Validation Report December 2016

Developmental validation of the Investigator® Lyse&Spin Basket Kit

The Investigator Lyse&Spin Baskets are used for pre-treatment of forensic samples in combination with manual and automated extraction kits, for example, QIAamp® DNA Investigator, EZ1® DNA Investigator, QIAsymphony® DNA Investigator, or Investigator STAR Lyse&Prep Kits. They allow the user to combine sample lysis and separation of solid sample substrates, for example, buccal or surface swabs, fabrics, cigarette butt paper and other, in one simple procedure. No sample transfer is required to obtain a cleared lysate.

The basket retains the lysis buffer during the lysis step of the forensic sample. During centrifugation in an ordinary benchtop centrifugation system, holes in the bottom of the basket open up and let the sample lysate pass through. The lysate containing the nucleic acids is efficiently recovered and collected in the 2 ml collection tube provided whereas the solid particles remain in the basket. Used baskets with solid sample substrates can optionally be stored in the second collection tube provided with the 250-prep kit (cat. no. 19598).

Optimal lysis conditions were established, and the effects of variations in those conditions were assessed. The performance of the Investigator Lyse&Spin Basket Kit was evaluated with regards to sensitivity and reproducibility. Various sample types were processed and potential cross-contamination was tested.

The assays used in this study make use of well-established methodologies for forensic DNA analysis. The validation of the assays is dealt with in detail in their respective validation reports that can be found on the respective QIAGEN® product pages.

Results of developmental validation

The validation study was performed by the QIAGEN R&D department. DNA extraction was performed with the EZ1 DNA Investigator Kit on the EZ1 Advanced XL instrument running the Large-Volume protocol or the QIAamp DNA Investigator Kit. DNA quantification was performed using the Investigator Quantiplex Kit on the Rotor-Gene® Q instrument, or the Applied Biosystems® 7500 Real-Time PCR System for Human Identification.

DNA profiles were obtained using the Investigator ESSplex SE Plus Kit according to handbook instructions. All electropherograms shown were generated on an Applied Biosystems 3500 Genetic Analyzer. The standard conditions specified in the respective kit handbooks were used for all experimental steps unless stated otherwise. A GeneAmp® PCR System 9700 with Gold-plated Silver 96-Well Block was used for amplification. Data were analyzed using Applied Biosystems GeneMapper® ID-X software (v1.2). Samples used were simulated casework samples where mentioned, as real casework samples are not readily available for product development processes.

Lysis conditions

Different incubation times at 56°C and shaking speeds were compared using an Eppendorf® ThermoMixer®. Aliquots of 50, 10, 1 and 0.1 µl blood were applied to swabs in a total volume of 50 µl and let dry. Swabs were incubated in the Investigator Lyse&Spin Baskets with buffer G2 and proteinase K for 1 or 2 hours shaking at 450 or 900 rpm (Figure 1). Overall shaking at 900 rpm resulted in higher yields compared to 450 rpm. Increasing the incubation to 2 hours compensated for some of the shaking reduction. We recommend using at least 750 rpm as shaking speed. Incubation times of 15 minutes and 60 minutes in lysis buffer ATL or G2 and proteinase K at 56°C with shaking at 900 rpm were compared using 1:10 blood dilutions. Higher and more consistent yields were obtained with 60 minutes incubation (Figure 2).

