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The ParaDNA® Screening System - A case study in bringing forensic R&D to market

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Abstract

The creation of new technologies and their application to forensic science is key to the field's development. Rapid DNA profiling is one such area of research which has grown in response to a desire from enforcement authorities for in-house forensic DNA processing and rapid access to forensic genetic intelligence. However, introducing novel technologies into the forensics market must be carefully monitored and controlled as the success or failure of any technology ultimately has long standing implications for victims, suspects, and also to Police and forensic practitioners. This article outlines the research, development, validation and implementation of the ParaDNA® Screening System as a case study in taking forensic research and development to market.

Keywords: Forensic science, Research, Development, R&D, Rapid DNA, Screening

1. Introduction

A key topic of discussion at Forensic Horizons 2013 was taking research and development (R&D) to market with many different stakeholders identifying the need to make this process easier. Launching new DNA based products and platforms into the forensics market place can be complicated and can take an extended period of time. A large amount of time is inevitably spent in development but there is also a large amount of time spent generating data to support the technology and its proposed application. Such data may be generated to test the scientific underpinning of the technology in question whilst additional data may be generated to address the requirements of stakeholders. The large number of stakeholders that exist in the forensic community includes government and non-government parties, private industry, academia, the Criminal Justice System and ultimately the general public. Given the diversity of the interested parties involved there are inevitably a number of equally valid, but not always similarly prioritised requirements and concerns

that need addressing which takes time. Having some awareness of the stakeholder groups and their requirements will facilitate the movement of technology from research through development and ultimately into operational deployment in the Criminal Justice System, intelligence gathering, and defence and security applications [1].

The ParaDNA Screening System has recently undergone such a transition from proof of concept to operationally deployed product. The ParaDNA Screening System consists of a sampling device, pre-loaded reaction plates and detection instrument. The test uses direct PCR with fluorescent HyBeacon™ probe detection [2] of PCR amplicons to identify the presence of human DNA on an evidence item and gender identification in approximately 75min. This simple-to-use design allows objective data to be acquired by both trained DNA analysts and non-specialist personnel, to enable more informed submission decisions to be made.

For any product to be deemed a success it has to fulfil a basic need. In 2010 the Association of Chief Police Officers (ACPO) commissioned the National Policing Improvement Agency (NPIA), via Forensics21, to investigate the feasibility of Accelerated DNA Profiling Technology (ADAPT) and its associated benefits. The overall aim and vision of the project was to introduce a technology capable of producing a full DNA profile compatible for loading to the NDNAD close to or at the point of sampling within an hour [3]. Under this programme the ParaDNA Screening System was categorised as a Level 1 DNA Screening Solution meeting the following requirements:

- It should be a basic tool to indicate whether or not human DNA is present within a crime stain sample, i.e. it should be a test for DNA.
- It should provide police forces with the capability for a more robust and reliable decision making method.
- It should make it faster for forces to gain early intelligence.
- It should be easy to use by non-scientific personnel.
- The results output should be simple enough to be interpreted by personnel with minimal training.

The ADAPT Initiative reported a potential cost saving of up to £3.25 million per annum if samples that did not contain human DNA were successfully screened out [3]. In the interest of continuing the important discussion regarding the controlled and careful release of research and development ideas into the field of forensics the following article uses the ParaDNA System as a case study in taking R&D to market.

2. Materials and methods

The ParaDNA Screening System consists of the following components:

Sample collector — A disposable plastic device (Fig. 1a) used in a similar manner as a traditional cotton swab. Collection of cellular material occurs through adsorption onto the four plastic tips of the device. Material can be recovered from both evidential swabs (indirect sampling — Fig. 1b) or directly from an evidence item (direct sampling). After sampling, the collector is inserted into the 4-well PCR plate (Fig. 1c), introducing the DNA template whilst simultaneously sealing the PCR wells.

