Research in Performance Comparison Among A27Plex Plus and Two Similar Imported Kits

Abstract: **Objective:** To compare the performance of A27Plex Plus Kit with two similar imported kits, namely, GlobalFiler™ and PowerPlex® Fusion 6C System. **Method:** Design a parallel experiment to compare the differences among A27Plex Plus, GlobalFiler™ and PowerPlex® Fusion 6C System in sensitivity and tolerance to PCR inhibitors. **Results:** Under the condition of 25 μL reaction system and 29-cycle amplification, the sensitivity of A27Plex Plus, GlobalFiler™ and Fusion 6C were all 0.125ng, and A27Plex Plus topped the detection rate of alleles, while GlobalFiler™ topped the detected peak height; A27Plex Plus exhibited the best hemoglobin tolerance, while Fusion 6C showed the best tolerance to heme; indigo and humic acid had little impact on the amplification using the above three kits; in terms of ETDA-2Na, the performance of A27Plex Plus was significantly superior to the other two kits. **Conclusions:** There is no significant advantage in the performance of the three kits, and all of them are suitable for the forensic identification.

Key words: Forensic biological evidence; Short tandem repeat (STR) typing; A27Plex Plus; GlobalFiler™; PowerPlex® Fusion 6C System.

PCR typing of STR loci is the most important technology in forensic individual identification and paternity identification in China and internationally. According to the EU Forensic DNA Typing Standardization Committee, although new technologies and new genetic markers are emerging, STR will remain the most important genetic marker of autosomes and sex chromosomes for a long foreseeable time[1]. In 2012, American Applied Biosystems (ABI) launched the GlobalFiler™ STR Typing Kit, which contains 21 autosomal STR loci, 1 Y-STR (DYS391), and two gender loci, namely, Amelogenin and Yindel. At the same time, the PowerPlex® Fusion 6C System launched by Promega, another famous manufacturer of STR typing kit in the United States, uses 6-color multiplex amplification detection system, which can amplify 27 loci at one time. Most of the domestic STR typing kits manufacturers are located in East China. Among them, the A27Plex Plus STR Typing Kit launched by Jiangsu Superbio Biomedical in 2018 contains 24 autosomal STR loci (D3S1358, vWA, D12S391, CSF1PO, Penta E, D2S441, D16S539, D7S820, D13S317, D2S1338, Penta D, D22S1045, D19S433, D18S51, D6S1043, D8S1179, D5S818, D21S11, FGA, D10S1248, TH01, D1S1656, TPOX, and SE33), 1 Y-STR (DYS391), 1 gender locus Amelogenin and 1 Y-insertion/deletion locus Y-indel (RefSNP: rs199815934), 23 of which contain the latest CODIS core loci. In order to provide reference for forensic examiners and to verify the forensic value of domestic kits, this paper compares the performance differences among A27Plex Plus, GlobalFiler™ and Fusion 6C in terms of sensitivity and tolerance.

1. Materials and Methods

1.1 DNA sample

Positive control 9948

1.2 Main instruments and reagents

Harris Stopper Borer, Applied Biosystems Veriti Thermal Cycle, 3500 Genetic Analyzer (ABI, USA), GlobalFiler™ PCR Amplification Kit (Thermo Fisher, USA), PowerPlex Fusion 6C System (Promega, USA), A27Plex Plus Kit (Jiangsu Superbio Biomedical Co., Ltd.), TE, heme, hemoglobin, EDTA-2Na and indigo (Sangon Biotech (Shanghai) Co., Ltd.), humic acid (Sigma-Aldrich, Inc)

1.3 PCR amplification and capillary electrophoresis

Use TE buffer to dilute the 1ng/μL 9948 positive control into 0.03125 ng/μL and 0.0625ng/μL two concentrations. The amplification system of sensitivity test is 25μL, and the amplification system of tolerance is 10μL. In order to reduce the error, the dosage of 9948 positive control in sensitivity test is 2μL, and the dosage of other components shall be operated according to the instructions. PCR amplification parameters of A27Plex Plus: 95 ℃ for 2min; 94 ℃ for 10s, 60 ℃ for 1.5min, 29 cycles; 60 ℃ for 10min; 4 ℃ for storage. The amplification parameters of GlobalFiler™: 95 ℃ for 1min; 94 ℃ for 10s, 58 ℃ for 1.5min, 29 cycles; 60 ℃ for 10min; 4 ℃ for storage. The amplification parameters of Fusion 6C: 96 ℃ for 1min; 96 ℃ for 5s, 60 ℃ for 1min, 29 cycles; 60 ℃ for 10min; 4 ℃ for storage. Take 1μL of PCR amplification product and add in 10μL HIDI formamide (containing internal standards), and analyze the data by GeneMapper ID-X software after being tested by 3500 Genetic Analyzer.