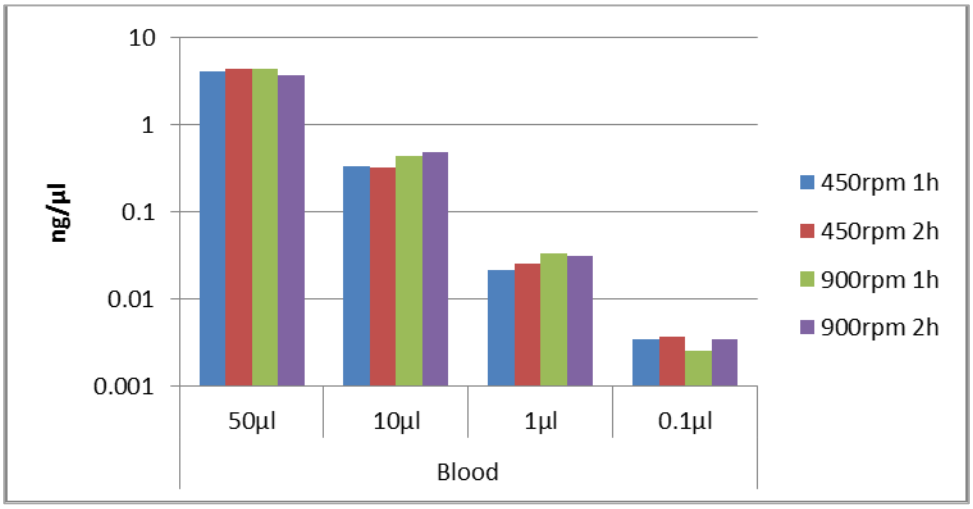

Figure 1. Comparison of lysis conditions.

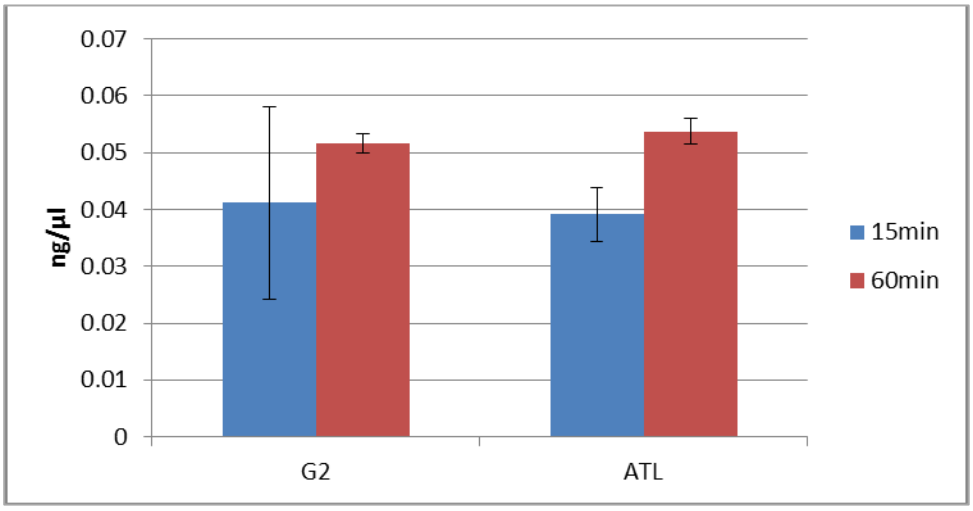

Figure 2. Comparison of lysis time and buffers.

Linearity and sensitivity

Dilutions of blood and saliva were applied to Sarstedt® Forensic Swabs (No. 80.629.001) and allowed to dry. Samples were lysed, using the Investigator Lyse&Spin Baskets or in 2 ml sample tubes as a reference, in buffer G2 and proteinase K with a final volume of 500 µl for 1 hour shaking at 900 rpm. For samples lysed in tubes, the entire lysate was recovered from the swabs by centrifugation in a QIAshredder column. Samples were extracted on the EZ1 Advanced XL.

The experiments showed linearity for both sample types over the range of input material tested. Note that the lowest sample amounts of the dilution series result in DNA quantification values that are at the detection limit (Figures 3 and 4).

