Assessing the impact of common forensic presumptive tests on the ability to obtain results using a novel rapid DNA platform

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Abstract

The rise of DNA evidence to the forefront of forensic science has led to high sample numbers being submitted for profiling by investigators to casework laboratories: bottleneck effects are often seen resulting in slow turnaround times and sample backlog. The ParaDNA® Screening and Intelligence Tests have been designed to guide investigators on the viability of potential sources of DNA allowing them to determine which samples should be sent for full DNA analysis. Both tests are designed to augment the arsenal of available forensic tests for end users and be used concurrently to those commonly available. Therefore, assessing the impact that common forensic tests have on such novel technology is important to measure. The systems were tested against various potential inhibitors to which samples may be exposed as part of the investigative process. Presumptive test agents for biological materials (blood, semen and saliva) and those used as fingerprint enhancement agents were both used. The Screening Test showed a drop in performance following application of aluminium powder and cyanoacrylate (CNA) on fingerprints samples; however this drop in performance was not replicated with high template DNA. No significant effect was observed for any agent using the Intelligence Test. Therefore, both tests stand up well to the chemical agents applied and can be used by investigators with confidence that system performance will be maintained.

Keywords: DNA Screening, Triage, Presumptive Tests, PCR inhibitors, Direct PCR, HyBeacon™ probes

1. Introduction

The primary purpose of performing presumptive forensic tests such as body fluid identification and fingerprint enhancement techniques is to identify suspects and/or link suspects to crime scenes for the purpose of supporting/refuting witness testimony. However, a secondary benefit is realised before the evidence even reaches court; performing such tests allows the crime scene investigator or forensic practitioner to prioritise items collected at crime scene for downstream analyses. However, the disadvantage of many presumptive tests is that they show poor specificity to the human biological/chemical target [1,2] while touch DNA items often fail to produce a corresponding STR profile [3,4] due to low amounts of template material available on these items and/or PCR inhibition.

Recently, there have been a number of rapid DNA profiling and detection platforms released that allow the analyses and prioritisation of items by non-expert users outside the traditional laboratory setting [5–9]. The development and release of novel technology into the forensic arena is both carefully controlled and monitored [10]. Efforts in this area generally include a thorough validation of the technology performance parameters [11] and in cases where the technology is not a stand-alone process, an assessment of how new and existing processes interact. With the advent of rapid DNA systems the latter becomes increasingly important as many novel technologies look to combine existing processes and thereby save time and money. For example, all rapid DNA systems look to combine the three current stand-alone processes of DNA extraction, PCR and STR detection steps [e.g. 5–9]. The move out of the laboratory brings additional risks as samples get processed earlier after collection and the boundaries between forensic processes begin to merge [10].

The ParaDNA System is a non-expert user system comprising a thermocycling instrument with fluorescent detection capabilities, sample collection device and associated chemistry. The chemistry exists in two forms, one for rapid screening and a second for rapid intelligence. The system is designed to improve the efficiency of sample submissions as well as speed up the investigative process as a whole. Given the intended use of the systems by end users is out of the laboratory environment, it becomes necessary to establish whether the use of the system is impacted by existing forensic tests, performed by investigators during examination of exhibits such as presumptive biological tests and fingerprint enhancement techniques. The data presented below assess the impact of treating biological stains and fingerprints with a selection of relevant presumptive tests and fingerprint enhancement agents, then sampling and processing with both Screening and Intelligence Tests.

2. Materials and method

The ParaDNA Screening Unit is connected to a laptop computer or the integrated Field Portable Unit with the ParaDNA software necessary to interpret and present results to the user. Both units contain four heads allowing four samples to be run simultaneously and independently; each head uses a thermal block allowing a direct PCR protocol to be carried out within a short time frame (75 min). The Screening Test assesses the quality and relative quantity of DNA detected to provide users with a DNA Detection Score, presented in the form of 0–100% which acts as a presumptive test for the presence of DNA on forensic evidence items. The Intelligence Test provides a five STR locus profile in addition to the amelogenin (gender determining) marker and offers the user the possibility to use this profile to search national and local databases. Both provide their result within seventy-five minutes.

Ten replicate blood samples and semen samples (both10ml) were pipetted separately onto cotton fabric and left to dry overnight before treatment with the relevant test agent. A breakdown of all the tests performed is provided in Figs. 1 a and 2 a. Given the relatively large volumes of reagent used in the presumptive testing, the impact of the process was assessed through the collection of a 'chemical control' sample using the same biological stain and undergoing to same methodological process but using water instead of reagent.

To assess the impact of Phadebas testing, ten replicate saliva samples (100ml) were pipetted onto cotton fabric and left to dry overnight. The stains were then blotted with Phadebas forensic CR paper (Magle Life Sciences). Brown paper was chosen as a chemical control sample to assess the impact of the chemically impregnated Phadebas paper. Both sample types were wetted with 3–4 sprays of deionised water, pressed together firmly using a clear plastic sheet and a weight for application of pressure. Stains were left to react for 45 min then papers removed and allowed to dry.

