Internal validation of RapidHIT™ 200 – DNA analysis system for automated human identification

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Summary

RapidHIT™ 200 by IntegenX® (Thermo Fisher Scientific) is an integrated *system* for fast human identification based on STR analysis, providing a number of advantages over traditional DNA profiling methods in terms of automation, speed of analysis and mobility. However, before *rapid DNA* technology could be implemented into routine forensic applications, both workflow and its results would have to be rigorously validated for reliability, performance and compatibility with data obtained from capillary electrophoresis, through an internal validation. As we were primarily interested in the RapidHIT™ 200's ability to efficiently generate DNA profiles from samples collected at crime scenes and to upload this data to the CODIS database (*Combined DNA Index System*), the evaluation study focused mainly on such samples. The results show that RapidHIT™ 200 can be a useful tool to complement conventional identification methods used in forensic genetics.

Key words: *rapid DNA*, forensic genetics, RapidHIT™ 200, STR analysis

Introduction

Genetic identification analysis based on STR (*short tandem repeat*) markers involve a series of consecutive steps: extraction of DNA from a biological sample, quantification of extracted DNA, amplification of specific STR markers by *polymerase chain reaction* (PCR), capillary electrophoresis of PCR products and analysis of resulting DNA profiles (Butler et al., 2004). Due to the multi-steps nature of this process, there is a significant time lag between the collection of a biological sample and the interpretation of the results. Furthermore, conventional genotyping takes place in an environmentally controlled laboratory setting, where the equipment and particular laboratory analysis stages should be separated. These constraints for the DNA profiling process limit expeditious processing of the results and do not allow for performing tests directly at the crime scene.

In recent years active development of technologies to couple all stages of STR analysis has resulted in development so-called *rapid DNA* technology equipment and reagent kits. *Rapid DNA* is a term used to describe a fully automated, fast forensic DNA analysis (Gangano et al, 2013; Tan et al, 2013; Morgan et al, 2019; Kartasinska et al, 2020). One of the pioneering devices working with using *rapid DNA* technology is the RapidHIT™ 200 Human Identification System (IntegenX® by Thermo Fisher Scientific). It is an automated mobile platform in which all steps of STR typing process are integrated and DNA profiles are generated from biological samples in approximately 90 minutes (Hennessy et al, 2013; Verheij et al, 2013; Holland, Wendt, 2015; Jovanovich et al, 2015). The instrument uses two sample cartridges with a total of eight wells for genotyping. The cartridges are activated simultaneously and allow for the analysis from one to seven samples, with the remaining single well reserved for the allelic ladder. Each piece of evidence or a sample taken from evidence is placed directly into an independent well of the cartridge, where it is processed through individual channels for amplification with GlobalFiler® Express or AmpFLSTR™ NGM SElect™ Express reagents. A separate module in the instrument containing capillary electrophoresis and detection system components separates fragments and collects raw data. The process is fully automated and the user is only required to put sample cartridges and ready-to-use reagent cartridges into the instrument.

The data obtained is initially processed by analysis software integrated with the RapidHIT™ 200, which allows DNA profiles to be interpreted without user intervention. However, this ‚on-board software' provides minimum information displayed on the screen of the instrument. Data files can then be exported from the instrument for further DNA profiles analysis by the

qualified analysts using GeneMarker® HID software (Alshehhi, Roy, 2015).

Profiles from the instrument can also be viewed in the RapidLINK™ application, which serves as a database for collecting DNA profiles generated with the instrument, searching them against each other and reporting the results for data concordance. It is also possible to import DNA profiles into RapidLINK™ in the form of CMF files generated by the CODIS system. In addition, using the GeneMarker® HID application, which works with RapidLINK™, it is possible to create DNA profile import files which can be uploaded into the CODIS system. The DNA profiles imported into the CODIS system can be searched against national and international DNA databases.

This study presents the results of an internal validation of the RapidHIT™ 200. Validation study included analysis of the accuracy and the sensitivity of the method to different amounts of biological material, as well as testing for potential cross-contamination. The effectiveness of the instrument in generating DNA profiles from samples routinely collected in criminal cases was also evaluated. In addition, the validation tests included the evaluation of the results concordance and comparison of the quality of DNA profiles obtained with the RapidHIT™ 200 with those generated with the traditional STR typing method.

Materials

Buccal swabs were collected in two sets on cotton swab sticks (Hagmed). Undiluted and diluted blood samples were applied to 4N6 FLOQSwabs® Crime Scene swabs (Copan Diagnostics). All swabs were allowed to dry completely at room temperature. Peripheral blood samples were collected into Vacutest® tubes containing EDTA (Kima SAS Di Chiarin Renzo & C).

Methods

RapidHIT™ 200 protocols and data analysis

Samples were processed with the RapidHIT™ 200 DNA Human Identification System, serial number RH200-0044, integrated with GeneMarker® HID Auto software v. 2.7.3 (SoftGenetics® for IntegenX), using the GlobalFiler® Express -7sample cartridge kit (Thermo Fisher Scientific) amplifying 24 STR markers. The process of sample genotyping with the RapidHIT™ 200 was performed according to the manufacturer's instructions using the ‚Run Other Samples' protocol, except for buccal swabs, for which the ‚Run Buccal' protocol was selected. The ‚Run Other Samples' protocol has two more rounds of PCR cycles than the ‚Run Buccal' protocol in order to increase the efficiency of genotyping of biological traces which often contain lower amount of template DNA compared to buccal swabs samples (Thong et al., 2015). Data analysis was performed using GeneMarker® HID software v. 2.9.5 (SoftGenetics® for IntegenX). STR-validator ver. 2.2.0 (RStudio package ver. 3.6.0) was used for statistical analysis of the results, including prediction of drop-out probability. In establishing the *analytical threshold* (AT), the baseline in the negative control samples was investigated individually for each fluorescent dye channels. In all colour channels same pull-up peaks were observed, which could be attributed to the high signal of the size standard in the absence of competing STR amplicons. After manual analysis, these peaks were deleted and not included in the calculation. Based on the *limit of quantitation* (LOQ) which is the mean of background noise plus ten standard deviations, the minimum analytical threshold to distinguish a true allelic peak from baseline noise was determined (Gilder et al., 2007). The minimum threshold were thus determined: 23 RFU for 6-FAM™, 28 RFU for VIC™, 33 RFU for NED™, 28 RFU for TAZ™ and 44 RFU for SID™. The analytical threshold was conservatively set at a common level of 50 RFU for all dye channels for the manual analysis conducted in this validation study.

Traditional STR typing protocols and data analysis

To compare the results generated with the RapidHIT™ 200 system, samples were processed using standard STR typing methods involving isolation with the AutoMate Express™ Forensic DNA Extraction System and the PrepFiler Express™ Forensic DNA Extraction Kit (Applied Biosystems), giving a final extract volume of 50 µl. Each sample was quantified in duplicate using the Plexor® HY System kit (Promega). Amplification was carried out using the GlobalFiler® PCR Amplification Kit (Thermo Fisher Scientific) and electrophoretic separation was carried out using the ABI PRISM® 3130xL Genetic Analyser (Applied Biosystems). DNA profile analysis was carried out using GeneMapper® ID-X v1.4 (Applied Biosystems), with the 50 RFU analytical threshold.

