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Review

# From crime scene to courtroom: A review of the current bioanalytical evidence workflows used in rape and sexual assault investigations in the United Kingdom

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ABSTRACT

Sexual assault casework requires the collaboration of multiple agency staff to formalise an investigative pipeline running from crime scene to court. While the same could be said of many other forensic investigations, few require the additional support of health care staff and the combined forensic involvement of body-fluid examiners, DNA experts and analytical chemists. The sheer amount of collaborative effort between agencies is laid out through a detailed examination of the investigative workflow from crime scene to courtroom with each step in the pipelines detailed and discussed. Beginning with a review of sexual assault legislation in the United Kingdom this article details how sexual assault investigations are initiated by police and supported by sexual assault referral centre (SARC) staff who are often the first responders providing primary healthcare and patient support to victims while simultaneously collecting and assessing forensic evidence. Detailing the myriad of evidential material that can be documented and collected at the SARC, the review identifies and categorises key forensic tests to first detect and identify body-fluids recovered from evidence through to the secondary analysis of DNA to help identify the suspect. This review also focusses on the collection and analysis of biological material used to support the allegation that the sexual activity was non-consensual and provides a breakdown of common marks and trauma as well as a review of common analytical methods used to infer Drug Facilitated Sexual Assault (DFSA). The culmination of the investigative pipeline is discussed by reviewing the Rape and Serious Sexual Assault (RASSO) workflow used by the Crown Prosecution Service before providing our thoughts on the future of forensic analysis and possible changes to the described workflows.

## 1. Introduction

The criminal investigation of sexual assault is multidisciplinary in nature and requires the support of numerous agencies including sexual assault nursing staff, Police, forensic scientists, health care specialists, UK Prosecution Services and wider criminal justice community. Each agency will have their own field of expertise and are well represented by research and reviews exploring field-specific areas of involvement. Such literature may be inaccessible to those outside the target audience due to a lack of background knowledge. This may result in a loss of engagement and opportunities for inter-disciplinary collaboration. As such the use of a holistic approach could facilitate a greater understanding between of these audiences. Therefore, this literature review seeks to provide a condense overview of the workflows within each of the key agency groups including key analytical methods used to collect and analyse evidence to support sexual assault investigations. The methodology used to build this holistic literature review followed a traditional approach and combined a number of evidence gathering methods including the identification of relevant stakeholders in the field; a review of UK government and policing sexual offence related policy and guidance documents; breakdown of the overarching investigative framework; identification through UKAS of common forensic methods used in the analysis of sexual assault evidence by UK Forensic Service Providers; and a trawl of the existing scientific literature of the most common methods.

### 1.1. Sexual offence legislation in the United Kingdom

Sexual offences in the United Kingdom (UK) are detailed in a number of different legislative acts, namely the Sexual Offences Act 2003

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(covering England and Wales) [1], The Sexual Offences (Northern Ireland) Order 2008 [2], and The Sexual Offences (Scotland) Act 2009 [3]. The legal entities responsible for prosecuting cases in each jurisdiction are the Crown Prosecution Service (CPS) for England and Wales [4], Public Prosecution Service (PPS) for Northern Ireland [5] and the Crown Office and Procurator Fiscal Service (COPFS) for Scotland [6]. The ***Sexual Offences Act (2003)*** came into force on the 1st May 2004 and applies to all offences committed on or after that date [7]. The Act is divided into two parts. Part One details the sexual offences covered by the Act. Part Two details the notification requirements (sometimes referred to as the sex offenders register) and the preventative orders allowed under the Act. Non-consensual offences including rape, assault by penetration, sexual assault and causing someone to engage in sexual activity without their consent are legislated against under Sections 1–4 of the Act (Table 1). Also covered by the 2003 Act are further offences that can also include those detailed above but are considered separate offences due to the age, and/or mental status of the victim and/or the relationship between the victim and offender. The ***Sexual Offences (Northern Ireland) Order 2008*** came into force on 2nd February 2009 [2] incorporating changes to the categorisation of sexual offences and to clarify issues around consent [8]. The 2008 Order remains similar to the 2003 Act albeit with different offence codes and content order. For example, Part One covers interpretation and definitions of 'consent' and 'sexual' which remain consistent with the Sexual Offences Act 2003, while Part Two covers non-consensual sexual offences including rape, assault by penetration, sexual assault, and causing sexual activity without consent (sections 5–11 detailed in Table 1). The ***Sexual Offences (Scotland) Act 2009*** came into force on the 1st December 2010 and applies to all offences committed on or after that date [3]. Like the legislation enforceable under English and Irish law, the Act updates and supersedes previous legislation namely the Sexual Offences (Scotland) Act 1976. The 2009 Act is divided into Seven Parts with the language, terminology and running order substantially different from both the English, Welsh and Northern Irish legislation. Part One details rape and associated non-consensual acts (sections 1–11 detailed in Table 1) while Part Two details the meaning of consent and reasonable belief. Further offences are covered within additional sections. As a consequence of the relatively recent nature of these Acts, historical sexual assaults committed prior to these Acts becoming law are prosecuted under the previous relevant legislation.

While there are some differences in each act, they reflect and uphold the overarching legislative goal of providing protection to victims of sexual abuse whether they are adults, children or vulnerable individuals. In such instances where an allegation of sexual abuse has been levelled and sufficient evidence exists to warrant charges being brought, the local investigating authority, under advisement of from the regional legal service, will select the most appropriate section of the act in which to charge. Some of the most difficult charges to level involve the non-consensual offences covered in Table 1 as they require some evidence that the defendant (A) has taken steps to ascertain that the complainant (B) does not consent but continues with the sexual offence regardless.

### 1.2. Starting a criminal investigation

Investigating sexual offences requires a multi-faceted approach with a number of different individuals and organisations involved in the process (Fig. 1). Principally the Police are responsible for overseeing the early stages of an investigation and are responsible for taking initial statements. Interviews should be conducted in private and the victim can ask for a female officer if they prefer [9]. Where possible the initial interview is performed by a Specially Trained Officer (STO) who has expertise in sexual assault casework. Such officers are also termed Sexual Offences Investigative Techniques trained officers (SOIT Officers) or Police Sexual Offence Liaison Officer (SOLO) [10-12] who will be a single point of contact during the case. Following the initial interview the STO is required to take a formal statement in the form of a written account or a video recording from the victim which may be used as evidence later. The victim will also be offered the chance to provide a Victim Personal Statement, which may be given to the prosecution team to inform the court about how the crime personally affect the victim. A suspect may be initially identified if they are known to the victim, or where they are unknown, be identified using CCTV evidence, mug shots, fingerprint evidence or DNA database searching. The case is also assigned an Officer In the Case (OIC) who has responsibility for ensuring that the case is progressed correctly and has operational oversight, while the STO deals with the day to day investigation. It is the OIC who decides whether there is sufficient evidence to arrest the suspect who may then be formally identified through an identity parade. It is also the OIC's responsibility to collect and preserve any evidence relevant to the case, which can involve conducting witness interviews, visiting the crime scene to collect evidence, arrange for a forensic medical examination (see Section 2), determine which evidence to send for forensic analysis (see Section 3, 4, and 5). The STO also acts as a point of contact for the prosecution (see Section 6) and is also responsible for directing the victim to any support services required.

## 2. Evidence collection

### 2.1. Types of evidence

The most common class of evidence sought during the forensic medical exam is biological material, specifically body fluids originating from the complainant and/or defendant to support the allegation that a sexual assault has occurred (Table 2).

***Seminal material*** is the most common body fluid of interest after a sexual assault as it is directly attributable to the male suspect and strongly indicative of a sexual act having occurred. Semen is composed of a fluid component called seminal plasma and spermatozoa. The fluid contains a mixture of salts, sugars, lipids, enzymes (Acid Phosphatase), nutrients, proteins (p30, Prostate-Specific Antigen), hormones, basic amines (spermine), and flavins. DNA in semen is found in the spermatozoa together with 'free DNA' from sloughed epithelial cells. Individual spermatozoa are commonly observed in sexual assault casework and there is some discrepancy in the literature regarding their maximum persistence within the vaginal cavity with observations between 3 and 12 days recorded [13]. While there appears to be a large amount of variation in retention times, the recommendations from the Faculty of Forensic and Legal Medicine of the Royal College of Physicians suggests semen collection should be within 48 h from the victims mouth, 72 h from the victims anus, and seven days from vicitms vagina (Table 2).