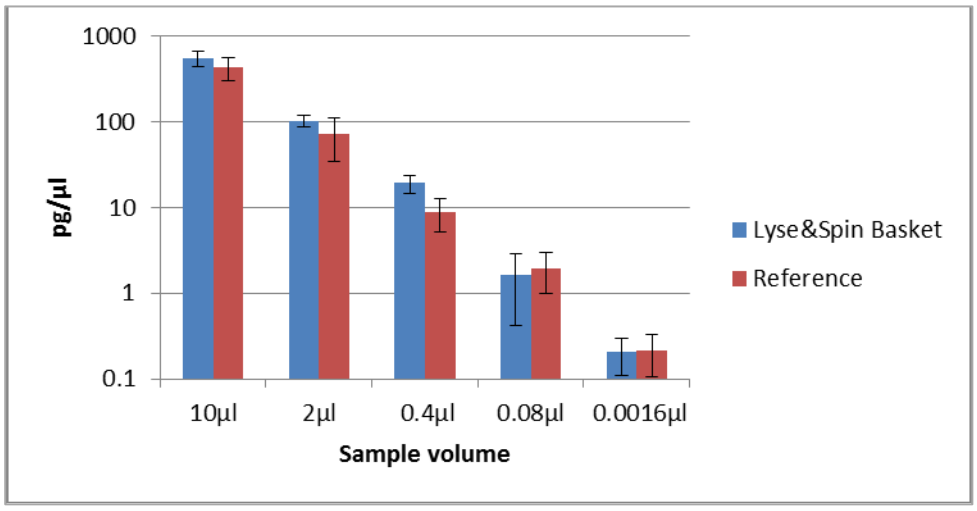

Figure 3. Sensitivity and linearity with dilutions of blood. Samples were diluted, and 50 µl containing the stated amount of blood was applied to swabs in 4 replicates each.

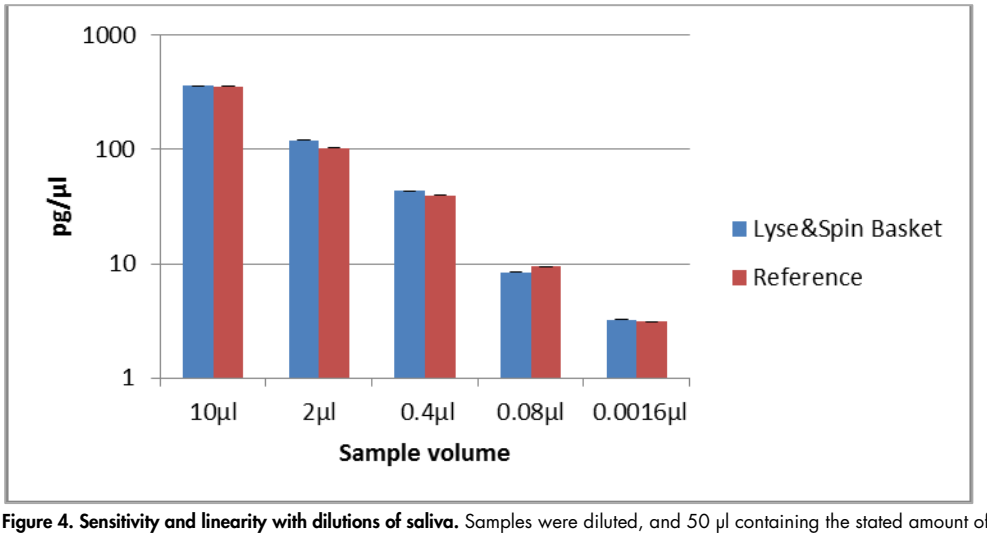

Figure 4. Sensitivity and linearity with dilutions of saliva. Samples were diluted, and 50 µl containing the stated amount of saliva was applied to swabs in 4 replicates each.

Reproducibility

Blood or saliva dilutions equivalent to 1 µl or 0.01 µl neat samples were applied to swabs in a total volume of 100 µl and let dry. 12 replicates of each sample were processed. Samples were lysed using the Investigator Lyse&Spin Baskets in buffer ATL and proteinase K with a final volume

of 500 µl for 1 hour shaking at 900 rpm. Samples were extracted using the QIAamp DNA Investigator Kit with an elution volume of 50 µl (Figure 5). Furthermore, extended incubations for 16 hours shaking at 900 rpm using different lysis buffers were tested for blood samples (Figure 6). The observed variation in yields is within the expected range introduced through the extraction and quantification process.