Test kit — Four independent PCR assays pre-loaded into the custom designed 4-well PCR plate (Fig. 1c). The assays use HyBeacon Technology to amplify and detect 2 STRs and the Amelogenin gender marker.

Analyses software — Controls the instrument (Fig. 1d), analyses the data and displays the screening result. The software analyses changes in fluorescence (ΔRFU) as a HyBeacon probe melts away from an its amplified allele at a specific melting temperature (TM) between 20 °C and 70 °C. The temperature at which this fluorescence change occurs varies with the length of the amplified allele. This temperature separation enables the software to attribute a proportion of the overall fluorescence change to each possible allele and converts this data into an easily interpretable colour-coded 'DNA Detection' result as follows:

■ Red— No DNA detected. Fluorescence change consistent with negative control data.
■ Green — DNA detected. Fluorescence change consistent with positive control data.

In addition to the red/green DNA Detection result, a percentage score provides a relative quantitative assessment of the amount of DNA loaded into the reaction, allowing the user to select which item to preferentially submit for laboratory analysis if multiple items from the same case are obtained. The software also provides gender information if enough template material is amplified.

In order to assess the ParaDNA Screening System against the NPIA's published specification a number of separate structured assessments of the technology were organised under the ADAPT programme. Initial trials (beginning March 2011) were followed by a review of documentary evidence provided by LGC which measured the acceptance criteria set by the NPIA against specification of the Screening System. Concurrent with these trials the technology was undergoing continued development and improvement followed by a rigorous forensic validation according to published Scientific Working Group on DNA Analyses Methods (SWGDAM) guidelines [4]. Independent technical evaluation was then performed by the Centre of Applied Science and Technology (CAST) group through the Food and Environment Research Agency (FERA) (July 2012).

The final part of the ADAPT initiative was assessment of the validated technology in several Police Force pilots in the United Kingdom.

A time line of the development process and external assessments performed is provided in Fig. 2. Information regarding the configurations under test at each stage is provided in Table 1. Product changes resulting from developmental improvements underwent strict equivalence testing to demonstrate a continuity between the original validation data and any newly introduced design improvements.

2.1. NPIA initial assessment

The initial ADAPT trials (February–March 2011) followed a simple side by side format whereby a single Scene of Crime Investigator (CSI) from a selected UK Police Force assessed the technology alongside a member of the LGC development team. Three trials took place, and each time a different Police Force and CSI were invited to take part. Mock samples types were tested comprising: blood on glass, blood on denim, cigarette butt, drinks bottle, tools, clothing (sock), lighter, and fingerprint on a glass slide. Both indirect and direct methods of collection were assessed. At this point of development there was no software to perform analyses and the data was acquired on a Bio-Rad CFX96 Real-Time PCR Detection System.

2.2. Validation

Validation experiments were designed to address the guidelines laid out by the Scientific Working Group on DNA Analysis Methods (SWGDAM) [4]. Experiments to characterise each aspect of the performance of the ParaDNA Screening System were performed at LGC Forensics, with the inter-laboratory reproducibility trials performed in collaboration with Florida International University (FIU) and the University of Central Florida (UCF). All data were analysed using the ParaDNA software v 1.0.1.0 except the species specificity experiments which were performed on a Bio-Rad CFX96 Real-Time PCR Detection System. The success of the Screening System was assessed based on how often a positive or negative screening result from the ParaDNA System correlated with a useable SGM+ profile when the sample was submitted downstream. Using the successful amplification of 14 or more alleles as a benchmark indicator that the SGMPlus profile was ‘usable’, samples were categorised as true positives, true negatives, false positives, or false negatives. The data has recently been accepted for publication [5].