***Saliva*** can support the allegation of sexual assault between the complainant and the defendant if suspect saliva is detected on intimate swabs taken during the medical examination or if victim saliva is detected on the defendants underwear and vice-versa. Saliva is 99.5% water with the remaining components being mucus, white blood cells, epithelial cells, enzymes (amylase and lipase) and secretory agents such as IgA and lysozymes. DNA in saliva is primarily found in the white blood cells together with sloughed cells from the inner lining of the mouth. Salivary amylase indicative of saliva has been observed on 25% of penile swabs and 32% of vaginal swabs during a review of 400 sexual assault cases [14], although recent research using an alternative testing method was only able to confirmed the presence of amylase on 8% of vaginal swabs [15]. Guidence from the Faculty of Forensic and Legal Medicine suggests saliva collection should be within 72 h from the victims anus, 3 days from the sucpects penis and seven days from vicitms vagina/vulva (Table 2).

***Blood*** is one of the most common body fluids found at crime scene and is important in understanding the issue of consent in sexual assault casework. Blood is made up of liquid (plasma) and solid parts including red blood cells (containing the haemoglobin protein), white blood cells (containing DNA) and platelets (containing glycoproteins, microtubules and clotting mediators). The presence of peripheral blood (as opposed to

**Table 1**
Non-consenting sexual acts as defined by relevant UK legislation.

| Offence | Overarching Legislative Definition | Relevant Legislative Act | Section number under relevant Act | Possible Penalty / Outcome |
|---|---|---|---|---|
| Rape | A person (A) commits an offence if -<br>(a) he intentionally penetrates the vagina, anus or mouth of another person (B) with his penis,<br>(b) B does not consent[X] to the penetration, and<br>(c) A does not reasonably believe[Z] that B consents[X]. | Sexual Offences Act 2003Sexual Offences (Norther Ireland) Order 2008 | 15 | Assuming that section 75 and 76 apply* (section 9 and 10 for Northern Ireland), a person guilty of an offence under this section is liable, on conviction on indictment, to imprisonment for life. |
| | If a person ("A"), with A's penis—<br>(a) without another person ("B") consenting[W], and<br>(b) without any reasonable belief that B consents[W],<br>penetrates to any extent, either intending to do so or reckless as to whether there is penetration, the vagina, anus or mouth of B then A commits an offence | The Sexual Offences (Scotland) Act 2009 | 1 | A person guilty of an offence under this section is liable, on conviction on indictment, to Life imprisonment and a fine |
| Assault by penetration | A person (A) commits an offence if -<br>(a) he intentionally penetrates the vagina or anus of another person (B) with a part of his body or anything else,<br>(b) the penetration is sexual[Y],<br>(c) B does not consent[X] to the penetration, and<br>(d) A does not reasonably[Z] believe that B consents[X]. | Sexual Offences Act 2003Sexual Offences (Norther Ireland) Order 2008The Sexual Offences (Scotland) Act 2009 | 26 | Assuming that section 75 and 76 apply* (section 9 and 10 for Northern Ireland), a person guilty of an offence under this section is liable, on conviction on indictment, to imprisonment for life.A person guilty of an offence under this section is liable, on conviction on indictment, to Life imprisonment and a fine |
| | If a person ("A"), with any part of A's body or anything else—<br>(a) without another person ("B") consenting[W], and<br>(b) without any reasonable belief that B consents[W],<br>penetrates sexually to any extent, either intending to do so or reckless as to whether there is penetration, the vagina or anus of B then A commits an offence, | | 2 | |
| Sexual assault | A person (A) commits an offence if -<br>(a) he intentionally touches another person (B),<br>(b) the touching is sexual[Y],<br>(c) B does not consent[X] to the touching, and<br>(d) A does not reasonably[Z] believe that B consents[X]. | Sexual Offences Act 2003Sexual Offences (Norther Ireland) Order 2008 | 37 | Assuming that section 75 and 76 apply* (section 9 and 10 for Northern Ireland), a person guilty of an offence under this section is liable (a) on summary conviction, to imprisonment for a term not exceeding 6 months or a fine not exceeding the statutory maximum or both; (b) on conviction on indictment, to imprisonment for a term not exceeding 10 years. |
| | If a person ("A")—<br>(a) without another person ("B") consenting[W], and<br><br>(b) without any reasonable belief that B consents[W],<br>does any of the things mentioned in subsection 3.2*, then A commits an offence | The Sexual Offences (Scotland) Act 2009 | 3 | A person guilty of an offence under this section is liable, on summary conviction, to Imprisonment for a term not exceeding 12 months or a fine not exceeding the statutory maximum (or both)<br>A person guilty of an offence under this section is liable, on conviction on indictment, to life imprisonment or a fine (or both) |
| Causing a person to engage in sexual activity without consent | A person (A) commits an offence if -<br>(a) he intentionally causes another person (B) to engage in an activity,<br>(b) the activity is sexual[Y],<br>(c) B does not consent[X] to engaging in the activity, and | Sexual Offences Act 2003Sexual Offences (Norther Ireland) Order 2008 | 48 | Assuming that section 75 and 76 apply* (section 9 and 10 for Norther Ireland), a person guilty of an offence under this section is liable (a) on summary conviction, to imprisonment for a term not exceeding 6 months or a fine not exceeding the statutory maximum or both; (b) on conviction on indictment, to imprisonment for a term not exceeding 10 years. If section 75, 76 and subsection 4.4* appy (section 9 |

(*continued on next page*)

**Table 1** (*continued*)

| Offence | Overarching Legislative Definition | Relevant Legislative Act | Section number under relevant Act | Possible Penalty / Outcome |
|---|---|---|---|---|
| | (d) A does not reasonably[Z] believe that B consents[X]. | | | and 10 and subsection 8.4* for Norther Ireland) a person guilty of an offence under this section, is liable, on conviction on indictment, to imprisonment for life. |
| Sexual coercion | If a person ("A")—<br>(a) without another person ("B") consenting[W] to participate in a sexual activity, and<br>(b) without any reasonable belief that B consents[W] to participating in that activity,<br>intentionally causes B to participate in that activity, then A commits an offence | The Sexual Offences (Scotland) Act 2009 | 4 | A person guilty of an offence under this section is liable, on summary conviction, to Imprisonment for a term not exceeding 12 months or a fine not exceeding the statutory maximum (or both)<br>A person guilty of an offence under this section is liable, on conviction on indictment, to life imprisonment or a fine (or both) |

[W] - "Consent" - Under Scots Law "consent" means free agreement.

[X] - Under English and Northern Irish Law a person consents if he agrees by choice, and has the freedom and capacity to make that choice.

[Y] - "Sexual" - penetration, touching or any other activity is sexual if a reasonable person would consider that (a) whatever its circumstances or any person's purpose in relation to it, it is because of its nature sexual, or (b) because of its nature it may be sexual and because of its circumstances or the purpose of any person in relation to it (or both) it is sexual.

[Z] - Whether a belief is reasonable is to be determined having regard to all the circumstances, including any steps A has taken to ascertain whether B consents.

* Subsection 3.2 - Those things are, that A (a) penetrates sexually, by any means and to any extent, either intending to do so or reckless as to whether there is penetration, the vagina, anus or mouth of B, (b) intentionally or recklessly touches B sexually, (c) engages in any other form of sexual activity in which A, intentionally or recklessly, has physical contact (whether bodily contact or contact by means of an implement and whether or not through clothing) with B, (d) intentionally or recklessly ejaculates semen onto B, (e) intentionally or recklessly emits urine or saliva onto B sexually.

* Subsection 4.4 (8.4 for Northern Ireland) - A person guilty of an offence under this section, if the activity caused involved (a) penetration of B's anus or vagina, (b) penetration of B's mouth with a person's penis, (c) penetration of a person's anus or vagina with a part of B's body or by B with anything else, or (d) penetration of a person's mouth with B's penis, is liable, on conviction on indictment, to imprisonment for life.

* Section 75 (9 for Northern Ireland) - If in proceedings for an offence to which this section applies it is proved that the defendant did the relevant act, and knew that (a) any person was, at the time of the relevant act or immediately before it began, using violence against the complainant or causing the complainant to fear that immediate violence would be used against him; (b) any person was, at the time of the relevant act or immediately before it began, causing the complainant to fear that violence was being used, or that immediate violence would be used, against another person; (c) the complainant was, and the defendant was not, unlawfully detained at the time of the relevant act; (d) the complainant was asleep or otherwise unconscious at the time of the relevant act; (e) because of the complainant's physical disability, the complainant would not have been able at the time of the relevant act to communicate to the defendant whether the complainant consented; (f) any person had administered to or caused to be taken by the complainant, without the complainant's consent, a substance which, having regard to when it was administered or taken, was capable of causing or enabling the complainant to be stupefied or overpowered at the time of the relevant act; the complainant is to be taken not to have consented to the relevant act.