1.4 Comparison of performance indexes

1.4.1 Sensitivity test

Take 0.03125 ng, 0.0625 ng and 0.125 ng of 9948 positive control to perform PCR amplification using the three kits, and repeat three times in parallel.

1.4.2 Tolerance test

The tolerance of three kits to five common PCR inhibitors, namely, heme, hemoglobin, indigo, humic acid and EDTA-2Na, is tested. Heme: take 9948 of 0.5ng template amount and add heme to make its final concentrations into 400, 500, 600, 700, 800 μM; the final concentrations of hemoglobin are 500, 600, 700, 800, 900 μM; the final concentrations of indigo are 8, 10, 12, 14, 15mM; the final concentrations of humic acid are 30, 40, 50, 60, 70ng/μL; the final concentrations of EDTA-2Na are 0.8, 1.0, 1.2, 1.4, 1.5mM. Perform PCR amplification using the three kits and repeat 3 times in parallel.

1.4.3 Calculation method of each test index

Alleles detected: peak heights are higher than 50 RFU, positions are correct, peak shapes are sharp

and clear, which can be clearly identified as the correctly typed peaks.

Allele detection rate = quantity of alleles detected / quantity of alleles that should be detected theoretically

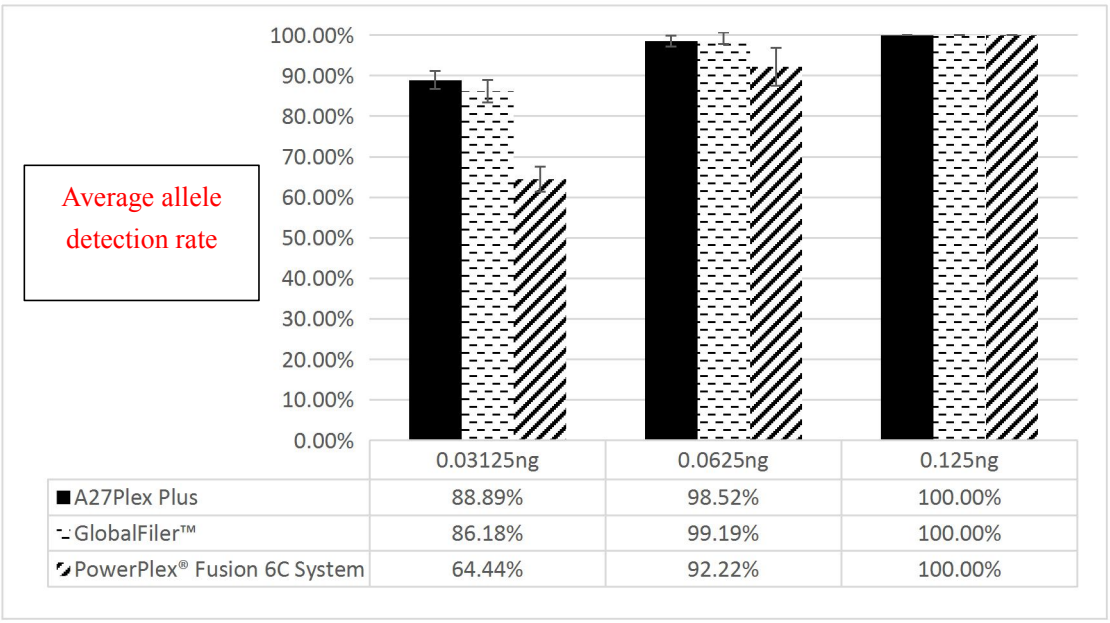
Peak height = sum of peak heights of alleles detected / quantity of alleles detected