2.3. NPIA usability study and CAST testing

The NPIA with the assistance of three Police Forces undertook usability testing of the ParaDNA device in 2012. After training by LGC staff the test user group consisting of both novice and experienced Crime Scene Investigators (CSIs) performed a number of tests on the system using samples prepared by the NPIA. Whilst mocked-up evidence samples were included in the analyses, the purpose of the testing was to assess the usability of the system by non-specialists and not the accuracy, sensitivity or reproducibility of data. Testers consisted of staff with varying levels of experience and skills. These included non-scientific background administration staff, submissions managers and highly experienced CSIs and each completed a questionnaire provided by the NPIA.
A formal independent assessment of the ParaDNA Screening System was performed by CAST through FERA in 2012. This laboratory evaluation analysed experimental samples providing a range of amounts of DNA, comprising: buccal swabs, blood on denim, drinking vessel, negative buccal, non-human species. The National DNA Database Accreditation Service (NAS) generated the samples. All samples were stored in a freezer at −20 °C during transportation and when not in use. All data were analysed using final specification chemistry, PCR plastics and Collection Device with success measured as whether the ParaDNA Screening System detected DNA on items that were known to contain template material.

2.4. Pilots

LGC, under the supervision of NPIA, undertook field test pilots with several Police Forces for ParaDNA technology. These pilots were planned in 4 phases to validate the future operating procedure for ParaDNA technology in police hands (Fig. 3). Two Police Forces took part in phases I–III, whilst a single Police Force was selected to perform phase IV on live case work. The four phases of the UK Police pilot programme had predetermined acceptance criteria (Table 2). Mock evidence samples for the pilots were provided from both LGC and UK Police Forces. All data was analysed using final specification chemistry, PCR plastics and Collection Device. As with the ParaDNA Developmental Validation, the successful amplification of 14 or more alleles was used as a benchmark indicator that the SGMPlus profile was ‘usable’. Samples were again categorised as true positives, true negatives, false positives, or false negatives.

3. Results and discussion

The results below present a brief overview of the data obtained at each strategic stage in the process. The data shows an evolution of the technology from early testing through to deployment and provides an account of the ParaDNA Screening Systems route to market.

3.1. NPIA initial assessment

The more template material there is on an evidence item the more chance it has of entering each of the four tubes of the ParaDNA Screening Test. In short, the presence of DNA can be measured crudely by a count of the number of tubes seen amplifying. The combined data generated at the initial ADAPT trials shows that there is a strong correlation between the number of tubes amplifying and the number of SGM+ peaks obtained from the sample after submission to a Forensic Service provider (FSP) (Fig. 4). The data also showed little difference in the results generated from the two users (LGC and CSI — data not shown). This data shows the ease of technology transfer and also confirms that there is a relationship between the amount of DNA template material on an evidence item, the amount of amplification product after ParaDNA amplification and the number of peaks seen in the subsequent DNA profile. After the initial trial, the NPIA provided a set of end user requirements for their level 1 product [3] which were used as the ParaDNA product target specifications during the continuing development of the Screening System. Further optimisation of the system and software was performed with the main change being the creation of the ParaDNA software which sums the area of the amplification peaks observed and provides a DNA Detection Score (%).

3.2. Validation

The validation studies performed at LGC marks the first point where the product was in a robust and testable format. The data demonstrates that the ParaDNA Screening System detected human DNA from purified DNA samples and swabbed, mocked-up evidence items with similar sensitivity to that demonstrated by commonly used STR profiling products. In addition, the ease of use of the ParaDNA Screening system by specialist and non-specialist users in several laboratories was demonstrated. The production of positive DNA scores from a variety of substrate and swab types and in the presence of inhibitors was observed. The validation data has been accepted for publication [5] and therefore cannot be duplicated for inclusion in this overview.

3.3. NPIA usability study and CAST testing

The technology underwent usability testing organised by the NPIA comprising of a questionnaire and assessment of the processing speed. The time taken from opening and examining the evidence item to obtaining a sample result took 81 min, which was more than the hour initially required on the ADAPT initiative specification. In all, nine users from different forces took part providing various feedback to the NPIA questionnaire. Upon completion of the usability testing the independent

assessment by CAST showed the ParaDNA system passed the evaluation with all blood, buccal and drinks giving positive results whilst the negative swabs and animal samples did not amplify.

