Application Note

Investigator® Casework GO! Kit as a novel approach for contact traces and body fluids

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Introduction

The most important role in genetic testing of traces collected at crime scenes is played by the quality of the genetic profiles. Factors that have a direct influence on quality of the profiles are: amount of DNA, level of DNA degradation and the presence of PCR inhibitors in the sample. Currently, forensic laboratories are struggling with the rapidly increasing number of samples submitted for genetic testing. The main growth among these samples is occurring in the area of contact traces. A characteristic of these traces is their diverse composition,with the presence, in varying proportions, of cell-free DNA, cell fragments, anucleate corneocytes and transferred nucleated cells. In most cases, these traces are collected with sterile swabs. Past publications have shown that specific adhesive tapes can be used as an alternative for collecting contact traces from solid or fabric surfaces. Increasing the throughput of laboratories is possible due to the introduction of new procedures that shorten analysis time by simplifiying applied analytical methods and automating the whole process, while at the same time maintaining the necessary sensitivity. One of the procedures that fulfills these criteria is a simplified process of quick lysis, quantitative evaluation and amplification.

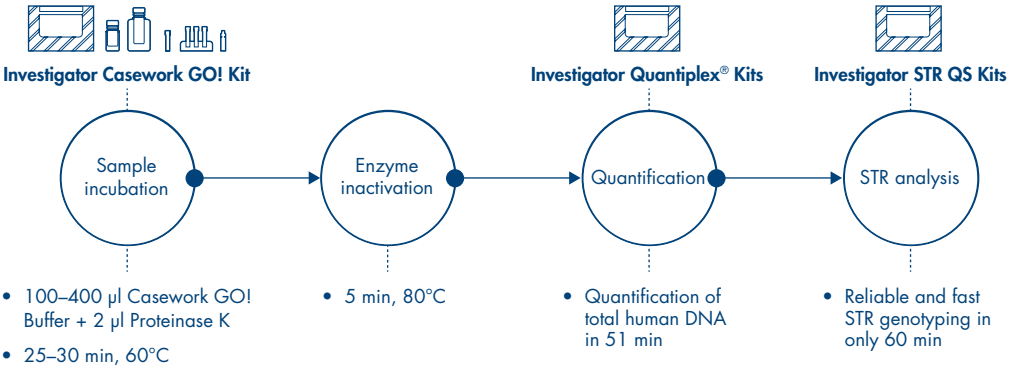

Figure 1. The Investigator Casework GO! Kit workflow.

The Investigator Casework GO! Kit

The Investigator Casework GO! Kit solves the problems associated with DNA isolation from biological samples that are collected at crime scenes, including contact traces, pieces of fabric, paper, cigarette butts, chewing gum, skin flakes, sexual assault swabs, and other sample types. Lysates generated by the Investigator Casework GO! Kit are compatible with all Investigator Quantiplex® and Investigator STR QS systems. This study evaluates the performance of an innovative method for the processing and analysis of typical biological fluids and touch traces from criminal events and compares it against the automated magnetic beads extraction method validated for routine use.

Methods

Sample collection

To evaluate the sensitivity of DNA extraction using the Investigator Casework GO! Kit, blood from one donor was collected and a serial dilution (1:1, 1:10, 1:100, 1:100, 1:1000) prepared in duplicate with low TE (10 mM Tris-HCl, 0.1 mM EDTA). A volume of 25 µl of each sample dilution was applied directly to the isolation tube.

To evaluate the performance of the Investigator Casework GO! Kit in extracting DNA from biological fluids in different substrates, 3 sample replicates of blood, saliva and semen (1:10 water dilution) were prepared on sterile cotton fabric, a sterile plastic surface and a microscope slide, simulating typical biological traces collected at crime scenes. A volume of 50 µl of body fluid was added to each substrate. Prepared samples were then stored for 24 hours for drying in a chamber with laminar airflow and subsequently resampled by collection with cotton swabs (HAGMED).

For the contact traces study, 22 samples from 2 individuals were prepared using 2 different collection methods (cotton swab and low-adhesive tape) from substrates common to the forensic routine: computer keyboard, mobile phone, landline phone, pocket knife, computer mouse and hair.

Sample processing

All samples were processed using the Investigator Casework GO! Kit. Lysis reagent mix was prepared as per manufacturer's instructions and 200 µl was added to each sample. Samples were incubated at 60°C for 25 minutes then at 80°C for 5 minutes for enzyme deactivation. Additionally, for comparison purposes, 11 representative samples were extracted by the BioRobot® M48 instrument (QIAGEN) using magnetic bead technology; elution volume was 75µl. The final concentration of samples processed with the Investigator Casework GO! Kit was corrected to represent the DNA yield in 75 µl.