2. Results

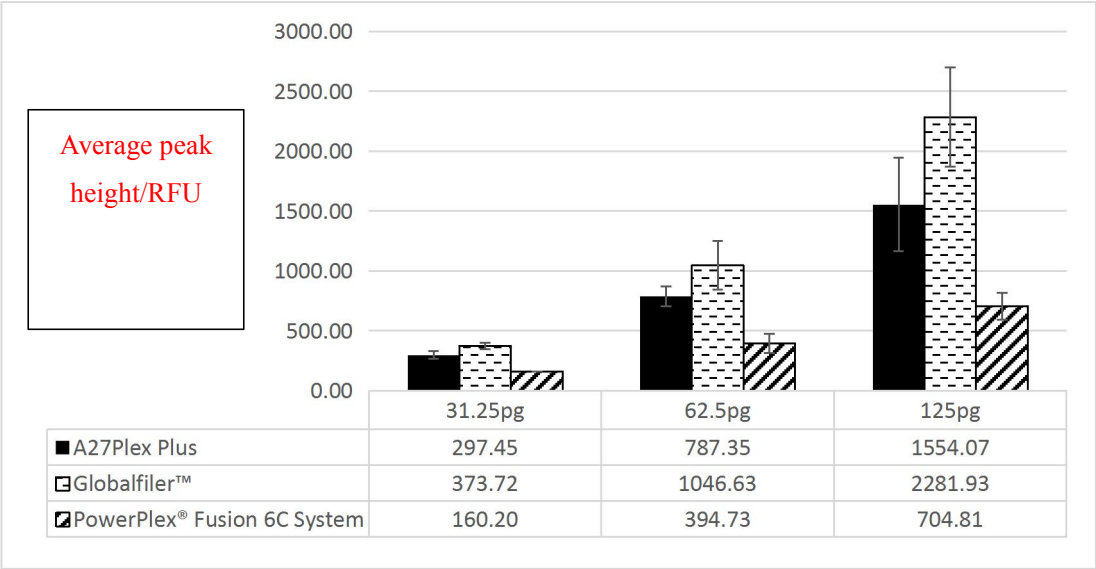
2.1 Sensitivity

When the amount of DNA template added in is 0.125ng, all the three kits can obtain complete STR typing maps, indicating that the sensitivity can reach 0.125ng. The average peak height of A27Plex Plus is 1554.07, that of GlobalFiler™ is 2281.93, and that of Fusion 6C is 704.81, which indicates that when the template amount is 0.125 ng, the average peak height of A27Plex Plus is slightly lower than that of GlobalFiler™, which is about twice higher than that of Fusion 6C; when the DNA template amount added in is 0.0625 ng, the average allele detection rate of A27Plex Plus is 98.52%, that of GlobalFiler™ is 99.19%, and that of Fusion 6C is 92.22%, which indicates that the average allele detection rate of Fusion 6C is the lowest, and its standard deviation is also the worst among the three kits, suggesting that the random effect of allele peak is more severe when Fusion 6C is used to amplify a small amount of DNA; when the amount of DNA template added in is 0.03125 ng, the average allele detection rate of A27Plex Plus is 88.89%, that of GlobalFiler™ is 86.18%, and that of Fusion 6C is 64.44%, respectively.

Although the number of loci contained in the three kits is different, the average allele typing results of A27Plex Plus (39.2) and GlobalFiler™ (35.3) are also more than Fusion 6C (29.0), indicating that A27Plex Plus and GlobalFiler™ are capable of acquiring more genetic information than Fusion 6C (Figure 1).



	0.03125ng	0.0625ng	0.125ng
A27Plex Plus	88.89%	98.52%	100.00%
GlobalFiler™	86.18%	99.19%	100.00%
PowerPlex® Fusion 6C System	64.44%	92.22%	100.00%

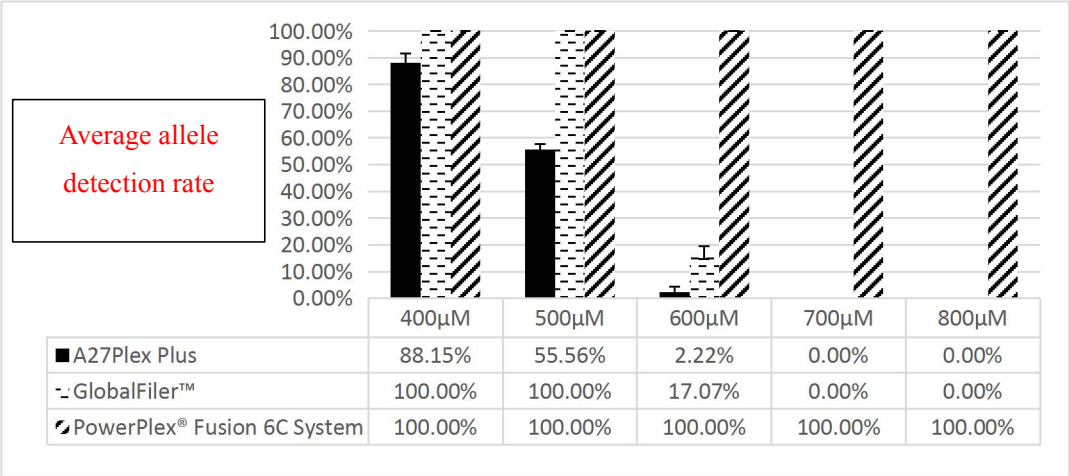


	31.25pg	62.5pg	125pg
A27Plex Plus	297.45	787.35	1554.07
Globalfiler™	373.72	1046.63	2281.93
PowerPlex® Fusion 6C System	160.20	394.73	704.81