Six replicate fingerprints (as a source of touch DNA) were obtained from donors by application of medium pressure for 5–10 s onto the relevant substrate (glass slides for CAN and aluminium powder treatment and filter paper for ninhydrin treatment) before treatment with the relevant test agent. A breakdown of all the tests performed is provided in Figs. 1b and 2b. Due to the inherent variability in DNA content of fingerprint samples the observed effects were verified by analysing three replicate saliva samples (50ml on glass) which were spiked with each test agent.

All samples described above were prepared in duplicate for use with the ParaDNA Screening Test and also the ParaDNA Intelligence Test. All presumptive tests were noted to have given a positive result before being photographed. After the presumptive testing, all items were left to dry before being sampled by one minute of continuous rubbing using the ParaDNA Sample Collector and being loaded into a reaction plate which was then placed in the ParaDNA Screening Unit. Control samples

that did not undergo presumptive testing consisted of spikes of body fluid on relevant background materials. All biological materials were provided by donors in accordance with the University of Strathclyde and LGC code of ethics. All data was checked for normality before testing for a significant difference between the control sample and the treatment.

3. Results and discussion

The importance of performing a thorough evaluation of the robustness of a novel forensic test is well documented. Often tests form part of a compartmentalised process (e.g. DNA extraction and purification, PCR, quantification, CE analyses) and are commonly assessed/validated independent of the other parts of the method. This is often acceptable, but with the advent of rapid DNA systems that seek to put laboratory based processes into the hands of non-expert users, a careful evaluation is required to assess the impact of running different tests in succession. All presumptive tests for body fluids generated positive results and all fingerprints showed clear ridges and furrows after application of each enhancement agent (data not shown). High DNA Detection Scores were obtained when using the Screening Test for blood, semen and saliva samples and there was no significant difference between the DNA Detection Scores of the untreated control, treated sample and chemical control (Fig. 1a). This suggests the presumptive tests had no impact on the ability of the test to provide a result. For fingerprint samples, no significant difference was observed between the control sample and the ninhydrin treated sample. A significant difference was observed between the control treatment and the aluminium powder ($p = 0.00$) and CNA on glass ($p = 0.02$) (Fig. 1b). It is possible that the difference observed between the control and these treated samples is due to a variable amount of DNA in the initial fingerprint sample rather than an effect of the enhancement agent. This was tested by repeating the enhancement on saliva stains which confirms that the aluminium powder has a negative impact on the DNA Detection Score observed (Fig. 1b), although the low number of replicates (three) prevent any statistical analyses from being performed. Previous data shows that aluminium powder has little impact on the ability to perform DNA typing [12] although the study cited did not use a direct PCR approach as used by the ParaDNA system. The impact of CNA was not observed on the saliva spikes, suggesting the significance seen in the fingerprint samples may be due to a variable amount of template collected from the fingerprints.
Approximately 90% of alleles were called when using the ParaDNA Intelligence Test and there was no significant difference observed between control and treated samples for any of the blood, semen or saliva samples (Fig.2a).This suggests the presumptive tests had no impact on the ability of the ParaDNA Intelligence Test to provide a result. There was also no significant difference observed between the control and treated fingerprint samples which was mirrored with tests on the high

template saliva samples (Fig.2b). The impact of aluminium powder seen on the Screening Test was not observed in the intelligence test. It is considered that this is possibly due to the larger volume of enzyme associated with the latter test increasing the test robustness and tolerance to inhibitors.
The impact of fluorescent presumptive test agents (luminol and Bluestar® Forensic) was particularly important to assess given the basis of the ParaDNA System is the fluorescent detection of a HyBeacon™ probe. These agents are commonly applied at the crime scene to help identify a potential area for further testing, so would always be applied prior to DNA sampling being carried out. No interference was observed in this study which can largely be explained by examination of the emission spectra of the fluorophores attached to the HyBeacon probes utilised by the system. Fluorescein has an emission range of 485–650 nanometres (nm) peakingat517nm[13]; luminol fromaround350–580 nm peaking at 420 nm[14].Despite some overlap between the two, peak emissions lie distinct from each other thus causing no interference to the operation of the system. Bluestar Forensic is a derivative of luminol and emits blue light so peak emission should fall around 430nm, explaining the lack of interference also observed with this test.
STR profiling success rates are affected by both DNA template amount [3,4] and carry through of inhibitors that can reduce DNA recovery and also prevent PCR [15]. This study did not assess the downstream STR success rate of the mock evidence items although data generated during the development of the ParaDNA technology shows there was no measurable impact in the quantification results between samples that underwent ParaDNA treatment and those that did not [5,7,16]. In these studies, mock evidence items did not undergo any form of presumptive testing; i.e. only the effect of low template was investigated. This would suggest that the success rates observed may be further reduced in samples that have undergone presumptive testing if inhibitors are carried over into the PCR process. Previous studies investigating the impact of presumptive tests on downstream analyses have shown that for some pre-treatments inhibition is not often observed [17,18]. If it is, protocols are developed that remove the inhibitors during the DNA recovery [e.g.19]. There is some evidence to suggest that inhibition has a greater impact on extremely low template samples [20] or is observed as a function of time between presumptive testing and STR profiling [21,22]. Given these observations, the data presented supports the use of ParaDNA technology on high template and medium template samples, which in the context of the present study would include the blood, saliva and semen stains.
Practitioners wanting to use the technology on touch DNA items, such as single finger-prints, may want to consider the success rate of the untreated samples as published in the developmental validation study of both tests [5,7] and also assess the impact of their own in-house practices on any results derived. Further work in this area should look to assess additional pre-treatments.

