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Results of the GHEP-ISFG collaborative exercise for the taxonomic identification of forensic samples using the SPInDel method

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ABSTRACT

The Species Identification by Insertions/Deletions (SPInDel) multiplex PCR allows the identification of species by the generation of numeric profiles combining the lengths of six mitochondrial ribosomal RNA (rRNA) gene regions. Here, we describe a collaborative exercise for the taxonomic identification of forensic samples using the SPInDel kit carried out in 2014 by the GHEP-ISFG. A total of 24 laboratories from 10 countries were supplied with a SPInDel primer mix and control samples of the 10 target species needed to perform genotyping of 11 samples from previous GHEP-ISFG Intercomparison Exercises. Overall, correct identifications were reported by 22 of the 24 laboratories. The errors were concentrated in a few laboratories, with one laboratory reporting errors in all profiles. The success rate in the identification of species with the SPInDel kit was 100% in 8 of the 11 samples. The level of concordance in identifications was always higher than 93%, including in samples with low amounts of DNA (hair shafts) and mixtures of saliva and blood. When considering all cases together, 98.6% of the reported profiles yielded correct species identifications. The frequency of wrong (5.8%) and missing (2.4%) alleles was low and did not interfere with the correct species identification, mainly because the SPInDel method relies on the analysis of multiple loci. In summary, the SPInDel method was easily implemented by different laboratories and genotyping platforms and the interpretation of results was straightforward. The method proved to be efficient in the identification of species in diverse forensic samples.

1. Introduction

The identification of species in casework samples is highly relevant for forensic investigations such as the illegal trade of protected species, poaching, traffic accidents with animals, animal or plant traces in murder scenes, among others [1]. The identification is usually achieved by DNA sequencing, although most sequencing methods are unable to provide identifications in the presence of complex mixtures or in degraded samples. The identification of species with the SPInDel method is achieved by the generation of a diagnostic profile combining the lengths of six mitochondrial ribosomal RNA (rRNA) gene regions amplified using highly conserved PCR primers [2,3]. Therefore, each species is identified by a unique numeric profile of fragment lengths resulting from the combination of the length of hypervariable regions (Fig. 1). We carried out an interlaboratory collaborative exercise within the GHEP-ISFG to evaluate the suitability of the SPInDel method for species identification in casework samples.

2. Material and methods

The number of laboratories participating in the collaborative exercise was 24:7 from Spain, 5 from Portugal and Argentina, and one from Brazil, Colombia, Costa Rica, Ecuador, Italy, Mexico and Czech Republic. The laboratories were supplied with a SPInDel primer mix needed to perform genotyping of samples M1–M8 of the 2014 GHEP-ISFG Intercomparison Exercise, and optionally 3 other samples from previous exercises already known to have non-human contribution. The study includes forensic samples

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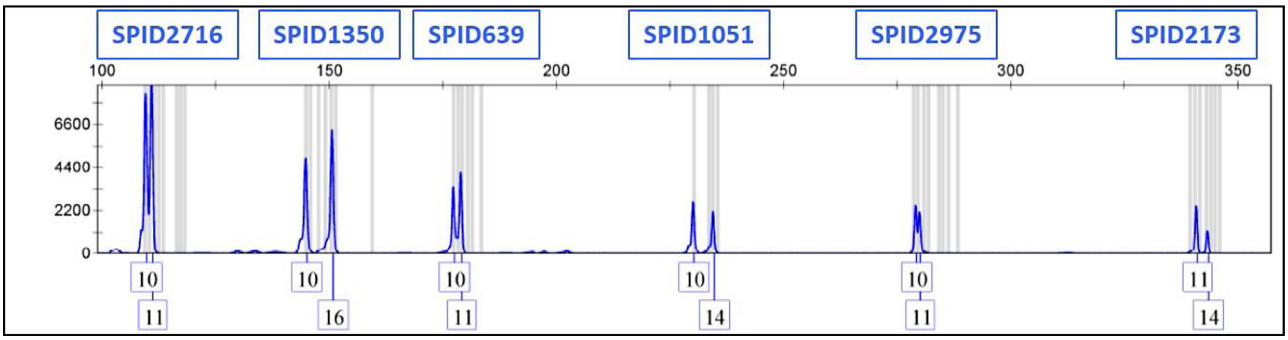

Fig. 1. Example of an electrophoregram obtained with the SPInDel method from a mixture sample (M7-2014) containing human saliva and blood from a cow.

with low quantity and/or quality DNA. For instance, M4 (2014) is a mixture of saliva and blood on a paper napkin, M5 (2014) is a hair shaft and M7 (2014) is a mixture of human saliva and blood from a cow on a swab. The laboratories were also supplied with 10 control samples for all target species and respective profiles, panels and bins for GeneMapper automatic analysis, and a software for automatic determination of the species—SPInDel Workbench version 1.3 portable (http://www.portugene.com/SPInDel/SPInDel_web.html).

3. Results and discussion

Ten samples (out of 13) were correctly identified by all participating laboratories. The percentage of correct identifications was higher than 93% for all samples. From a total of 208 reported profiles, 205 (98.6%) yielded correct species identifications. There were 3 cases of wrong or missing identifications: in sample M5 (2014), which is *Homo sapiens*, one laboratory reported *Capra hircus*; in sample M4 (2014), which is *H. sapiens*, one laboratory reported a mixture of *H. sapiens* and *C. hircus*; in sample M8 (2013), which is a mixture of *H. sapiens* with *Equus caballus*, one laboratory only reported *H. sapiens*. The first two errors occurred in the same laboratory.

The percentage of complete profiles with all correct alleles was higher than 81% per sample. From a total of 208 reported profiles: 12 had wrong alleles (5.8% of the total) and 5 had missing alleles (2.4% of the total). Most reported errors were from four laboratories (each with 2 or more errors). A single laboratory reported errors in six profiles. 14 laboratories provided results with no errors. 16 out of 1248 reported alleles were wrong (1.3% of the total). Wrong alleles were reported in 3 markers: SPID2716, SPID639 and SPID2173. The marker SPID639 had the largest number of wrong alleles, with 8 reported cases. Thirteen out of 1248 reported alleles were missing (1.0% of the total). The 4 missing alleles for sample M2 (2014) were reported in a single profile. Nevertheless, a correct identification (*H. sapiens*) was possible.

4. Conclusion

The SPInDel method proved to be efficient in the identification of species in diverse forensic samples, including mixtures. The method was easily implemented by different laboratories and genotyping platforms, the interpretation of results was straightforward and a positive feedback was provided by most laboratories, who demonstrated interest in using the method regularly.

Conflict of interest

None.

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