Sensitivity and stochasticism studies

Sensitivity of the method was evaluated by processing of blood samples in dilutions of equivalent to 0.5 µl, 0.25 µl, 0.125 µl of blood per sample and undiluted blood samples of: 6 µl, 5 µl, 4 µl, 2 µl and 1 µl. The test was performed in several replicates, following as shown in Table 1.

Blood samples were collected from two individuals (samples A and B) and the respective volumes were applied to cotton swabs and allowed to dry completely overnight. To determine the average quantity of DNA input in each blood volume, a similar set of samples was extracted with a traditional method, using the PrepFiler™ Forensic DNA Extraction Kit, and the amount of DNA was determined in three replicates, using the Plexor® HY System kit (Promega), with the Applied Biosystems™ 7500 Real-Time PCR instrument according to the manufacturer's protocols. The above dilutions were used to determine the smallest amount of blood sufficient to generate a full DNA

profile with the RapidHIT™ 200, and to evaluate the stochastic effects resulting from low DNA template used for PCR. Allelic drop-out in profiles was observed and average peak height per locus was determined. *Peak height ratio* (PHR) between heterozygous alleles was calculated by dividing the lower allele peak height by the higher allele peak height.

Tab. 1. Blood sample dilutions performed in the sensitivity study

Blood volume per sample [µl]	Number of replicates for sample A	Number of replicates for sample B
6	2	-
5	3	2
4	3	3
2	3	2
1	3	2
0.5	3	2
0.25	2	2
0.125	2	2

DNA mixture study

A mixture study was performed to verify if the GeneMarker® analytical software integrated with the RapidHIT™ 200 would appropriately flag a sample that might be a mixture of genetic material. Mixture of two blood samples from a man and a woman was examined at the following ratios: 1:1, 1:2, 1:4 and 1:8, while maintaining the total blood volume constant at 5 µl for all mixtures. Each mixture was tested in three replicates.

Precision and cross-contamination studies

Precision study was performed by 11-fold processing a blood sample (50 µl each) from one individual over three consecutive runs and by analysis of 10 allelic ladders generated from 10 separate runs. As an acceptance criterion for precision a standard deviation for size of each allele was used, which should not exceed 0.5 bp (ENFSI, 2010; SWGDAM, 2016). To assess the possibility of potential cross-contamination between cartridge channels and consecutive instrument runs, blood samples were arranged alternately, separated by negative control samples, as shown in Table 2.

Efficiency and concordance studies

Blood swabs, sperm swabs, saliva swabs, hair, chewing gum, cigarette butts, swabs from the necks of empty water bottles, postage stamps, swabs from mobile phones, swabs from firearms owned by police officers (who were laboratory staff) and swabs from the inside of used gloves were used to evaluate the effectiveness of generating DNA profiles from different biological sample types. From each type of the above listed types of materials five samples were collected and the traces on particular type of material originated from a single donor. Three samples from each set were processed with the RapidHIT™ 200 and two samples, for comparison, were tested in parallel using standard protocol, determining the percentage of alleles detected for each type of material. Dried chewing gums were whole placed in the wells. The tipping paper of the cigarette butts and postage stamps measuring 3 cm x 2 cm (previously licked and dried) were cut into 0.5 cm x 0.5 cm fragments, pricked on sterile needles and placed in the sample wells. Fragments of cigarette butts and postage stamps measuring 1 cm x 1 cm were collected for processing according to the traditional protocol. The chewing gum was cut in half, and both halves were examined separately. Swabs from the entire surface of mobile phones, firearms, the inside of gloves and from the necks of bottles were collected using moistened swabs and were allowed to dry completely at room temperature. To further evaluate concordance of the results, 34 buccal swabs were profiled in parallel using the RapidHIT™ 200 and according to the standard protocol.

Tab. 2. Arrangement of samples for a cross-contamination study performed with the RapidHIT™ 200 system

RUN	well 1	well 2	well 3	well 4	well 5	well 6	well 7	well 8
1	50 µl of blood	negative control	50 µl of blood	negative control	50 µl of blood	allelic ladder	50 µl of blood	negative control
2	negative control	50 µl of blood	negative control	50 µl of blood	negative control	allelic ladder	negative control	50 µl of blood
3	50 µl of blood	negative control	50 µl of blood	negative control	50 µl of blood	allelic ladder	50 µl of blood	negative control

Stutter

Complete, good-quality DNA profiles generated from the concordance and efficiency studies, were used to calculate stutter ratio to parent peak in each locus. To detect stutter peak heights, the AT threshold in GeneMarker® was set at 50 RFU and the stutter filters were lowered to 1%. The proportion of stutter product relative to the main allele was measured by dividing the height of the stutter peak by the height of the associated allele peak, and the result expressed as a percentage.

RESULTS

Sensitivity study

The sensitivity study consisted of determining the minimum volume of blood needed to generate a full DNA profile, with RFU of alleles above the designated analytical threshold. Table 3 shows the average DNA concentration in each of the blood volumes tested.

Tab. 3. Results of the quantitative evaluation of DNA in each of the blood volumes tested in the sensitivity study

Blood volume (µl)	Average DNA concentration ng/µl
6	7.6
5	7
4	5.5
2	3
1	1.5
0.5	1.2
0.25	0.5
0.125	0.3

As shown in Figure 1, complete autosomal STR profiles were generated with 2 µl, 5 µl and 6 µl of blood, corresponding to an average of 3 ng, 7 ng and 7.6 ng of DNA per sample. Among the six results of 4 µl blood, one dropout was observed. However, it should be noted that complete profiles could be obtained with as little as 1 µl of blood (1.5 ng DNA) (Figure 2). For the range of 0.5 - 0.125 µl blood, an increase in the number of undetected alleles, i.e. amplified below the accepted analytical threshold, was observed.

Peak height ratio

The peak height ratio at each heterozygous locus was determined based on complete heterozygous loci generated from different volumes of peripheral bloods ranging from 6 µl to 0.125 µl and plotted (Figure 3) against blood volume (µl).