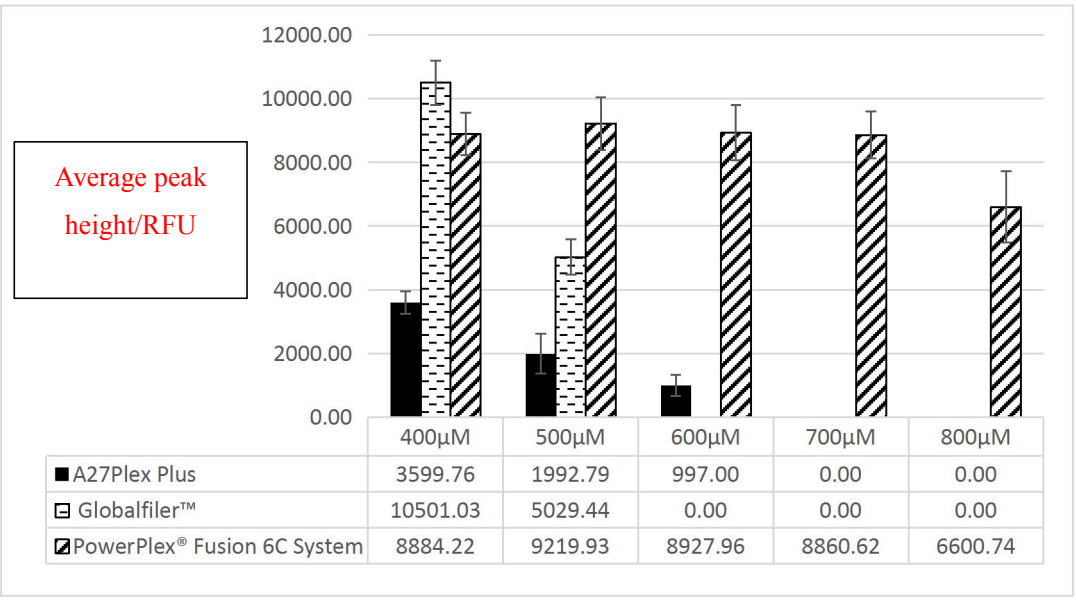
Figure 1: Sensitivity test results of 3 kits

2.2 Tolerance

(1) With the increase of heme content, the average allele detection rate of A27Plex Plus decreases gradually. When the heme concentration reaches 700 μM, the PCR amplification reaction is completely inhibited, and the negative results are obtained. GlobalFiler™ can obtain complete STR typing maps when the heme concentrations reach 400 μM and 500 μM, but when the heme concentration reaches 600 μM, the alleles of GlobalFiler™ are largely lost, only 6-8 alleles are detected. Fusion 6C can detect all alleles between the heme concentration of 400 μM and 800 μM; (2) A27Plex Plus has the best tolerance to hemoglobin. When the hemoglobin concentrations are 500 μM, 600 μM, 700 μM, 800 μM and 900 μM, the average allele detection rate can reach 100%. The average allele detection rate of the other two imported kits will be reduced to different degrees. Among them, D13S1338, vWA, TPOX, Yindel, Amel, DYS391, D2S441, TH01, D10S1248, D1S1656 and D12S391, 11 alleles of GlobalFiler™ have split peaks; (3) the three kits have similar tolerance to the inhibitor indigo, when the concentration of indigo is 8mm-15mm, all alleles are detected, and when the concentration of humic acid reaches 60ng/μL, the average allele detection rate reaches 100%. Although the detection rate of A27Plex Plus does not reach 100% when humic acid concentration increases to 70ng/μL, 89.63% of the loci are successfully detected; (4) when EDTA-2Na concentration is 0.8mM, only A27Plex Plus and PowerPlex® Fusion 6C System can obtain complete STR typing maps. With the increase of EDTA-2Na concentration, the average allele detection rate of the three kits deceases. GlobalFiler™ can not detect any allele at EDTA-2Na concentration of 1.2 mm, Fusion6C only detects 0.7 alleles at EDTA-2Na concentration of 1.5 mm, and A27PlexPlus can still successfully detect 19.7 alleles (Figure 2).

