NEXT GENERATION STR KITS APPLIED TO POST-MORTEM DNA EXTRACTS

***Amory S.[1], Huel R.[1], Bilić A.[1], Parsons T.J.[1]**

[1]**I**nternational **C**ommission on **M**issing **P**ersons, Sarajevo, Bosnia and Herzegovina.

* e-mail: sylvain.amory@ic-mp.org

Introduction

The International Commission on Missing Persons is involved in the identification of persons missing as a result of armed conflict or mass disasters. The samples collected in those circumstances are often bone fragments or teeth. Depending on the environmental and taphonomic conditions the state of preservation of the DNA molecules can vary greatly and complicates STR typing. PCR amplification of post-mortem samples can also been hindered by the presence of various inhibitory compounds or an excess of fungal / bacterial DNA.

With the growing demand for kits allowing for the successful typing of more challenging samples, several kits have been upgraded and new ones have been released in the past few years. These kits are meant to be more robust against inhibitors and more sensitive due to different buffer compositions combined with new *Taq* polymerases. The aim of this study is to assess how two new kits compare to their former versions and to test one new STR kit that contains the new ESS loci using DNA extracts obtained from bone samples.

Material and Methods

All experiments were performed using remaining DNA extracts obtained from bone fragments of previously identified individuals.

Five kits were tested: PowerPlex® 16, PowerPlex® 16 HS, PowerPlex® ESX17 (Promega), AmpFℓSTR® Identifiler and AmpFℓSTR® Identifiler Plus (Applied Biosystems).

• General STR profile quality

- The same 30 DNA extracts were amplified according to the manufacturer's recommendations to assess the overall quality of STR profiles.
- Balance between loci was assessed by calculating the median peak height value across all loci and by measuring the deviation from this value for each locus.

• Sensitivity

- 3 DNA extracts for which full profiles were obtained were diluted down to 5, 12.5 and 50pg/µl and amplified in triplicate using 10pg, 25pg and 100pg input.
- Allelic drop-outs, locus drop-outs and drop-ins were reported for each locus and summarized by calculating the relative proportion of each type of result.

• Inhibition

- 2 batches of 7 samples showing high level of inhibition (Quantifiler Human Ct IPC>40) were amplified in duplicate using 5ul of extract (batch1: PP16, PP16HS, ESX17 – batch2: Identifiler, Identifiler Plus).
- The average peak height for each locus was calculated and compiled in a chart corresponding to each kit.

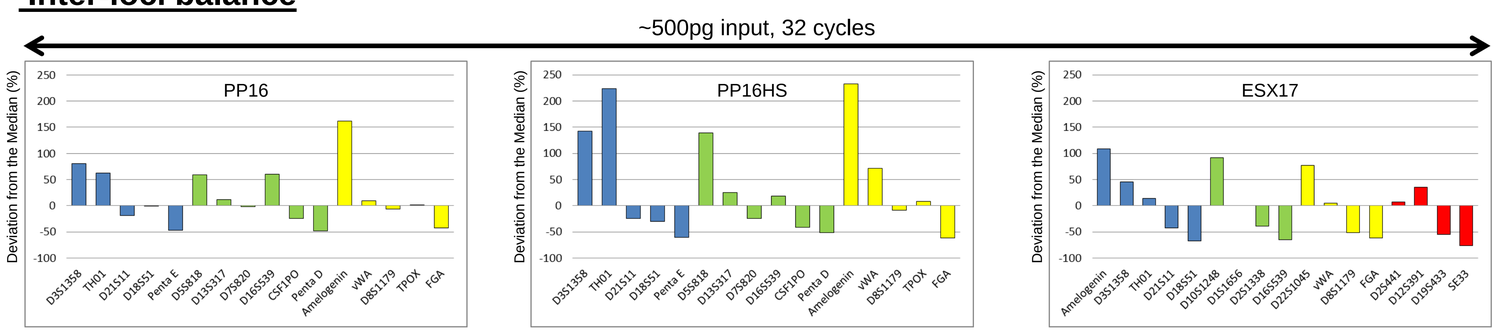
• Bacterial DNA:

- 7 extracts previously typed with PP16 and known to contain bacterial/fungal DNA were amplified in duplicate with each kit under the manufacturer's recommended conditions.
- The occurrence of bacterial peaks and their size were monitored for each kit.

