A155 Interpretation Challenges Using the PowerPlex® 16 HS Kit for Forensic Casework

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The goal of this presentation is to educate the forensic DNA community on interpretation challenges faced, especially in mixtures, when amplifying forensic casework samples with PowerPlex® 16 HS.

This presentation will impact the forensic science community by increasing the knowledge of additional common artifacts and anomalies seen in casework samples amplified with PowerPlex® 16 HS that may not be noted in technical manuals or validation studies, which will help during interpretation, comparisons, and reporting.

With the evolution of next generation amplification kits, manufacturers are competing to deliver products that meet the demands of the forensic DNA community. This includes overcoming inhibition, increasing sensitivity, adding discrimination power, and reducing overall amplification time. Although amplification kits undergo developmental and internal validations, once applied to actual unknown samples they may not behave as predicted. Bode internally validated PowerPlex® 16 HS in a 25µl reaction with 30 total cycles and found it to be reliable, accurate, reproducible, and precise. An examination of mixtures, contamination, and artifact assessment was also performed. During validation, this kit outperformed other amplification kits in its efficiency to overcome inhibition. However, optimization of reagents to increase robustness in the PowerPlex® 16 HS amplification kit has led to an increase in amplification artifacts and anomalies that can make interpretation challenging.

An assessment of artifacts during validation supported an increase in n-4 stutter over the manufacturer's recommendations at D3S1358 (increase from 11.22% to 13.7%) and CSF1PO (increase from 8.15% to 11%). In general, excessive stutter occurred more frequently when amplification reactions generated profiles with alleles greater than 3000 RFU or when n-4 and n+4 peaks were acting on the same stutter artifact. With the exception of the documented dye artifact occurring at 172 base pairs (bp) in the ILS 600, no other dye artifacts were noted.

Once the kit was implemented for use on casework samples, previously unidentified artifacts/anomalies were noted in many samples. This includes a dye artifact at Penta D which ranges from 450bp-500bp and varies in total relative florescent units (RFU) from 300 to 1000 RFU. Other loci displayed uncommon non-specific artifacts including peaks in the n-20 position at TPOX, the n-12 position at TH01, and the n-17 position at Penta D. Occasionally, these peaks fall into sizing bins and can cause confusion in mixture interpretation; however, the most challenging new artifact is an increase in possible bacterial peaks. The addition of a third amplicon of equal or greater height (in RFU) than other alleles present at the same location has been noted in several different casework samples at the following loci: TH01, D18S51, D5S818, D7S820, D16S539, and CSF1PO. These artifacts occurred in sexual assault samples processed within the same year they were collected. The sample types ranged from rectal swabs, tampons, outer labia swabs, peritoneum swabs, and oral swabs. These additional peaks are assumed to be non-human in nature and can be rectified by confirming data with other amplification kits. Finally, discordance between samples has also been noted at Penta E between PowerPlex® 16, PowerPlex® 16 HS, and/or PowerPlex® 18D. Collections of these artifacts led to an internal investigation and discussion ultimately resulting in several interpretation changes needed to effectively interpret samples amplified with this kit.

Case examples, displays of artifacts/anomalies, and changes to interpretation guidelines to accommodate casework processing will be provided.

PowerPlex® 16 HS, Artifacts, Interpretation

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