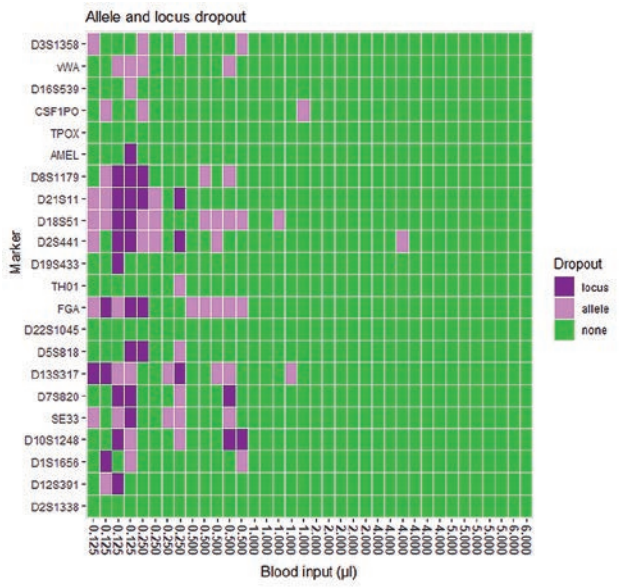

Fig. 1. Heat map showing the observed allele and locus drop-out events by amount of blood input noted in profiles obtained from different volumes of bloods ranging from 6 µl to 0.125 µl on the RapidHIT™ 200 run with GlobalFiler™ Express Kit chemistry. Each column represents a result for a single sample. Rows represent results for a given locus

A calculated peak height ratio at each heterozygous locus below 60% was considered a significant increase in disproportion (ENFSI, 2010). This threshold was marked on the diagram with a blue line, thus separating observed loci with high PHR (above the line) from loci with low PHR (below the line). The results indicate that for a sample blood volume range between 5 and 6 µl, all determined loci showed PHR at the recommended level. With 4 µl of blood in the sample, approximately 12% of loci showed a degree of imbalance in the PHR, 24% of loci were imbalanced - with 2 µl of blood in the sample. With 0.5 µl of blood 39% of loci were observed to be imbalanced. The above observations give rise to the conclusion that, as DNA template levels decrease, uncertainty of the genotype determination increases, because a pair of alleles may be represented unequally, due to the phenomenon of preferential amplification of lower molecular weight alleles.

The inter-locus balance, also referred to as locus balance (*Lb*), is an important feature of a DNA typing kit and is defined for all loci in the kit. In a perfectly balanced kit, the total peak height at each locus in a single source DNA profile should be equal. Hence, it is easier to discover and interpret e.g. rare alleles that fall outside the marker ranges, drop-out phenomena or degraded DNA (Hansson et al. 2014). The inter-locus balance was calculated by dividing the sum of peak heights at a locus by the sum of all peak heights in a given profile. The average *Lb* per marker ranged from 0.02 to 0.12 (D8S1179 and TH01). In contrast, the optimal *Lb* for GlobalFiler® Express System should be

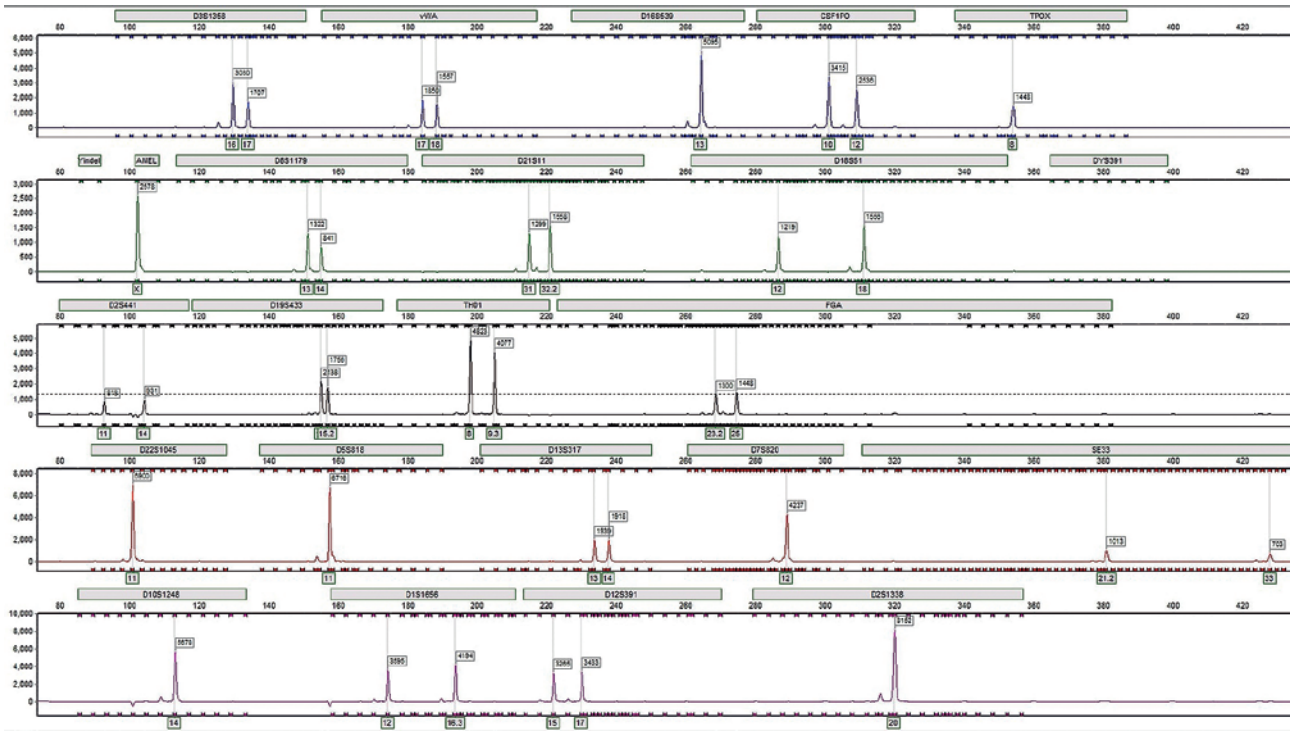

Fig. 2. A complete STR profile obtained from 1 µl of blood processed on the RapidHIT™ 200 using GlobalFiler™ Express kit

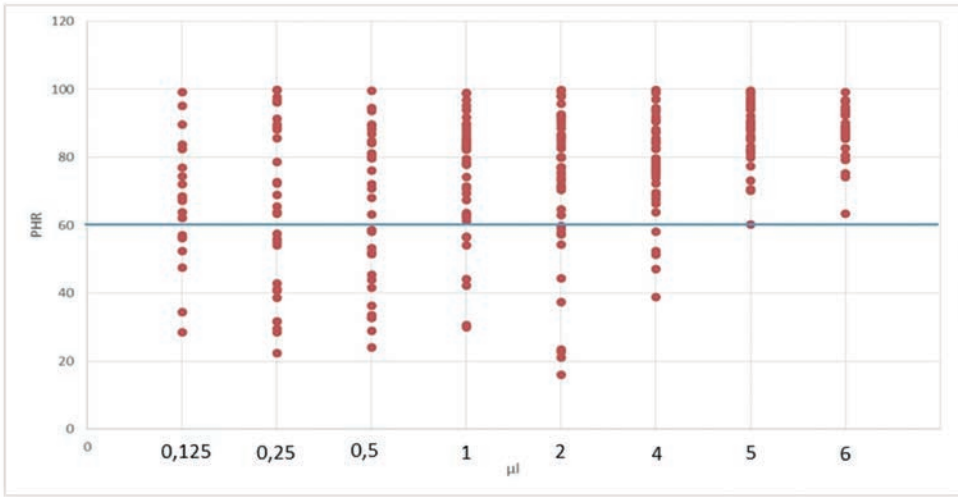

Fig. 3. Figure showing peak height ratio at each heterozygous locus plotted by the amount of blood in the sample (µl) tested within the range of 6 µl to 0.125 µl. *PHR* is presented as percentage. Data come from 279 heterozygous loci analyzed on the RapidHIT™ 200. The horizontal blue line represents acceptable minimum *PHR*

0.047 (1/21). Fig. 4 shows the *Lb* by the average peak height of a given locus, while Fig. 5 shows the *Lb* by box plots representing the individual loci. In these diagrams, it can be observed that the alleles of the TH01 and D22S1045 amplify with higher efficiency compared to the alleles of the other loci in the profile, especially for samples with low levels of DNA.