* Section 76 (section 9 for Northern Ireland) - If in proceedings for an offence to which this section applies it is proved that the defendant did the relevant act and that (a) the defendant intentionally deceived the complainant as to the nature or purpose of the relevant act; (b) the defendant intentionally induced the complainant to consent to the relevant act by impersonating a person known personally to the complainant, it is to be conclusively presumed that the complainant did not consent to the relevant act, and that the defendant did not believe that the complainant consented to the relevant act.

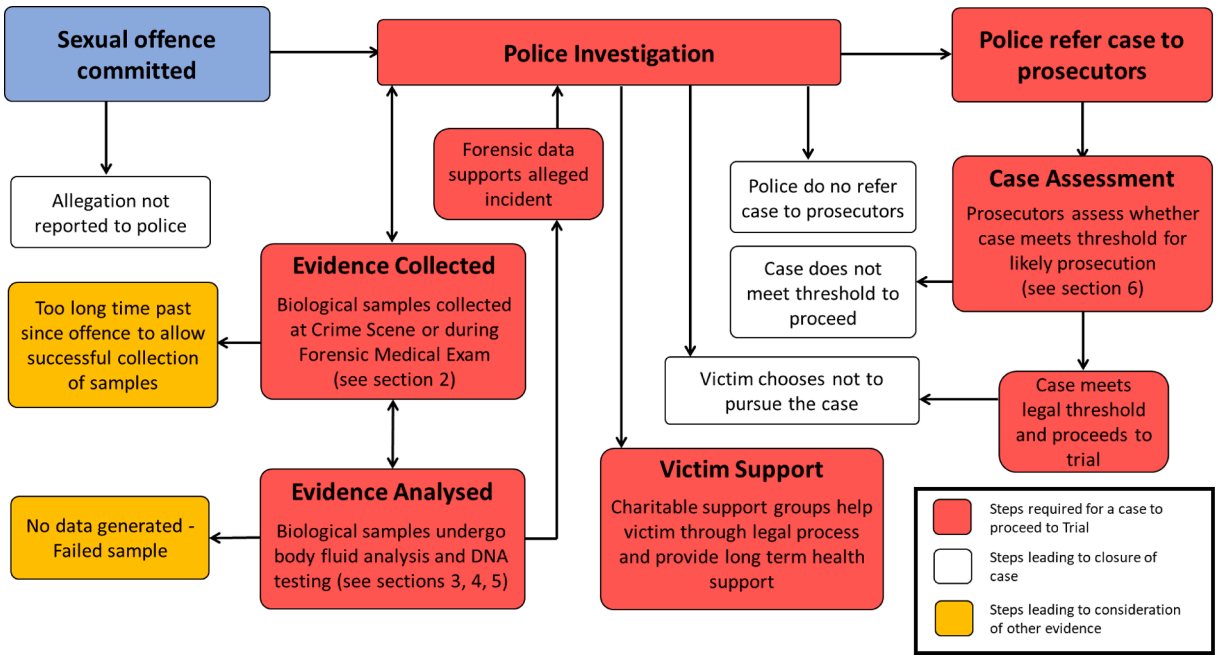


**Fig. 1.** Workflow detailing key elements of a sexual offence investigation. Developed from refs [10-12].

menstrual blood) recovered from vaginal swabs post assault can indicate trauma suggesting a violent and non-consensual act [16] with genital injury cited as occurring in up to 45% of female rape cases [17]. Guidence from the Faculty of Forensic and Legal Medicine make no specific recommendations around the collection times associayed with blood samples. (Table 2).

***Vaginal fluid*** is also relevant in sexual assault casework and the detection of victim vaginal fluid on the suspect's penis or penetrative object (i.e. finger, bottle, sex toy) supports the allegation that a sexual act has occurred [18]. Vaginal fluid is composed primarily of cervical mucus (water, mucin proteins and soluble proteins), vaginal and cervical cells, and bacteria (predominantly lactobacillus). This sample type has historically been identified using microscopic approaches with data suggesting that vaginal epithelial cells from penile swabs were detected in 50% of samples five days after intercourse [19], with a more recent study detecting cells on 38% samples up to 72 h [20]. Guidence from the Faculty of Forensic and Legal Medicine make no specific recommendations around the collection times associayed with vaginal fluid samples. (Table 2).

***Hair*** is an important forensic sample type to consider and is comprised of 65–95% keratin protein, 15–35% water, 1–9% lipids and < 1% minerals. Transfer of pubic hair from the suspect to the victim of interest for DNA testing with research showing that transfer occurs more frequently from men to women (23.6%) than from women to men (10.9%) [21]. Hair collected from the back of the victims head is also taken for toxicological analysis with data showing common chemicals used in Drug Facilitated Sexual Assault (DFSA) being detected months after exposure [22]. Guidence from the Faculty of Forensic and Legal Medicine suggest routinely collecting transferred hairs while hair for toxicicological analysis should be taken a minimum of 4–6 weeks after the date of interest.

### 2.2. Collection by sexual assault referral centre

In sexual assault investigations the victim's body becomes akin to the crime scene itself and the victim can request or be advised to seek a forensic medical examination by specially trained nurses working within a Sexual Assault Referral Centre (SARC). Such centres can be funded directly by the National Health Service (NHS), e.g. The Havens [23] and Saint Mary's [24] or be run privately but in collaboration with the NHS, e.g. Mountain Healthcare [25] or G4S [26]. The Forensic Medical Examination performed within these centres follow guidance outlined by the Faculty of Forensic and Legal Medicine of the Royal College of Physicians [27,28] who provide proforma examination templates to use during the assessment of both complainants [29] and suspects [30]. The guidance states which sample types should be considered, provides a time limit for collection based on time passed since the assault, states the reason for the collection and analysis, and briefly explains how to sample, package and store the evidence after collection [28]. Collection typically involves a series of swabs of the skin surface, mouth area, and ano-genital area, while the collection of victim hair and nail clipping is common to test for the presence of chemicals commonly used in Drug Facilitated Sexual Assault (DFSA) [31,32]. Photographic evidence of any trauma are also collected as evidence of non-consensual behaviour (see Section 5). Clothing worn at the time of the assault can also be collected together with bedding, condoms and objects used in the assault if available. If the victim prefers, both Police and SARCs can also provide the victim with an early evidence kit for self swabbing at home. Such kits contain swabs, containers and evidence bags for the collection of urine and cavity samples following similar proceedures to those followed by SRAC staff. Once secured the evidence is transferred to laboratories for further examination.

### 2.3. Collection by forensic laboratory

Once an evidence item enters a forensic laboratory it is logged and input into the Laboratory Management Information System (LIMS) and a Reporting Officer (RO) is assigned (Fig. 2). Unless the evidence is provided by the SARC in the form of a swab, there may be a requirement for further evidence examination. For example, clothing or bed linen may undergo a 'first sweep' visual examination followed by a 'secondary sweep' with a magnifying examination lamp and sometimes a fluorescent light to improve contrast and aid detection. The biological materials most readily identified at this stage are visible stains (blood, semen, saliva) and trace evidence (hairs and hair shafts). Identified stains are typically swabbed directly to recover the biological material [33-36], but may also undergo 'scraping' if the stain is dried [37]. The recovery of the material aims to a) identify the nature of the biological material and

