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A procedure to detect 6 basic STSs and 11 extended STSs in the AZF region using multiplex PCR

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Abstract:

Microdeletions of Y chromosomes frequently occur in 3 subregions of the AZF, namely, AZFa, AZFb, and AZFc, with 6 basic STS marker sequences, which are sY84, sY86 (AZFa), sY127, sY134 (AZFb), and sY254, sY255 (AZFc). According to EAA/EMNQ guidelines, 11 additional AZFabc marker sequences should be used to determine the extent of the microdeletion in the AZF region of infertile men, which is known as 11 extended STSs. By applying mPCR, the authors develop an optimal detection procedure for the 6 basic STS and 11 extended STS using 3 multiplex PCR reactions. The first multiplex PCR reaction includes 6 basic STS plus the 2 control sequences sex-determining region Y (SRY) and zinc finger protein X/Y-linked (ZFX/Y). The second multiplex PCR reaction includes the 6 extended STS sY88, sY1182, sY105, sY121, sY1191, and sY1291 and the 2 control sequences SRY and ZFX/Y. The third multiplex PCR reaction includes the 5 extended STS sY153, sY160, sY82, sY143, and sY83 and the 2 control sequences SRY and ZFX/Y. Six basic primer sequences and eleven extended primer sequences are redesigned to simultaneously pair and amplify STS in the same multiplex reaction: set of 8 primers for 6 basic STS: 6 basic STS + 2 (SRY, ZFX/Y), 8 extension primers set E1: 6 extended STS + 2 (SRY, ZFX/Y), and 7 extension primers set E2: 5 extended STS + 2 (SRY, ZFX/Y). We successfully designed primer pairs with high specificity and stability and successfully amplified 6 basic STS and 11 extended STS, which ensures that the STSs have the correct sequence as recommended by EAA/EMQN and are consistent with the NCBI gene bank. This study has successfully developed a procedure to simultaneously detect 17 STSs, including 6 basic STSs and 11 extended STSs in the AZF region using 3 multiplex PCR reactions.

Keywords: **AZF, azoospermia, male infertility, microdeletion, multiplex PCR, oligospermia.**

Classification number: **3.2**

Introduction

Microdeletions of Y chromosomes account for about 10-15% of male infertility [1-3]. On the long arm of the male sex Y chromosome (Yq11) exists a region called AZF (azoospermia factor) that can be classified into three subregions, AZFa, AZFb, and AZFc, which contain genes that regulate sperm production and differentiation. Microdeletion mutations frequently occur in these three subregions. To diagnose microdeletions on the AZF region of the Y chromosome, the European Academy of Andrology (EAA) and European Molecular Genetics Quality Network (EMQN) recommend using at least 2 sequences in each subregion: AZFa (sY84, sY86), AZFb (sY127, sY134), and AZFc (sY254, sY255). These are the basic sequences to examine and determine any microdeletions are present. In addition to these basic STS sequences, examination of extended STS sequences are also recommended, namely, 4 additional sequences in the AZFa subregion, 4 additional sequences in the AZFb subregion, and 3 additional sequences in AZFc subregion [4, 5]. The eleven recommended STSs are sY88, sY1182,

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sY105, sY121, sY1191, sY1291, sY153, sY160, sY82, sY143, and sY83. The use of multiple sequences in the AZF subregions helps to avoid missing microdeletion mutations when determining the cause of male infertility. The present techniques applied to simultaneously find multiple STSs are capillary electrophoresis and multiplex PCR. While capillary electrophoresis requires a sequencing system, multiplex PCR is simpler and does not require a lot of expensive equipment. However, several problems arise when pairing multiple primers to simultaneously detect many STSs because the difference between STS sizes is insignificant. Therefore, this study deployed a new design to primer sequences and simultaneously amplify multiple STSs in a multiplex PCR reaction. This study intends to integrate 17 STSs into three multiplex PCR reactions. The first multiplex PCR reaction includes 6 basic STSs plus the 2 control sequences SRY and ZFX/Y. The second multiplex PCR reaction includes the 6 extended STSs: sY88, sY1182, sY105, sY121, sY1191, and sY1291 and the 2 control sequences SRY and ZFX/Y. The third multiplex PCR reaction includes 5 extended STSs, namely, sY153, sY160, sY82, sY143, and sY83 and the 2 control sequences SRY and ZFX/Y.