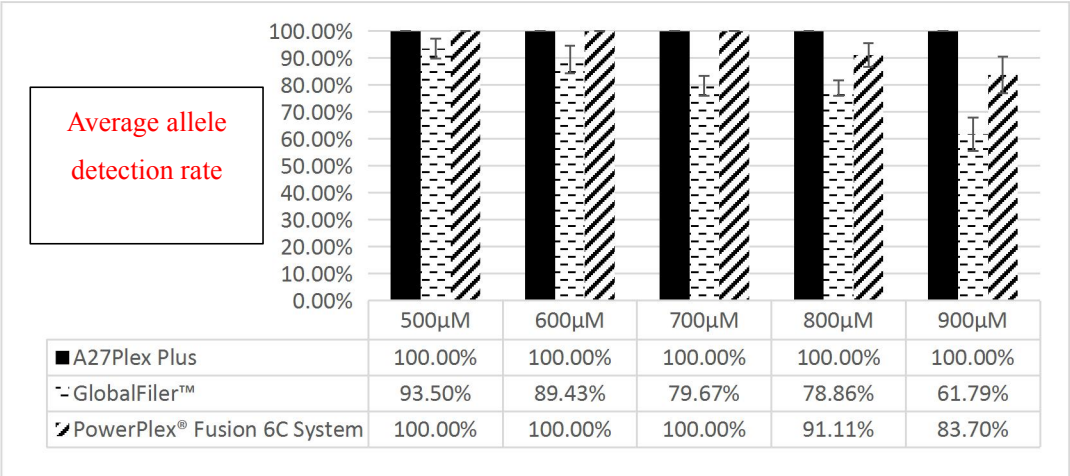


	400μM	500μM	600μM	700μM	800μM
A27Plex Plus	88.15%	55.56%	2.22%	0.00%	0.00%
GlobalFiler™	100.00%	100.00%	17.07%	0.00%	0.00%
PowerPlex® Fusion 6C System	100.00%	100.00%	100.00%	100.00%	100.00%

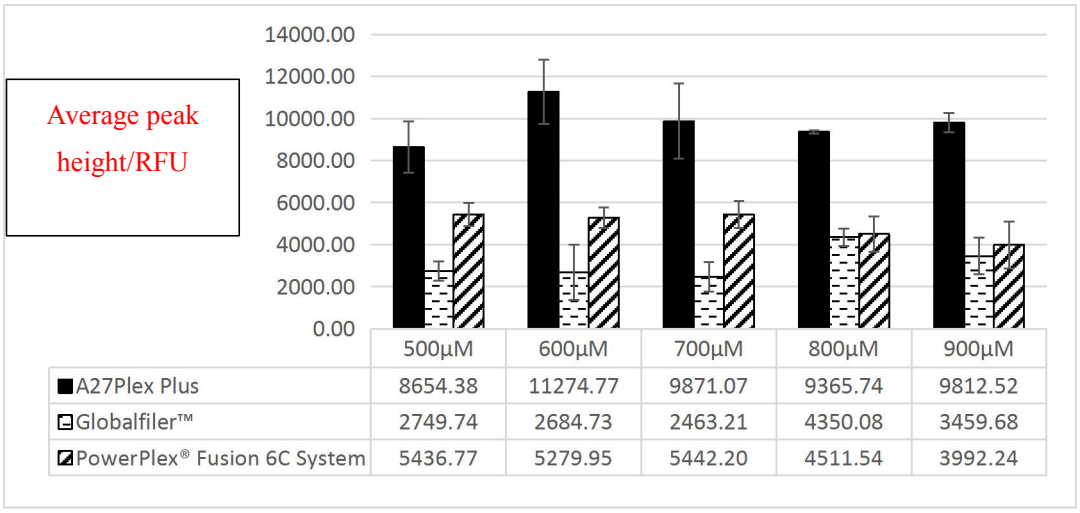


	400μM	500μM	600μM	700μM	800μM
A27Plex Plus	3599.76	1992.79	997.00	0.00	0.00
Globalfiler™	10501.03	5029.44	0.00	0.00	0.00
PowerPlex® Fusion 6C System	8884.22	9219.93	8927.96	8860.62	6600.74