4. Summary

The direct PCR approach of the ParaDNA System mean robustness is essential for the assays to effectively perform their function. The ParaDNA Screening and Intelligence Systems appear robust to many common crime scene presumptive tests as neither showed inhibition except for the aluminium powder when used in conjunction with the Screening Test. This data allows crime scene investigators to use existing presumptive tests in confidence that they will not impact the sensitivity or specificity of ParaDNA tests or will allow the crime scene investigator (as in the case of aluminium powder and the ParaDNA Screening Test) to take the decision to either not process the sample or caveat the results in the context of the data presented.

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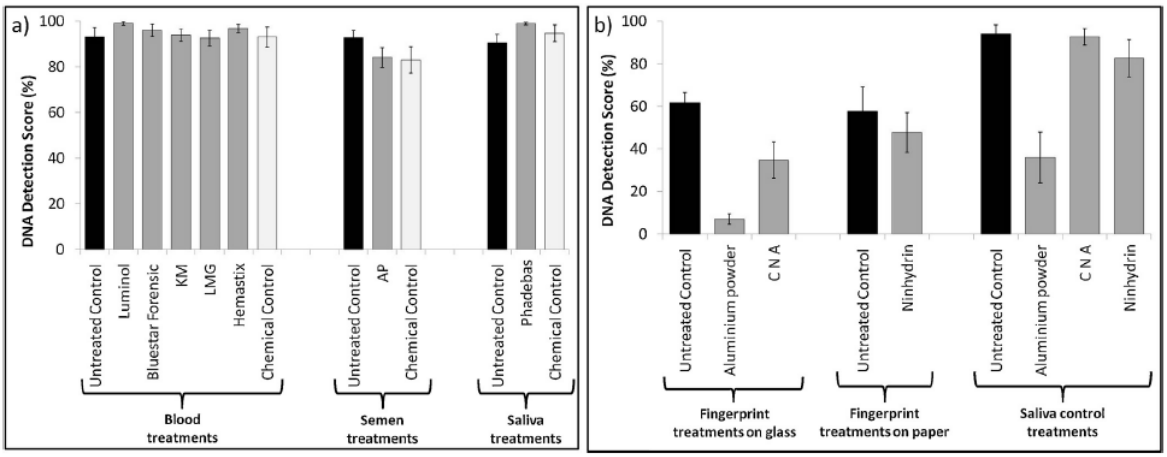

Fig.1. Results showing impact of presumptive tests for blood, semen and saliva (a) and fingerprint enhancement agents (b) on ParaDNA Screening Test. Standard error shown.

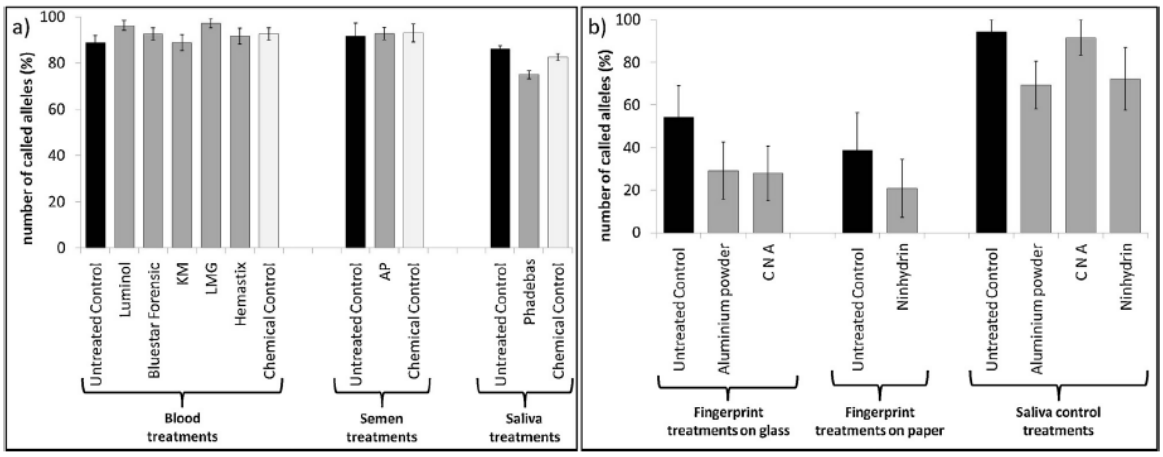

Fig. 2. Results showing impact of presumptive tests for blood, semen and saliva (a) and fingerprint enhancement agents (b) on ParaDNA Intelligence Test. Standard error shown.