Materials and methods

Materials

The primers help the amplification of the STSs on the AZF region of the Y chromosome. DNA samples of normal men and normal woman were extracted from EDTA anticoagulant blood.

DNA samples were extracted from EDTA anticoagulant blood of male patients with severe oligospermia (sperm density ≤5 million/ml semen) and azoospermia (no sperm in semen). The samples were analysed to detect microdeletions with Sweden's Devyser v2 and the Extension test kit, and then compared to the result after applying the multiplex PCR technique.

Methods

One set of seventeen primers has been designed for multiplex PCR. Six primers were used to amplify the six basic STSs including sY84 and sY86 (AZFa), sY127 and sY134 (AZFb), sY254 and sY255 (AZFc). Then, 11 primers were used to amplify 11 extended STSs including sY82, sY83, sY88, and sY1182 (in AZFa), sY105, sY121, sY143, and sY153 (in AZFb), sY160, sY1191, and sY1291 (in AZFc), and 2 primers to amplify 2 sequences used as internal controls, namely, SRY and ZFX/Y. The primer sequences refer to the design of EAA/EMQN. These primers are divided into 3 groups to create 3 multiplex PCR reactions. The first multiplex PCR reaction includes the 6 basic STSs plus the 2 control sequences SRY and ZFX/Y. The second multiplex PCR reaction includes the 6 extended STSs: sY88, sY1182, sY105, sY121, sY1191, and sY1291 and the 2 control sequences SRY and ZFX/Y. The third multiplex PCR reaction includes the 5 extended STSs: sY153, sY160, sY82, sY143, and sY83 and the 2 control sequences SRY and ZFX/Y. To check the accuracy of the primers, a single primer PCR reaction was used. With a total volume of 20 µl, the Master Mix included a forward primer, reverse primer (2 µl), template DNA (2 µl), Master Mix 2X (10 µl), and dH2O (6 µl). A heat cycle of 95°C for 15 min, then 35 cycles of [95°C-30 s; 57°C-30 s; 72°C-45 s], then 72°C for 10 min.

Three multiplex PCR reactions of 11 STSs in the AZF region and the 2 internal control sequences (SRY and ZFX/Y) were optimised.

Nineteen sequences (17 STSs in the AZF region and the 2 internal control sequences SRY and ZFX/Y) were transferred into the cloning vector. The vector pBT, of *E. coli* DH5α [end A1 rec A1 hsd R17 sup E44 gyp A96 thi-1 relA1Δ lac U169 (ϕ80 lacZM15)] from the Invitrogen company was used to clone. After the purified PCR products are transferred into the cloning vector, the plasmid DNA is transformed into *E. coli* cells by heat shock. Then, PCR is directly performed from the *E. coli* colony and the plasmids are extracted from the *E. coli* cells.

The 17 STSs of the AZF region and 2 internal control sequences SRY and ZFX/Y were sequenced from the plasmids.