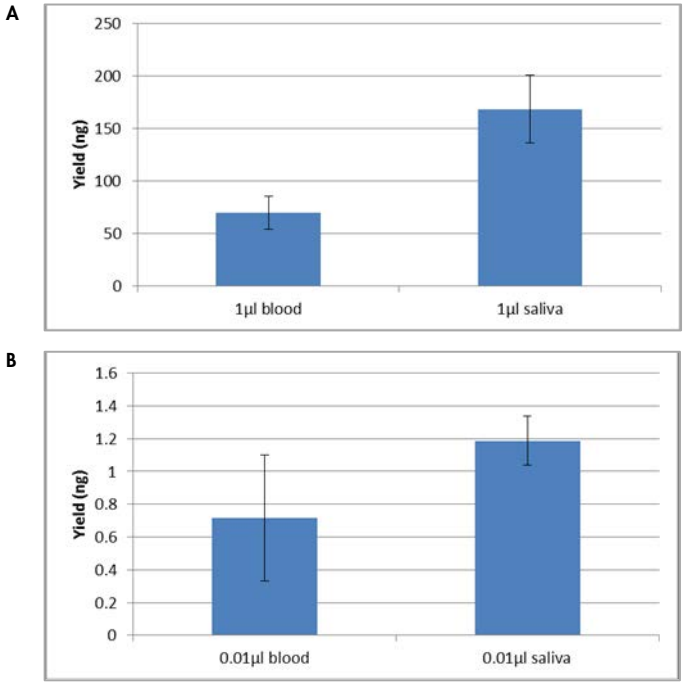

Figure 5. Reproducibility with dilutions of blood and saliva. A: corresponding to 1 µl of neat sample; **B**: corresponding to 0.01 µl of neat sample.

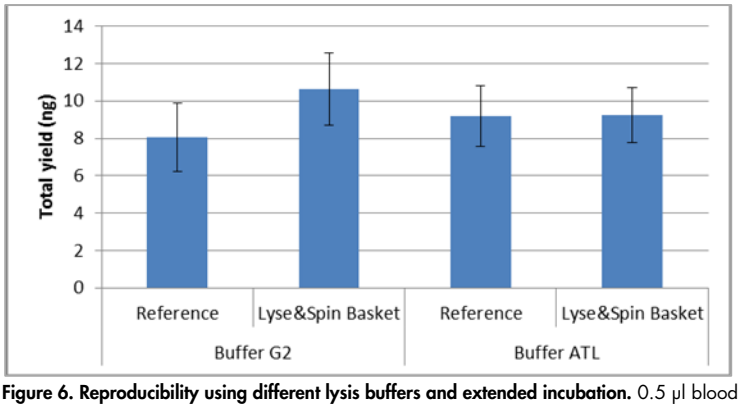

Figure 6. Reproducibility using different lysis buffers and extended incubation. 0.5 µl blood on swabs were incubated for 16 hours in buffer ATL or G2 plus proteinase K. As a reference, samples were incubated in tubes and swabs removed by centrifugation through QIAshredder columns. Samples were extracted on the EZ1 Advanced XL.

Absence of contaminating human DNA

Investigator Lyse&Spin Baskets are treated with ethylene oxide (EO) to ensure no potential human DNA contamination is present in the final product. In order to show absence of exogenous human DNA, 24 negative samples were processed. Swabs free of sample were incubated in lysis buffer G2 and proteinase K for 1 hour shaking at 900 rpm. After extraction on an EZ1 Advanced XL, samples were quantified and used for STR analysis with the maximum template volume of 15 µl. None of the samples yielded a positive quantification result or a DNA profile (example in Figure 7).

