Developmental validation of the QIAamp® DNA Investigator® Kit

The QIAamp DNA Investigator® Kit (cat. no. 56504) is designed for manual or automated purification of total DNA from samples encountered in forensic, human identity, and biosecurity applications. Proven silica membrane technology provides high-quality DNA, which is suitable for direct use in downstream applications, such as quantitative PCR amplification reactions or STR analyses, or for storage for later use. Purified DNA is free of proteins, nucleases, and inhibitors. Pretreatment protocols are available for various typical casework or reference sample types. Automated protocols for extraction on the QIAcube®* have been developed, converting the established manual procedures into hands-off workflows. The use of QIAamp MinElute® spin columns allow the elution of purified DNA in as little as 20 µl, providing maximum sensitivity for challenging samples. The performance of the QIAamp DNA Investigator Kit, both on the QIAcube instrument and manually, was evaluated with regard to various sample types and conditions commonly encountered in forensic and parentage laboratories. Sensitivity, reproducibility, and freedom from cross-contamination were tested. Typical casework sample types were extracted.

Results of developmental validation

The validation study was performed by the QIAGEN R&D department. For DNA extraction, protocols were followed as described in the QIAamp DNA Investigator Handbook, www.qiagen.com/HB-0355, unless stated otherwise. Automated extraction on the QIAcube was performed using standard forensic protocols available for corresponding sample types. Quantification of human genomic DNA was performed using a SYBR® Green based real-time PCR assay, amplifying highly repetitive Alu sequences. The assay was run on a Rotor-Gene® 6000† instrument. The samples used were simulated casework samples where mentioned, because real casework samples are not readily available for product development processes.

Linearity and sensitivity

The Investigator STR kits (www.qiagen.com/STR-technology) are designed to work robustly over a range of DNA quantities. The recommended amount of input DNA to yield good quality STR profiles is 500 pg, based on real-time PCR quantification of human DNA. In particular, for heavily degraded DNA, the use of increased template amounts may improve results. Extraction using the QIAamp

* QIAcube Connect (cat. no. 9002840) makes use of protocols that have been transferred from the QIAcube instrument.
† Rotor-Gene 6000 is the precursor of Rotor-Gene Q.
DNA Investigator Kit was tested for relevant sample types with expected DNA yield in a range below and above the ideal input amounts for Investigator STR kits. Log dilutions of blood and saliva were extracted using the protocol for small volumes of blood or saliva on the QIAcube (n=3). DNA was eluted in 50 μl ATE, and real-time PCR quantification shows linear correlation of input sample amount and purified DNA (Figure 1). Observed yields were comparable to EZ1® Advanced and QIAsymphony® SP (cat. no. 9001297) results using the corresponding Investigator kits and protocols (data not shown). The experiments showed linearity for both sample types over the range of input material tested.

![Figure 1. Linear correlation of input sample amount and purified DNA.](image)

Reproducibility

To test reproducibility of results obtained with the QIAamp DNA Investigator Kit, extraction of blood on FTA® was carried out manually and automated on the QIAcube. For each preparation, 3 punches (3 mm diameter) of an FTA card with the dried blood of a single donor were used. According to the protocol for FTA and Guthrie Cards described in the QIAamp DNA Investigator Handbook, 12 samples were processed manually. In 3 individual runs, 36 samples were processed with the QIAcube. Sample lysis and purification were performed on the instrument. DNA was eluted in 100 μl buffer ATE and quantified by real-time PCR (Figure 2). Comparable yields were obtained within – as well as between – individual batches of samples. No significant differences were observed between manual and automated extractions.

![Figure 2. Manual and automated extraction of blood on FTA paper.](image)
Freedom from cross-contamination

In forensic applications, cross-contamination must be avoided at all cost. To confirm the absence of foreign human DNA in the buffers and spin columns, as well as to test for cross-contamination of the automated extraction protocol, alternating patterns of strong positive and negative samples were run. The positions of positive and negative samples were switched within each of the 4 individual runs (Figure 3). Positive samples were 100 µl blood samples from a single donor. For negative samples, lysis buffer ATL and proteinase K were used. Samples were extracted using 100 µl elution and the protocol “Isolation of Total DNA from Small Volumes of Blood or Saliva” in the QIAamp DNA Investigator Handbook. Eluates were quantified using real-time PCR. The blood samples, on average, yielded 966 ng DNA, whereas no DNA above the detection limit was observed in any negative sample.

Figure 3. Cross-contamination study on a QIAcube instrument.

Stability: recovery of degraded DNA

Another typical problem encountered in casework analysis is degradation of DNA due to adverse environmental conditions. The protocols have been optimized for efficient recovery of DNA fragments that can still be used to create an STR profile. To simulate degraded DNA, defined DNA ladders were used as spiked samples and were extracted by applying protocols with different binding stringencies triggered by the ratio of ethanol to binding buffer. For the final protocol, 0.5 volumes of ethanol were chosen to allow full recovery of 200 bp long fragments (Figure 4).
Casework and reference samples

The QIAamp DNA Investigator Kit has been developed specifically for forensic casework and reference samples. The extraction was tested using various types of simulated casework samples. Therefore, sample types commonly faced in forensic casework were processed, including blood stains, chewing gums, cigarette butts, surface swabs, hair, and buccal swabs. All samples represent simulated casework items that were created in the lab. Casework samples were extracted using a 60 μl elution and protocols as outlined in the kit handbook for the corresponding sample types. Eluates were quantified using real-time PCR. As expected, touch DNA items in particular showed high variation between individual samples. Buccal swabs were extracted as a typical example of a reference sample. Therefore, 1 swab each was taken from 6 different donors in duplicate. Reference samples were extracted using 60 μl elution with the “Isolation of Total DNA from Surface and Buccal Swab” protocol in the QIAamp DNA Investigator Handbook. Eluates from the manual or automated purification were quantified using real-time PCR.

Figure 4. Recovery of degraded DNA. GelPilot® 1 kb DNA ladder was reextracted using protocols with decreasing ethanol volumes in the binding step. Lanes 1, 5, 9, and 13: Ladder before extraction. Lanes 2–4: Ratio 1:1 buffer AL:ethanol. Lanes 6–8: Ratio 1:0.5 buffer AL:ethanol. Lanes 10–12: Ratio 1:0.25 buffer AL:ethanol. Lanes 14–16: Only buffer AL, no ethanol.
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Figure 5. Extraction of simulated casework samples. Hair: 1 hair strand each from different donors (n=7); no telogen hairs were used. Chewing gum: Approximately 30 mg of different donors (n=8). Cigarette butts: A quarter of a filter paper, different donors (n=6). Blood swabs: 1 µl of blood was applied and allowed to dry (n=4). Saliva swabs: 0.1 µl of saliva was applied (n=4). Blood on denim: 1 µl of blood was applied to a piece of approximately 1 cm² (n=4). Touch DNA samples: Moist swabs were taken from the surface (n=3 each).

Figure 6. Extraction of buccal swab samples. One swab each was taken from different donors in duplicate (n=6). Samples were processed manually or automated on the QIAcube using the protocol “Surface and Buccal Swabs”.

QIAcube Connect

QIAcube Connect (cat. no. 9002840) makes use of protocols that have been transferred from the QIAcube instrument. Equivalence of protocol procedures and performance has been proven across a large number of applications.
Document Revision History

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<th>Date</th>
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<tr>
<td>10/2019</td>
<td>Added text references to QIAcube Connect.</td>
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