May 2022

Validation Report

Developmental Validation of the Investigator® Casework GO! Kit

The Investigator Casework GO! Kit can be used for direct amplification of casework samples and thus enables an accelerated workflow in casework analysis. It allows the rapid processing of swabs from casework samples, including cuttings of sexual assault swabs or other materials, without the need of further purification. The lysate can be used directly for quantification with all Investigator Quantiplex® assays or for DNA profiling using the Investigator STR assays (unless data from the quantification indicates the presence of possible PCR inhibitors).

In sexual assault screening analysis, the Investigator Casework GO! workflow can facilitate the sample processing decision by male-quantification and Auto/Y ratio.

The validation study was based on the recommendations of the European Network of Forensic Science Institutes (ENFSI) (1) and, where applicable, on the Revised Validation Guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM) (2).

The sensitivity studies for the Investigator Casework GO! Kit are given on page 2. A repeatability (page 6) and robustness study (page 7) were also performed. The sample lysates were tested for stability (page 8), and the kit's compatibility with different collection devices was assessed (page 9). Moreover, various casework sample types (page 11), the sexual assault screening (page 13), and a differential wash protocol with mock sexual assault samples (page 15) were tested.

Principle and procedure

The Investigator Casework GO! Kit is used for direct amplification of casework samples and thus enables an accelerated workflow in casework analysis. It enables rapid processing of swabs from casework samples, including cuttings of sexual assault swabs, pieces of fabric, paper, cigarette butts, chewing gum and other sample types, without the need for further purification. The lysate can be used directly for quantification using the Investigator Quantiplex Pro Kit (QIAGEN, cat. no. 387216)and Quantiplex Pro RGQ Kit (QIAGEN, cat. no. 387316)or in STR amplification using all Investigator STR assays, like Investigator 24plex QS Kit (QIAGEN, cat. nos. 382415 and 382417), ESSplex SE QS Kit (QIAGEN, cat. no. 381575 and 381577) or 26plex QS Kit (QIAGEN, cat. no. 382615 and 382617), unless data from quantification indicates the presence of possible PCR inhibitors.

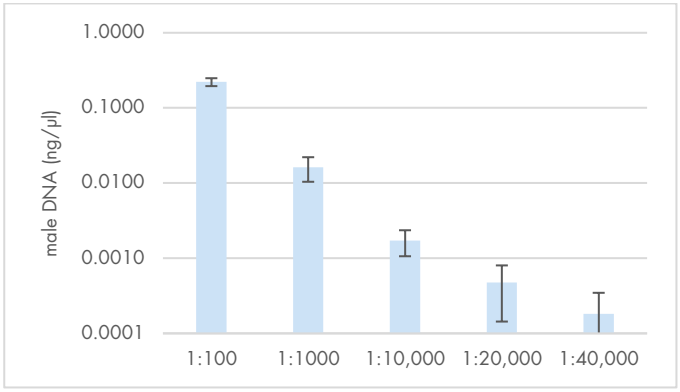
All swabs and fabric cuttings were processed using 2 ml safe-lock tubes (Sarstedt). A master mix containing Casework GO! Lysis Buffer, Proteinase K, and DTT was freshly prepared according to the Quick Start Protocol for the Investigator Casework GO! Kit. A volume of 100 to 400 microliters of master mix was added to the samples. Samples were briefly vortexed and then incubated in a heater shaker at 60°C for 25 minutes and 900 rpm. Finally, samples were heated to 80°C for 5 minutes in order to inactivate the Proteinase K. The lysates were directly used for quantification with the Investigator Quantiplex Pro Kit and STR profiling with the Investigator 24plex QS Kit according to the handbook.

Results of developmental validation

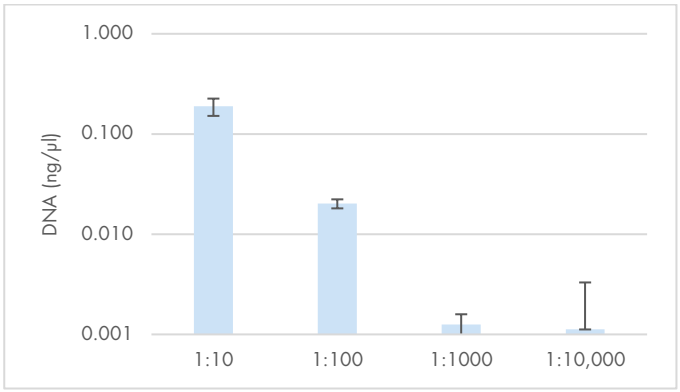
Sensitivity study

Three male samples (blood, saliva, and semen) were used in the sensitivity study. Serial dilutions of neat body fluid in ATE (1:100, 1:1,000, 1:10,000, 1:20,000, and 1:40,000 with semen and 1:10, 1:100, 1:1,000, and 1:10,000 with blood and saliva) were prepared and 25 µl of diluted sample was applied to a whole cotton swab and was dried. Each condition was tested in sextuplicates and was extracted with 200 µl master mix. Samples were quantified using the Investigator Quantiplex Pro Kit on the Applied Biosystems 7500 Real-Time PCR System; 1 ng/reaction or 15 µl of sample was used for STR analysis with the Investigator 24plex QS Kit and Investigator Argus Y-12 QS Kit (QIAGEN, cat. nos. 38315 and 383617) on the Applied Biosystems 3500 Genetic Analyzer. Data shows high correlation between the input volume of body fluid and the resulting DNA concentration (Figure 1) and peak heights in 24plex QS reactions (Figure 2) for all 3 types of serially diluted samples, as well as high allele coverage (Figure 3).