**Table 2**
Common samples and forensic tests used in body fluid identification.

| Sample of interest | Location of sample | Time scale for collection post assault | Supports allegation of: | Forensic tests available to detect sample of interest | 'Reactive' component in sample of interest |
|---|---|---|---|---|---|
| Suspect's semen | Victim's vagina | 7 days | Rape | Acid Phosphatase test | Acid Phosphatase |
| | Victim's vulva | 7 days | Sexual Assault | Florence Iodine test | Choline |
| | Victim's anus | 72 hrs | Rape | ABA card p30 | Prostate specific antigen (PSA) |
| | Victim's mouth | 48 hrs | Rape | PSA Semiquant | Prostate specific antigen (PSA) |
| | Victims hands/ fingernails | 48 hrs – 168 hrs | Sexual Assault | Microscopy | Single sperm heads |
| | Victim's underwear | No time limit specified | Sexual Assault | RSID™ Semen | Semenogelin |
| | Victim's sanitary towls/ tampons | No time limit specified | Sexual Assault | mRNA based methods | e.g. PSA, PRM1, PRM2, SEMG1, TGM4 |
| | Victim's clothing | No time limit specified | Sexual Assault | microRNA based methods | e.g. miR10a, miR10b, miR135a, miR135b, miR888, miR891a |
| | Bedding/covers | No time limit specified | Sexual Assault | | |
| | Condom | No time limit specified | Sexual Assault | | |
| Saliva | Suspect's saliva from: | | | Phadebas Test | Amylase |
| | • Victim's vagina | 7 days | Rape | Seretec Amylase test | Alpha-amylase |
| | • Victim's vulva | 7 days | Sexual Assault | RSID™ Saliva | Human salivary-Amylase Antigen |
| | • Victim's anus | 72 hrs | Rape | mRNA based methods | e.g. HTN3, STATH, MUC7 |
| | • Victim's underwear | No time limit specified | Sexual Assault | microRNA based methods | e.g. miR205, miR658, miR583, miR518c, miR208b |
| | Victim's saliva from suspect's penis | 3 days | Rape | | |
| Victim's blood | Victim's vagina | No time limit specified | Intercourse was violent | Kastle mayer | Hemoglobin |
| | Victim's vulva | | | Hemeastix | Hemoglobin |
| | Victim's anus | | | ABA card Hematrace | Hemoglobin |
| | Victim's underwear | | | Leucomalachite green | Human Glycophorin A Antigen |
| | Bedding/covers | | | BlueStar OBTI | Hemoglobin and monoclonal anti-human Hb antibodies |
| | Suspect's penis | | | RSID™ Blood | Human Glycophorin A Antigen |
| | Condom | | | mRNA based methods | e.g. HBB, SPTB, PBGD, ALAS2 |
| | suspects underwear | | | microRNA based methods | e.g. miR20a, miR106a, miR185, miR451, miR16, |
| | Penetrative object, i.e. bottle | | | | |
| Victim's menstrual blood | As for arterial blood | No time limit specified | Used to differentiate with real blood | Seretec PMB | human hemoglobin and D-dimer |
| | | | | mRNA based methods | e.g. MMPP7, MMP11, |
| | | | | microRNA based methods | e.g. miR185, miR144, miR451, miR412, miR214 |
| Victim's vaginal fluid | Suspect's penis | No time limit specified | Rape | mRNA based methods | e.g. MMP11, HBD1, MUC4, CYP2B7P1, MYOZ1 |
| | Condom | | Rape | microRNA based methods | e.g. miR617, miR891a, miR124a, miR372, |
| | suspects underwear | | Sexual Assault | | |
| | Penetrative object (finger, bottle, etc) | | Assault by penetration | | |
| Suspect's skin cells | Victim's vagina | 48 hrs | Assault by penetration | mRNA based methods | e.g. CDSN, LOR, KRT9 |
| | Victim's anus | 48 hrs | | microRNA based methods | e.g. miR205, miR203a |

Time frames for collection are taken from Faculty of Forensic and Legal Medicine Recommendations for the collection of forensic specimens from complainants and suspects [28].

assess whether it's presence/absence supports/refutes the sexual activity alleged, b) identify whether the DNA from the suspect matches the DNA recovered from the evidence, and c) assess what evidence there is to suggest the act was non-consensual.

## 3. Body fluid identification methods used to infer alleged sexual offence

Identifying whether the evidence collected contains the presence of a specific body fluid can begin to add weight to the hypotheses that an alleged sexual offence has occurred. The field of forensic body fluid identification is well established and routinely used in sexual assault investigation with tests either be described as presumptive or confirmatory.

### 3.1. Presumptive body fluid testing

Presumptive tests are quick, easy and cheap to use and give the forensic analyst some initial information about the sample in question [38]. They remain a popular part of a forensic analyst's tool-kit as they can help screen samples and inform the forensic workflow (Fig. 2). Presumptive tests are often chemical in nature and are designed to undergo some form of colour-change reaction when in the presence of the body fluid of interest. However, they may also cross-react with other substances or be unable to detect the sample of interest at very low levels. Consequently, presumptive test results may not necessarily be treated as '*of evidential standard*' [39], but can inform the forensic process.

There are a large number of presumptive tests available for use

(Table 2) with [40] providing an excellent review on specific tests. For this review, we have limited our discussion to those body fluid tests most commonly used by forensic bioanalytical scientists after checking these methods are validated for application by the three main Forensic Providers in England (Eurofins Forensic Services Ltd [41], Cellmark Ltd [42] and Key Forensic Services Ltd [43]) on the UK Accreditation Services (UKAS) website [44]. The full list of validated tests provided by each Forensic laboratory can be found on the UKAS page [45].

### 3.1.1. Semen testing

The Acid Phosphatase (AP) test for seminal plasma (Table 2) has been in routine use as early as the 1940 s [46]. This component of semen is produced by the prostate but other body fluids such as blood, saliva, vaginal secretions also contain this protein but at much lower levels [47], meaning that weak, false positive reactions can occur in these tissues without semen being present [38,40]. The chemical reaction uses the chemicals α-napthyl acid phosphate and diazo blue dye in a buffered solution. Application of these two reagents will produce a purple colour in the presence of AP (Fig. 3c). The strength of the reaction will depend on the amount of AP, with a dark purple indicating a strong reaction. The test is cheap and easy to perform although it is advised that the sample is not tested directly because the chemicals used in the test may damage DNA required for downstream analysis. Therefore, the sample is either swabbed or transferred to filter paper for testing. Research data shows that while popular, the AP test is a low predictor for the presence of sperm on intimate swabs suggesting that Time Since Intercourse [TSI] guidelines should be used when considering the use of presumptive tests [48]. Furthermore, false negative AP test results were reported in 3% of casework samples that subsequently underwent microscopic analysis [49]. An alternative presumptive test for semen used by Forensic Service Providers in the United Kingdom is the Florence Iodine Test [50] which reacts to the presence of choline which occurs in seminal fluid in high concentrations. Iodine reacts with choline to form characteristic brown choline periodide crystals. More crystals form with a greater amount of choline meaning that a spectrum of strong and weak reactions can be observed [51]. Detection of crystals occurs through microscopic approaches and shows that the test is sensitive but is less specific than AP testing as choline naturally occurs in a variety of food stuffs [51].

### 3.1.2. Saliva testing

A standard chemical test for the detection of saliva is the Phadebas® Press Test (Table 2), which detects the α-amylase component present in saliva [52]. Forensic analysts can either formulate their own Phadebas suspension using dissolvable tablets in a test-tube for swab analysis [53] or purchase as a pre-treated paper to perform a press test against clothing [54]. The chemical reaction works by binding a blue dye to a bio-degradable starch microsphere (DSM) which is insoluble in water when bound. In the presence of α-amylase the DSM is degraded and the blue dye is released (Fig. 3d). The test is relatively specific although amylase is found in other body fluids including faecal material [52], mucous [55], breast milk, vaginal secretion [56], semen [57] and urine and sweat [58]. It is therefore recommended that a substrate control sample (one taken from another area) also be submitted for downstream analysis if a positive result is obtained from the samples. In addition to cross reactivity, studies have demonstrated that the presence of saliva can also occur due to secondary transfer and does not always suggest a sexual assault has occurred [59,60]. Research has also looked to define specific validation criteria to evaluate the performance of Phadebas as a screening tool for triaging evidential samples [61], although data suggests it is not sensitive enough and cross-reacts with other forensically relevant body fluids. While this may prevent to use of the approach in this manner, the tests does not inhibit downstream DNA profiling [62] and continues to be used as a presumptive test.

### 3.1.3. Blood testing

A standard test for the detection of blood is the Kastle-Meyers (KM) test, which can be directly applied to suspected bloodstains at the crime scene or in the laboratory (Table 2). This method is most useful when the stain is already identifiable by eye and has been shown to be less sensitive than luminol formulations [63]. Despite these apparent limitations the test remains popular due to its ease of use. The chemical reaction works through the chemical phenolphthalein which reacts with the haemoglobin in the blood stain. Again, using hydrogen peroxide as an activator to catalyze the oxidation of phenolphthalin into