Stochastic threshold and drop-out probability study

The stochastic nature of PCR inevitably lead to an imbalance between the two amplified alleles of heterozygous loci. An extreme effect of heterozygous imbalance (resulting from the so-called 'stochastic effects') is the drop-out phenomenon (Gill et al., 2012). This phenomenon refers to the absence of one (allele drop-out) or both alleles (locus drop-out) in one or more loci in the

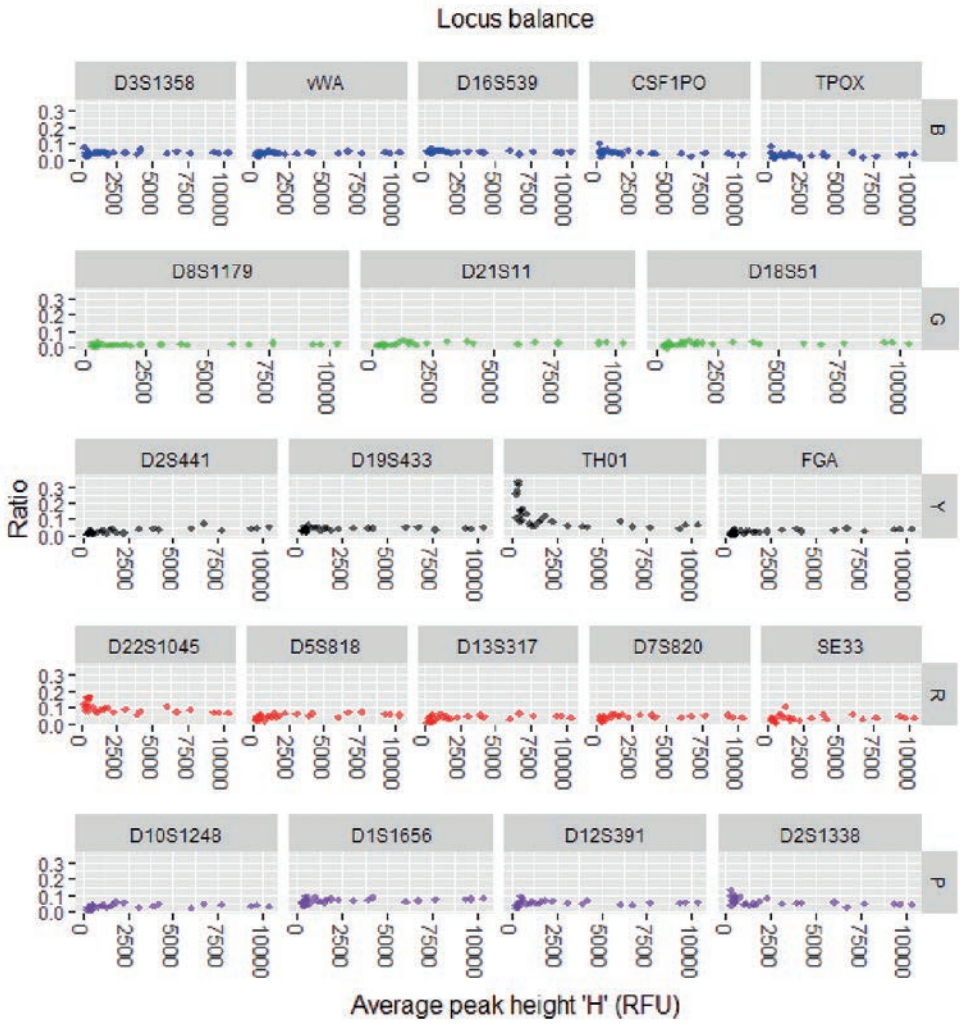

Fig. 4. Inter-locus balance calculated as a proportion of the total locus peak height and the total profile peak height plotted by the average peak height of the locus. Data were derived from complete profiles obtained from different volumes of bloods ranging from 6 µl to 1 µl on the RapidHIT™ 200 run with GlobalFiler® Express Kit chemistry. DYS391 and AMEL were excluded from the test

DNA profile. A drop-out phenomenon can occur due to the absence or presence of too few DNA template molecules in PCR to elicit a signal in the capillary electrophoresis or to exceed the laboratory-established Limit of Quantification (LOQ).

In Figure 6, heterozygous alleles with drop-out of the sister allele are marked with dots. The highest peaks with drop-out of the sister allele were: 279 RFU for blue dye (observed in CSF1PO), 302 RFU for green dye (observed in D18S51), 593 RFU for yellow dye (observed in D2S441), 363 RFU for red dye (observed in D13S317) and 200 RFU for purple dye (observed in D1S1656).

Using the scored drop-out events and the peak heights of the surviving alleles in the heterozygous loci, the probability of drop-out was modeled using logistic regression (Figure 7). This way, a *stochastic threshold* (ST) was determined, which minimizes the risk of erroneous designation of a heterozygous locus as a homozygote. At a probability of dropout 1%, the stochastic threshold is 552 RFU. A conservative stochastic threshold was set at 827 RFU. Above this threshold, it is reasonable to assume that no dropout of the sister allele has occurred at the locus. The difference between the analytical and stochastic threshold determined is that a peak with a signal above the accepted *AT* is attributed to an allele of the STR locus, rather than a non-allelic peak derived from baseline noise, whereas a peak with a signal above *ST* at the STR locus is identified as the homozygous genotype for that locus.

Mixture study

Analysis of the mixtures of blood samples from a man and woman, showed that the samples were determined correctly as mixtures of genetic material, thus requiring further expert review. Both components of the DNA mixtures combined in a 1:1 ratio were identified with

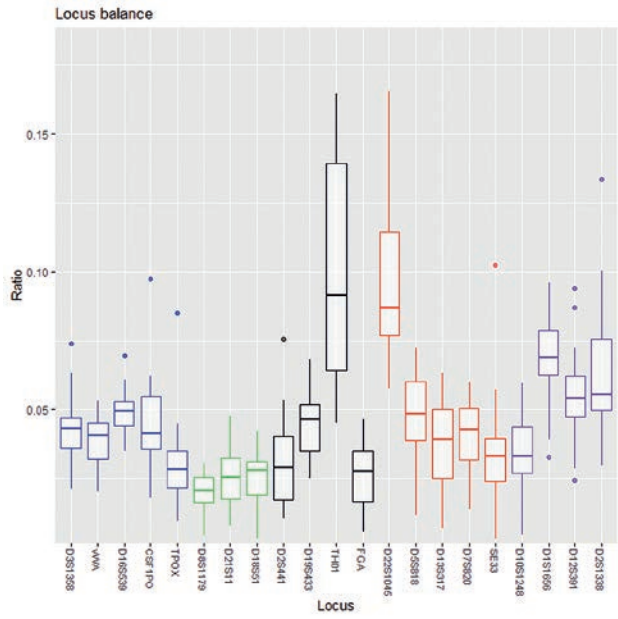

Fig. 5. Box plots showing the interquartile distributions of inter-locus balance for each STR locus analyzed on the RapidHIT™ 200. Data were derived from complete profiles generated from different volumes of bloods ranging from 6 µl to 1 µl with GlobalFiler™ Express Kit chemistry