Semen



Saliva



Blood

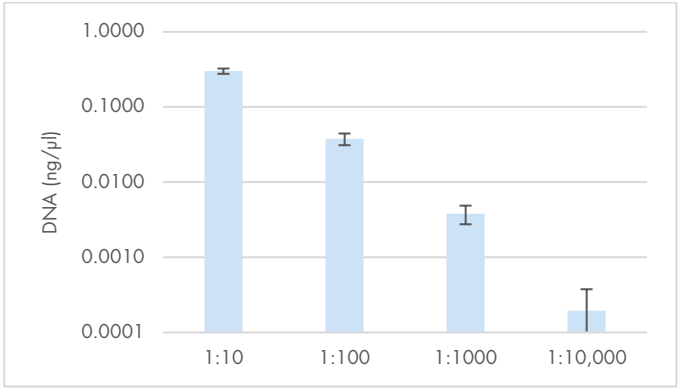
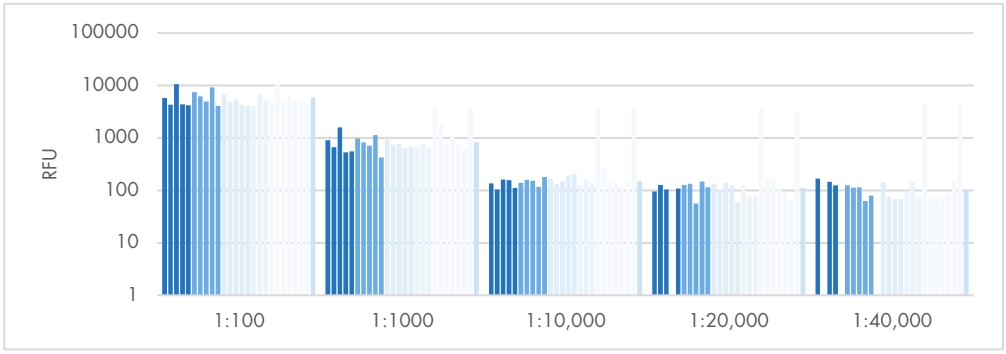
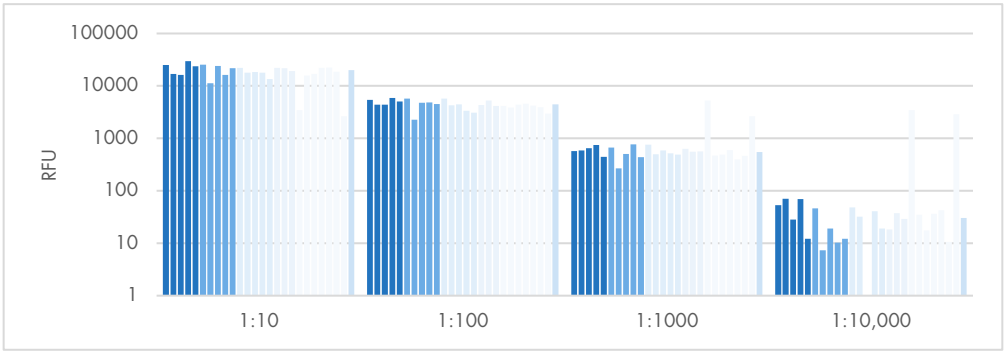


Figure 1. Quantification results for serially diluted semen, saliva, and blood samples processed with Investigator Casework GO!. The figure shows the average male DNA quantification (ng/µl) ± standard deviation.

Semen



Saliva



Blood

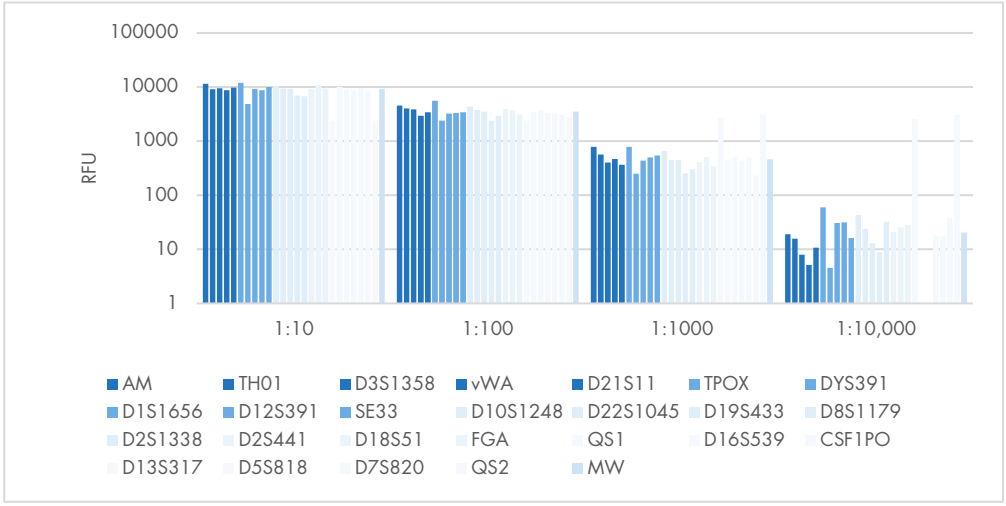
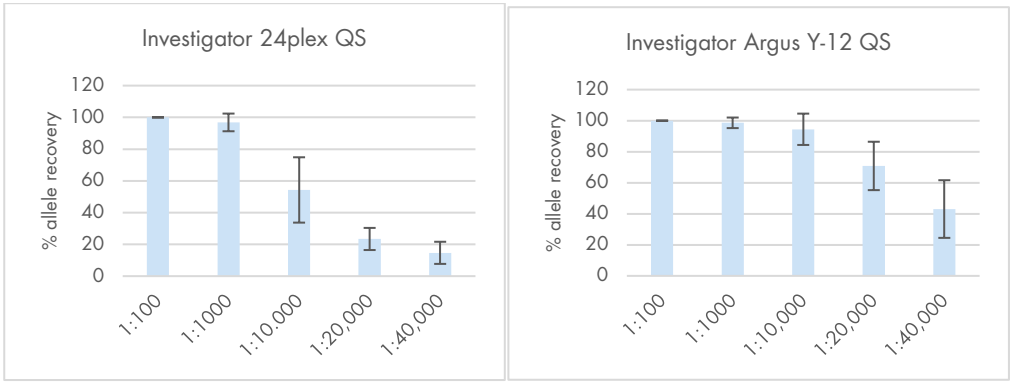


Figure 2. STR results showing the mean peak height in RFU for serially diluted semen, saliva , and blood samples processed with Investigator Casework GO! and amplified with the Investigator 24plex QS Kit.