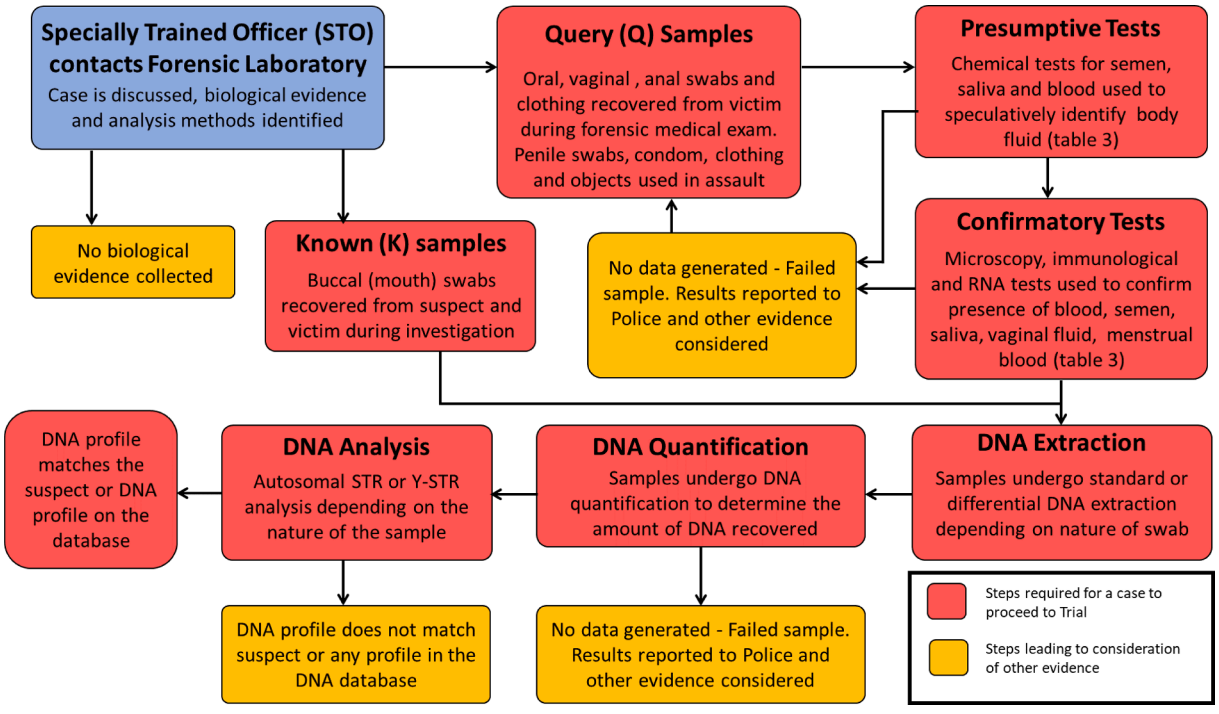


**Fig. 2.** Forensic Laboratory workflow to determine the body fluid present on the sample and the identity of the individual based on DNA typing.

phenolphthalein. This is observed as a colour change from 'clear' to 'bright pink' (Fig. 3a). The KM test shows low cross reactivity with other body fluids but does react with menstrual blood and further false positives have been noted with some chemical oxidants and fruit or vegetable peroxidases [40]. Further disadvantages of the test is that it is time sensitive and waiting for longer than 30 s will result in most samples gradually turning pink. It is therefore important to run negative and positive samples and have a maximum amount of time identified through validation studies. Broadly speaking, the KM test shows sensitivity and ease of use in line with a number of other presumptive tests for blood, hence its continued use [64]. Hemastix are a commercially available plastic strip detection system for blood which uses the peroxidase-like activity of haemoglobin to catalyse the reaction of 3,3′, 5,5′-tetramethylbenzidine from its reduced colourless state to its oxidised coloured form. The presence of di-isopropylbenzene di-hydroperoxide on the plastic strip reacts with the haemoglobin to cause a colour change (Fig. 3b). Research shows that the Hemastix approach is easy and safe to use, sensitive at extremely low levels of blood [65] but can cause a reduction in the amount of DNA recovered from the blood under some circumstances [66,67]. As such it is necessary to test a subsample of the identified stain by first removing a small amount using a moistened swab. The test also shows cross-reactivity with menstrual blood as well as non-forensic sample types [64].

### 3.2. Confirmatory body fluid testing

Confirmatory tests, as their name suggests, confirm the results of early presumptive tests and are designed to provide a highly accurate result that can be presented as evidence in court. Consequently, these tests may be more labour intensive, require more expertise to perform and have higher associated costs. Current confirmatory tests for body fluids typically use either microscopy or immunological strip tests with research into mRNA and microRNA based methods struggling to be routinely employed.

#### 3.2.1. Microscopy

The use of microscopy to confirm the presence of spermatozoa on sexual assault evidence is considered one of the gold standard confirmatory methods even when other methods are also used [71] (Table 2). Prior to visualisation, seminal material is transferred to a microscope slide by wetting the swab head and wiping the swab across the slide before the slide is stained to help visualise the individual spermatozoa. Common staining methods include hematoxylin-eosin [72,73], nuclear fast red and picroindigocarmine (Christmas tree staining) [74,75], alkaline fuchsin [76,77] and Baecchi's staining [78]. In these methods, the cells are observed with chemical dyes, allowing spermatozoa to be differentiated from other cells based on their physical structure. Problems may occur however when forensic samples are degraded, resulting in a loss of the tail from the sperm head, or when there is a mixture of sperm epithelial cells and microorganisms [71]. The process is performed by a trained laboratory scientist with specialism in microscopy and research suggests that the Christmas tree staining is the most appropriate for use [71] (Fig. 4a). Comparison of the Christmas tree technique to AP testing and PSA testing (using the One Step ABA card PSA™) showed a degree of concordance in detection sensitivity between microscopy and AP testing, while the PSA testing gave positives at low levels of seminal material which were not detectable by the Christmas tree staining. Given that microscopic approaches are considered the gold standard the results suggest that use of the PSA test may give 'false positives' in some instances and highlights that having an extremely low level of detection is only useful if it shows concordance with techniques used later in the workflow [79]. The last ten years has seen the release of a commercial staining kit termed SPERM HY-LITER™ [80] which uses 4′6-diamidino-2-phenylindole (DAPI) staining for nuclei of the sperm cells as well as a fluorescently labelled antibody that stains a protein that is specifically expressed in the human sperm head [81]. This approach means that the spermatozoa of other species are not detected. The recent release of the SPERM HY-LITER Express™ kit has increased the sensitivity of detection using this method by using a different fluorescently labelled antibody [82]. Combination staining using HY-LITER and other

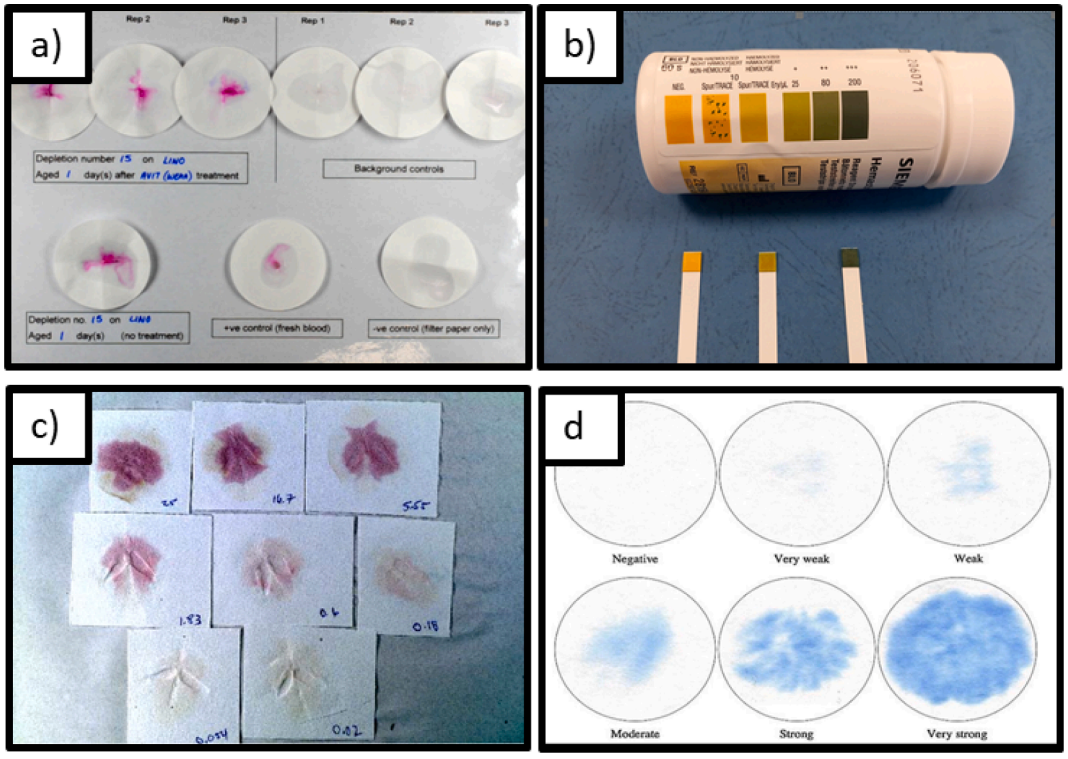


**Fig. 3.** Results from different presumptive forensic tests used in the analysis of sexual assault samples with a) KM test (image taken from [68]), b) Hemastix, c) Acid Phosphatase test (image taken from [69]), and d) Phadebas test image (taken from [70]).

approaches is also possible. After visual detection, images are recorded for the case file and based on the results the swab is sent for DNA testing (see Section 4). In instances when there are very few visualised sperm heads or the victim cells may be in much greater numbers than the suspect cells (e.g. intimate swabs from the forensic medical examination), it may be necessary to recover individual spermatozoa from the sample using Laser Capture Microdissection (LCM). This method combines existing light microscopic instrumentation with laser beam technology allowing the separation of cells into separate tubes for direct DNA analysis [83]. The two developed systems either use ultraviolet cutting systems [84] or infrared capture systems [85] and avoids the use of differential DNA extraction. It is a more powerful approach than the presumptive tests described above with research showing that microscopic methods capable of detecting sperm heads after six washes while other method ceased to detected the presence of seminal material after two washes [86].

