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Original Research Article

Rapid and simultaneous screening of 47,XXY and *AZF* microdeletions by quadruplex real-time polymerase chain reaction

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ABSTRACT

We developed a quadruplex real-time PCR assay that allows rapid and simultaneous detection of 47,XXY and azoospermia factor (AZF) microdeletions on Y chromosome. The quadruplex assay consisted of four hydrolysis probes and primer sets. Three probes and the corresponding primers were used to qualitatively detect AZFa, AZFb, and AZFc deletions. For the detection of 47,XXY, the hydrolysis probe-mediated melting analysis was conducted to analyze the relative amounts of X and Y chromosomes. The quadruplex assay for detecting 47,XXY was characterized by very high analytical specificity (100%) in a wide template DNA range (2–100 ng). The detection limit of the assay was 2 ng of genomic DNA, and the optimal template DNA amount for the detection of 47,XXY was 25 ng. The quadruplex assay for detecting 47,XXY and AZF microdeletions has also demonstrated very high diagnostic sensitivity and specificity (100%). The assay was found to be rapid, sensitive, reliable, and inexpensive. This method is suggested to be applied as a first-step tool in genetic screening of patients with non-obstructive azoospermia and severe oligospermia.

1. Introduction

Genetic abnormalities, including cytogenetic and molecular abnormalities, account for 15–30% of male infertility by affecting spermatogenesis and sperm transport [1]. The identification of certain genetic abnormalities not only helps to clarify the causes of infertility but also helps to guide treatment options (e.g., assisted reproduction) and evaluate prognosis. Nowadays, it is well recognized that men with non-obstructive azoospermia and severe oligospermia (NOASO) should undergo karyotyping and Y-chromosomal

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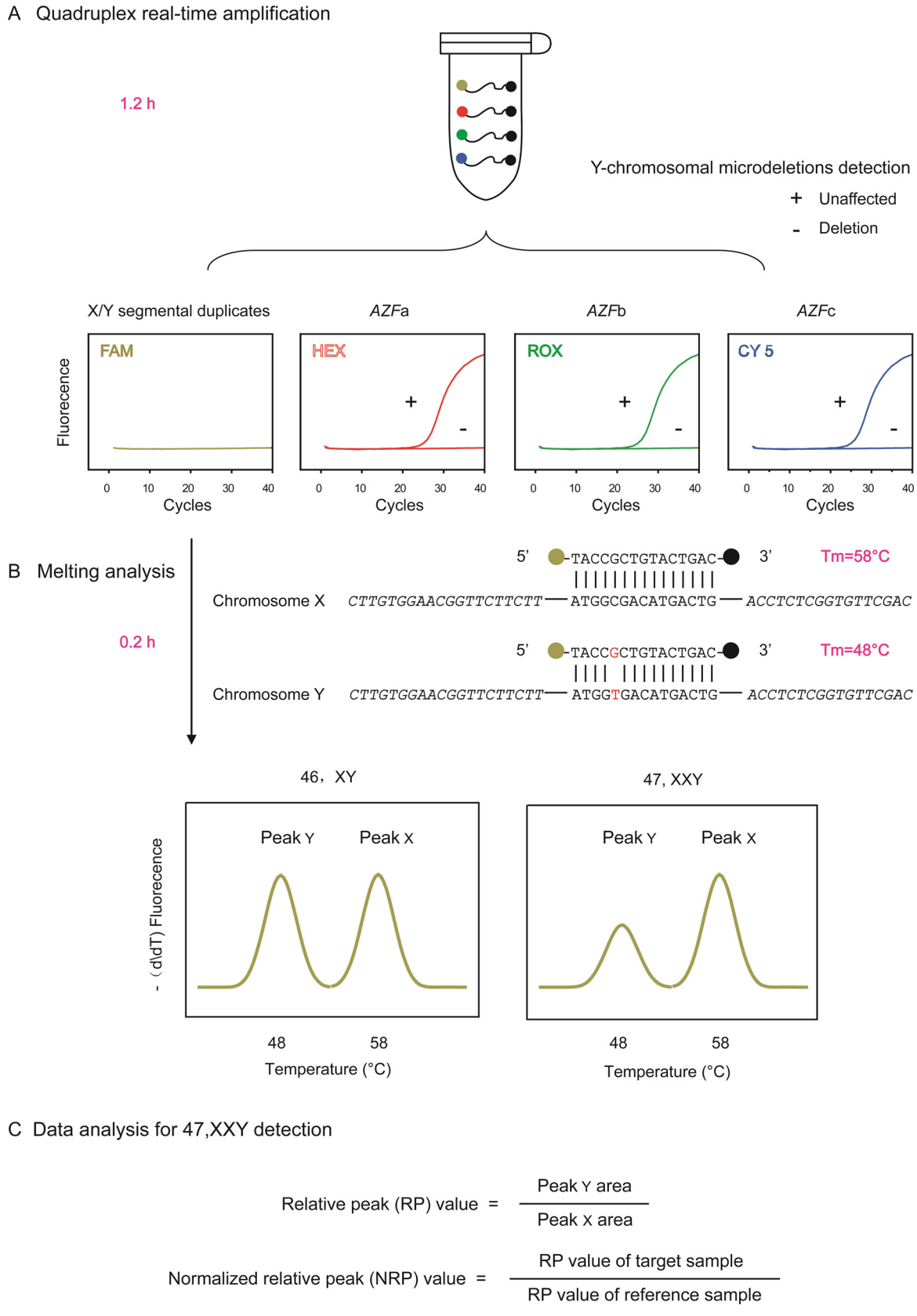

Fig. 1 – Principle of quadruplex real-time PCR assay. (A) Quadruplex real-time amplification with hydrolysis probe detection. This process takes approximately 1.2 h. Different colors indicate different fluorescent labeled hydrolysis probes. Information on *Azoospermia factor* (*AZF*) microdeletions was obtained from the HEX (for AZFa), ROX (for AZFb) and CY5 (for AZFc) channels. (B) Melting analysis of segmental duplicates for the detection of 47,XXY. This process takes approximately 0.2 h. Specific peak profiles were generated in FAM channel by melting analysis. Theoretically, the ratio of the two peak areas indirectly