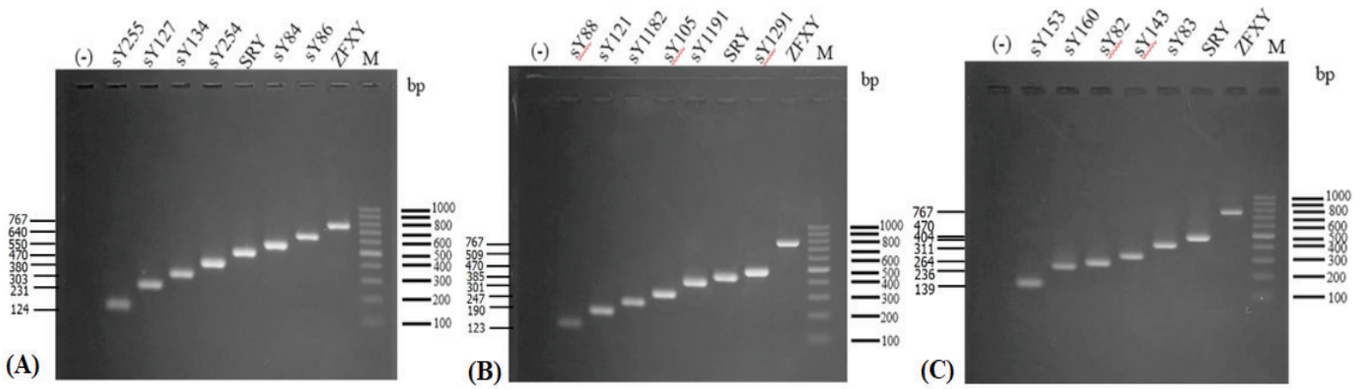

Fig. 1. Agarose gel electrophoresis image of 8 STS sequences after single primer PCR.
Notes: M: marker, DNA 100 bp ladder, (-): negative control, distilled water. **(A)**: set of 6 single basic STSs primer and 2 control sequences: sY255: 124 bp, sY127: 231 bp, sY134: 303 bp, sY254: 470 bp, SRY: 470 bp, sY84: 550 bp, sY86: 640 bp, ZFX/Y: 767 bp; **(B)**: extended set E1 primers with 6 extended STSs and 2 control sequences: sY88: 123 bp, sY121: 190 bp, sY1182: 247 bp, sY105: 301 bp, sY1191: 385 bp, sY1291: 509 bp, SRY: 470 bp, ZFX/Y: 767 bp; **(C)**: extensive set E2 primers with 5 extended STSs and 2 control sequences: sY153: 139 bp, sY160: 236 bp, sY82: 264 bp, sY143: 311 bp, sY83: 404 bp, SRY: 470 bp, ZFX/Y: 767 bp.

A set of 3 multiplex PCR reactions were completed to simultaneously detect microdeletions in the 17 STSs of the AZF region.

DNA samples from normal men, normal women, and severe oligospermia or azoospermia men were used to complete the AZF microdeletion(s) detection procedure.

Main chemicals and equipment required: QIAgen HotStarTaq DNA polymerase (5000U); dNTP Mix, PCR Grade (200 µl); QIAquick PCR purification kit (250); QIAquick gel extraction kit (250); GelPilot Plus Ladder 100 bp, Devyser v2 and Extension test kit (Sweden). A PCR system (BioRad CX96), basic agarose electrophoresis system, agarose gel camera, and an ABI3500 system were the equipment used.

Results

The accuracy of the primers sequences

Nineteen primers designed for amplification were optimised by single primer reactions for each sequence of the 17 AZF STSs and the 2 internal controls SRY and ZFX/Y. The AZF sequences were amplified by single primer PCR, which are the 6 basic STS of the AZFabc region: sY84 and sY86 (AZFa), sY127 and sY134 (AZFb), sY254 and sY255 (AZFc) and 11 extended STS of the AZFabc region: sY82, sY83, sY88, and sY1182 (in AZFa), sY105, sY121, sY143, and sY153 (in AZFb), and sY160, sY1191, and sY1291 (in AZFc).

The results from the 3 designed primer sets showed that 19 PCR products (including the 17 STSs in AZF region and the 2 control sequences SRY and ZFX/Y) were obtained when electrophoresis on agarose gel were of the correct sizes (Fig. 1). Initially, the newly designed primer sequences are suitable to amplify the 17 STS sequences of AZF region and the 2 internal controls SRY and ZFX/Y.

Transfer of sequences into cloning vector

The entire PCR product of the 8 STSs, after being purified, was transferred into the pBT cloning vector by a splicing reaction. Then, the product was transformed into *E. coli* DH5α cell. The results of the PCR products directly from the colonies were obtained through electrophoresis images as seen in Fig. 2.

The electrophoresis results showed that the obtained PCR products were of correct size. Thus, 17 STSs were successfully cloned in three PCR reaction sets. The recombinant pBT plasmids were then extracted, purified, and used to sequence the STSs.