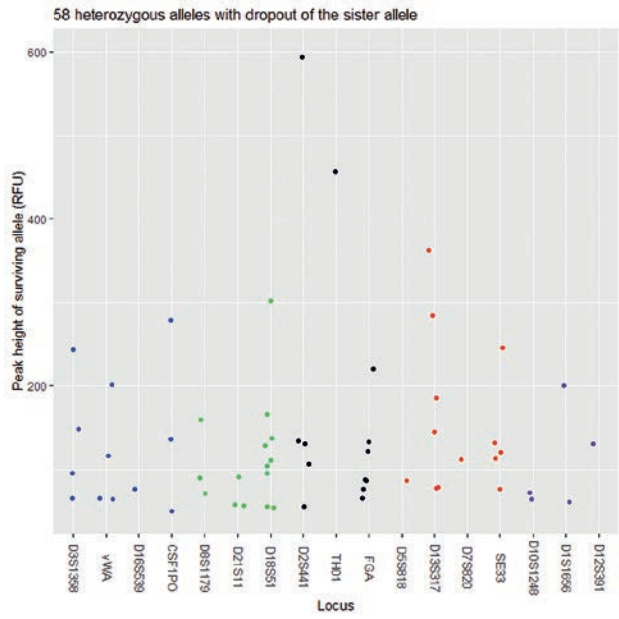

Fig. 6. Dot plot showing drop-out events for 58 heterozygous locus where the phenomenon occurred. The dots indicate the peak heights of heterozygous alleles which drop-out of the sister allele. Data were derived from profiles generated on the RapidHIT™ 200 from blood tested within a range of 6 µl to 0,125 µl using the GlobalFiler™ Express kit

100% efficiency at each locus. The minor component with male genotype was detected in all other mixtures at 96.7%. Figure 8 illustrates the results of mixture study of two blood samples combined in ratios of 1:1, 1:2, 1:4, 1:8, with alleles of the minor component indicated with an asterisk.

Precision study

Precision of the method was analyzed by assessing the variation in results when the same sample and allelic ladder were repeatedly determined, and included assessment of the accuracy and reliability of genotyping. This study was conducted by generating 11 replicates from one known blood samples. For each allele, the standard deviation of its size did not exceed the recommended value of 0.5 bp and ranged from 0.03 to 0.11 bp. All measured alleles were within a ±0,5 bp window around the measured size for corresponding alleles in the allelic ladder. Analysis of 10 allelic ladders confirmed that all measured alleles repeatedly reached similar size, with a recommended accuracy of 0.5 bp (Figure 9). Microvariants differing by a single base pair at smaller fragments (e.g. D2S441 - 95.6, 96.5 bp) and microvariants differing by two bases at larger fragments (e.g. SE33 - 372.6, 374.6 bp) were clearly resolved. The allelic set in the allelic ladder for larger markers, e.g. SE33 and FGA, does not include account microvariants that differ by a single base, which remains the standard for allelic ladder design in the STR multiplex systems.

Cross-contamination study

Spatial separation of pre-amplification and post-amplification areas of a forensic DNA typing laboratory is a common and effective method to reduce contamination. The fact that the RapidHIT™ 200 contains all parts of the process within a single instrument means that pre- and post-amplification areas exist in the same physical space. Therefore, tests were carried out to check for potential contamination due to material transfer between individual cartridge channels, as well as between individual runs of the instrument. The results showed no called alleles in any of the blank channels demonstrating no cross contamination occurs within a run or from run-to-run. No evidence of contamination occurred in any of the remaining parts of the validation study for negative controls or DNA containing samples.

Efficiency and concordance studies

To assess the effectiveness of generating DNA profiles from different crime scene samples, a set of commonly encountered forensic samples was tested. As shown in Figure 10, RapidHIT™ 200 was able to process forensic samples, which generally contain large amounts

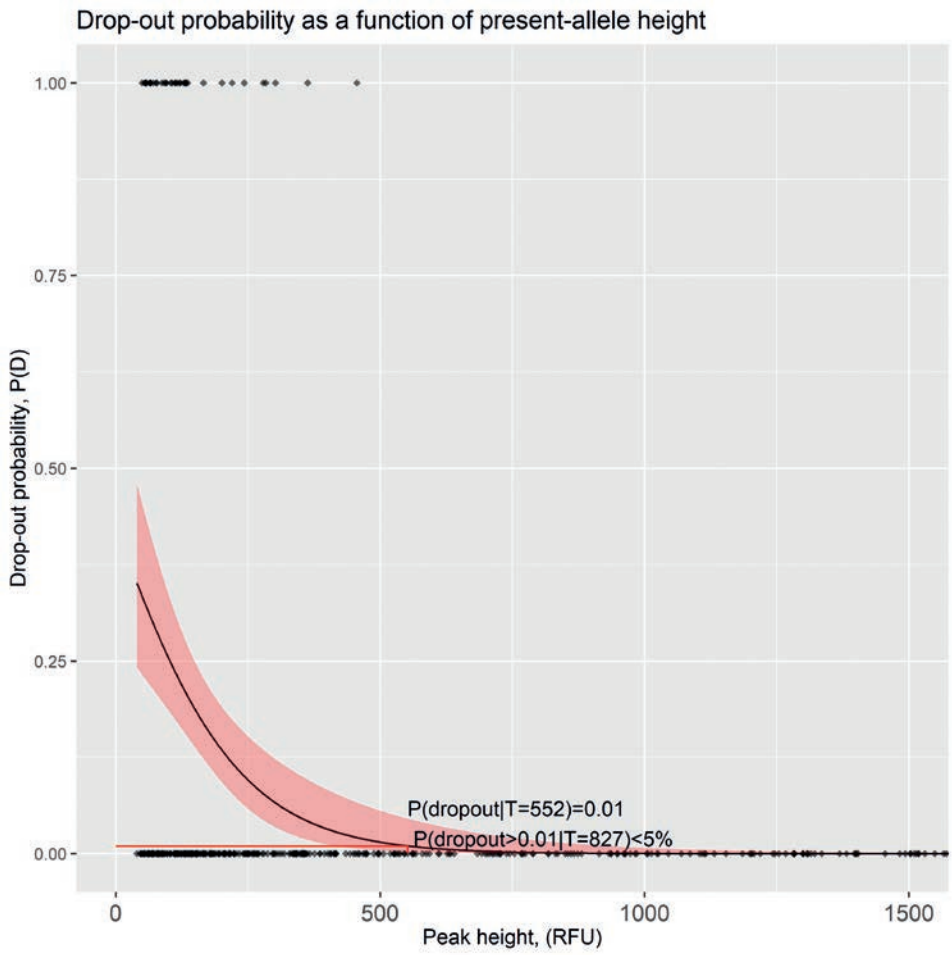

Fig. 7. Predicted drop-out probability as a function of peak height of the present-allele height from a pair of sister alleles in a heterozygous locus as modelled by logistic regression. The red shaded part marks the 95% confidence interval of the drop-out probability. Observations of heterozygous loci without drop-out of the partner allele are shown as points at P = 0, while observations of heterozygous loci with drop-out of the partner allele are shown as points at P = 1