3.4. Pilots

The UK Police pilot programme began in October 2012. Acceptance criteria for phases I and II were all met. Template rich buccal swabs generated high percentage calls together with gender calls. The saliva dilution samples showed some variation between users, although the data still demonstrated successful technology transfer. The mock evidence items tested in phase III showed that the ParaDNA screening system could be successfully incorporated into a routine evidence triage and DNA submission process (Table 3), although data from one participating Force gave a higher than expected false negative rate. Upon investigation this highlighted a key process step not being followed by the end user and the erroneous data was excluded from further analyses with the permission of the regulatory bodies. The assessment was repeated with results falling into specified pass criteria. For the total data set the false positive and false negative values fell within the expected range based on results of the development validation [5]. Phase IV was on live casework items (Table 3) selected by the participating Police Force. ParaDNA correctly determined samples as having sufficient DNA present to generate a useful profile in 76% of cases (38/50) of which 53% (20/38) generated correct gender calls. 14% of all samples would have been correctly screened out. The relatively low number of true negatives detected is likely due to the larger number of 'template rich' items (blood and saliva) screened. Indeed it is clear that the rate of identification of true positives and negatives will vary with the sample type highlighted by the fact that no true negatives blood samples were identified whilst 26% of the touch samples were successfully screened out. 4% of samples were false positives and 6% of samples were false negatives. This proportion of false negatives and false positives fell within the products specified performance. The ParaDNA technology did not compromise live case work exhibits and the data generated could be used to develop a detailed business case which demonstrates the financial benefits of the ParaDNA Screening System.

Recent data from one Police Force who have validated and implemented the ParaDNA Screening System for its use as part of their DNA submissions screening procedures have calculated savings in the region of £37,000 p.a. in the first year from their operational testing of ParaDNA Screening process. Indeed challenging touch evidence items are now pre-screened more frequently alongside commonplace cellular samples leading to cost-saving benefits and improved success rates, and the screening in of crucial evidence that will have previously not been prioritised for DNA profiling.

4. Summary

To date, many of the novel technologies or developments in forensic science have come from the wider scientific community and been applied to the field rather than from innovation within the field itself. The case study presented here represents a product that from its very inception was intended to be deployed in the forensics arena. The route to market briefly described above represents the combined efforts of a number of different bodies including, research laboratories, industry groups, government departments and academic institutions. It has taken more than three years to move from a proof of concept to an operationally deployed product with the official launch of the ParaDNA Screening System held at the American Academy of Forensic Sciences in March 2013. In addition to the work outlined in this manuscript there have been ongoing validation and pilot work held in the United States [6].

It is wholly recognised by all those involved in this endeavour that novel technology developed for use in the forensic market needs vigorous testing and careful implementation. It should be recognised that forensic science is the application of science to the law and as such the peer reviewed publication and acceptance of the underlying principals and interpretation of data should ultimately determine if a process is scientifically valid. At the same time it is only through cooperation with end users, i.e. the Criminal Justice System, that a sound scientific process can be critically assessed and implemented. Therefore for successful development to occur industry research scientists, academic bodies and development teams need to fully interact with the wider forensic community both to aid in their understanding and acceptance of novel scientific principles and for end users to critically assess any new advances in the field of forensic science.

