Examination of Proposed Manufacturing Standards Using Low Template DNA

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Abstract

Forensic DNA laboratories rely on reagent and plastics manufacturers to supply high-quality products with minimal interference from contaminating DNA. With the increasing sensitivity of short tandem repeat (STR) amplification systems, levels of DNA that were previously undetected may now generate partial profiles. To address the concern of laboratories worldwide, accrediting bodies in the United Kingdom and Australia proposed guidelines, PAS377 and AS5483, respectively. As a result of these initiatives, a new international ISO standard has been drafted. ISO18385 will be an internationally applicable ISO standard for forensic consumable manufacturers if/when approved. Therefore, as a manufacturer, we need to understand the limit of detection for the analysis methods currently being used and what level of contaminating DNA would interfere with analysis in customer labs.

To determine the sensitivity of STR analysis, we analyzed the sensitivity of its two major components: the capillary electrophoresis (CE) instrument and the STR reagents.

Next, we explored proposed definitions for Forensic Grade certification. Detection at both a single cell and a single allele peak were achieved and compared to levels of contamination that would interfere in forensic laboratories.

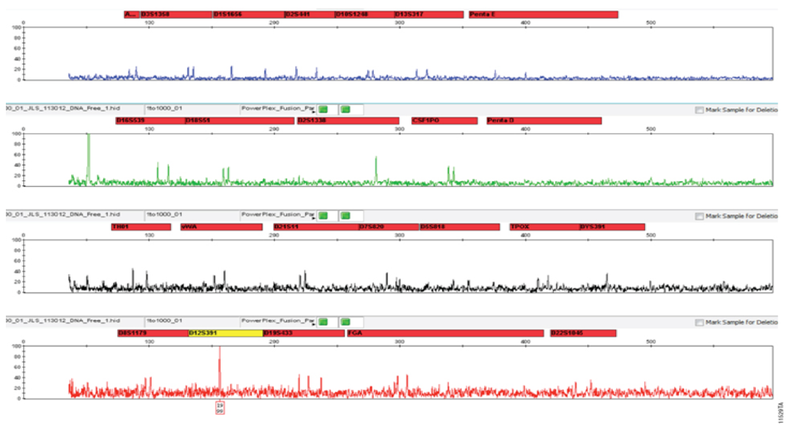
Finally, we compared sensitivity of STR analysis to qPCR analysis. PAS377 only allows manufacturers to analyze reagents and consumables via STR analysis while ISO 18385 allows for both. The utility of both methods is discussed.

Capillary Electrophoresis Instrument Sensitivity

500pg DNA amplified with PowerPlex® Fusion System → Serially dilute amplification product → Triplicate injections

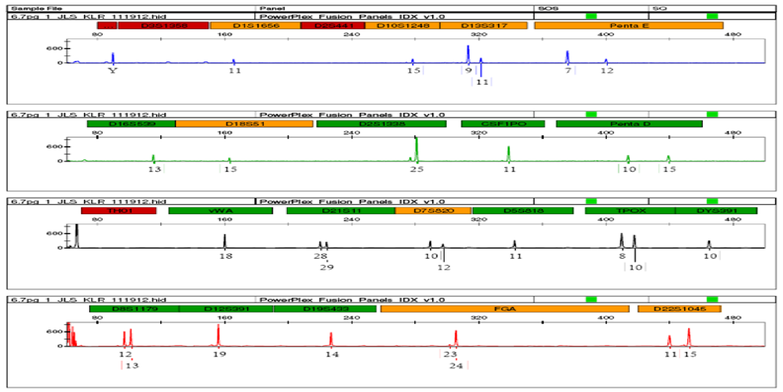
Condition	3500 CE
Default Injection	15sec, 1.2kV
Enhanced Injection	24sec, 1.2kV
Default Threshold	175RFU
Enhanced Threshold	90RFU

Dilution	% Alleles Called			
	15sec 175RFU	15sec 90RFU	24sec 175RFU	24sec 90RFU
1:10	100	100	100	100
1:50	73	97	95	100
1:100	11	83	61	96
1:200	2.9	20	5.1	67
1:300	0	2.9	2.2	18
1:400	0	2.2	0.7	8.9
1:500	0	0.7	0	2.2
1:1000	0	0	0	2.2
1:5000	0	0	0	0

