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Evaluation of "in house" criteria for PCR-based analysis in immigration casework

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Abstract

"In house" criteria for PCR-based analysis in immigration casework have been evaluated in 28 immigration families. The isolated DNA was amplified using the multiplex systems SGM+ (Applied Biosystems), CTTv, FFFL and PowerPlex 16 (Promega). PCR products were separated on the ABI 310 analyzer. The one and two parental indices (*I*) were calculated using the DNA.VIEW software package (C. Brenner, Oakland, USA). DNA was isolated twice to reduce mistakes. Combining the results of the multiplex STR systems, used in this evaluation, appeared to be successful in analysing immigration casework. From the results of this evaluation, it appeared that the involvement of other family members in immigration casework has to be critically evaluated.

Keywords: Immigration casework; PCR analysis

1. Introduction

Predefined criteria, concerning strategy for testing, exclusions, the calculation of the one and two parental index (*I*) and consequences of mutations, have been evaluated in 156 one-parent child combinations and 105 two-parent child combinations, from 28 immigration families.

Immigration casework criteria were predefined. DNA from the same person was isolated in duplicate using two different buccal swabs. Amplification was performed using the multiplex system SGM+ (Applied Biosystems) with DNA from one isolation and the multiplex systems CTTv and FFFL (Promega) on the other isolated DNA sample

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from the same person. Results had to be obtained from at least 14 out of 16 STR systems, including the overlapping systems HUMTH01 and vWA. An exclusion is reported if three or more mismatches are observed. Supplementary investigation has to be performed, if only one or two mismatches are seen. Consequently, if no exclusion can be reported, the calculated *I* value has to be corrected [1,3]. If no exclusion is found, the calculated one-parent *I* value has to be 1.000 or more and the calculated two-parent *I* value has to be 10.000 or more. Further investigation has to be performed if these values are not obtained.

In addition, all cases were tested with PowerPlex 16 (Promega), to investigate the contribution in immigration casework. By testing for SGM+, CTTv, FFFL and PowerPlex 16 (PP), typing for 21 different STR loci were performed.

2. Materials and methods

Genomic DNA was isolated from buccal swabs according to the QIAamp Spin procedure (Qiagen). The isolated DNA was amplified using the fluorescent STR multiplex systems SGM+, CTTv, FFFL and PowerPlex 16. The amplification setup of the CTTv and the FFFL was according to the manufacturer's recommendation [4]. The setup of the SGM+ kit was modified by reducing the amplification mix per sample to 10 μl and a template volume of 1 μl [2]. The PowerPlex 16 setup was modified by reducing the mix per sample to 10 μl, with 1:2 diluted 10* primer pair mix and the use of template 1 μl [5]. After STR analysis, using the ABI310 analyzer, based on capillary electrophoresis, the one and two parental indices (*I*) were calculated using the DNA.VIEW software package (C. Brenner, Oakland, USA).

3. Results and discussion

3.1. Mismatch

In this evaluation, 156 meioses out of 28 families were tested. After testing for SGM+, CTTv and FFFL, in 16 cases, an exclusion could be reported. In 14 cases, one or two mismatches were observed. After additional testing with PowerPlex 16, in 10 out of those 14 cases, no exclusion could be reported. Mutations were assumed and the corrected *I*

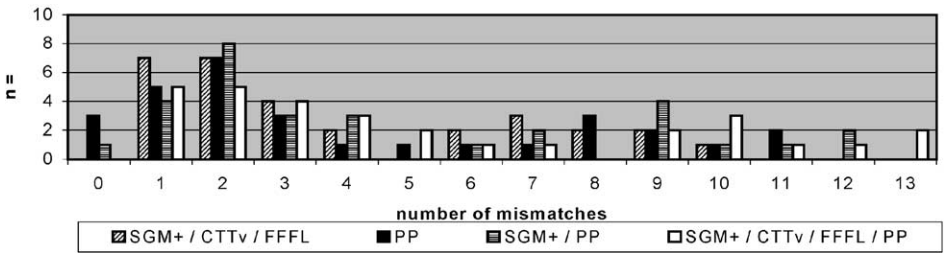

Fig. 1. Observed mismatches in STR systems (n=30 cases).