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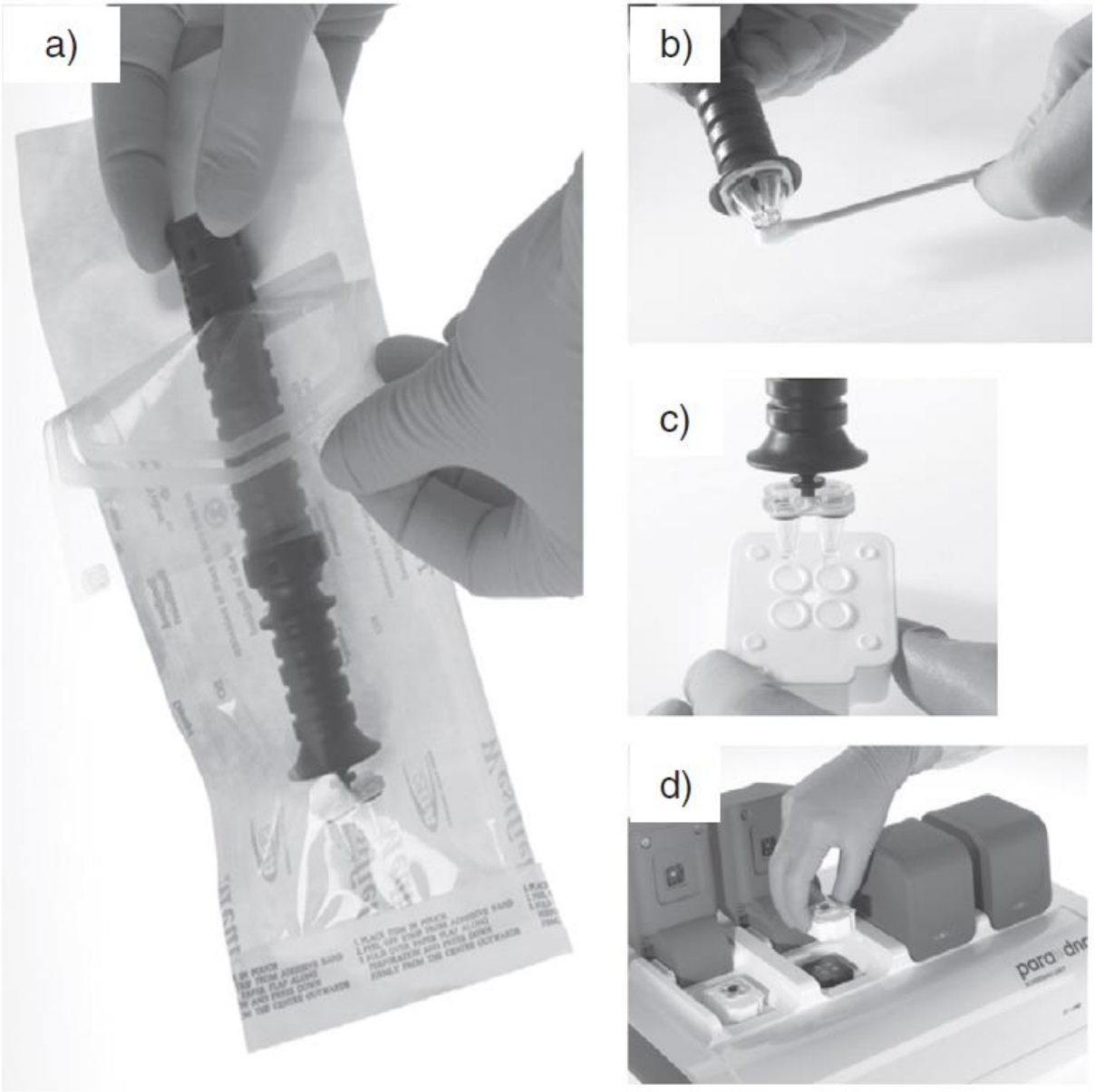

Fig. 1. To use the ParaDNA Screening System simply (a)open the disposable ParaDNA sample collector; (b) recover the cellular material from an evidence item; (c) introduce the template material into the PCR consumable containing the assay mix; and (d) load on the instrument.