A: Effect of different concentrations of heme on three kinds of kits

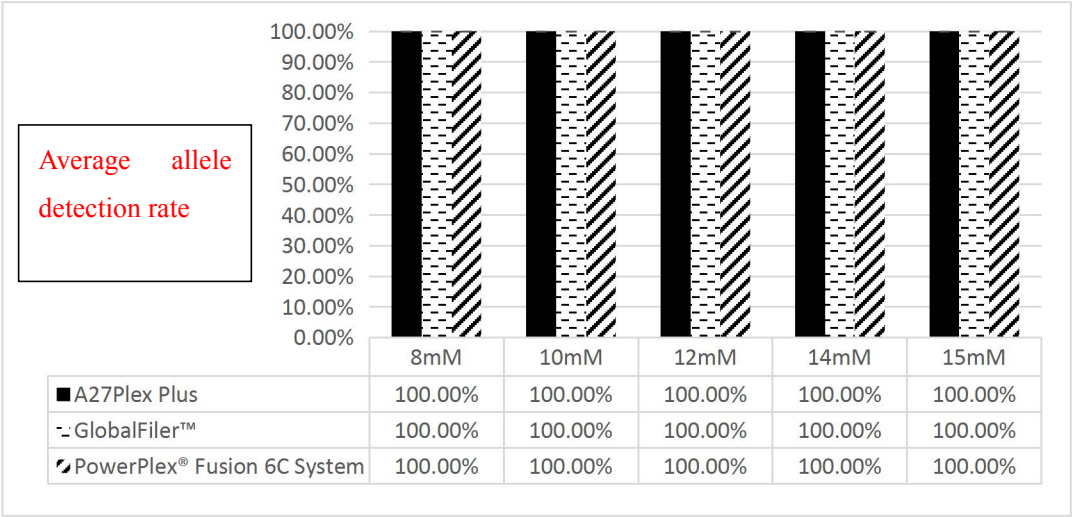


	500μM	600μM	700μM	800μM	900μM
A27Plex Plus	100.00%	100.00%	100.00%	100.00%	100.00%
GlobalFiler™	93.50%	89.43%	79.67%	78.86%	61.79%
PowerPlex® Fusion 6C System	100.00%	100.00%	100.00%	91.11%	83.70%

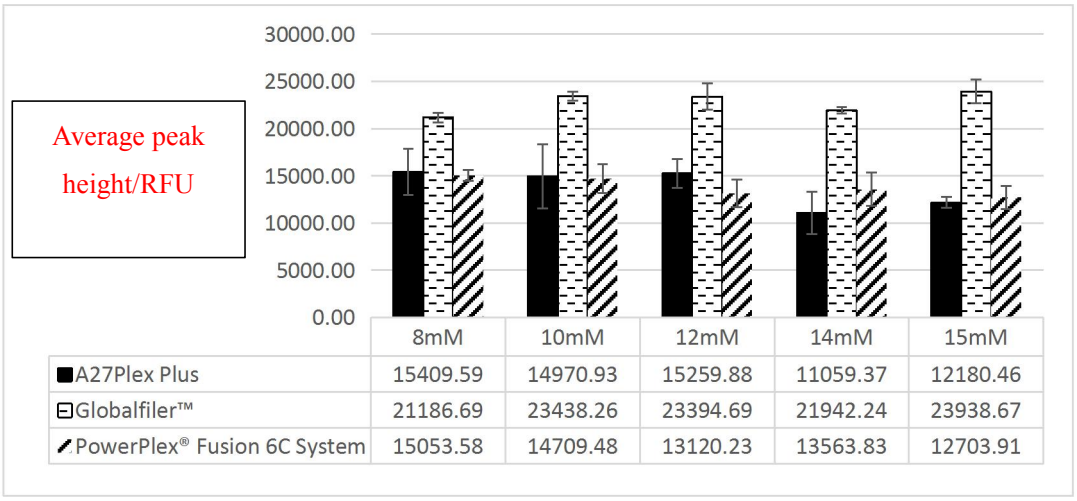


	500μM	600μM	700μM	800μM	900μM
A27Plex Plus	8654.38	11274.77	9871.07	9365.74	9812.52
Globalfiler™	2749.74	2684.73	2463.21	4350.08	3459.68
PowerPlex® Fusion 6C System	5436.77	5279.95	5442.20	4511.54	3992.24

B: Effect of different concentrations of hemoglobin on three kinds of kits

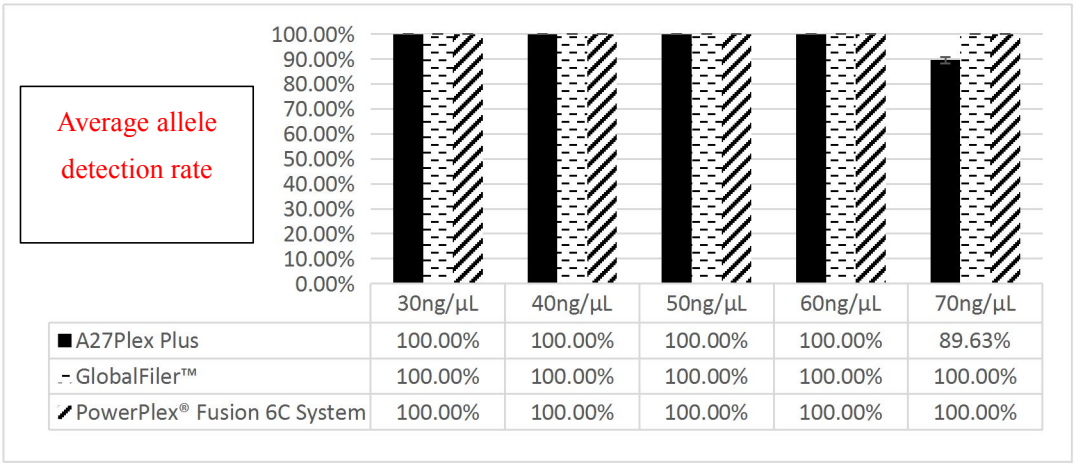


	8mM	10mM	12mM	14mM	15mM
A27Plex Plus	100.00%	100.00%	100.00%	100.00%	100.00%
GlobalFiler™	100.00%	100.00%	100.00%	100.00%	100.00%
PowerPlex® Fusion 6C System	100.00%	100.00%	100.00%	100.00%	100.00%