Semen



Saliva **Blood**

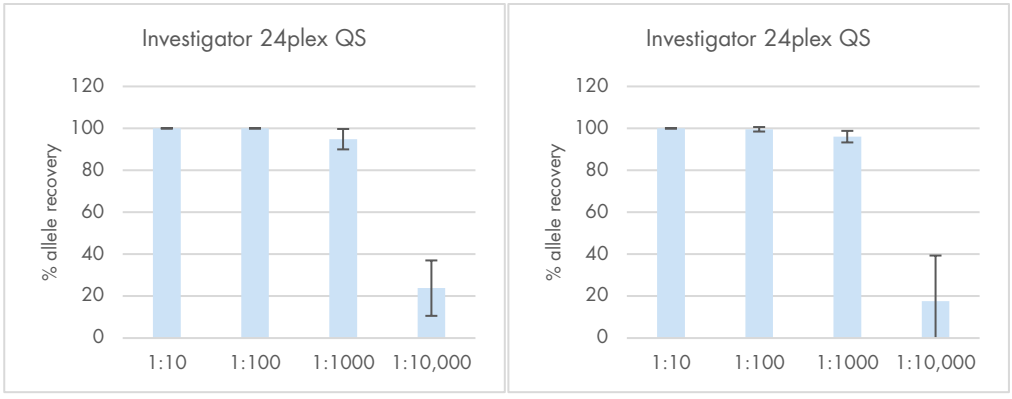


Figure 3. Allele recovery rates for serially diluted semen, saliva, and blood samples processed with Investigator Casework GO! and amplified with the Investigator 24plex QS and/ or Investigator Argus Y-12 QS Kit.

Repeatability Study

Blood from a male donor was diluted serially with ATE (1:10, 1:100, 1:1,000, 1:10,000), and 25 µl of diluted sample applied to a whole cotton swab and dried. Each sample was prepared in triplicate and extracted in 3 separate extraction sets for a total of 9 replicates per dilution. Each sample was lysed with 200 µl of master mix. The Casework GO! lysates were quantified with the Investigator Quantiplex Pro Kit and STR profiles were created with the Investigator 24plex QS Kit. Figure 4 demonstrates high repeatability for the 3 independent runs in terms of DNA concentration (A), allele recovery (B) and peak heights (C) in a wide range of sample concentrations.

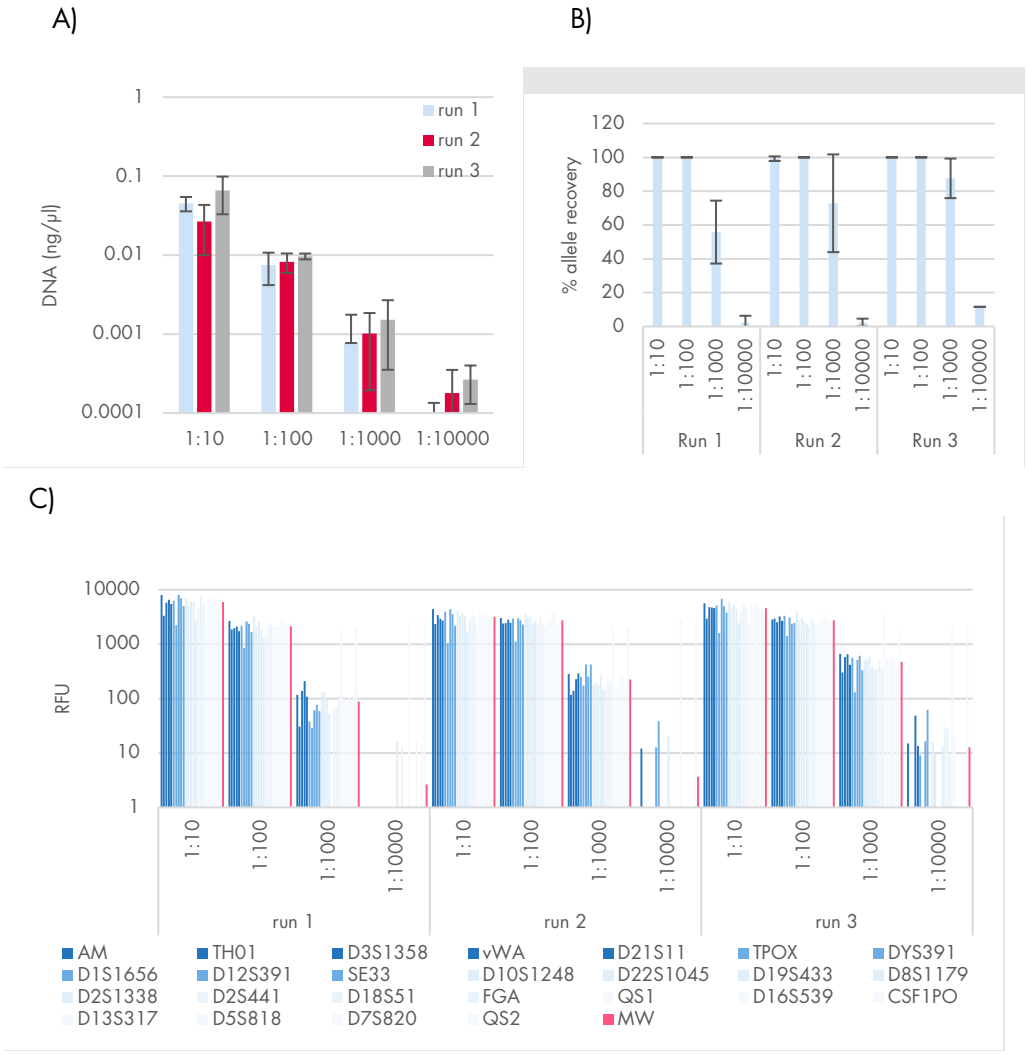
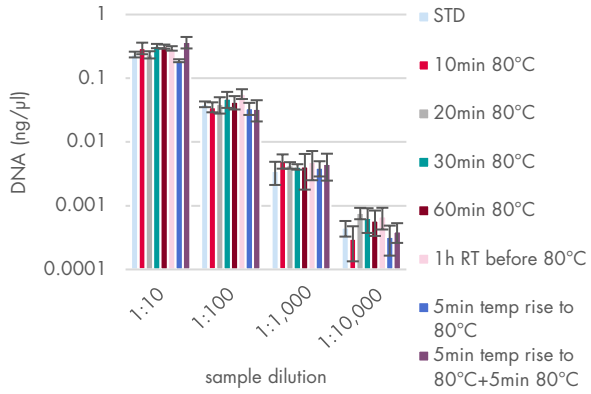


Figure 4. Results of repeatability study with blood samples processed in 3 independent runs with the Investigator Casework GO! Kit. The figure shows A) the average quantification (ng/µl) with Investigator Quantiplex Pro ± standard deviation, B) allele recovery rates (%) ± standard deviation, and C) average STR peak heights (RFU) for samples amplified with the Investigator 24plex QS Kit.