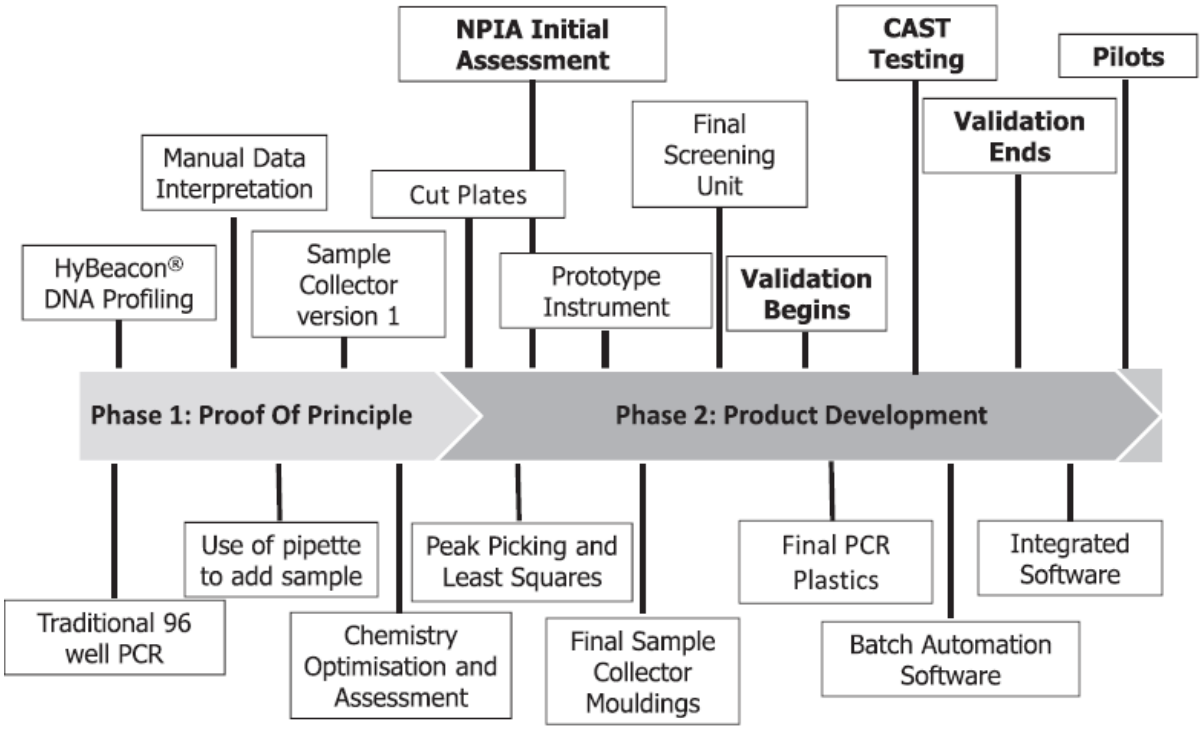

Fig. 2. The development of the ParaDNA Screening System from proof of principle through product development with key stages identified.

