Evaluating the sensitivity of presumptive and confirmatory tests for body fluids

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Abstract

Most exhibits received in the forensic genetics laboratory of Ras Al Khaimah (United Arab Emirates) are swabs or clothing with stains suspected of being either blood, semen or saliva; swabs from touch DNA are also reasonably common. Routine practice has been to use presumptive tests to characterise the materials before DNA extraction.

In this study we evaluated the presumptive methods currently used for blood (Hemastix® and Kastle-Meyer), semen (Phosphatesmo KM) and saliva (Phadebas®) in comparison to the confirmatory tests OBTI Hexagon and RSID™ Blood, RSID™ Semen and RSID Saliva™.

Results from this study showed that, as expected, presumptive tests were generally more sensitive in detecting body-fluids than confirmatory tests. The greater sensitivity of presumptive tests is largely due to the abundance of the target molecule/enzyme in the respective body-fluids, whereas confirmatory tests target more specific antibodies that are generally present in body-fluids at lower quantities. The presumptive tests were at least two-fold more sensitive than the RSID™ Blood, Semen and Saliva confirmatory tests. However, the OBTI Hexagon test showed comparable sensitivity to Kastle-Meyer and Hemastix for the detection of blood; this test utilizes an anti-haemoglobin antibody, which enables the high level of sensitivity.

DNA as extracted from different dilutions and quantified using real-time PCR with the Quantifiler Human kit. Except for the RSID™Blood and Saliva, the limit of detection for the tests was at dilutions where recovery of sufficient DNA for STR analysis was not likely. Therefore, positive presumptive/confirmatory test results could potentially be followed by negative DNA profiling.

1. Introduction

Both confirmatory and presumptive tests save time and money by prioritizing for DNA analysis [1].However, a positive reaction in a presumptive or confirmatory test does not necessarily mean that a DNA profile can be generated; DNA may be present in insufficient quantities or in a highlydegraded state.

This study was conducted to evaluate and establish the "sensitivity limit" of detection of four presumptive and four confirmatory tests in relation to their ability to detect three different bodyfluids: blood, semen, and saliva. We also aimed to identify the DNA quantity present at the point of the sensitivity limit for each bodyfluid tested.

2. Materials and Methods

2.1 Serial dilutions

All samples were taken from a single male to minimize inter-sample variation. Dilutions were made using deionised water past the manufacturers' sensitivity claims. When no kit was available for the test, dilutions were prepared to the sensitivity reported in published papers.

Blood dilutions were prepared from stock liquid blood in EDTA in the following dilutions 1:200, 1:300, 1:400, 1:500, 1:700, 1:10000, 1:15000, 1:20000, 1:25000, 1:150000, 1:200000 and 1:250000. Semen dilutions were prepared from stock seminal fluid in the following dilutions 1:2000, 1:3000, 1:4000 and 1:5000. Saliva dilutions were prepared from stock saliva sample in the following dilutions 1:350, 1:400, 1:450, 1:500, 1:900, 1:1000 and 1:1100.

2.2Presumptive and confirmatory tests

Four presumptive tests: the Kastle-Meyer (Phenolphthalein) test and the Hemastix® test for blood,the Phosphatesmo KM® test for semen, and the Phadebas® test for saliva were all performed following manufacturers' instructions with a cut-off time of 2 min for positive results readings. When applicable, samples were applied directly on the test materials rather than using an intermediate cotton swab.

Four confirmatory tests: the RSID™-Blood and the Hexagon® OBTI for Blood, the RSID™-Semen for semen and the RSID™-Saliva for saliva samples, were all performed following manufacturers' instructions and cut-off times.

2.3 DNA extraction and quantification

All sample types were extracted using the chelex-100 method. To each tube containing 100 µl of diluents, 175 µl of 10% Chelex (Sigma-Aldrich, Germany) and 20 µl proteinase K (10mg/mL) (Sigma-Aldrich, USA) were added. In addition, 7µl of DTT (10mM) (Sigma-Aldrich, USA) were added to semen samples. The mixture was then placed in a water bath at 56 °C for 2 h followed by 8 min at 100 °C. The samples were then centrifuged for 3 min at 13000*g*and the supernatant was then transferred to a separate 1.5 tube ready for DNA quantification.

DNA quantification was carried out on all body fluids extractions using the Quantifiler™ Human DNA Quantification kit on an ABI 7500 real-time PCR machine (Thermo Fisher Scientific) following the manufacturer's recommendations.