Robustness Study

The Investigator Casework GO! Kit was tested for its robustness to a variety of protocol modifications that might be required for implementation in an automated or manual forensic testing procedure (e.g. if only 1 heater shaker is available). Dilutions of semen in ATE (1:10, 1:100, 1:1,000, 1:10,000) were applied to whole cotton swabs (25 µl per swab) and were dried. Each sample was extracted with 200 µl of master mix. Following protocol modifications were tested in triplicates: (I) Prolonged 80°C step (5, 10, 20, 30 and 60 mins), (II) samples cooled down to room temperature for 60 min before 80°C step, (III) samples kept in heat block during temperature increase from 60 to 80°C (timer set for 5 min), (III) samples kept in heat block during temperature increase from 60 to 80°C (timer set for 5 min) + 5min 80°C. Figure 5 demonstrates that the chemistry is very robust and is able to compensate protocol modifications to some extent.

A)



B)

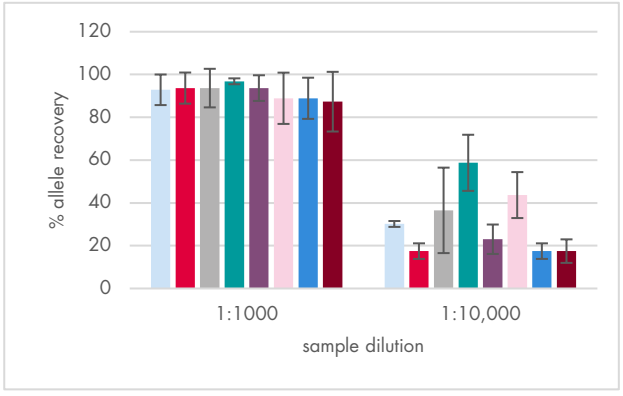


Figure 5. Results of robustness study. The figure shows A) the average quantification (ng/µl) with Investigator Quantiplex Pro ± standard deviation and B) the average allele recovery rates (%) ± standard deviation for samples amplified with the Investigator 24plex QS kit.

Stability study of Casework GO! lysates

Lysates from various sample types (saliva, blood, chewing gum, cigarette butts) that underwent the Casework GO! procedure, were kept in the fridge (2 to 8°C) for a period of 25 months. After 1, 2, 4, 5, 6, 10, 13, 18, and 25 months of storage, lysates were quantified with the Investigator Quantiplex Pro Kit. Figure 6 demonstrates that samples can be kept in the fridge for at least 2 years without significant loss of DNA.

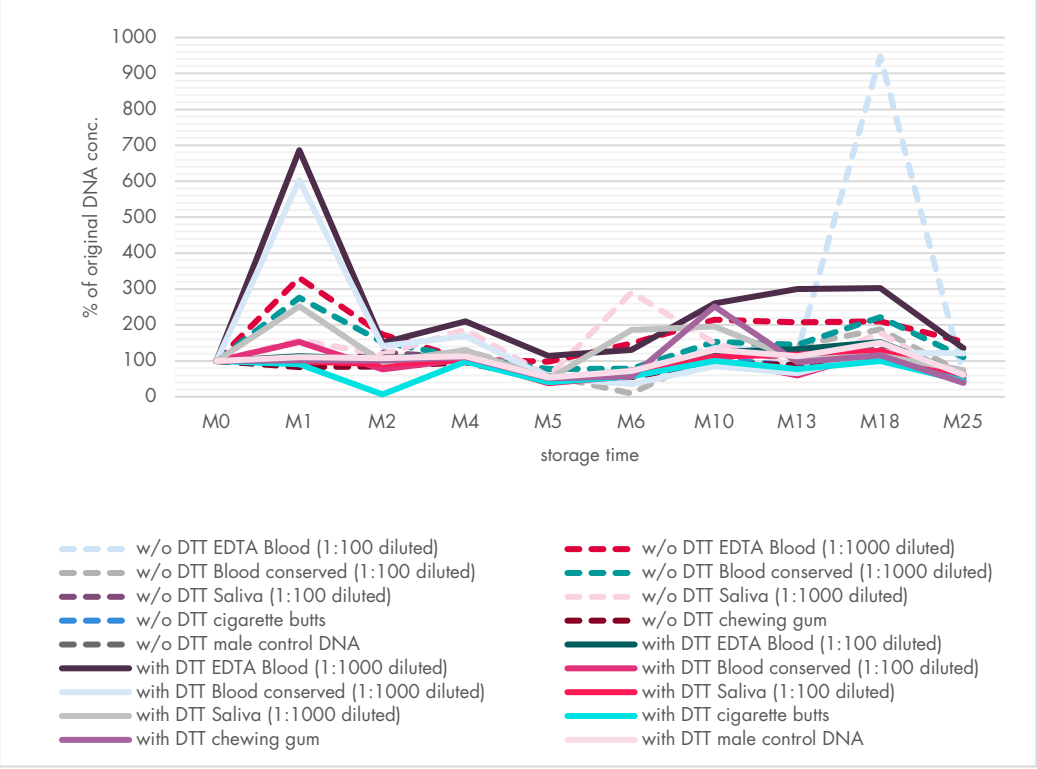


Figure 6. Results of stability study for Casework GO! lysates. The figure shows the percent change in measured DNA concentration from baseline values at time point M0.

Compatibility study for different collection devices

Male DNA (20 ng per sample) was applied to different collection devices and was dried. Each condition was tested in triplicate. Samples were lysed using 200 µl of master mix according to the Casework GO! protocol. Data shows that the kit is compatible with all tested devices independent if they are cotton tipped, polyester tipped or flocked swabs, except 1 flocked swab from Copan (Figure 7).