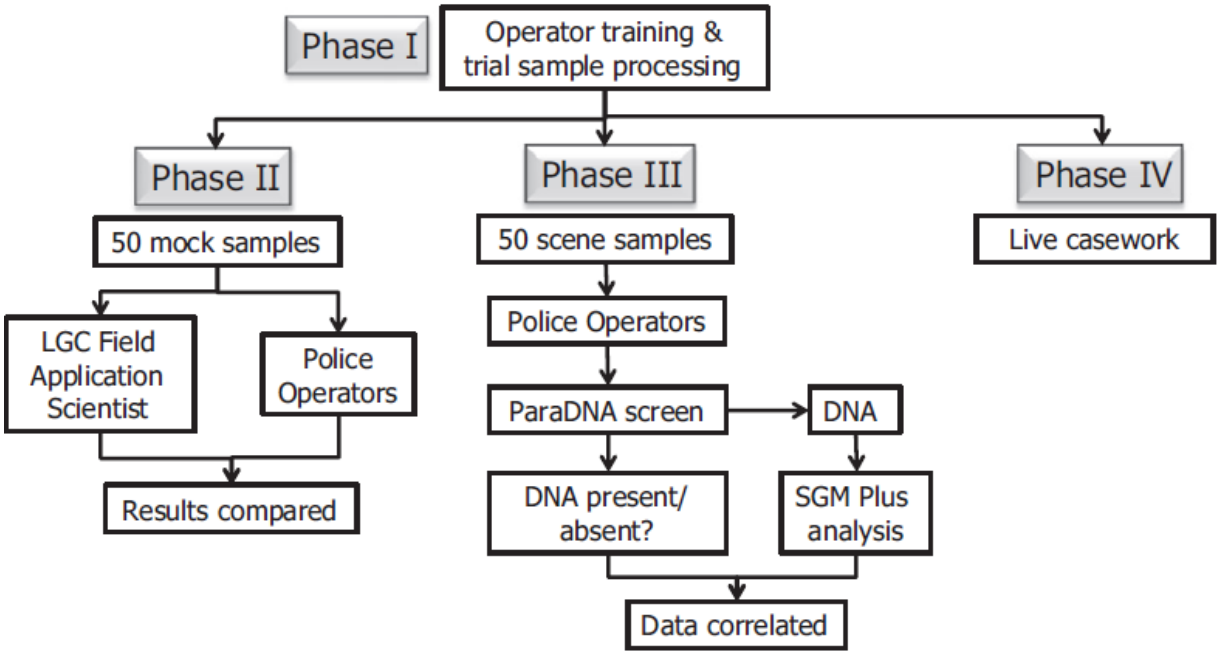

Fig. 3. Plan designed to be followed by UK Police pilots.