For all samples and processing methods, DNA quantification was performed using the Investigator Quantiplex Pro RGQ Kit, according to the manufacturer's instructions on the Rotor-Gene® Q. Samples were amplified on a Veriti® Thermal Cycler using the Investigator ESSplex SE QS Kit and subjected to electrophoresis with an ABI® 3500xL Genetic Analyzer. GeneMapper® ID-X v1.5 was used for data analysis.

Results and discussion

Sensitivity test

Blood dilutions of 1:10 prepared with the Investigator Casework GO! Kit generated full STR profiles with no indication of inhibition (average DNA concentration of 0.027 ng/µl). The 1:100 dilution resulted in non-inhibited partial profiles (average DNA concentration of 0.002 ng/µl) The undiluted blood sample showed inhibitor presence, confirmed by the complete dropout of the Quality Sensor 2, and provided only a partial STR profile.

Biological fluids

Saliva was the biological fluid that yielded the highest DNA concentrations following lysis using the Investigator Casework GO! Kit (Figure 2). There was no evidence of inhibition. The different substrates had no impact on the results.

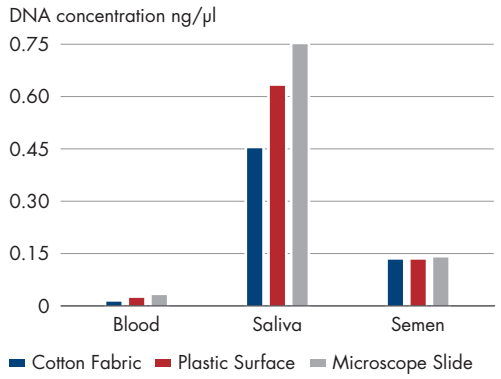

Figure 2. DNA concentration of sample replicates of blood, saliva and semen (1:10 dilution) collected with cotton swabs from cotton fabric, a sterile plastic surface and a microscope slide then processed with the Investigator Casework GO! Kit.

Contact traces

Figure 3 shows the DNA concentrations of contact traces obtained from common substrates with the Investigator Casework GO! Kit. At least 45% of the samples resulted in full STR profiles after amplification with the Investigator ESSplex SE QS Kit. This STR kit has an ideal concentration of 0.5 ng and a flexible DNA input volume of 15 µl. In addition, the Investigator Casework GO! Kit proved to be efficient in extracting DNA from contact traces collected with adhesive tape with an average concentration of 0.011ng/µl. It was not possible to obtain DNA from hair, showing the need of a protocol modification for this sample type. The present study worked with a lysis volume of 200 µl. Using the Investigator Casework GO! Kit, it is possible to adopt a lower lysis volume (e.g., 50 µl) to concentrate the DNA obtained from contact traces, thereby increasing the chances of obtaininga full STR profile. It must be noted that inhibitors present in the sample will also be concentrated, resulting in an elevated risk of inhibitory effects in the PCR.

Comparison with magnetic bead technology

Standard procedures of DNA isolation using magnetic beads guarantee the removal of contaminants from the sample. A disadvantage is that DNA loss during magnetic bead purification steps may contribute to incomplete genetic profiles leading to a negative result. This situation was observed in this study (Figure 4). Samples extracted with the Investigator Casework GO! Kit had higher DNA concentrations (average of 0.56 ng/µl) than duplicate samples extracted with the magnetic bead BioRobot M48 instrument (average of 0.11 ng/µl). PCR amplification showed that inhibitors were not present in samples extracted with the Investigator Casework GO! Kit and full profiles were obtained. ▷