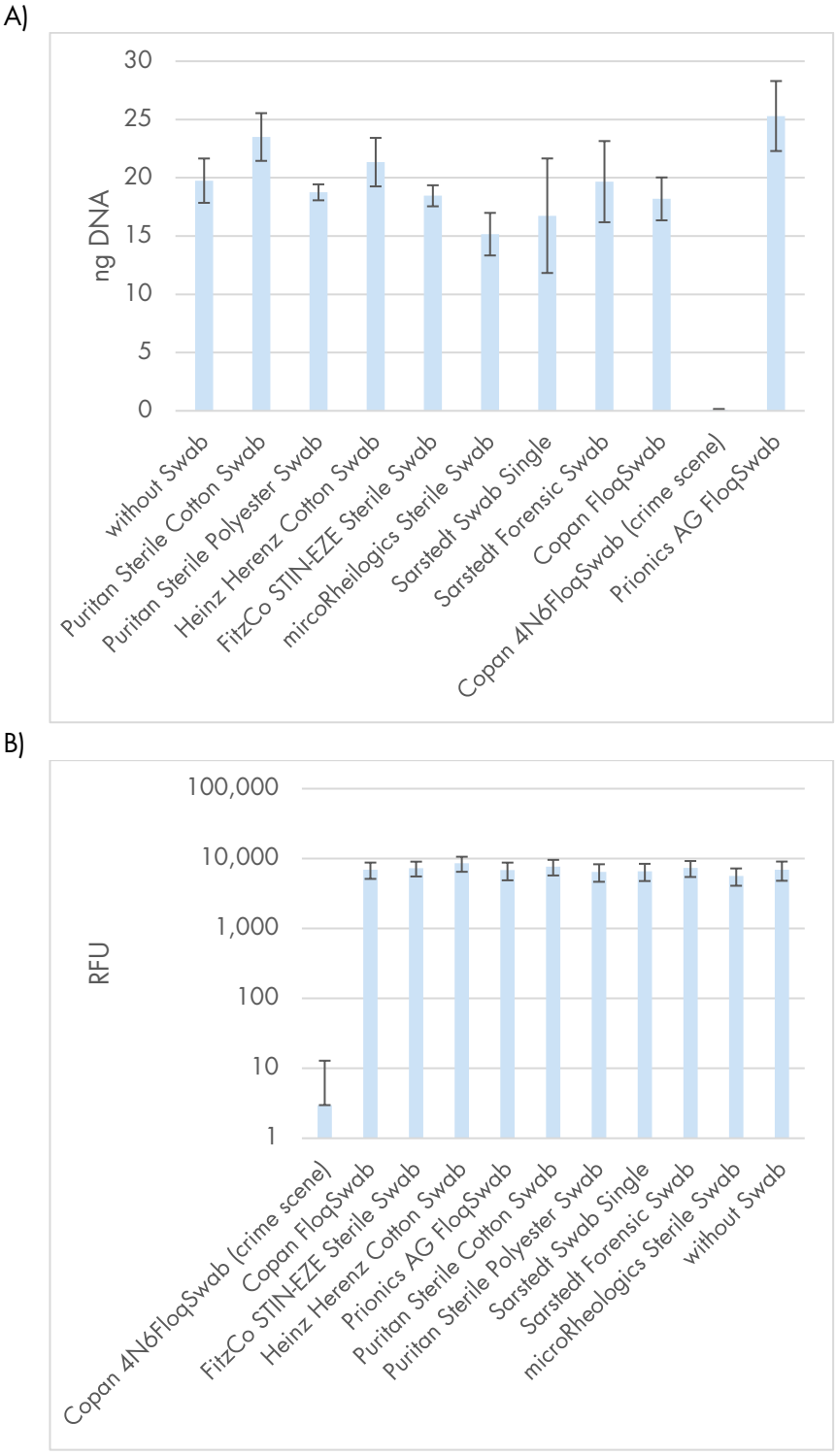


Figure 7. Compatibility of investigator Casework GO! with different collection devices. The figure shows the average DNA yield (A) with Quantiplex Pro and STR peak heights with Investigator 24plex QS (B) for each collection device.

Casework Samples

A variety of mock evidence samples, such as cigarette butts, chewing gums, touch skin cell samples, and fabric cuttings were extracted with 200 µl of master mix. In parallel, samples were processed according to the EZ1 large volume protocol with elution in 100 µl TE on an EZ1 Advanced XL Instrument.

Cigarette butts: The filter paper of cigarette butts from various donors was removed and cut into 1 cm² pieces. Figure 8A gives the resulting template DNA for 27 different cigarette butts and demonstrates high resulting allele coverage. Results of the direct approach are comparable to sample preparation workflow using the EZ1&2 DNA Investigator Kit (QIAGEN, cat. no. 952034) (Figure 9B).

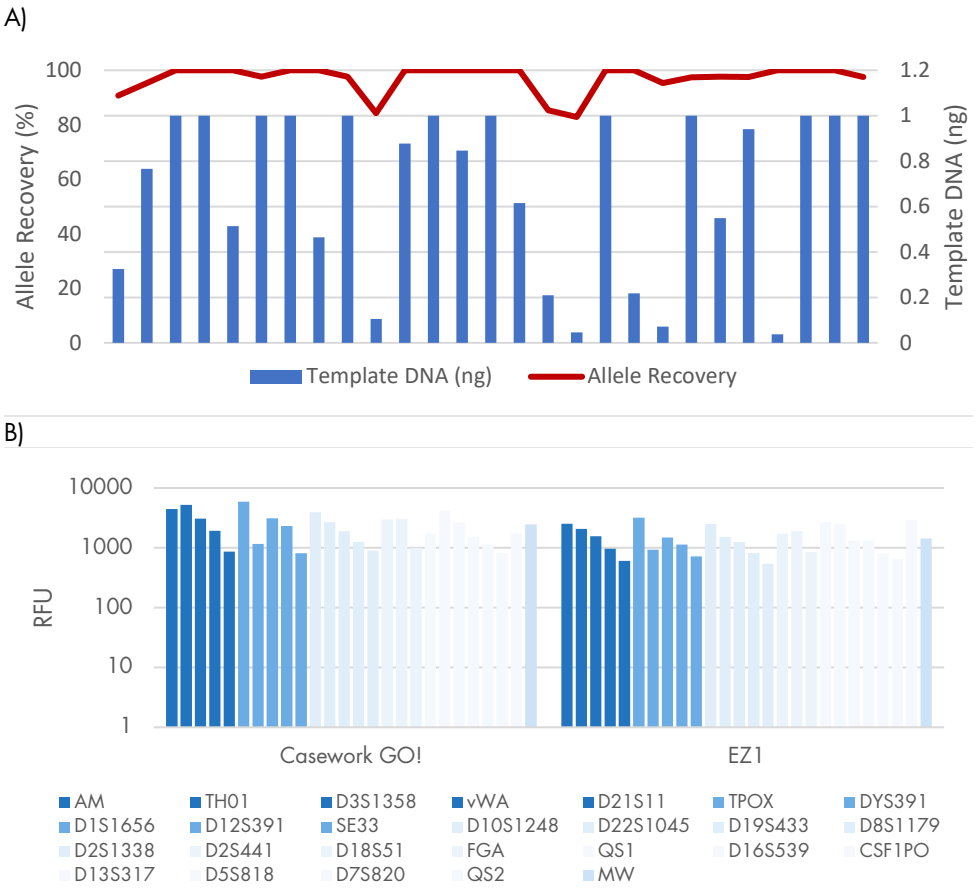


Figure 8. Processing of cigarette filter paper with Investigator Casework GO!. A) Correlation between template DNA for STR profiling and allele recovery (B) Peak heights of same samples processed with the direct approach and the EZ1&2 DNA Investigator Kit.