Results

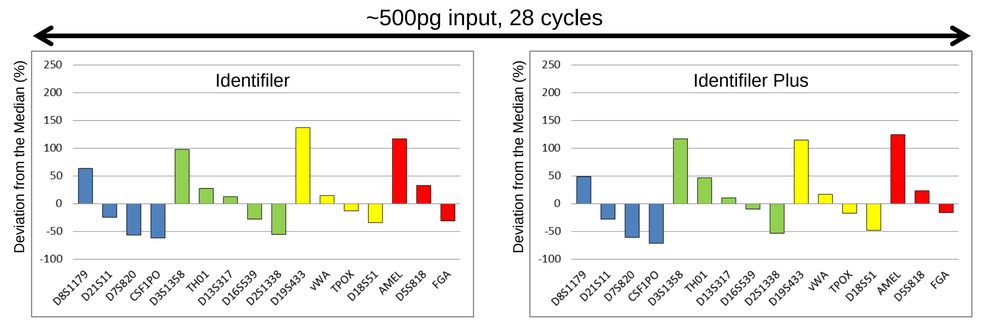
• Inter-loci balance

~500pg input, 32 cycles



➢ **Over amplification of short loci with PP16HS**

~500pg input, 28 cycles



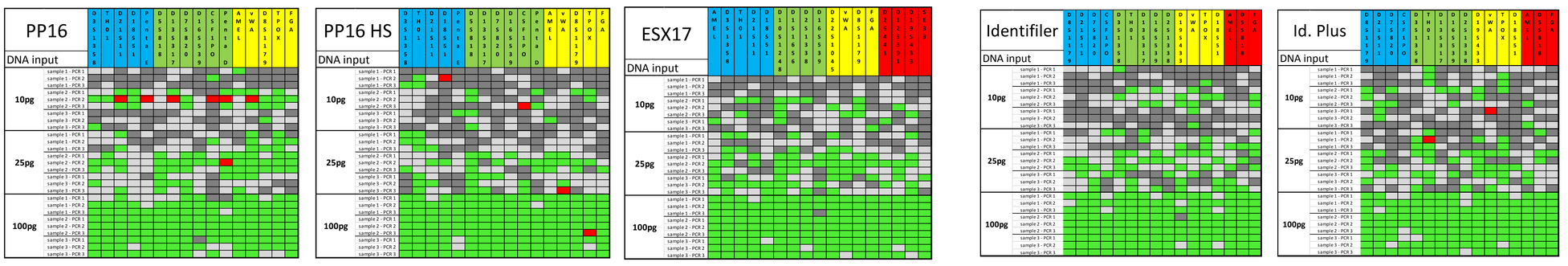
➢ **Equivalent inter-loci balance with both kits**

• Sensitivity

34 cycles

29 cycles

DNA input	Typing result	Typing results per kit (%)				
		PP16	PP16HS	ESX17	Identifiler	Id. Plus
10pg	Correct	17.4	10.4	14.0	11.8	13.9
	Allele DO	35.4	29.2	33.1	34.7	34.7
	Locus DO	43.1	59.0	52.9	53.5	50.7
	Drop-in	4.2	1.4	0.0	0.0	0.7
25pg	Correct	38.9	27.8	41.8	36.1	30.6
	Allele DO	43.1	43.8	39.2	41.0	33.3
	Locus DO	17.4	27.8	19.0	22.9	35.4
	Drop-in	0.7	0.7	0.0	0.0	0.7
100pg	Correct	86.8	95.8	95.4	93.1	91.7
	Allele DO	11.1	3.5	3.9	6.3	8.3
	Locus DO	2.1	0.0	0.7	0.7	0.0
	Drop-in	0.0	0.7	0.0	0.0	0.0

