Analytic and diagnostic evaluation of a real-time PCR based Malaria-screening assay

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Introduction

Malaria is a vector-borne infectious disease caused by eukaryotic apicomplexan parasites of the genus Plasmodium. Within this genus, there are five species pathogenic to humans: Plasmodium falciparum, Plasmodium vivax, Plasmodium knowlesi, Plasmodium malariae and Plasmodium ovale. The parasites are transmitted during the blood meal of an infected female Mosquito of the genus Anopheles. Malaria is endemic in most tropical countries worldwide and provoke over 200 million clinical cases each year, mainly in Sub-Saharan Africa. Early symptoms are not specific and indistinguishable from other tropical or fever diseases. Therefore, fast and reliable screening assays for the detection of Plasmodium spp. are highly needed.

Aim of the Study

We developed and validated a real-time PCR-based Malaria-screening assay. Analytical validation, including analytical sensitivity (Limit of Detection: LoD) and specificity (cross-reactivity), was realised by altona Diagnostics GmbH (Hamburg, Germany). In cooperation with the Bernhard Nocht Institute for Tropical Medicine (Hamburg, Germany) a retrospective and a prospective study with 103 and 120 specimens, respectively, were performed for diagnostic evaluation of the RealStar® Malaria PCR Kit 1.0.

Material & Methods

For the retrospective Study pretested specimens were analysed with the RealStar® Malaria PCR Kit 1.0. Pretesting was carried out with the gold standard method for Malaria diagnostic, the Giemsa-stained thick blood smear. Out of the 103 pretested specimens, 83 were Malaria positive (P. falciparum, P. vivax, P. malariae and P. ovale, 6 mixed infections) and 20 Malaria negative. DNA of these blood samples was extracted using different extraction methods and stored at -20°C for different period of time. During the prospective study 120 specimen, send in for routine Malaria diagnostics in a four week period were analyzed by "Thick Smear" microscopy at the Bernhard Nocht Institute for Tropical Medicine and parallel at altona Diagnostics GmbH using the RealStar® Malaria PCR Kit 1.0. Nucleic acid was extracted manually using QiAmp DNA Blood Kit (QIAGEN) while adding the Internal Control (IC).

The RealStar® Malaria PCR Kit 1.0 detects Plasmodium spp. DNA in the FAM-Channel (Fig. 1A) and an Internal Control (IC) in the JOE Channel (Fig. 1B) of the real-time PCR instrument.

Results

Analytical Sensitivity

The analytical sensitivity (Limit of Detection: LoD) of the RealStar® Malaria PCR Kit 1.0 is defined as the concentration (IU per µl of the eluate) of Plasmodium spp. specific DNA molecules that can be detected with a positivity rate of ≥ 95%. The analytical sensitivity was determined by analysis of dilution series of “1st WHO International Standard for Plasmodium falciparum DNA Nucleic Acid Amplification Techniques”. Analytical sensitivity of the RealStar® Malaria PCR Kit 1.0 determined by Probit analysis is 1.27 IU/µl [95 % confidence interval: 0.57 - 5.42 IU/µl].

Analytical Specificity

The specificity of the RealStar® Malaria PCR Kit 1.0 was evaluated by analysing different Plasmodium species, as well as a panel of genomic DNA/RNA extracted from species related to Plasmodium, other blood-borne pathogens and pathogens causing similar symptoms (Table 1).

Table 1: Organisms tested to demonstrate the analytical specificity of the RealStar® Malaria PCR Kit 1.0

All samples were analysed with the same result as with the Golden Standard in Malaria diagnostic (Thick Smear Microscopy), using the RealStar® Malaria PCR Kit 1.0.

Conclusion

The RealStar® Malaria PCR Kit 1.0 turned out to be more sensitive and at least as specific as “Thick Smear” microscopy. Therefore, the RealStar® Malaria PCR Kit 1.0 is a useful assay in Malaria diagnostics.

Contact

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