Chewing gums: Used chewing gums from various donors and of different brands were dried and cut into small pieces (around 3x3x3 mm). Figure 9 shows that the direct protocol results in comparable DNA concentrations (A) and peak heights (B) than the workflow with the EZ1&2 DNA Investigator Kit.

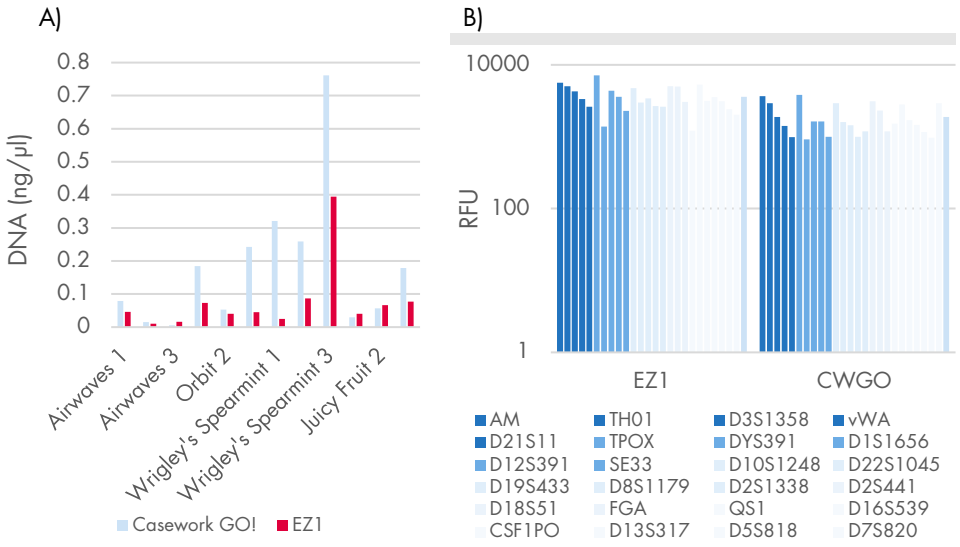


Figure 9. Casework GO! vs. EZ1Adv X with EZ1&2 DNA Investigator Kit for processing chewing gums. The figure shows A) the DNA concentration for same samples processed with both methods and the resulting STR peak heights (B) in the Investigator 24plex QS reaction when 15 µl sample, or up to 1 ng DNA was applied.

Touch skin cell samples: Different surfaces (chair arm rests, mobile phones, phones, key boards and spoons) were swabbed evenly with pre-moistened cotton swabs (n=5). The direct protocol results in comparable DNA concentrations and allele recovery rates than the workflow with the EZ1&2 DNA Investigator Kit (Figure 10).

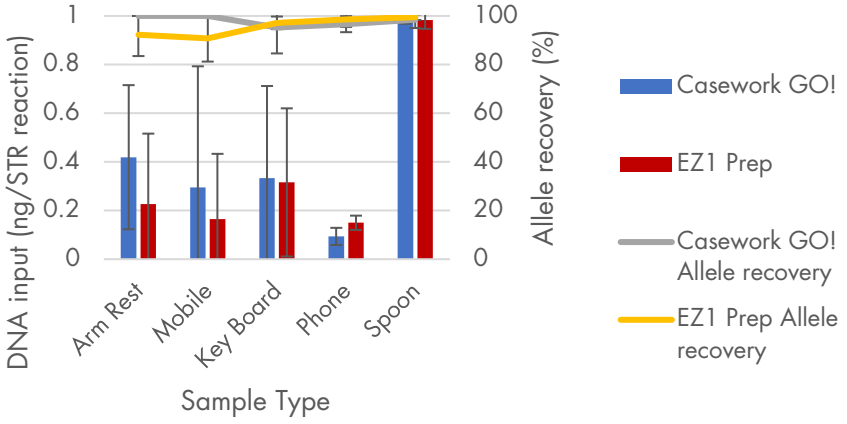


Figure 10. Casework GO! vs. EZ1Adv X with EZ1&2 DNA Investigator Kit for processing touch skin cell samples. The figure shows the correlation of DNA input per STR reaction which resulted when 15 µl sample or up to 1ng DNA were applied to Investigator 24plex QS and allele recovery rates.

Fabric cuttings: Different fabrics (jeans, cotton, polyester, stretch) were cut into equally sized pieces (0.5 x 0.5cm) and 25 µl diluted blood or semen samples (1:100 in ATE) were applied to the fabric and dried (n=6). Figure 11 shows that the direct protocol results in comparable or even higher DNA concentrations and allele recovery rates than the workflow with the EZ1&2 DNA Investigator Kit.

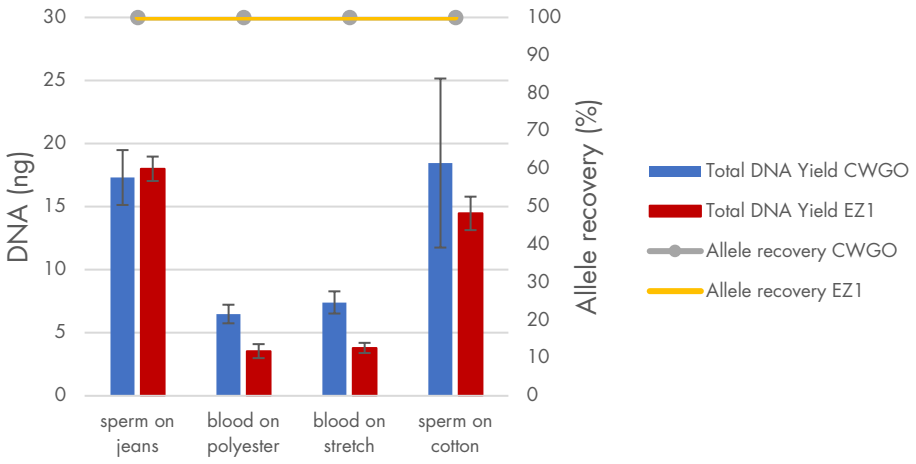


Figure 11. Casework GO! vs. EZ1Adv X with EZ1&2 DNA Investigator Kit for processing fabric cuttings. The figure shows the correlation of DNA input per STR reaction which resulted when 15 µl sample or up to 1ng DNA were applied to Investigator 24plex QS and allele recovery rates.