### 3.2.2. Immunological tests

There is some disagreement in the literature as to whether commonly available immunological tests satisfy the requirements of a confirmatory test or should instead be considered alongside other presumptive tests. This stems from observed instances of false positives and false negatives leading to low results' concordance between different testing methods. The authors have chosen to include the tests in this section to differentiate them from the presumptive chemical tests described in the previous section, as their relative performances are greatly improved. All immunological tests function in the same way, by producing artificial antibodies in a laboratory that match the analyte of interest (Table 2). The antibodies are affixed to a chromatographic test strip that changes colour when mixed with the sample of interest [87]. Swabs from the sample are first mixed with a supplied lysis buffer which can either be loaded directly onto the chromatographic strip well or be further mixed with a 'running' buffer before loading. These tests take minutes to use and yield results within 10–20 min. Tests have been developed for the forensic detection of semen (ABA card p30, PSA Semiquant, RSID™ Semen, Fig. 4 b, c and d respectively) with comparison studies kits suggesting each kit is more sensitive than the AP test under similar test conditions with little cross reactivity between body fluids [88,89].

Tests comparing different immunological methods suggest that the RSID™ Semen test is less sensitive than the ABA card p30 [94], although this may actually represent over-sensitivity with regards to the ABA card test leading to false positives [79]. Furthermore, studies have shown that there is a low level of cross reactivity with saliva swabs and vaginal swabs [95] and some of these tests cross react with energy drinks if the commercial buffer is changed with PBS [96]. Immuno-chromatographic tests have also been developed for the forensic detection of saliva (Seretec Amylase test, RSID™ Saliva) which work following the same process. Comparison studies for saliva test kits show that they are more sensitive than Phadebas although the Seretec Amylase test shows some cross reactivity (8%) with vaginal swabs [95] while the RSID test shows some cross reactivity with breast milk, urine and sweat [97,98]. Furthermore, time series experiments shows that salivary alpha amylase was detectable at 40 days [99,100] suggesting that such tests may be useful for screening samples that have been stored over time when there is a backlog. Immuno-chromatographic tests strips for blood (ABA card Hematrace, RSID™ Blood) show the RSID kit is less sensitive compared to both the ABAcard Hematrace and to KM testing [68,101]. Tests for menstrual blood such as the Seretec PMB show little cross reactivity to other relevant body fluids or to other species and is designed to simultaneously detect peripheral blood [16]. This 'multiplexing' approach is certainly attractive from an end user perspective and has led to the in-house construction of an immuno-chromatographic test strip for the detection of five body fluids simultaneously [102]. While not currently a commercial product, it represents a possible future developmental application for biotech industry scientists.

Despite ongoing research leading to the development of new

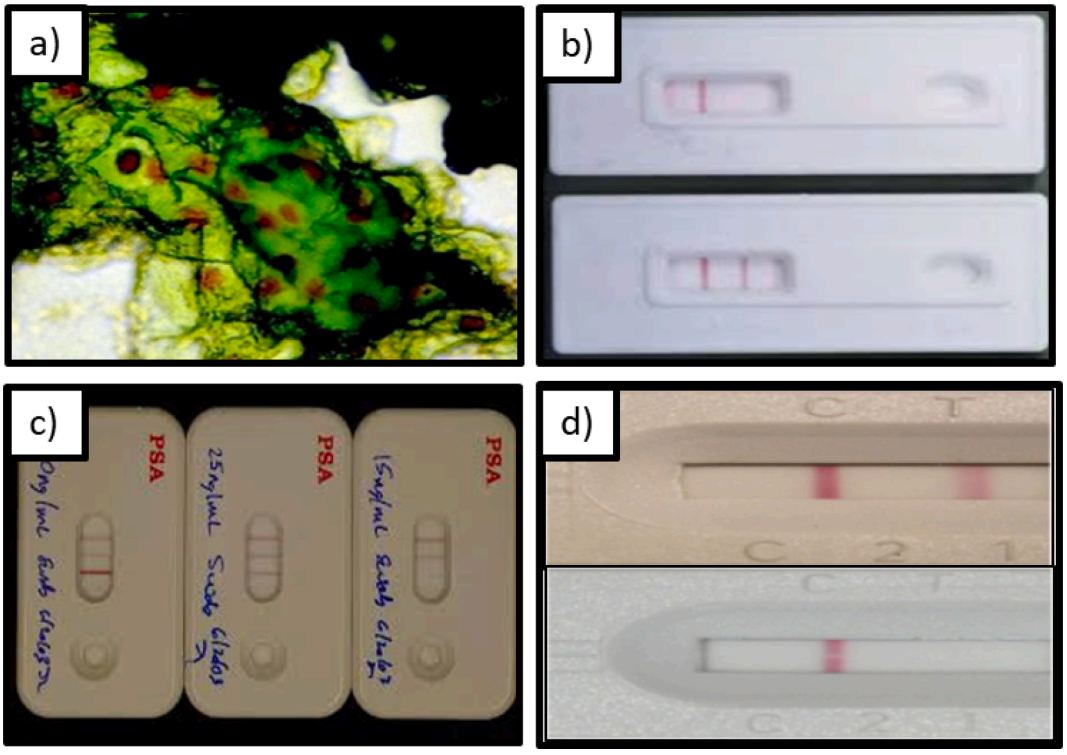


**Fig. 4.** Results from different forensic tests used in the analysis of sexual assault samples with a) Christmas tree stain showing pink spermatozoa (image taken [90]), b) ABAcard p30 showing native (above) and positive (below) test strips (image taken from [91]), c) Seretec PSA test showing strong positive results at two concentrations of semeinal material (image taken from [92]), and d) RSID semen test showing positive (above) and negative (below) test strips from a cross reactivity study (image taken from [93]). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 3**
Common DNA extraction, quantification and DNA amplification kits used in the processing of sexual assault samples.

| Analysis Stage | Example Forensic Consumables available to use | Supplier | Method of isolation/detection | Target |
|---|---|---|---|---|
| DNA Extraction | QIAamp DNA Investigator kit | Qiagen | Silica matrix column | Cellular DNA from victim and/or suspect |
| | Investigator STAR Lyse&Prep Kit | Qiagen | Magnetic bead capture | |
| | PrepFiler™ DNA Extraction kit | Thermo Fisher | Magnetic bead capture | |
| | ChargeSwitch™ Forensic DNA Purification Kit | Thermo Fisher | Magnetic bead capture | |
| | DNA IQ™ System | Promega | Magnetic resin capture | |
| | Differex™ System | Promega | Magnetic resin capture | |
| DNA Quantification | Investigator Quantiplex HYres Kit | Qiagen | Scorpion® probes | Automsoal target, male target |
| | Quantifiler™ Duo | Thermo Fisher | TaqMan® probes | |
| | Plexor® HY | Promega | Dabcyl-*iso*-dGNTP quenching | |
| | Quantifiler™ Trio | Thermo Fisher | TaqMan® probes | Large/small autosomal target, male target |
| | PowerQant® System | Promega | Hydrolysis probes | |
| | Investigator Quantiplex® Pro | Qiagen | TaqMan® probes | |
| | InnoQuant® HY-R | InnoGenomics Technologies | Probe based | |
| | Investigator Quantiplex® Pro RGQ | Qiagen | TaqMan® probes | Large/small autosomal target, large/small male target |
| DNA Amplification | Investigator ESSplex SE QS Kit | Qiagen | 6-FAM, BTG, BTY, BTR fluorescently labelled primers | 17 Automsomal STRs |
| | AmpFlSTR NGM SElect PCR Amplification Kit | Thermo Fisher | FAM,LIZ,NED,PET,VIC fluorescently labelled primers | |
| | PowerPlex® ESI 17 Fast System | Promega | Fluorescein, JOE, TMR-ET, CXR-ET and WEN labelled primers | |
| | Investigator Argus Y-12 QS Kit | Qiagen | 6-FAM, BTG, BTY fluorescently labelled primers | 12 Y-chromosomal loci |
| | AmpFLSTR™ Yfiler™ PCR Amplification Kit | Thermo Fisher | FAM,LIZ,NED,PET,VIC fluorescently labelled primers | 17 Y-chromosomal loci |
| | PowerPlex® Y23 System | Promega | Fluorescein, JOE, TMR-ET, CXR-ET and WEN labelled primers | 23 Y-chromosomal loci |
| DNA Size Separation | Applied Biosystems SeqStudio Genetic Analyzer | Thermo Fisher | Fragment analysis through polyacrylamide matix | Fluoresently labelled STR fragments |
| | Spectrum CE System | Promega | | |