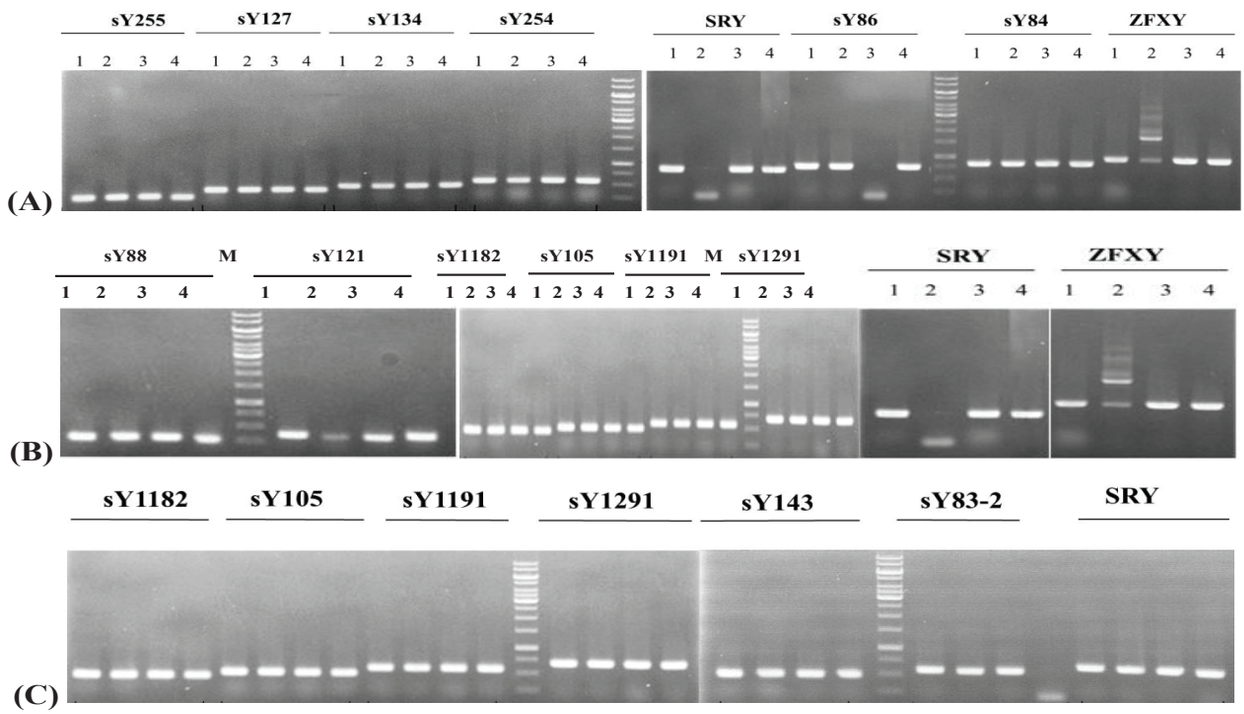

Fig. 2. Electrophoresis image of PCR products from colonies of 17 STSs.
Notes: **(A)**: cloning of 6 basic STSs: sY255, sY127, sY134, sY254, sY84, sY86; **(B)**: cloning of 6 extended STSs: sY88, sY121, sY1182, sY105, sY1191, sY1291; **(C)**: cloning of 5 extended STSs: sY153, sY160, sY82, sY143, sY83.

Sequencing the sequences transferred into the cloning vector

PCR products from the above cloning sequences were purified and then sequencing was performed on ABI 3500 system. Comparing the above sequences with gene bank references, the obtained sequences are found to be consistent with the reference sequences of the AZF region from the NCBI GenBank (National Center for Biotechnology Information) [6].

Optimising the detection of STSs in a single multiplex PCR reaction

The first multiplex PCR reaction consisted of the basic primer set of the 8 primers sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc); SRY and ZFX/Y in 1 multiplex mixture and then the detection of 8 sequences in 1 multiplex PCR reaction was optimised.

The second multiplex reaction consisted of the E1 extension primer set that allows the combination of the 8 primers sY88; sY121; sY1182; sY105; sY1191; sY1291; SRY and ZFX/Y in a multiplex mixture and then the detection of 8 sequences in 1 multiplex PCR reaction was optimised.

The third multiplex PCR reaction consisted of the E2 extension primer set that combines the 7 primers sY153; sY160; sY82; sY143; sY83; SRY and ZFX/Y in 1 multiplex mixture and then the detection of 7 sequences in 1 multiplex PCR reaction was optimised.

The primers to amplify the 17 STSs and 2 internal controls were combined with equal concentration. The optimal annealing temperature was 57°C for 70-90 s. The primers were diluted to 40 µM using Quiagen's Master Mix, which has all the basic ingredients for PCR (dNTPs, Taq polymerase, enzyme buffer). Mixtures for multiplex PCR reactions include: 200 ng of template DNA (4.0-6.5 µl), Master Mix (18.5 µl), and just enough distilled water to ensure the volume of multiplex PCR reaction mixture is 25 µl. The optimal thermal cycle is 95°C for 15 min, then a cycle of (94°C/30 s; 57°C/90 s; 72°C/60 s) for 35 cycles, and finally 72°C for 15 min. The PCR products were electrophoresis on 3% agarose gel at a voltage of 100 V for 85 min. The results are shown in Fig. 3.