3. Results and discussion

Analysis of common body-fluid screening tests indicates to a more sensitive nature of presumptive tests compared to confirmatory tests [2]; however, published data shows a large degree of discrepancy in the values for the sensitivities of such tests. Previously addressed by [3], variable sensitivity of tests was thought to be due to differences in reagent concentrations, methods of preparation of samples and reagents, and differences in the type of material containing the samples. Other studies further add that many of the discrepancies observed were probably due to the application methods of the test; for example, test reagents being added directly to a dilute bodyfluid solution rather than on a material containing the dilute bodyfluid [4]. These discrepancies in sensitivity values and uncertainty in the cause of such wide range values prompted the investigation to locally study the sensitivity of common presumptive and confirmatory tests used in our laboratory. Results from this current study provided a measure of the relative sensitivity of each test (Table 1).

Table 1: A comparative table showing the sensitivity of all tests, presumptive and confirmatory, carried out in this study, showing the number of positive samples at any given dilution. Each dilution was tested in triplicate.

	Reagent							
Dilution	**Presumptive**				**Confirmatory**			
	Hemastix®	*Kastle-Meyer*	*Phosphotesmo KM ®*	*Phadebas®*	*OBTI Hexagon ®*	*RSID™-blood*	*RSID™-semen*	*RSID™-saliva*
1:400	+++	+++	+++	+++	+++	++	+++	+++
1:500	+++	+++	+++	+++	+++	–	+++	–
1:900	+++	+++	+++	+++	+++	–	n/a	–
1:1000	+++	+++	+++	+	+++	–	n/a	–
1:2000	+++	+++	+++	–	+++	–	++	–
1:3000	+++	+++	+++	–	+++	–	–	–
1:4000	+++	+++	+++	–	+++	–	–	–
1:5000	+++	+++	+	–	+++	–	–	–
1:10000	+++	+++	–	–	+++	–	–	–
1:15000	+++	–	–	–	+++	–	–	–
1:200000	+++	–	–	–	–	–	–	–
1:250000	+	–	–	–	–	–	–	–

+++ = strong positive ++ = Positive + = Weak positive - = Negative

The greater sensitivity of presumptive tests can be attributed to their 'tissue-specific' target molecule; they have been developed to detect substrates that are abundant in their respective body-fluids. Whereas confirmatory tests target more specific antibodies that are generally present in body-fluids at lower quantities.

DNA extraction and quantification were carried out for the lowest concentration that was able to give a positive result for each of the bodyfluid, in order to establish a relationship between the limit of detection for each presumptive and confirmatory test and its corresponding DNA quantity as measured by real−time PCR.

The presumptive Hemastix® test was the most sensitive test for blood with its sensitivity limit established at dilution of 1:200 000, a measure at which real−time PCRwas not able to detect any DNA. The confirmatory Hexagon® OBTI test and Kastle-Meyer test showed comparable sensitivity (1:15 000and 1:10 000 respectively) and both had negligible levels of DNA (0.001ng/µl and 0.002ng/µl respectively), whereas the RSID™-Blood was the least sensitive at 1:400 dilution and yielded0.01ng/µl(SD 0.001) of DNA.

When the confirmatory RSID™-Semen was compared with the presumptive Phosphatesmo KM® test the latter showed twice the sensitivity for semen (1:2000 and 1:4000 dilution respectively). However, the RSID™-Semen was more likely to produce a DNA profile with a yield of 0.01ng/µl (SD 0.011) of DNA compared to 0.003 (SD 0.001) yielded from the Phosphatesmo KM® test

0.003ng/µl (SD 0.011) respectively).Similarly, when comparing the confirmatory RSID™-Saliva with the presumptive Phadebas® for saliva, the latter was the more sensitive at a dilution of 1:900 compared to 1:400 for the RSID™-Saliva (0.004ng/µl(SD 0.002) and 0.014ng/µl(SD 0.02) respectively).

The RSID™Blood and Saliva confirmatory tests'limit of sensitivity corresponded to quantities of DNA that could be successfully profiled, i.e.,10-14 pg/µl. For all other tests negligible quantities of DNA were recovered from the dilutions at the limit of sensitivity.

4. Conclusions

In many cases, the results of this current study question the need to perform enzymatic presumptive tests in laboratory conditions on forensic samples when confirmatory tests are available, especially with semen and saliva samples. Since most positive screening tests for bodyfluid are carried through for DNA analysis, current results showed that in most cases the sensitivity of both presumptive and confirmatory tests are beyond levels required for DNA analysis. Therefore, in many cases, correct identification of a certain body-fluid may fail to yield DNA that can identify the depositor of a bodyfluid. Although this is true for all test types, the confirmatory RSID™ kits' lower sensitivity reduces the likelihood of detecting body fluids that will not yield sufficient DNA.

While further studies are required to consolidate the current findings, the wide range of reported sensitivities in the literature may raise doubt on the reliability of these screening tests. More significantly, this study highlighted the need for a standardized method of application and communication for presumptive and confirmatory testing in order for results of different studies to be compared confidently.

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Conflict of interest statement

The authors declare no conflict of interests.

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