATAT-3′

The primers and Dual-Labeled Probe were chosen from within this target sequence. S. mansoni 1 uses the same regular primer pair from Hamburger et. al. (1998), but a new Dual-Labeled Probe sequence was designed for use with these primers using the Beacon Designer™ software from PREMIER Biosoft (see also Santo et. al. 2013, which describes the use of this and other S. mansoni assays in a hamster model).

*S. mansoni 1**

Forward Primer: 5′-GATCTGAATCCGACCAACCG-3′

Reverse Primer: 5′-ATATTAACGCCCACGCTCTC-3′

Dual-Labeled Probe: 5′-(6-FAM™)-CGTTGTATCTCCGAAACCACTGGACGG-(Y)-3′
where

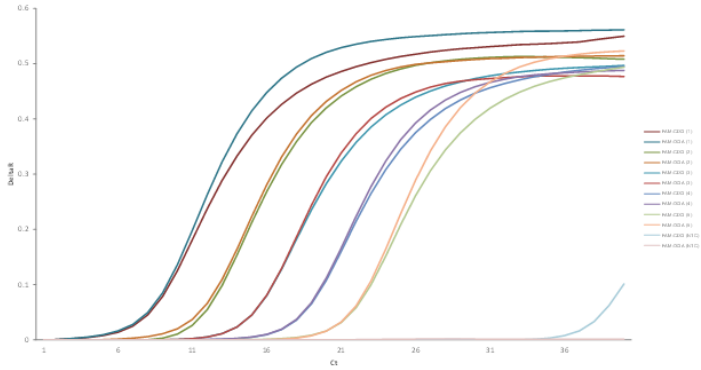
- Y = OQ™A (Onyx Quencher A) or a CDQ (Comparable Dark Quencher)

5′-FAM/3′-OQA and 5′-FAM/3′-CDQ Dual-Labeled Probes were run with Sigma's LuminoCt® qPCR ReadyMix™ with primers and probe at final concentrations of 300 nM each and 200 nM, respectively. The thermal profile was the following:

Step 1 1 x 95°C, 2:00 min

Step 2 40 x 95°C, 10 s
60°C 40 s

Step 3 1 x 40°C, 2:00 min



Baseline normalized data for FAM/CDQ and FAM/OQA probes. The sensitivity and end-point fluorescence are identical or slightly better with OQA for each dilution.

In conclusion, 5′-FAM//3′-OQA Dual-Labeled Probes are suitable for the Detection of Schistosomiasis via qPCR. More importantly, Onyx Quencher is an excellent alternative to other available dark quenchers.

*The primers and probes were made by SIGMA Custom Products, The Woodlands, Texas.

References

1. Markell and Voge's Medical Parasitology. Chapter 6 The Trematodes, pp. 166 - 206. Ed. David T. John and William A. Petri. Saunders Elsevier (2006).
2. Heath, A.R., Deluge, N., de Amorim, M.G., Dias-Neto, E. Development and use of qPCR assays for detection and study of neglected tropical and emerging infectious diseases. PCR Technology Current Innovations. Ed. Nolan, T and Bustin, S.A. CRC Press (2013).
3. Bergquist, R. et. al. Blueprint for schistosomiasis vaccine development. Acta. Trop. 82:183-192 (2002).
4. Hamburger J., et. al. Highly repeated short DNA sequences in the genome of Schistosoma mansoni recognized by a species-specific probe. Mol. Biochem. Parasitol. 44:73-80 (1991).
5. Hamburger, J. et. al. Development and laboratory evaluation of a polymerase chain reaction for monitoring Schistosoma mansoni infestation of water. Am. J. Trop. Med. Hyg. 59:468-473 (1998).
6. Santo, M., et. al. Early detection of Schistosoma mansoni infection by real-time PCR in a hamster model. Submitted for publication to PlOS (2013).
7. 6-FAM™ is a trademark of Applera Corporation or its subsidiaries in the U.S. and certain other countries.