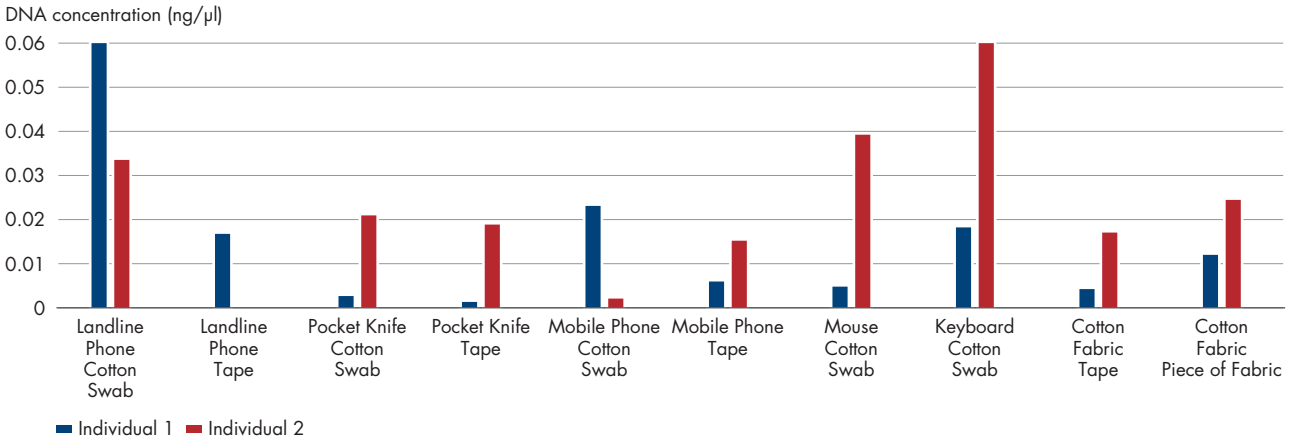

Figure 3. DNA concentration of touch traces of two individuals collected with different methods and processed with the Investigator Casework GO! Kit.

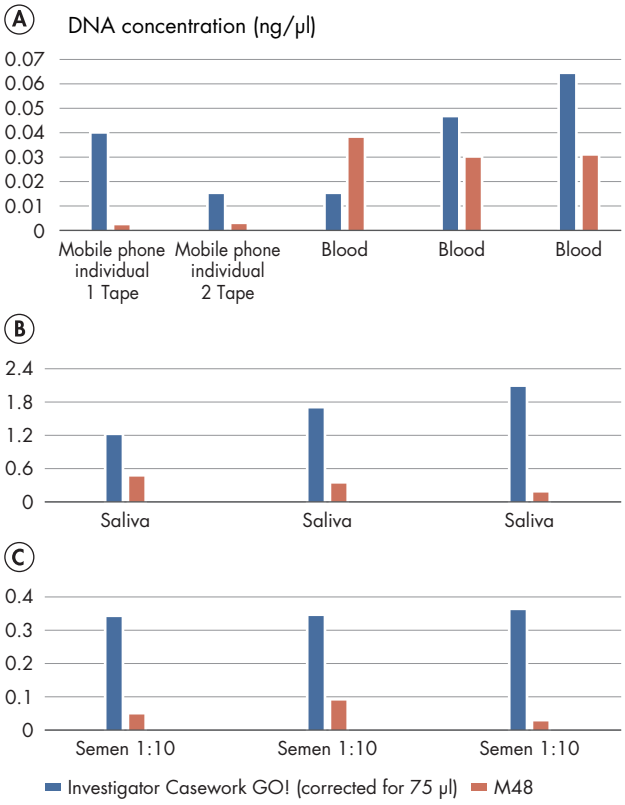

Figure 4. Comparison of DNA extraction methods in forensic samples: Investigator Casework GO! Kit versus magnetic bead extraction with the BioRobot M48 instrument. A Samples from mobile phone and blood samples; **B** Saliva samples; **C** Semen samples.

Conclusion

These preliminary results indicate the great potential of the Investigator Casework GO! Kit for use in the analysis of biological traces with an emphasis on the analysis of touch traces. The direct lysis approach of the Investigator Casework GO! Kit provided positive results for STR analysis of contact traces and body fluids. The quality controls from the STR kit did not indicate any influence of inhibitors on PCR efficiency. Samples extracted with the Investigator Casework GO! Kit resulted in a superior DNA yield compared to the automated magnetic bead extraction method.

Summary

Users of the Investigator Casework GO! Kit can directly amplify casework samples, including:

- Lyse forensic casework samples for subsequent use in direct amp workflows
- Maximize the chances of recovering DNA from a variety
- of body fluid and trace samples
- Use with QIAGEN assays for feedback on your PCR performance thanks to unique quality controls

Ordering Information

Product	Contents	Cat. no.
Investigator Casework GO! Kit	Casework GO! Lysis Buffer, Proteinase K Solution and nuclease-free water	386546
Related Products		
Investigator Quantiplex Pro RGQ Kit (200)	For use on QIAGEN Rotor-Gene Q Real-Time Systems: Quantiplex Pro RGQ Reaction Mix, Quantiplex Pro RGQ Primer Mix, Male Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387316

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.