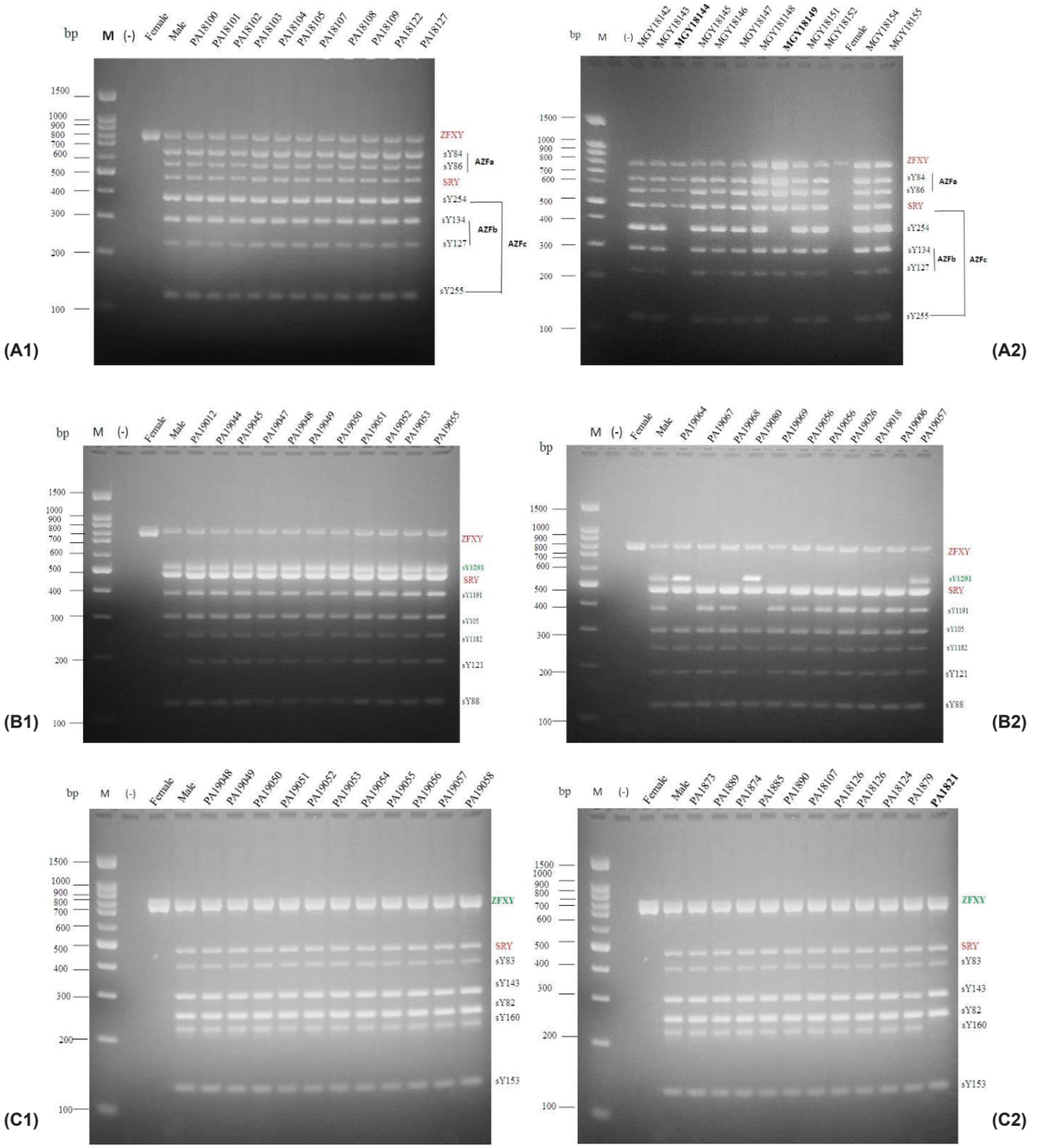

Fig. 3. Electrophoresis image of 3 multiplex PCR products: 17 STSs and 2 control sequences, SRRY, ZFX/Y.