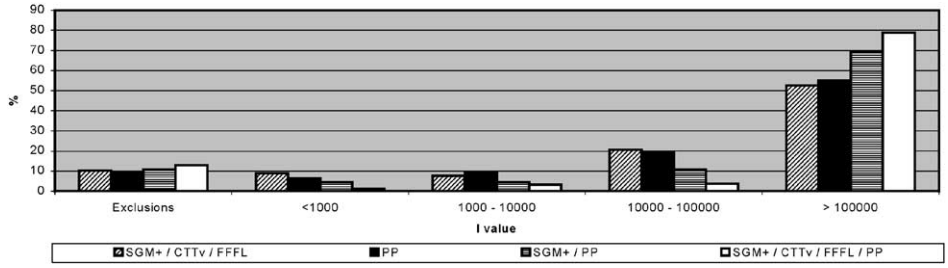

Fig. 2. Analysis of 156 one parent cases out of 28 immigration cases.

values were calculated. Testing only for PowerPlex 16 resulted in exclusion in 15 cases. In 12 cases, one or two mismatches were observed and in three cases, no mismatches were seen. No mismatches were observed in the other 126 meiosis (Fig. 1).

3.2. Non-exclusion

From 28 immigration cases, 156 one-parent indices, obtained with SGM+, CTTv and FFFL, were calculated. Also the PowerPlex 16 results were evaluated. After classification of the results, the data were summarised. Testing with SGM+, CTTv and FFFL, 9% of the calculated one-parent child indices did not meet the predefined criterion of 1.000 or more. After additional testing with the PowerPlex 16 system, 1 out of 156 of the one-parent child indices was under 1.000. Testing only with PowerPlex 16, 10% of the calculated indices were under 1.000 (Fig. 2).

The two-parent child index was calculated from 105 cases tested with the multiplex systems SGM+, CTTv and FFFL. The results are summarised in Fig. 3. The calculated two-parent child indices ranged from 10E7 to 10E21.

3.3. Mismatch correction

Testing the influence of the mismatch correction, 156 one-parent child cases with one mismatch were simulated. Using an average mismatch correction factor of 1/100 per mismatch and typing for SGM+, CTTv and FFFL, 48% of the calculated *I* values were under 1.000.

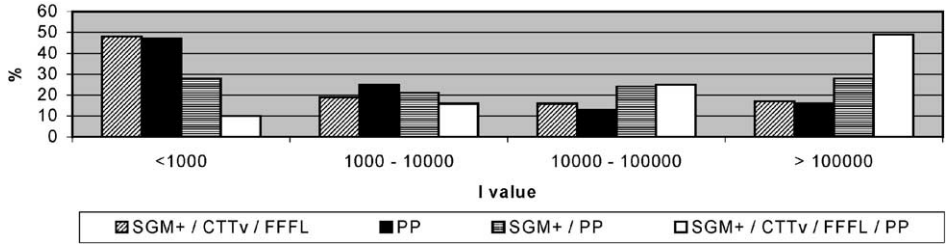

Fig. 3. Evaluation mismatch correction (n=156).

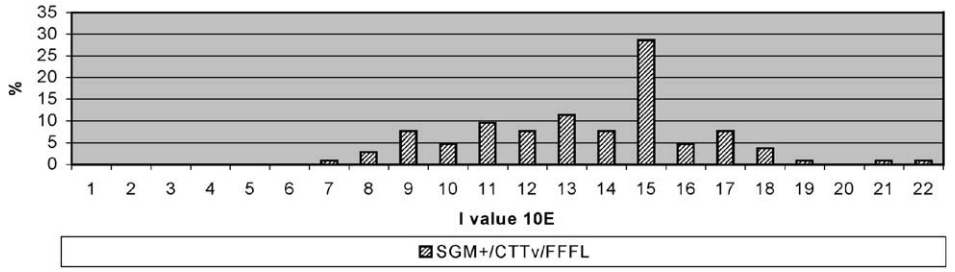

Fig. 4. Evaluation two-parent child indices (n=105).

With additional information obtained with the PowerPlex 16 system, this percentage was reduced to 10. When testing only with PowerPlex 16, 47% did not meet the criteria (Fig. 4).

4. Conclusion

A combination of the multiplex STR systems, used in this evaluation, appeared to be successful in analysing immigration casework. However, to be decisive in all cases, without reducing the quality of the results, more highly informative systems are needed. The predefined criterion, for the I value, 1.000 or more, in one-parent child combinations, is useful. However, the criteria in two-parent child combinations do not seem very meaningful and have to be considered critically.

Testing for 21 STR systems in 10 cases, only one or two mismatches were seen. This might be due to the involvement of other family members.

References

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