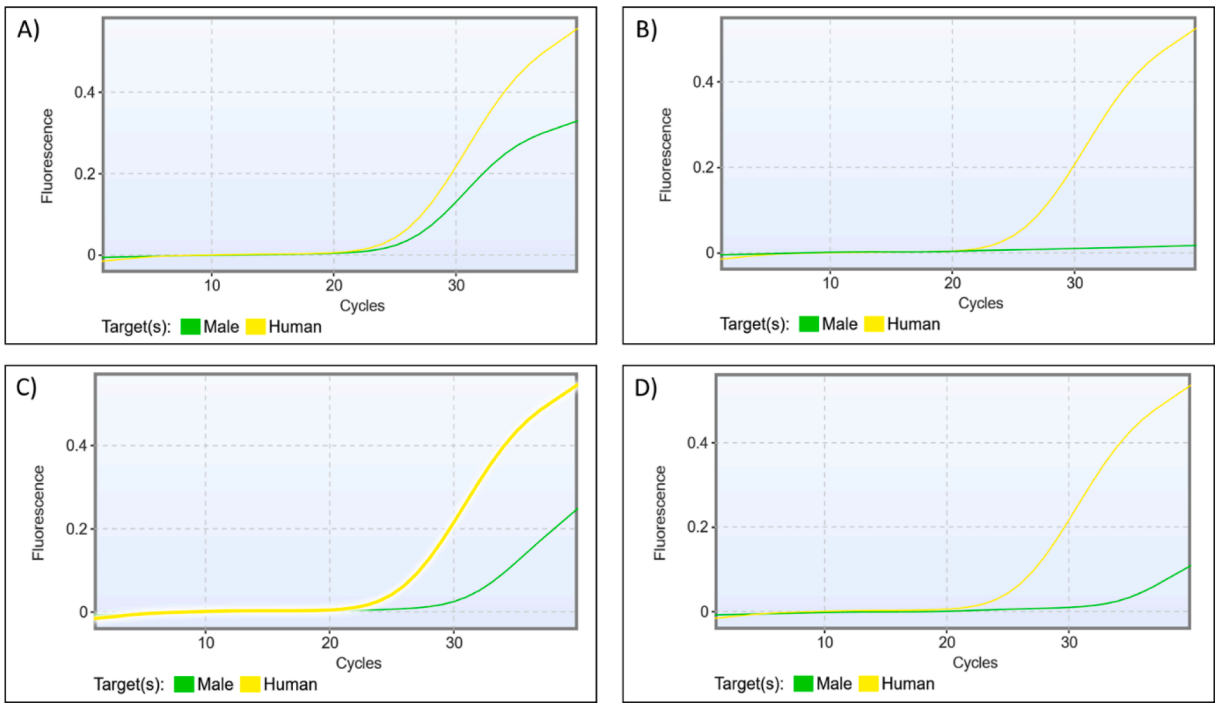


**Fig. 5.** qPCR amplification curves from the Quantiplex Pro-RGQ kit showing a) amplification of single source male DNA, b) single source female DNA, c) amplification of mixed male and female DNA in a 1:50 ratio (male:female), and d) in a 1:1000 ratio (male:female).

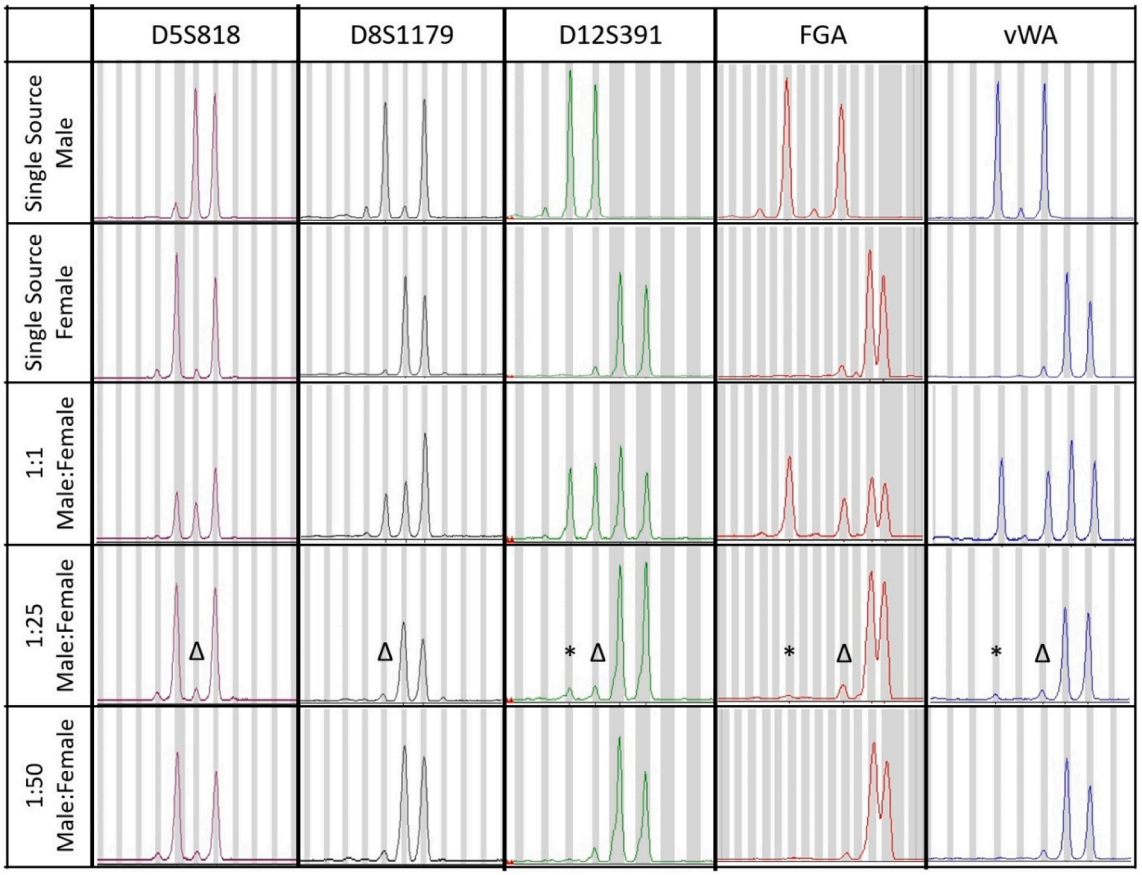


**Fig. 6.** Electropherogram of five representative loci amplified from various mixed DNA samples using the Investigator 24plex QS Kit. * Denotes instances of minor contributor alleles in 1:25 mixtures; Δ denotes instances where minor contributor alleles cannot be differentiated from allelic stutter. Image taken from [114].

commercially available presumptive tests for vaginal and menstrual blood, at the time of writing many of these tests are not routinely employed by UK forensic labs. Furthermore, research and development into the use of confirmatory mRNA analysis has yet to be fully realised and routinely employed in criminal casework in the United Kingdom and its inclusion in this review is therefore limited to Section 7 below.

## 4. DNA methods used to determine identity of individual leaving body fluid stain

The three most common commercial manufacturers of forensic DNA equipment in the United Kingdom are Qiagen [103], Thermo Fisher [104] and Promega [105]. Each biotechnology company provides its own brand kits and assays to support the Human Identification (HID) pipeline (Table 3).