Notes: M: marker, 100 bp DNA ladder; bp: base pair; (-): negative control (distilled water), female: normal female DNA; male: normal male DNA; **(A1**, **A2)**: 1st multiplex PCR reaction, samples PA 18100-18105,18107-18109, 18122, 18127, MGY18142-18144, 18145-18149, 18151-18152, 18154-18155. **(B1**, **B2)**: 2nd multiplex reaction, samples: PA19012, PA19044, PA19045, PA19047, PA19048, PA19049, PA19050, PA19051, PA19052, PA19053, PA19055, PA19064, PA19067, PA19068, PA19080, PA19069, PA19060, PA19056, PA19026, PA19018, PA19006 , PA19057. **(C1**, **C2)**: 3rd multiplex reaction: samples PA19048-19058, PA1873-74, PA1889, PA1885, PA1890, PA18107, PA18126, PA18124, PA1879, PA1821.

For each multiplex reaction, electrophoresis images of the multiplex PCR products showed electrophoresis bands that are clearly separated, the image is sharp, and is consistent with the theoretical calculated size. Each multiplex reaction is performed with the following controls: positive control (male), female control (female), negative control (distilled water). The results obtained indicate the positive control is male with all STSs, female control only has ZFX, and no product existing in the negative control.

For the first multiplex PCR reaction, 23 infertile male samples were analysed with 21 samples having 8 bands corresponding to the 8 sequences sY84, sY86, sY127, sY134, sY254, sY255, SRY, and ZFX/Y (without AZF microdeletion). The sample MGY18144 lacked electrophoresis bands at the sY127, sY137, sY254, and sY255 positions, indicating an AZFbc microdeletion, while sample MGY18149 lacked electrophoresis bands at sY254 and sY255 positions, which indicated an AZFc microdeletion.

For the second multiplex PCR reaction, 22 infertile male samples were analysed with 12 samples PA19012, PA19044, PA19045, PA19047, PA19048, PA19049, PA19050, PA19051, PA19052, PA19053, PA19055, and PA19057, which were similar to the male control and had 8 bands corresponding to the 8 sequences sY88, sY121, sY1182, sY105, sY1191, sY1291, SRY, and ZFX/Y. Samples PA19067, PA19068, PA19069, PA19060, PA19056, PA19026, PA19018, and PA19006 lacked electrophoresis bands at the sY1291 position, which demonstrates a sY1291 microdeletion. Sample PA19064 lacked an electrophoresis band at the sY1191 position, which indicated a sY1191 microdeletion. Sample PA19080 lacked an electrophoresis band at the sY1191 and sY121 positions, which indicated sY1191 and sY121 microdeletions.

For the third multiplex PCR reaction, 22 infertile male samples were analysed. There were 19 samples similar to the male control with 7 electrophoresis bands corresponding to the 7 sequences sY153, sY160, sY82, sY143, sY83, SRY, and ZFX/Y. Samples PA1889 and PA1879 lacked an electrophoresis band at the sY143 position and sample PA1821 lacked an electrophoresis band at the sY160 position.

These samples (67 samples with male and female controls) were analysed for AZF microdeletions using the Swedish Devyser v2 and Extension test kits. The results were similar between the two methods in their ability to detect and classify the microdeletions.

Discussion

PCR is the leading technique employed in molecular genetics and can be easily applied in many laboratories. Each multiplex PCR reaction currently being used in diagnostics can only detect up to 4 or 5 sequences [4]. The idea of this study is to design a multiplex PCR procedure to detect as many AZF-region STSs as possible in the diagnosis of Y chromosome microdeletions in order to solve the problem. These sequences are just the basic STSs of AZF regions a, b, and c [7]. Combining multiple primers in a multiplex PCR reaction requires the primers to have a suitable melting temperature and the primers should not have a complementary sequence to each other, especially at the 3' end. The DNA bands must also be significantly different in size to be easily separated on electrophoresis gel, but the differences should not be too large to be easily amplified under the same PCR cycle. The capillary electrophoresis technique can overcome the shortcomings of multiplex PCR as it can pair multiple primers to simultaneously detect multiple sequences, but this method requires a genetic sequencing system and thus has an expensive test cost.