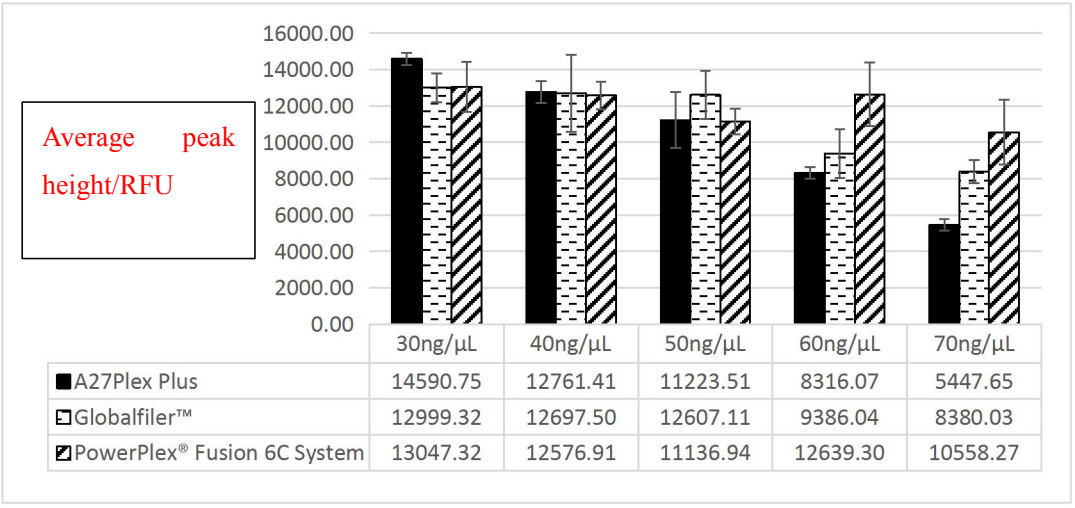


	8mM	10mM	12mM	14mM	15mM
A27Plex Plus	15409.59	14970.93	15259.88	11059.37	12180.46
Globalfiler™	21186.69	23438.26	23394.69	21942.24	23938.67
PowerPlex® Fusion 6C System	15053.58	14709.48	13120.23	13563.83	12703.91

C: Effect of different concentrations of indigo on three kinds of kits

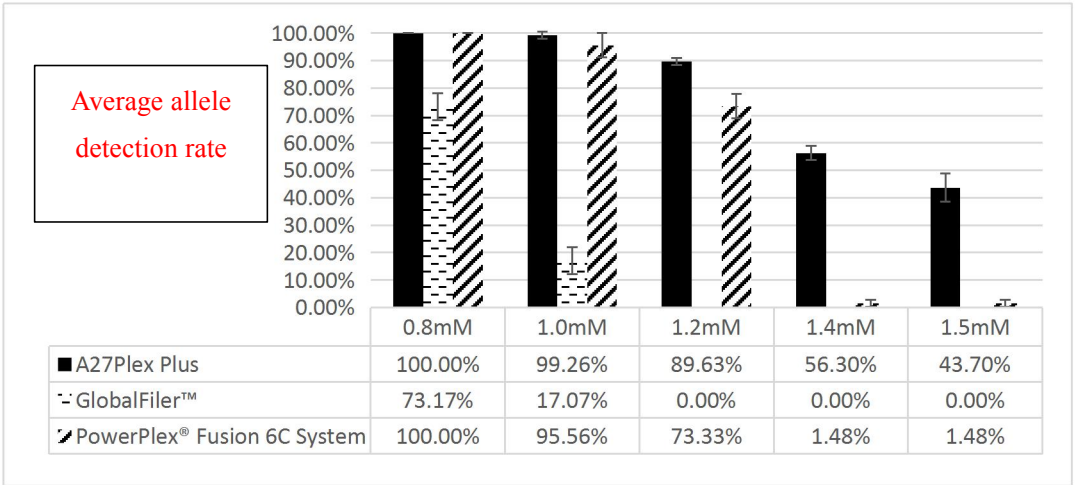


	30ng/μL	40ng/μL	50ng/μL	60ng/μL	70ng/μL
A27Plex Plus	100.00%	100.00%	100.00%	100.00%	89.63%
GlobalFiler™	100.00%	100.00%	100.00%	100.00%	100.00%
PowerPlex® Fusion 6C System	100.00%	100.00%	100.00%	100.00%	100.00%