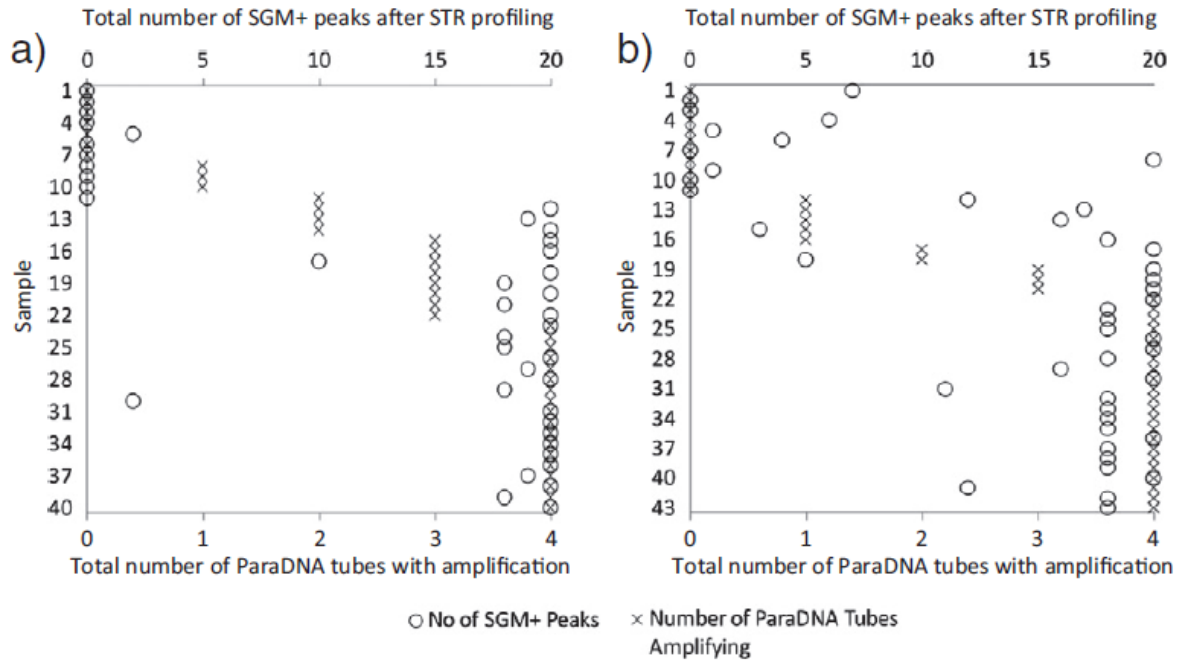

Fig. 4. Results from first ADAPT trial showing correlation between number of peaks observed in SGM+ profile and number of ParaDNA tubes amplifying for both (a) direct sampling and (b) indirect sampling of mock evidence items. The measurement metric evolved from a number of tubes amplifying to % DNA Score during the subsequent development process.

Table 1

Configurations under assessment during each stage of the ADAPT initiative.

	Chemistry Version	PCR Consumables	Sampling Device	PCR Unit	Metric used to assess data
Initial ADAPT trial	1.0.0 and prior	Cut PCR plates	Prototype v 1	Bio-Rad CFX	Number of tubes amplifying
LGC developmental validation	4.2.0	Cut PCR plates*	PARA-050	Bio-Rad CFX	DNA Detection Score (%)
		LGCSTK005657		PARA-010	
CAST testing	4.2.0	LGCSTK005657	PARA-050	PARA-010	DNA Detection Score (%)
NPIA usability trial	4.2.0	LGCSTK005657	PARA-050	PARA-010	DNA Detection Score (%)
UK Police pilots	4.2.0	LGCSTK005657	PARA-050	PARA-010	DNA Detection Score (%)

*Equivalence shown between cut PCR plates and LGC mouldings during ParaDNA validation

Table 2

Acceptance criteria proposed for UK Police pilot programme.

Phase	Acceptance criteria	Samples	Number
I - Training	Demonstrate the straightforward training and transfer of technology to Police Operators with no major operator issues encountered. Obtain posiive feedback on the usability of the system	Buccal swabs	One per user
II - Proficiency	Users to be able to effectively obtain expected DNA results from a range of evidence types using the ParaDNA Screening System	Saliva dilutions spiked in swabs	3-4 swab replicates per user
III - Mock case Samples	The successful incorporation of the ParaDNA Screening System by police Staff into their routine evidence triage and DNA submissions process The ParaDNA Screening results correlate to the STR profile success. The results from the testing demonstrate that the false negative and positives were within tge expected range based on the developmental validation for the total data	Blood Saliva Touch	92 items
IV - Live case work	Show correlation between the screening results and the STR results. Demonstrate that the technology does not compromise live casework exhibits	Blood, saliva, touch, DNA swabs, training shoes, tape lifts	50 items

Table 3

Results of UK Police pilots' phases III and IV.

Pilot study	Category	Blood	n	Saliva	n	Touch	n	Total data set	n
Phase III	True positive	100%	14	88.90%	48	33.30%	8	76.10%	70
	False positive	0%	0	0%	0	41.60%	10	10.90%	20
	True negative	0%	0	0%	0	25%	6	6.50%	6
	False negative	0%	0	11.10%	6	0%	0	6.50%	6
Phase IV	True positive	100%	5	82%	18	65.20%	15	76%	38
	False positive	0%	0	4.50%	1	4.30%	1	4%	2
	True negative	0%	0	4.50%	1	26.20%	6	14%	7
	False negative	0%	0	9.00%	2	4.30%	1	6%	3

Successful profile defined as returning 14 or more alleles. True positives (ParaDNA Detection Score was green and the STR profile yielded ≥ 14 alleles), true negatives (ParaDNA DNA Detection Score was red and the STR profile yielded < 14 alleles), false positives (ParaDNA Detection Score was green and the STR profile yielded <14 alleles), or false negatives (ParaDNA DNA Detection Score was red and the STR profile yielded ≥ 14 alleles).