Since 1999, according to the guidelines of the EAA and EMQN, the diagnosis of AZFa, b, and c microdeletions must use the 6 sequences sY84, sY86, sY127, sY134, sY254, and sY255 along with the two control sequences SRY and ZFX/Y, the latter being a gene found on both male and female sex chromosomes (X and Y). These 8 sequences are then combined into 2 multiplex PCR reactions (multiplex A and B) and the electrophoresis products took about 24 h not including sample preparation and PCR. Because the sequence sizes are close to each other, when under electrophoresis, a low voltage and a long separation time are required to form PCR products [4]. Since 2010, the Hanoi Medical University, based on the method of EAA/EMQN, improved the multiplex PCR method to detect 6 STSs of AZF by dividing them into 3 multiplex PCR reactions (multiplex 1, 2 and 3), which separates similarly size sequences into different multiplexes for the purpose of shortening the electrophoresis time to only 30-45 min

each. Thus, these multiplex reactions can amplify the maximum simultaneous number of 3 STSs [8]. In 2015, the University of Science, Vietnam National University, Ho Chi Minh city, redesigned the primers of the above 6 sequences into 2 multiplex reactions (multiplex A and multiplex B). However, when redesigned, the sequences were significantly different in size and could be clearly separated in the same electrophoresis with the maximum of 3 STSs in a multiplex reaction [7].

Our study is the first study in Vietnam succeeding in optimising 17 STSs simultaneously in 3 multiplex PCR reactions with 5 to 6 STSs in each multiplex PCR reaction. This doubles the number of STSs in multiplex reactions compared to previous studies. For the 6 basic STSs of the AZF region, previous studies had to divide them into 2-3 multiplex PCR reactions [4, 7, 8], in this study, all 6 STSs were detected with only 1 multiplex PCR reaction. For the extended STSs, no studies exist using multiplex PCR to detect these, however, we have also successfully built 2 reactions to simultaneously detect these 11 extended STSs in the AZFabc region as recommended by EAA/EMQN. The use of extended STSs in the diagnosis of AZF microdeletions is recommended for infertile patients especially those with moderate and severe oligospermia. The STSs are selected so that they can be combined together. The most difficult part of this study was the redesign of the 17 STSs amplification primer sequences and the two control sequences, SRY and ZFX/Y, to ensure that each STS had a clear, well separated band on the electrophoresis gel.

In this study, the authors referenced several primer pairs designed in the basic and extended AZF test kits as recommended by EAA/EMQN. We also redesigned several primer pairs to easily identify STSs after electrophoresis. The principle of the primer redesign is to fit in all the respective sequences recommended by EAA/EMQN. According to EAA/EMQN guidelines, the basic and extended AZF test kits should have 17 STSs in the AZFa, AZFb, AZFc subregions and the 2 control sequences SRY and ZFX/Y. To develop a basic and extensive AZF test kit, the following sequences are chosen: sY84 (472 bp); sY86 (318 bp); sY127 (274 bp); sY134 (301 bp); sY254 (380 bp); sY255 (123 bp); sY88 (123 bp); sY121 (190 bp); sY1182 (247 bp); sY105 (301 bp); sY1191 (385 bp); sY1291 (527 bp), sY153 (139 bp); sY160 (236 bp); sY82 (264 bp); sY143 (311 bp); sY83 (275 bp); SRY (472 bp); ZFX/Y (495 bp) [4]. However, the sizes (shown in parentheses) of some STSs are not significantly different, making separation on agarose gel electrophoresis difficult.

Several STS primers sequences were redesigned to amplify as clearly identified products on electrophoresis gel. The sequences are detailed as follows: sY84 (640 bp); sY86 (550 bp); sY127 (231 bp); sY134 (303 bp); sY254 (380 bp); sY255 (124 bp); sY88 (123 bp); sY121 (190 bp); sY1182 (247 bp); sY105 (301 bp); sY1191 (385 bp); sY1291 (509 bp); sY153 (139 bp); sY160 (236 bp); sY82 (264 bp); sY143 (311 bp); sY83 (404 bp).

The separation of the two control sequences SRY (472 bp) and ZFX/Y (495 bp) was difficult, so ZFX/Y was redesigned with a 767 bp size. Some sequences had insignificant differences in size from reference, for example, SRY is now 470 bp (2 nucleotides different) and sY1291 is now 509 bp (18 nucleotide different). The single STS PCR products tested on 1.5% agarose gel in Fig. 1 show successful amplifications and clear electrophoresis bands for each sequence. However, to ensure that these amplified sequences are the STSs in the AZF region and the two control sequences SRY and ZFX/Y, they were purified and transferred into a pBT cloned vector and then transformed into *E. coli* DH5α cells to clone the sequences. The results in Fig. 2 show that we successfully cloned 17 STSs in the AZF region and the 2 control sequences. The electrophoresis results of the PCR products obtained are of the correct theoretical size (STS size plus 164 bp, 164 bp is the general size of the plasmid sequence). Thus, the STSs present in the basic and extended AZF test kits were successfully cloned. The recombinant pBT plasmids were extracted, purified, and used to sequence STSs in the basic and extended AZF test kit. These sequences were then compared with the NCBI GenBank references and found to be completely similar to those references sequences.