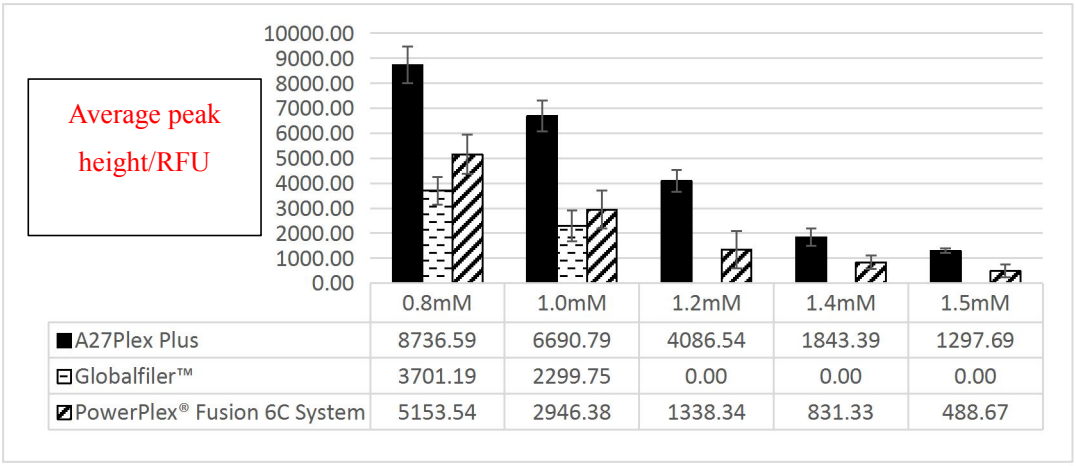


	30ng/μL	40ng/μL	50ng/μL	60ng/μL	70ng/μL
A27Plex Plus	14590.75	12761.41	11223.51	8316.07	5447.65
Globalfiler™	12999.32	12697.50	12607.11	9386.04	8380.03
PowerPlex® Fusion 6C System	13047.32	12576.91	11136.94	12639.30	10558.27

D: Effect of different concentrations of humic acid on three kinds of kits



	0.8mM	1.0mM	1.2mM	1.4mM	1.5mM
A27Plex Plus	100.00%	99.26%	89.63%	56.30%	43.70%
GlobalFiler™	73.17%	17.07%	0.00%	0.00%	0.00%
PowerPlex® Fusion 6C System	100.00%	95.56%	73.33%	1.48%	1.48%



	0.8mM	1.0mM	1.2mM	1.4mM	1.5mM
A27Plex Plus	8736.59	6690.79	4086.54	1843.39	1297.69
Globalfiler™	3701.19	2299.75	0.00	0.00	0.00
PowerPlex® Fusion 6C System	5153.54	2946.38	1338.34	831.33	488.67

E: Effect of different concentrations of EDTA-2Na on three kinds of kits

Figure 2 Effect of 5 different PCR inhibitors on three kinds of kits

3. Discussion

The results show that the sensitivity of A27Plex Plus, GlobalFiler™ and Fusion 6C is 0.125ng under the same experimental condition. Among them, the average peak height of GlobalFiler™ is the highest among the three. Whereas at DNA additions of 0.0625ng and 0.03125ng, , the detection rate of A27Plex Plus is slightly higher than that of the other two products. It shows that the sensitivity of the three kits is at the same level. In the amplification of samples containing different kinds of PCR inhibitors, the influence of inhibitors on the amplification can be reduced by using anti-inhibitor DNA polymerase or adding BSA, betaine and other substances into the PCR system. Because the components of DNA polymerase and buffer used in the three kits are different, the abilities of anti PCR inhibitors are quite different. Therefore, when the samples are difficult to be amplified, the kits being used together to obtain more genetic information could be considered.

To sum up, in the performance comparative study of sensitivity and tolerance of the three kits, A27Plex Plus and the other two imported similar kits have little difference in detection sensitivity, and have their own advantages in inhibitor tolerance, which means A27Plex Plus can meet the needs of forensic identification in theory. In the detection of some difficult samples, A27Plex Plus can be used together with these imported kits. The total number of loci and the number of autosomal loci detected by A27Plex Plus are the most among the three, which can provide more genetic information in a single test. In addition, A27Plex Plus contains 11 autosomal STR loci whose amplification length is less than 220bp (Mini STR), which is the most among the three kits. Therefore, A27Plex Plus has a higher success rate in the detection of degradation samples in theory, which makes A27Plex Plus have a high application value in DNA database construction and field cases.

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