of DNA, such as blood swabs, sperm swabs and saliva swabs with 100% success rate. With respect to chewing gum, cigarette butts, single plucked hairs, postage stamps and swabs from bottle necks, the RapidHIT™ 200 generated: 98%, 87%, 75%, 92% and 87% success rate, respectively, based on the number of alleles called. No genetic profiles were obtained from touch/contact traces such as swabs from mobile phones, swabs from firearms and swabs from the inside of latex gloves, compared to tests performed according to the standard genotyping protocol, where full genetic profiles were generated in each case.

The resultant profiles from forensic samples and reference samples proceeded with the RapidHIT™ 200 were 100% concordant with profiles generated with GlobalFiler® kit according to standard protocol.

Stutter

Stutter ratio was determined on 46 different individuals and their complete profiles (sex markers were excluded from the study). Figure 11 shows average and maximum '– repeat' stutter and '+ repeat' stutter reported as a percentage of the parent peak for each locus. The stutter range across all loci measured on the RapidHIT™ 200 was comparable to that indicated by the GlobalFiler® Express kit manufacturer. The heights stutter ratio were observed for '– repeat' stutter for D12S391 and SE33. In Figure 12, an overall increase stutter ratio can be observed with increasing allele size in individual STR markers. The average peak height ratio at heterozygous loci was about 88% and ranged from 82% to 93%.

CONCLUSIONS

The RapidHIT™ 200 is a mobile instrument that offers fast forensic DNA analysis within two hours, with minimal user intervention and in a location where an environmentally controlled laboratory setting is not available. As validation studies have shown, the instrument can produce quality STR profiles from buccal swabs

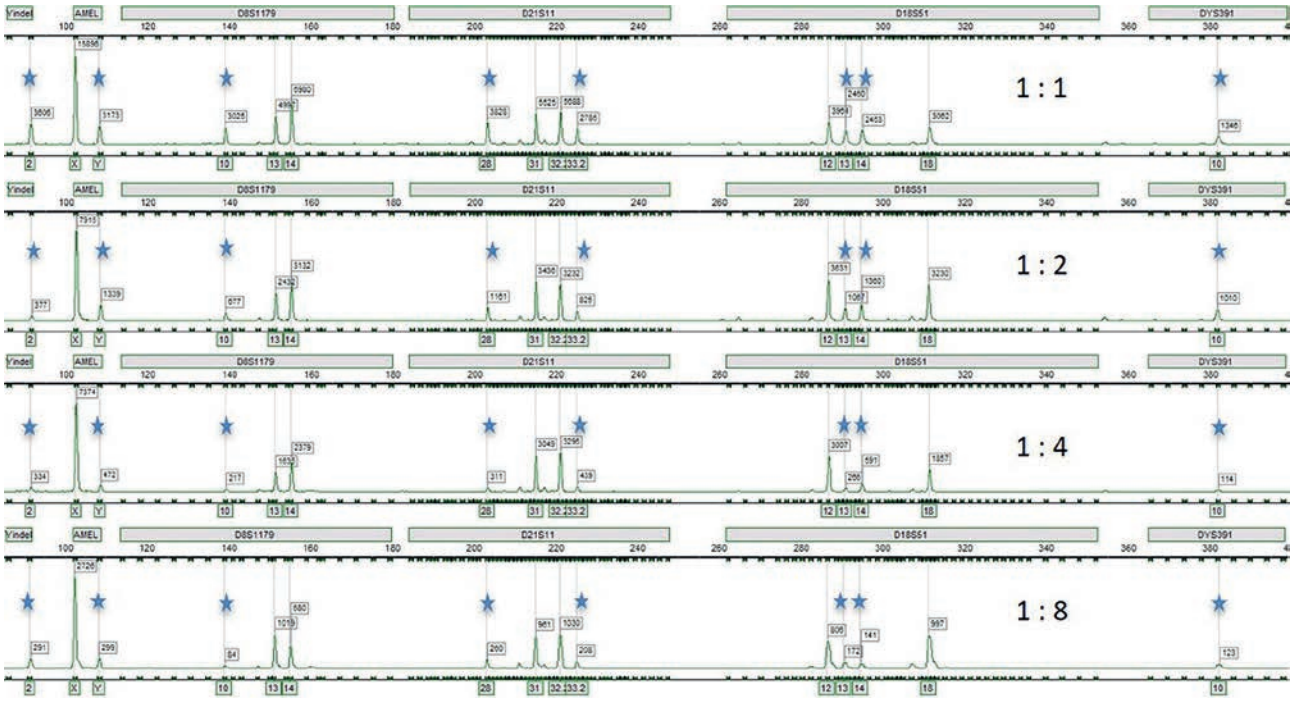

Fig. 8. Results of mixture study of two blood samples combined in ratios of 1:1, 1:2, 1:4, 1:8 for loci in green dye channel. Alleles of the minor component are indicated by the asterisk