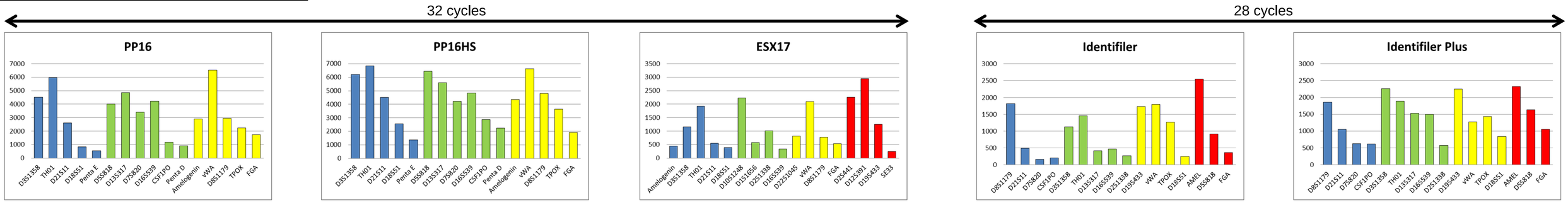


➢ **Comparable sensitivity for all kits with enhanced PCR parameters**

• Performance vs PCR inhibitors

32 cycles

28 cycles

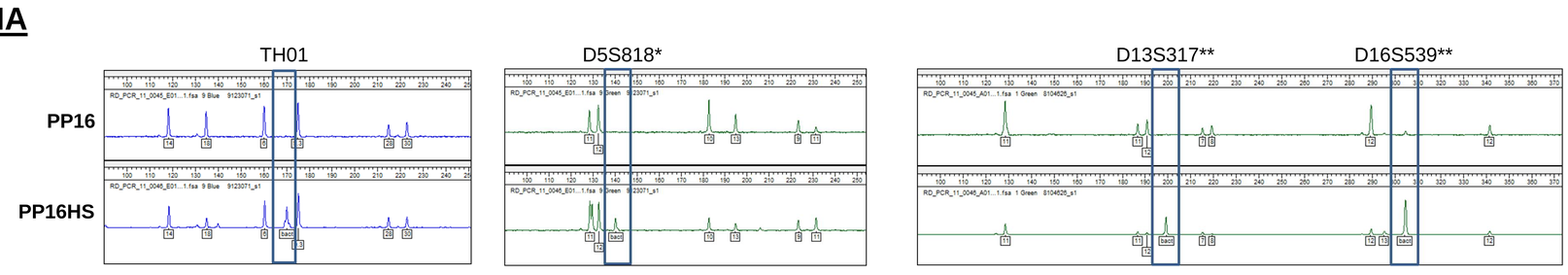


➢ **Amelogenin and large loci more affected by inhibitors, PP16HS more robust**

➢ **Larger loci affected, Identifiler Plus more robust**

• Non-specific amplification of bacterial/fungal DNA

Locus	PP16	PP16HS	ESX17	Identifiler	ID. Plus
TH01 ~170bp		✓			
D5S818 ~140bp		✓			
D13S317 ~199bp		✓			
D16S539 ~304bp	✓	✓			



* Reported in ICMP's caseworks

** Reported in Alonso et al. 2001, CMJ, 42(3):260-266

➢ **Non specific amplification related to PP16 primers, bacterial/fungal peaks over amplified with PP16HS, detected in two consecutive amplifications**

Discussion / Conclusion

In ICMP's perspective next generation kits could represent various potential advantages applicable to post-mortem samples typing. PP16HS and Identifiler Plus could be used as a replacement for the former kits versions in order to process more easily challenging samples. ESX17 brings additional markers that could help in cases of low threshold matches and can be used as a stand alone kit.

- Both PP16HS and Identifiler Plus benefited from the recent improvement in regards to their performance against inhibitors but ESX17 appeared to be more sensitive to PCR inhibition than the two aforementioned kits. Therefore a post-extraction clean-up of inhibited extracts represents the optimal solution in particular affected cases.
- The sensitivity of the 5 kits tested on diluted bone DNA extracts was equivalent and drop-out rates were similar.
- In the case of PP16HS low molecular weight markers were over amplified which led to a higher imbalance between small and large markers; this does not appear to diminish the amount of reliable genetic information that can be obtained, but can in some cases complicate the analysis procedure. Moreover, in comparison to PP16HS and Identifiler Plus, the classic versions of PP16 and Identifiler allowed a broader range of DNA inputs for a given PCR protocol before issues of over-amplification and bleed-through became significant problems. This represents a particular advantage when DNA quantification results are not accurate.
- Non-specific amplification of bacterial/fungal DNA is exacerbated with PP16HS when compared to the PP16 classic kit. These non-specific peaks were not detected in ESX17, Identifiler or Identifiler Plus due to the use of different primer sets. Future testing of samples with known bacterial/fungal peaks that affect Identifiler will be performed using both versions of this kit.

Although the next generation kits enhanced the results from inhibited samples, their overall performance are similar to the classic versions. The ICMP successfully typed more than 36,000 samples with PP16 and 2,300 samples with Identifiler showing the suitability of the classic kits for STR typing of bone samples. ESX17 introduces 8 additional loci compared to PP16 and 7 compared to Identifiler and showed comparable performance which make it a valuable tool for missing persons identification.

15 years

icmp International Commission on Missing Persons