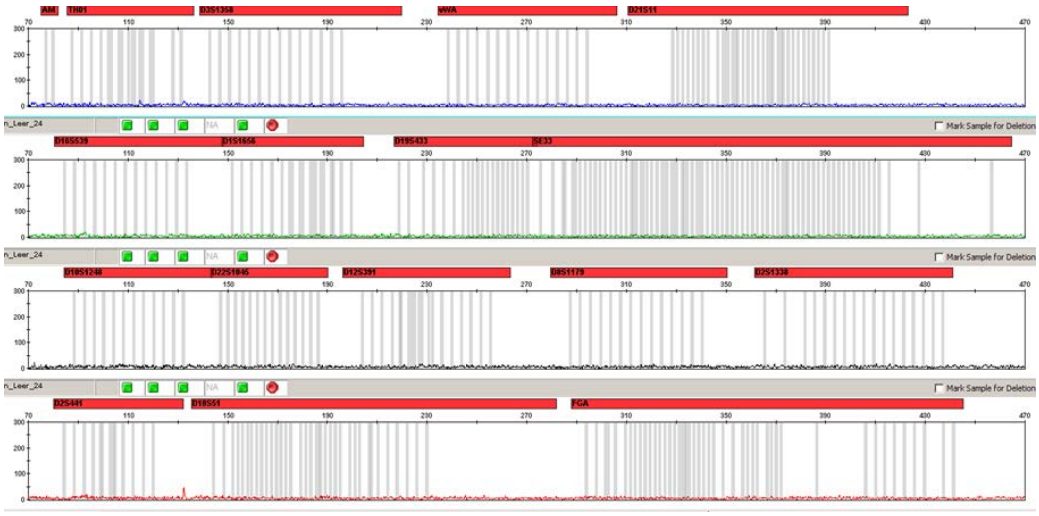

Figure 7. Example electropherogram of a negative sample.

Simulated casework samples

Different types of typical casework samples on solid substrates were tested:

- Cigarette butts
- Chewing gum
- Surface swabs (from computer keypad, mouse)
- Blood on fabric
- Saliva on paper (envelopes)

None of the samples caused clogging of the spin basket. Note however that some kinds of chewing gum may form a viscous lysate that blocks the spin basket during centrifugation. Average yields were comparable between the Investigator Lyse&Spin Basket Kit and the reference method. As expected, sample to sample variation of DNA content is considerable for these sample types (Figure 8). Typical DNA profiles are shown in Figures 9–12.

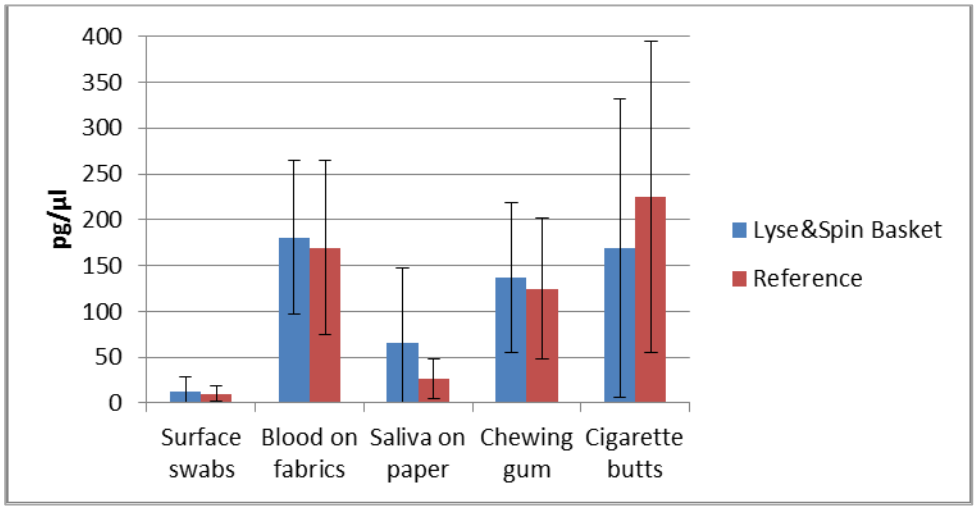

Figure 8. Various mock casework samples. 12 samples each were processed using the Investigator Lyse&Spin Basket Kit or the reference method. Samples were extracted on the EZ1 Advanced XL.