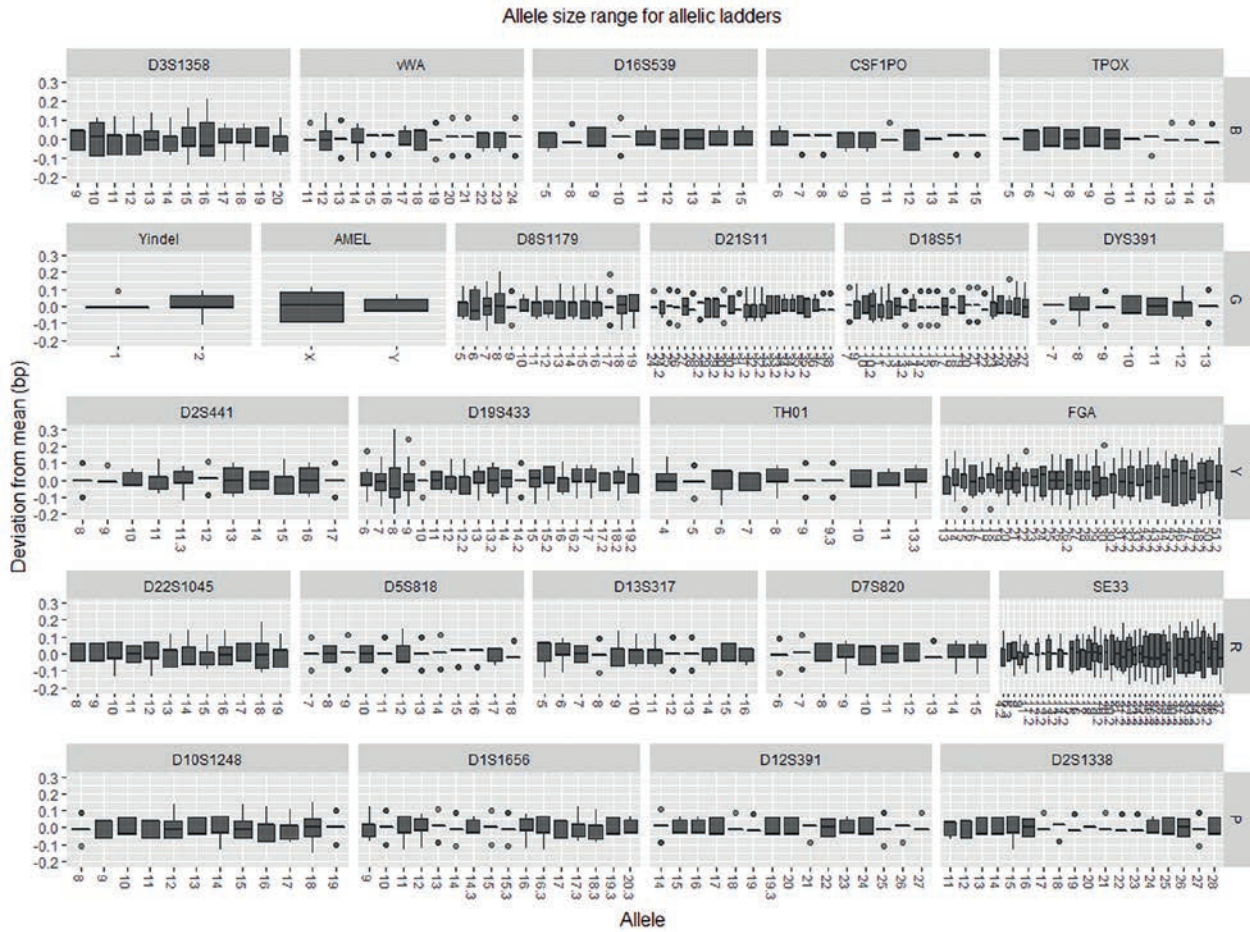

Fig. 9. Sizing precision plot for 10 GlobalFiler™ Express allelic ladders analyzed on the RapidHIT™ 200. Box plots show deviation from the mean (in base pairs) plotted by marker and allele

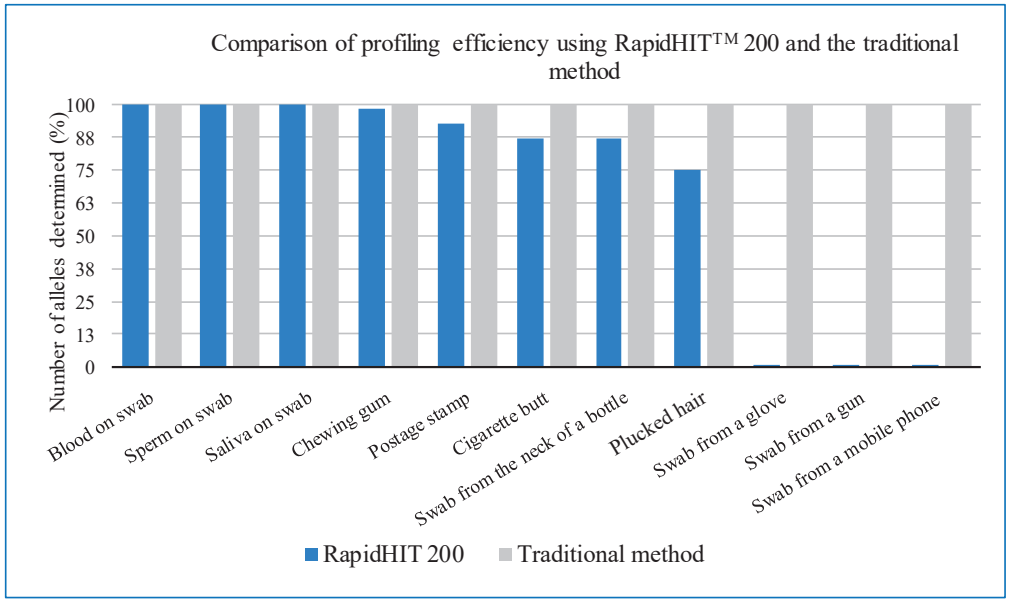

Fig. 10. Efficiency study using a variety of biological samples processed with the RapidHIT™ 200 and the standard protocol. Data are presented as percentage of determined alleles

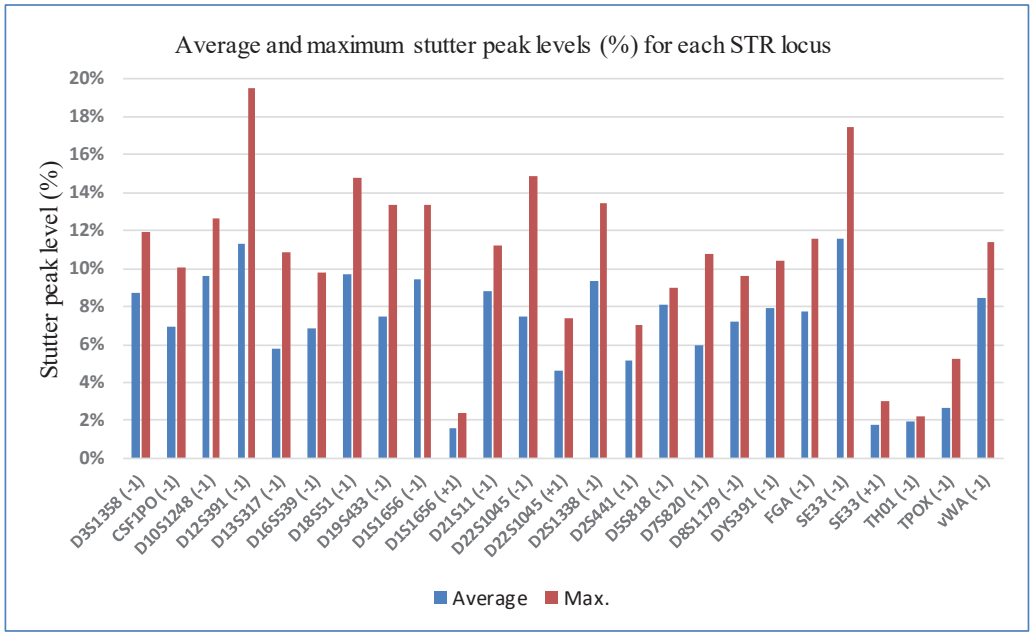

Fig. 11. Average and maximum stutter percentage for each STR locus of the GlobalFiler® Express determined with the RapidHIT™ 200.

