PCR INHIBITION

An Introduction to PCR Inhibitors

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INTRODUCTION

For as long as scientists have used the polymerase chain reaction (PCR), PCR inhibitors have been an obstacle to success. All who use PCR are likely to be impacted by inhibitors at some time, but the wide range of forensic sample types and variety of sampling conditions encountered make forensic scientists particularly vulnerable.

PCR inhibitors generally exert their effects through direct interaction with DNA or interference with thermostable DNA polymerases. Direct binding of agents to single-stranded or double-stranded DNA can prevent amplification and facilitate co-purification of inhibitor and DNA. Inhibitors can also interact directly with a DNA polymerase to block enzyme activity. DNA polymerases have cofactor requirements that can be the target of inhibition. Magnesium is a critical cofactor, and agents that reduce Mg^{2+} availability or interfere with binding of Mg^{2+} to the DNA polymerase can inhibit PCR.

The presence of inhibitors in samples has been the focus of much of the published literature. Common sample types known to contain inhibitors include blood, fabrics, tissues and soil (Table 1). Other important sources of inhibitors are the materials and reagents that come into contact with samples during processing or DNA purification. These include excess KCl, NaCl and other salts, ionic detergents such as sodium deoxycholate, sarkosyl and SDS (1), ethanol and isopropanol (2), phenol (3) and others.

Given the wide range of PCR inhibitor-laden sample types and the options available for handling them, a multi-faceted approach is the best solution for amplification failure.

APPROACHES TO OVERCOMING INHIBITION

The surest way to avoid PCR inhibition is to prevent the inhibitor from being processed with the sample. For inhibitors that are inherent to the sample, as is the case for blood and certain tissues, this is not possible. For casework samples on other materials, such as blood on denim or saliva on food items, the inhibitor-containing substrate may be avoided by using swab-transfer methods rather than processing cuttings or pieces of stained or contacted material.

DNA purification is the method used most often to remove inhibitors. A wide range of commercially available kits, such as the DNA IQ™ System(h), and home-brew methods are available to extract DNA, but only a few of these methods have been widely adopted in forensic laboratories because, in part, adoption of a new method requires labor-intensive validation. Validation should evaluate the method's ability to efficiently extract inhibitor-free DNA from a wide range of sample types. Extraction methods that are proven to eliminate inhibitors from the purified template DNA should be favored.

There are several options to overcome the effects of inhibitors that are not eliminated during extraction. The choice of DNA polymerase can have a large impact on resistance to inhibition (3,4). AmpliTaq Gold® DNA polymerase, which is the standard for use with commercial multiplex STR kits, is among the most sensitive to inhibition (5). This underscores the importance of sample handling and extraction and highlights an opportunity for future improvement. Increasing the amount of DNA polymerase in the reaction or using additives such as BSA, which provides some resistance to inhibitors in blood (6), are proven methods. BSA is included in the Promega PowerPlex® Systems. However, labs should be cautious about adding BSA to STR amplifications. BSA quality can vary greatly between sources, and material should be rigorously quality-tested. Finally, adding less DNA template to the amplification can often improve performance greatly, emphasizing STR kit sensitivity as a key advantage when generating profiles from templates that contain inhibitors.

PCR INHIBITION

Table 1. Known PCR Inhibitors.

Inhibitor	Source of Inhibitor	Reference
bile salts	feces	9*
complex polysaccharides	feces, plant material	10*
collagen	tissues	11*
heme	blood	12*
humic acid	soil, plant material	13*,14
melanin and eumelanin	hair, skin	15*,16
myoglobin	muscle tissue	17*
polysaccharides	plants	18*
proteinases	milk	19*
calcium ions	milk, bone	20*
urea	urine	21*
hemoglobin, lactoferrin	blood	22*
immunoglobin G (IgG)	blood	23*
indigo dye	denim	24

*Reviewed in reference 25.

DETECTION OF INHIBITORS

Inhibition of multiplex STR amplifications can result in reduced product yield or complete failure. When inhibited samples exhibit a partial profile, a specific pattern of locus dropout is common. Quite often, smaller loci in the kit are preferentially amplified. The same pattern is typical of highly degraded DNA templates, and very often, inhibited samples are mistakenly assumed to be degraded.

Use of multiplex real-time PCR to quantitate DNA provides an opportunity to use an internal positive control (IPC) to detect PCR inhibitors. For example, the Quantifiler® system uses an IPC. Real-time PCR data can also be used to detect inhibitors by analyzing target amplification efficiency (7). This IPC strategy has been used in combination with two autosomal targets of differing size to simultaneously assess both inhibitors and template degradation (8). The additional information about inhibition and degradation obtained by real-time quantitation systems allows laboratories to make better choices for sample processing and ultimately leads to higher amplification success rates and improved laboratory efficiency. However, the differential effects of inhibitors on DNA quantitation systems and STR amplification systems is a topic that has received little attention thus far but will undoubtedly become important as real-time PCR quantitation methods continue to be implemented.

CONCLUSION

Given the wide range of PCR inhibitor-laden sample types and the options available for handling them, a multi-faceted approach is the best solution for amplification failure. The best defense against STR amplification failure is to combine sound sample handling and processing techniques with extraction systems proven to efficiently purify inhibitor-free DNA. Despite those efforts, inhibitors may still be present, underlining the value of using quantitation systems capable of detecting them, and more importantly, emphasizing the importance of using sensitive and robust multiplex STR amplification systems.

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