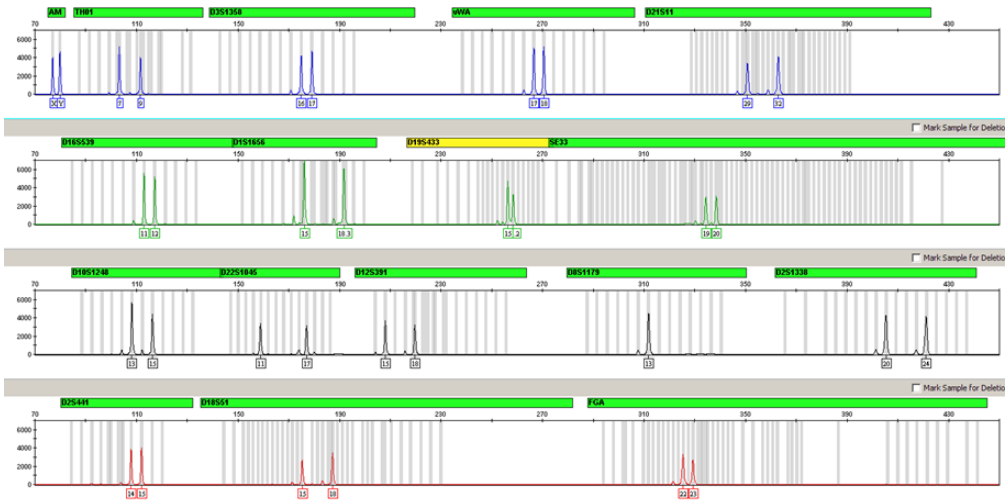

Figure 9. Example electropherogram of a blood sample on fabric. 500 pg template DNA was used for amplification.

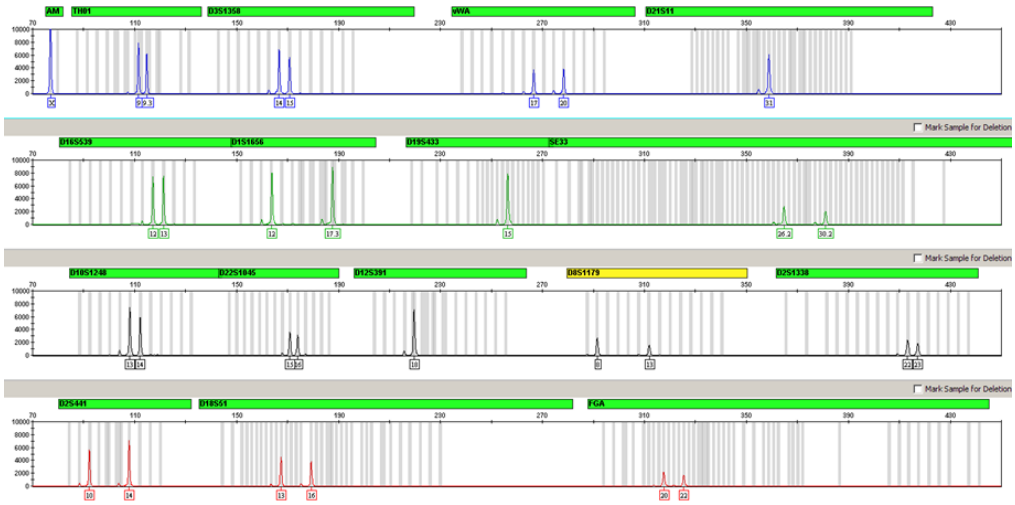

Figure 10. Example electropherogram of a cigarette butt sample. 500 pg template DNA was used for amplification.