and from various types of crime scene samples containing, in principle, large amounts of DNA, such as blood, sperm or saliva. The results of the blood dilution series showed that 5 µl of blood (approximately 7 ng of DNA) is needed to generate full autosomal STR profiles. Low peak height ratio between heterozygous alleles resulting in allelic dropouts, were observed only in case in which the volumes of blood were lower than noted above. GlobalFiler® Express data from profiles produced according to standard protocol were 100% concordant with those generated with the RapidHIT™ 200 using the 'Run Buccal' and the 'Run Other Samples' protocols. Evaluation of the profiles obtained from the RapidHIT™ 200 during precision and stutter

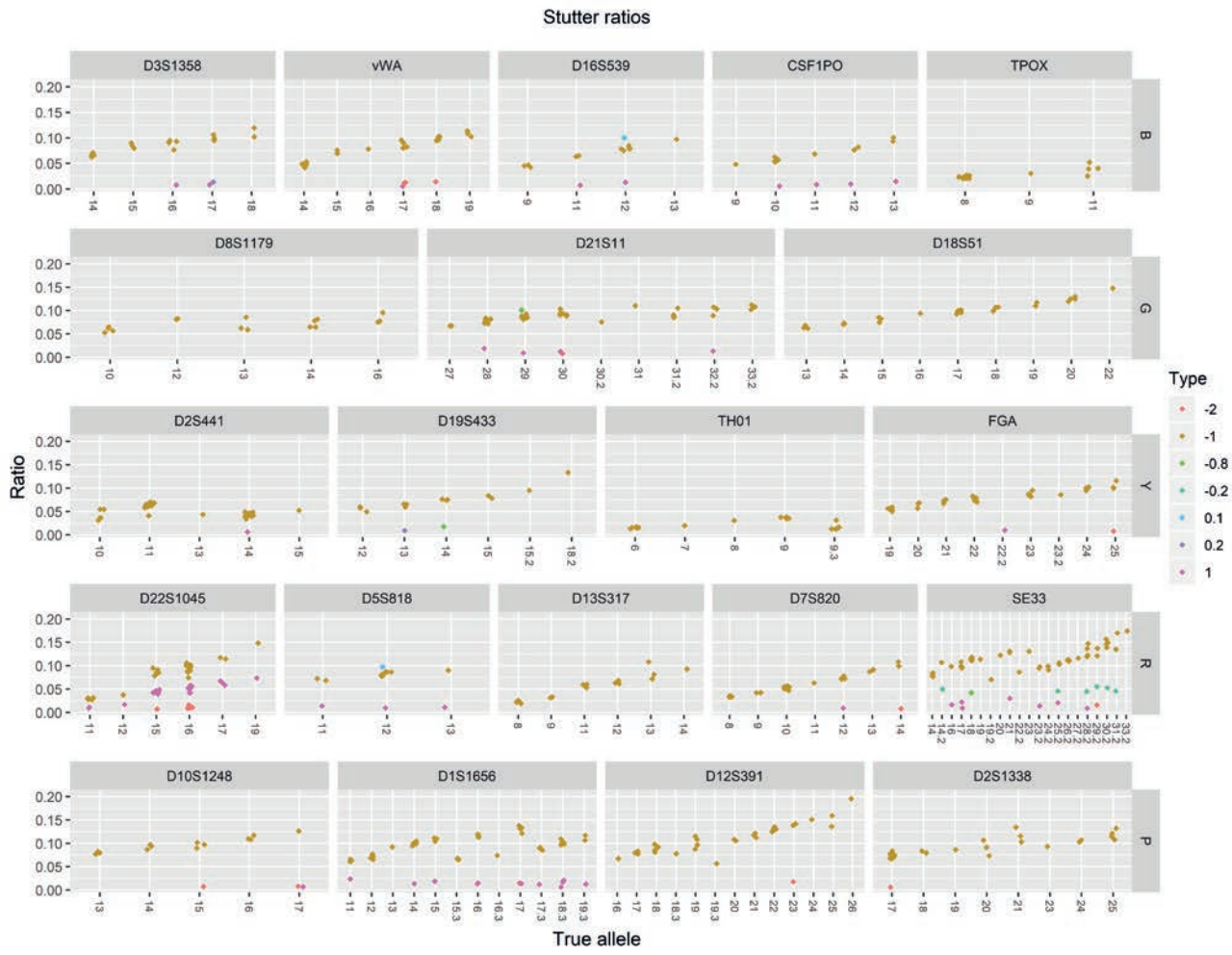

Fig. 12. Stutter ratio as a function of parent allele at each locus. GlobalFiler® Express data from 46 complete profiles generated with the RapidHIT™ 200

studies confirmed the ability of the instrument to generate profiles in a reproducible and reliable fashion with no evidence of contamination. The analytical software of the RapidHIT™ 200 correctly flagged DNA mixture at 1:8, which is sufficient to indicate when a sample may be a mixed profile.

However, the present study has demonstrated that the RapidHIT™ 200 does not exhibit equivalence in sensitivity compared to standard protocol when processing samples with low levels of genetic material, such as cigarette butts, postage stamps, plucked hair. The lower sensitivity of the instrument excludes its ability to analyze touch/contact DNA samples. No profiles were obtained from any trace swab collected by swabbing the entire surface of mobile phones, firearms and the inside surface of latex gloves, whereas the standard protocol generated 100% success rate for each of the above traces.

Given the limitations mentioned above, it must be concluded that the RapidHIT™ 200 instrument can be a useful tool to complement conventional identification methods used in accredited genetic laboratories, but it cannot replace them. However, in situations where it is extremely important to quickly determine DNA profiles, and, for example, to search a DNA database to identify a perpetrator, this instrument has advantages over conventional methods. The instrument can be operated by non-specialized personnel, following appropriate training to know how to insert samples into the cartridges and how to operate the instrument. Each time, a forensic genetic expert or qualified analyst should determine whether the DNA profile determined with the RapidHIT™ 200 is suitable for search against national and international DNA databases.

When assessing the possibility of accrediting this instrument as a mobile laboratory for the analysis of biological traces directly at the crime scene, the following provision of EN ISO/IEC 17025:2018 (clause 6.3.5) should be taken into account: “When the laboratory performs laboratory activities at sites or facilities outside its permanent control, it shall ensure that the requirements related to facilities and environmental

conditions of this document are met". This provision imposes the need to ensure that:

- the environmental conditions of conducting tests as well as laboratory facilities will not adversely affect the validity of results;
- the procedure will drawn up which will include requirements for ensuring environmental conditions necessary for carrying out tests at sites or facilities outside its permanent control;
- the laboratory will monitor, control and record environmental conditions in accordance with appropriate procedures when these affect the validity of results of tests carried out at sites or facilities outside its permanent control.

Consequently, extending the scope of method accreditation to include tests carried out directly at the site of an incident requires extending the scope of validation to include a number of additional parameters (e.g. with regard to robustness to external factors, determination of components affecting measurement uncertainty). Preparation of documentation on monitoring environmental conditions, ensuring the quality of test results and handling samples would be necessary, also.

Source of figures and tables: authors

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