The authors successfully combined 19 primers, including 17 primers to amplify the 17 STSs and 2 primers to amplify the 2 internal control sequences SRY and ZFX/Y, into 3 multiplex PCR reactions. When optimised, the redesigned sequence sizes are relatively different from each other, so after amplification and electrophoresis in 3% agarose gel, the STSs are clearly separated in each reaction (Fig. 3). The negative control

(distilled water) was bandless after electrophoresis and the normal female had 1 band of ZFX (767 bp) while the normal male had 8 basic AZF electrophoresis bands, 8 electrophoresis bands of AZF Extension set E1, and 7 electrophoresis bands of AZF Extension set E2. The 67 infertile male samples were analysed for microdeletions in the AZF region with the Devyser v2 and Extension test kit (Sweden). The obtained results were similar to our study's results. Thus, the optimised multiplex PCR technique to simultaneously detect 17 STSs in 3 multiplex PCR reactions is similar to the detection of AZF microdeletions by gene segment size analysis on an ABI 3500 sequencing system. The AZF region sequences were correctly detected and were stable when performed with patient and control DNA samples.

Conclusions

The authors have successfully designed primer pairs with high specificity and stability according to amplified 17 STSs that were clearly separated on agarose gel. Eight plasmids were also cloned successfully to carry DNA sequences from the AZF region, as well as SRY and ZFX/Y, which were used as reference DNA samples for the detection of microdeletions on Y chromosome.

A procedure to simultaneously detect 17 STSs in 3 multiplex PCR reactions was successfully developed with each multiplex PCR reaction designed to simultaneously detect up to 6 STSs and the 2 control sequences SRY and ZFX/Y. The first multiplex PCR reaction detects 6 basic STSs: sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc); SRY and ZFX/Y in a multiplex mixture and optimises detection of 8 sequences in a multiplex PCR reaction. The second multiplex reaction detected 6 extended STSs, namely, sY88, sY121, sY1182, sY105, sY1191, and sY1291 along with SRY and ZFX/Y in a multiplex PCR reaction and 8 sequences were optimised and detected in each. The third multiplex PCR reaction detected 5 extended STSs, namely, sY153, sY160, sY82, sY143, and sY83, as well as SRY and ZFX/Y in a multiplex PCR reaction and 7 sequences were optimised detected in each.

After testing 67 DNA samples of infertile males, a normal male, and a normal female, this procedure detected the above described AZF regions microdeletions with high sensitivity, specificity, and stability. The results were similar to that of using a sequencing system.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] L. Ting, S. Yu-Xin, J. Yong-Mei (2019), "Early detection of Y chromosome microdeletions in infertile men is helpful to guide clinical reproductive treatments in southwest of China", *Medicine*, **98(5)**, DOI: 10.1097/MD.0000000000014350.

[2] P. Taylor, R. Jaden, C. Ryan (2019), "The prevalence of Y-chromosome microdeletions in oligozoospermic men: a systematic review and meta-analysis of European and North American studies", *European Urology*, **76(5)**, pp.626-636.

[3] C. Stacy, M. Deepak (2018), "Genetics of the human Y chromosome and its association with male infertility", *Reproductive Biology and Endocrinology*, **16(14)**, DOI: 10.1186/s12958-018-0330-5.

[4] C. Krausz, et al. (2014), "EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013", *Andrology*, **2(1)**, pp.5-19.

[5] K. Sandeep, et al. (2016), "Gr/gr deletions on Y-chromosome correlate with male infertility: an original study, meta-analyses, and trial sequential analyses", *Scientific Reports*, **6**, DOI: 10.1038/srep19798.

[6] https://www.ncbi.nlm.nih.gov/.

[7] T.K.Ph. Huynh, et al. (2015), "Establishment of a multiplex - PCR protocol for detection of Y chromosome microdeletion in Azoospermia male patients", *Science and Technology Development Journal*, **18(5)**, pp.5-15.

[8] T.D. Phan, et al. (2013), "Complete multiplex PCR technique to detect microdeletion on the Y chromosome in male infertility patients", *Journal of Vietnamese Medicine*, **403**, pp.655-661.