Sexual Assault Screening

Dilutions of semen samples (1:5,000, 1:10,000, 1:20,000, 1:40,000) in ATE were applied to whole cotton swabs and dried in order to prepare mock sexual assault samples. A volume of 400 µl of Casework GO! Lysis Buffer was applied and swabs were rigorously vortexed and shaken at 1.200 rpm with a shaker mixer for 5 minutes in order to release the sperm into the buffer. For screening purposes, a 10% fraction (40 µl) was removed and lysed with 1.5 µl Proteinase K and 1.3 µl 10 mM DTT in a new 2 ml safe-lock tube. Samples were quantified with the Investigator Quantiplex Pro Kit. Samples with male DNA concentrations above 0.0625 pg/µl (25 pg in total sample) were processed further. For this purpose, 20 µl Proteinase K and 20 µl 1 M DTT were added to the remaining 360 µl sample and lysed at 60°C for 1 hour in presence of the swab. Thereafter, 400 µl Buffer MTL was added to the lysate and the supernatant was extracted without the swab head with the EZ1&2 DNA Investigator Kit using the large volume protocol and elution in 40 µl TE (Figure 12). Results demonstrate that there is only marginal DNA loss between the direct approach, and the DNA yield after sample purification with the EZ1 Advanced XL instrument. DNA input and allele recovery show a good correlation. Moreover, a very good sensitivity with the STR profiling can be achieved.

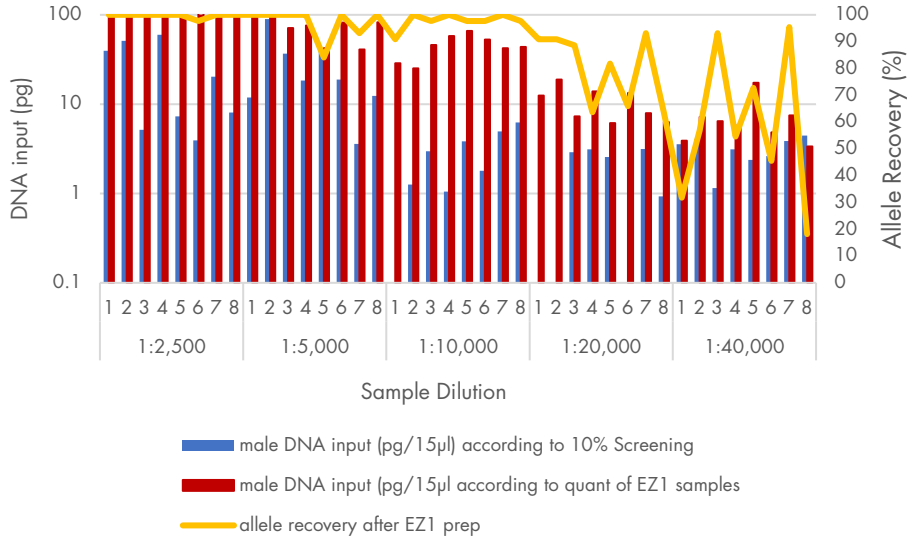


Figure 12. Sexual assault screening with Investigator Casework GO!. The figure shows the correlation of DNA input according to the quant results of the 10% screening and 90% testing fraction and allele recovery rate of the 90% testing fraction.

Differential wash protocol with mock sexual assault samples

Female buccal swabs (cotton) with and without 25 µl of 1:100 diluted semen samples and cotton swabs with only 25 µl of 1:100 diluted semen samples (n=3) were differentially washed using the Investigator Casework GO! Kit. Each sample was tested in triplicate. Therefore, swabs were placed in Sample i-SEP® DL (Biotype, cat. no. 60-00101-0250) and lysed analog to the published Biotype protocol for the i-SEP DL baskets twice with 300 µl Casework GO! Lysis Buffer containing Proteinase K (but without DTT). Lysis was performed at 60°C for 25 min and 900 rpm. After lysis, samples were collected by centrifugation at 5.000 rpm for 1 minute and the 2 ml collection tube was changed. For semen lysis 150 µl of master mix (containing both Proteinase K and DTT) was applied after the second lysis and samples were treated as described. All collected samples were finally heated at 80°C for 5 min in order to inactivate the Proteinase K. Samples were quantified with the Investigator Quantiplex Pro Kit (Figure 13 and Figure 14) and analyzed with the Investigator Kits Argus Y-12 QS (Figure 15). Figure 14 shows that male DNA could be found in all 3 collected flow throughs of the differential washing procedure. As anticipated, the biggest male proportion was obtained in the final fraction after lysis with DTT. This finding is also reflected by rising allele recovery rates with the Investigator Argus Y-12 QS Kit (Figure 15). The high proportion of female background DNA makes it impossible to perform an autosomal STR analysis.

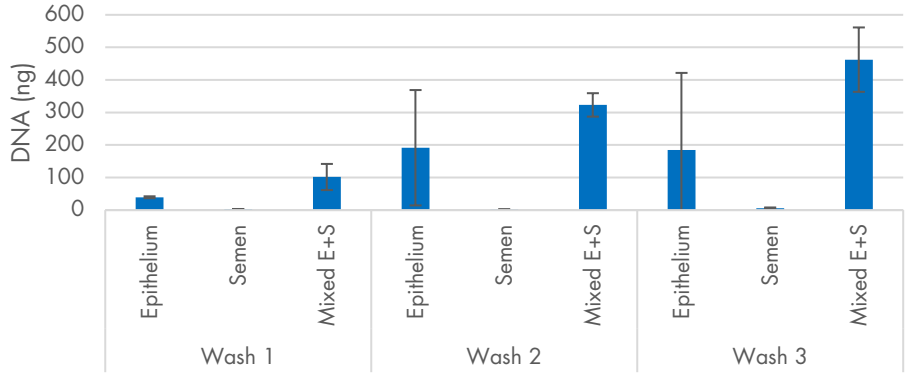


Figure 13. Total human DNA yield upon differential washing with Investigator Casework GO!.

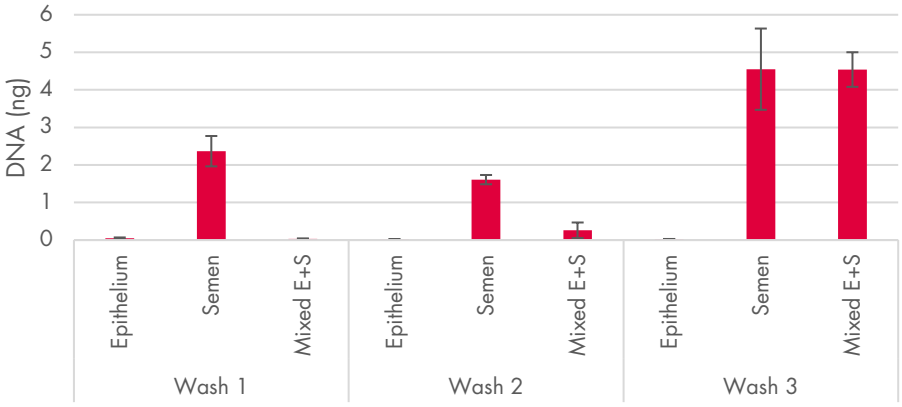


Figure 14. Total male DNA yield upon differential washing with Investigator Casework GO!.