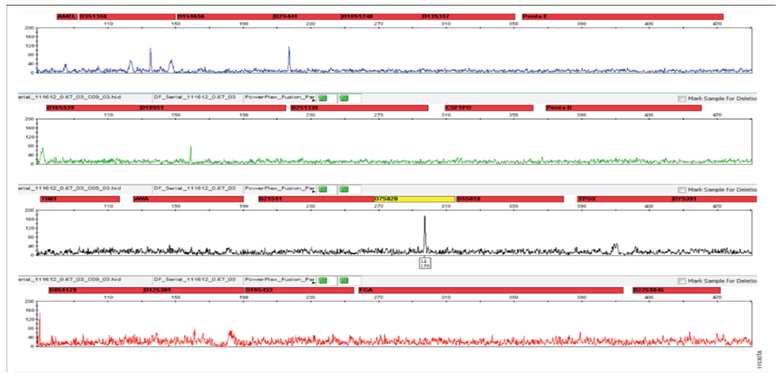


Representative electropherogram of the 1:1000 dilution with enhanced injection conditions.

Practical Definition of Forensic Grade

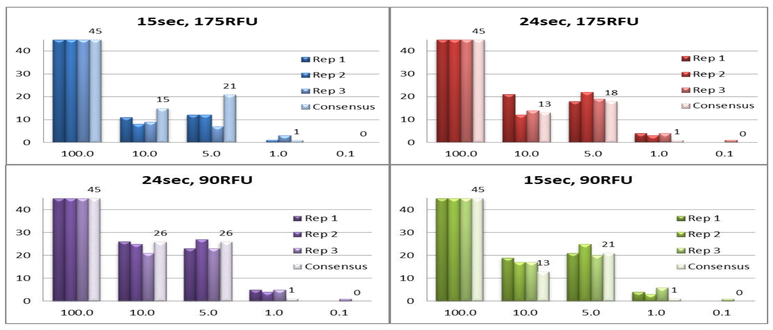


Representative electropherogram of one-cell equivalent (6.7pg) of DNA. 24 second, 1.2kV injection on AB3500 and 90 RFU analytical threshold.



Representative electropherogram with 0.67pg of input DNA (1/10th of a cell). This would be equivalent to the PAS377 requirement for no more than one allelic peak. 24 second, 1.2kV injection on AB3500 and 175 RFU analytical threshold.

qPCR vs. STR. What is Appropriate?



Total allele counts for various injection and threshold parameters with decreasing levels of Input DNA. Consensus alleles had to be observed in at least two of three replicates.

C_q Values Using 45 Cycles.			
	C_q Value		
DNA Amount	Replicate 1	Replicate 2	Replicate 3
250pg	25.4	25.3	25.5
25pg	29.0	28.9	28.6
2.5pg	33.7	33.5	33.2
1.25pg	33.7	33.5	34.2
0.25pg	37.1	37.4	37.1
0.025pg	39.1	ND	ND
0.0025pg	ND	ND	ND
0.00025pg	ND	ND	ND
ND = Not detected.			

The limit of detection with qPCR is 0.25pg. Compare to 1pg with STR, under enhanced injection conditions. LOD is less than 1 cell for both STR and qPCR analysis.

Summary

- "One cell equivalent" or 6.7pg resulted in STR profiles with up to 50% expected alleles.
- The amount of DNA equivalent to "one allele peak at an increased injection setting" was 0.1pg using non-replicate analysis and 1pg using replicate analysis; both amounts are significantly less than the amount of DNA present in one cell.
- qPCR is more sensitive than STR analysis. The limit of detection for STR analysis was 1pg. However, the limit of detection for qPCR analysis was 0.25pg. Both analysis methods should be available for manufacturer use in the new proposed standards. Each manufacturer should decide which method or combination of methods works best in their process flow.
- It is vital that manufactures be allowed to determine the best means to control their processes and identify areas where contamination can occur. In conjunction with these controlled processes, appropriate sampling of consumables and testing with a sensitive assay can provide a high degree of confidence regarding the "forensic DNA grade" nature of associated samples.
- We believe that continued discussion between the forensic community, manufacturers, laboratories and policy makers about acceptable and practical steps will help ensure the quality of reagents and consumables used for forensic DNA analysis.

References

The British Standards Institution and Home Office (2012) PAS 377:2012. Specification for consumables used in the collection, preservation and processing of material for forensic analysis.

Standards Australia (2012) AS 5483 -2012. Minimizing contamination in products used to collect and analyse biological material for forensic purposes.

Gill, P. *et al.* (2010) Manufacturer contamination of disposable plastic-ware and other reagents—an agreed position statement by ENFSI, SWGDAM and BSAG. *Forensic Sci. Int. Genet.* 4, 269–70.

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