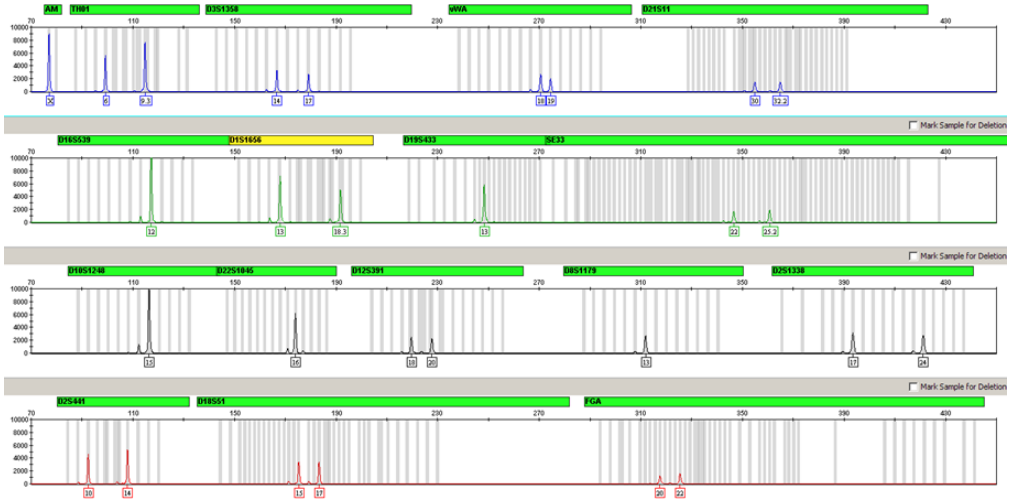

Figure 11. Example electropherogram of a chewing gum sample. 500 pg template DNA was used for amplification.

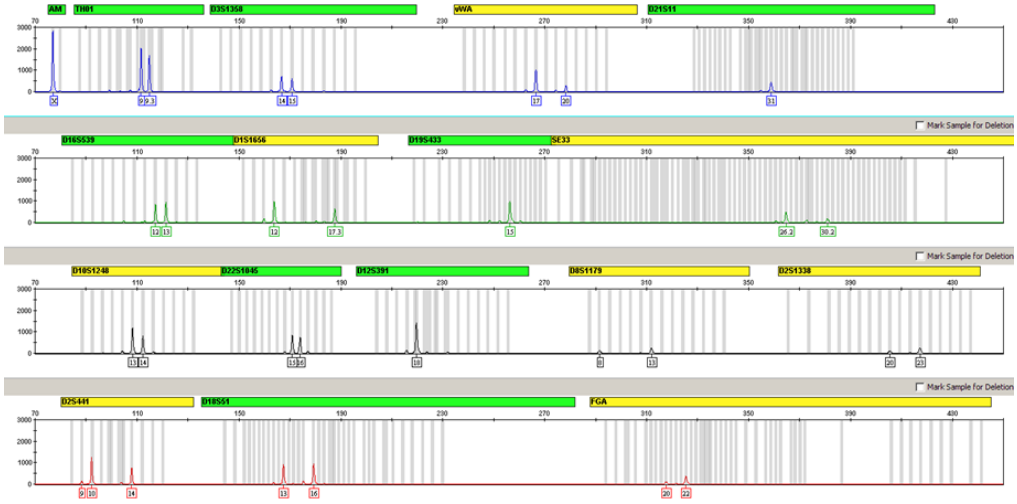

Figure 12. Example electropherogram of a surface swab sample. 108 pg template DNA was used for amplification.

Ordering Information

Product	Contents	Cat. no.
Investigator Lyse&Spin Basket Kit (50)	50 pouches containing 50 baskets and 100 collection tubes	19597
Investigator Lyse&Spin Basket Kit (250)	10 pouches containing 5 x 50 baskets and 5 x 50 collection tubes	19598

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 Service or your local distributor.

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