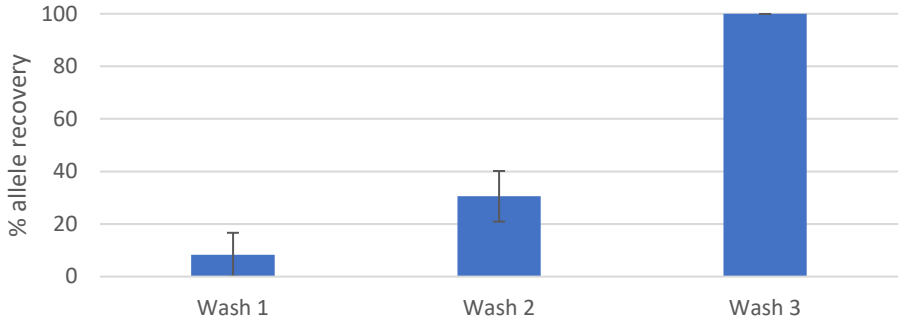


Figure 15. Allele recovery rates of mixed samples upon 3 differential wash steps.

Inhibition Study

To test the robustness of the Investigator Casework GO! Kit in the presence of inhibitors, it was run in the presence of the following inhibitors, chosen to mimic challenging forensic sample types: 25 µl of semen dilution (1:1,000) in ATE were mixed with 100 µl master mix and 10 µl inhibitor solution to obtain final inhibitor concentrations of 333.3 to 833 ng/µl humic acid and 1.666.7 to 4.167 µM hematin in the samples. Each condition was tested in quadruplicate (Table 1). Data demonstrates that the workflow can tolerate very high levels of inhibitor concentrations resulting in nearly no allelic drop-out. Additional purification of inhibitory samples with the EZ1&2 DNA Investigator Kit secures high performance of quant and STR PCRs without significant sample loss.

Table 1. Effect of inhibition in Investigator Casework GO! samples on Investigator Quantiplex Pro and Investigator 24plex QS.

	Inhibitor Series	Human (ng/µl)	Male (ng/µl)	Degradation (ng/µl)	IC Shift	Deg. Index	% Profile 24plex QS
Investigator Casework GO!	No Inhibitor	0.0750	0.0856	0.0679	0.2084	1.1	100
	333.3 ng/µl HA	0.1066	0.1047	0.0545	1.3004	2.9	100
	416.7 ng/µl HA	0.0970	0.0901	0.0195	2.4114	5.8	100
	625 ng/µl HA	0.0602	0.0798	-	8.2829	-	100
	833 ng/µl HA	0.0394	0.0581	-	11.8060	-	100
	1666.7 µM Hem	0.0056	0.0092	0.0010	0.9280	3.0	100
	2083.3 µM Hem	0.0064	0.0148	0.0018	1.3903	4.2	100
	3125 µM Hem	0.0045	0.0161	0.0004	3.6256	11.3	91
	4167 µM Hem	0.0054	0.0249	-	12.9779	-	89
Additional purification with EZ1&2 DNA Investigator Kit	No Inhibitor	0.0417	0.0510	0.0388	0.1168	1.1	100
	333.3 ng/µl HA	0.0563	0.0803	0.0548	0.0765	1.0	100
	416.7 ng/µl HA	0.0457	0.0533	0.0401	0.1319	1.1	100
	625 ng/µl HA	0.0476	0.0554	0.0439	0.2589	1.1	98
	833 ng/µl HA	0.0053	0.0077	0.0046	0.0241	1.2	100
	1666.7 µM Hem	0.0202	0.0281	0.0162	0.0834	1.2	100
	2083.3 µM Hem	0.0165	0.0204	0.0139	0.1742	1.2	100
	3125 µM Hem	0.0136	0.0167	0.0123	0.0755	1.1	99
	4167 µM Hem	0.0170	0.0168	0.0127	0.1731	1.3	99

References

1. ENFSI Standing Committee for Quality and Competence (QCC). (2006) Validation and Implementation of (New) Methods. Ref. Code: QCC-VAL-001, Issue No. 001, November 2006. http://www.enfsi.eu/get_doc.php?uid=144.
2. Forensic Science Communications. (2004) Revised Validation Guidelines of Scientific Working Group on DNA Analysis Methods (SWGDAM), **6 (3)**, July 2004. www.cstl.nist.gov/strbase/validation/SWGDAM_Validation.doc.
3. Gill, P., et al. (2006) The evolution of DNA databases-Recommendations

Ordering Information

Product	Contents	Cat. no.
Investigator Casework GO! Kit	Casework GO! Lysis Buffer, Proteinase K Solution, and Nuclease-Free Water	386546
Accessories		
DTT (1 ml)	1M DTT, forensic grade quality; for sperm cell lysis	1117316
HID-related products		
Investigator Quantiplex Pro Kit (200)	For use on Applied Biosystems 7500 or QuantStudio 5 Real-Time Systems: Quantiplex Pro Reaction Mix, Quantiplex Pro Primer Mix, Quantiplex Pro Control DNA M1, QuantiTect® Nucleic Acid Dilution Buffer	387216
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
Investigator 24plex QS Kit (100)*	Primer Mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder, DNA Size Standard, and Nuclease-free water	382415
Investigator 26plex QS Kit (100)*	Primer Mix, Fast Reaction Mix 3.0, Control DNA, allelic ladder, and Nuclease-free water	382615
Investigator ESSplex SE QS (100)*	Primer Mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder, DNA Size Standard, and Nuclease-free water	381575
Investigator Argus X-12 QS Kit (25)*	Primer Mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder, DNA Size Standard, and Nuclease-free water	383223
Investigator Argus Y-28 QS Kit (100)*	Primer Mix, Fast Reaction Mix 3.0, Control DNA, allelic ladder, DNA Size Standard, and Nuclease-free water	383625

* Larger package sizes are available.

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Revision History

Date	Changes
May 2022	Initial release.