### 4.1. DNA extraction

Most kits are sold for both manual and automatic sample processing depending on the laboratory throughput. Research data is somewhat conflicting with some showing little difference in the DNA yield between manual and automated extraction [106,107] while others show a significant difference [108-110]. Kit-to-kit comparison studies show that the leading kits perform better than traditional methods such as chelex [109,111,112] while difference between the leading kits can vary from experiment to experiment. All the available kits allow the processing of single source reference samples using a standard DNA extraction protocol or from mixed sexual assault samples that may contain multiple contributors using 'differential extraction' protocols for the separation of semen from epithelial (i.e. vaginal) cells. In the intial steps of this method, lysis buffers and proteinase K are added to the mixed sample containing spermatozoa (male contribution) and epithelial cells (female contribution), causing the female DNA in the epithelial cels to be released. The male DNA is not released due to the presence of sulphide bridges in the spermatozoa preventing cell lysis. Due to the differential weights between the lighter female DNA and the heavier male spermatozoa it is possible to separate each contributor through centrifugation. After which the supernatant (top layer) of the solution containing the lysed DNA (termed the Female Fraction) is removed and processed separately from the sperm pellet (Male Fraction) at the bottom of the tube. The further addition of dithiothreitol (DTT) to the sperm fraction which breaks the disulphide bonds releasing the male DNA associated with these cells. By using the differential extraction method a single swab can yield both suspect (male) and victim (female or male) DNA samples.

### 4.2. DNA quantification

After extraction, DNA is quantified to determine its concentration. This allows the reporting officer to review how successful the item extraction has been and also allows the DNA analyst to determine the correct volume of DNA extract required for STR amplification. There are a number of different commercial kits available for use (Table 3) each including specific PCR primers to amplify both autosomal and Y-chromosome (male specific) DNA targets. Comparison studies looking at kit-to-kit performance show that all kits were comparable in determining the quantity of high quality DNA at the sub-picogram level [113] but each kit may vary slightly in respect to each of its performance parameters. The InnoQuant(R) HY kit showed the highest precision while the Investigator® Quantiplex® Pro Kit was the most tolerant to PCR inhibitors.

Another benefit of performing DNA quantification is that it provides one of the earliest assessments of whether the sample is single source or contains mixed male/female DNA (Fig. 5) allowing the analyst to determine whether the sperm isolation technique of choice has worked and consider next steps for analysis. The number of autosomal and male target copies in a single source male sample will be similar (Fig. 5a), while in a mixed female:male sample there will be a larger number of autosomal targets (Fig. 5c). Recent research has shown that the additional amplification of long DNA fragment markers for both autosomal and male targets allow the analyst to determine the 'degredation index' of the sample and it's relative components. In fresh material the ratio of short verses long DNA fragments is approximately 1:1, while the loss of long target fragments through DNA degredation skews the ratio leading to the degredation index increasing [114]. The ability to add such qualitative information to the quantitative information derived from this steo can help inform cold casework working from degraded samples.

The quantification of DNA recovered from samples also allows forensic researchers to assess the impact that presumptive tests may have on downstream analysis which in turn can inform forensic practice. For example, research has shown that the buffer used in the RSID-Semen test can itself undergo DNA extraction, preventing the need to collect a secondary swab or test a sub-sample of the primary swab after confirming the presence of seminal material [115]. As such, it becomes possible to streamline the entire process from stain identification through the DNA extraction.

### 4.3. STR amplification

The regions of DNA that are targeted during forensic DNA analysis are termed Short Tandem Repeats (STRs), each existing at a different region (locus) of DNA in the genome. STR's can occur on autosomal chromosomes (common to both male and females) but may occur on the Y chromosome (therefore unique to males). Each autosomal STR locus exists in two allelic forms, one inherited from the paternal line and one from the maternal lines. While individuals may share some autosomal alleles at a single locus, the chance of sharing all alleles across seventeen autosomal STR loci (UK standard) is greater than a billion to one. Y-STR loci only exist in one allelic form, being uni-parentally inherited from the father. As such the Y-STR profile from a male suspect will also match their father and any brother they have. STR amplification kits are available from either Qiagen, Thermo Fisher (formerly Applied Bio-Systems) and Promga (Table 3) and are sold for either autosomal STR amplification (if single source DNA is expected) or Y-STR amplification (if samples contain cells from both victim and suspect but cannot be separated, i.e, in cases of digital penetration when differential extraction wont separate male and female epithelial cells). Each STR locus is amplified through the process of Polymerase Chain Reaction (PCR) leading to an exponential increase in the copy number of the target DNA over successive cycles. This is of great benefit when the amount of genetic material collected is relatively small and/or degraded. To visualise the fluorescently labelled STR fragments it is necessary to run the amplified sample through a Genetic Analyser (Table 3). The platform

**Table 4**
Table of key feature to note during injury examination. Adapted from [136].

| Feature | Notes |
|---|---|
| Site | Record the anatomical position of the wound |
| Size | The dimension of the wound should be measured |
| Shape | Describe the shape of the wound (e.g. linear, curved) |
| Surrounds | Note the condition of the surrounding tissue (e.g. bruised, swollen) |
| Colour | Observation of colour is particularly relevant when describing bruises |
| Course | Comment on the apparent direction of the force applied |
| Contents | Note the presence of any foreign material in the wound (e.g. dirt, glass) |
| Age | Comment on any evidence of healing. Note that accurate ageing is impossible and great caution is required when commenting on this aspect |
| Borders | The characteristics of the edges of the wound may provide a clue as to the weapon used |
| Classification | Use accepted terminology wherever possible |
| Depth | Give an indication of the depth of the wound |

uses Capillary Electrophoresis (CE) as a method to separate the amplified STR fragments by passing them through a matrix of poly-acrylamide [116]. Smaller more mobile STR fragments pass toward the positive anode at a faster rate than larger fragments. Fluorescence is detected and expressed as a series of peaks on an electropherogram with locus alleles being identified based on their colour and size relative to a series of standards (Fig. 6).

### 4.4. DNA match statistics

After size separation on the Genetic Analyser the STR profile generated from the forensic evidence (termed the Query (Q) sample) is compared to the STR profile generated from the victim or suspect reference sample (termed the Known (K) sample). If a suspect has not been identified and there is no K sample to match with the Q sample, the National DNA database may be searched to identify the whether a matching K sample can be found. There can be three outcomes associated with sample matching; i) in the event that the Q and K profiles don't match an 'exclusion result' is declared, ii) in the event that the Q and K sample match an 'inclusion result' is declared, iii) in the event that the Q profile contains multiple alleles a 'complex result' is declared. When performing STR profiling using autosomal markers, the probability of observing a match has to be calculated. In this instance allele frequency data from each of the amplified loci in the Query profile are determined from data representing the ethnic population matching the suspect (i.e. UK Caucasian, UK Indo-Pakistasni; or UK Afro-Carribean) [117]. These allele frequency data are used to generate a match probability (MP) following established mathematical formula [118]. The match probability is presented in the form of a likelihood ratio (LR) and is expressed as '*the probability of selecting an unrelated individual at random who has an STR profile matching the Query sample*'. The analysis of STR data and generation of likelihood ratio's has been achieved using both manual (binary) and software (probabalistic) methods [119] and there exists a number of software available for mixed profile analysis including LRmix [120] and STRmix™ [121]. When performing Y-STR profiling, the probability of observing a match by chance is a lot higher given the uniparental mode of inheritance of the Y-STRs. As such it is possible that two genetically related individuals will share the same Y-STR profile meaning that establishing a match is done by looking at the rarity of the specific profile in a Y haplotype database following the methods outlined in [122].

The success rate of STR profiling of sexual assault samples can vary depending on sample type, although research data has shown that full STR profiles can be obtained from post-coital swab samples taken from the vaginal cavity [123], buccal swab/semen mixtures [124], mouth, [125], underwear [60,126], skin surface [36], condom [127], penis [128,129] and finger after digital penetration [130]. Hair may only yield mtDNA from the hair shaft (unsuitable for STR profiling) and only a small amount of nuclear DNA when the hair follicle is present. Across all sample types the STR profiling success rate declines over time, correlating with Time Since Intercourse (TSI) measurements [125,131] and worsens if the victim washes before evidence is recovered [132,133]. However, these observations have to be considered alongside other research which has shown that DNA profiles from semen were only recovered from 70% of the underwear and 60% of the swabs taken during the forensic medical examination [134] with other casework reviews report no seminal material being detected [135].

## 5. Biological evidence used to refute sexual act was consensual

In Rape and Serious Sexual Offence (RASSO) investigations it is often the case that both parties (complainant and defendant) both agree that a sexual act occurred, but differ in their interpretation of whether the act was consensual. Consent is defined by section 74 of the Sexual Offences Act 2003 [1] and section 3 of the The Sexual Offences (Northern Ireland) Order 2008 [2] as if 'they agree by choice, and have the freedom and capacity to make that choice' and by part 2 of the Sexual Offences (Scotland) Act 2009) as 'having free agreement' [3]. Often it becomes necessary to prove beyond reasonable doubt that an act was not consensual by determining whether the complainant was under the effect of alcohol or any other substance; whether violence was used or threatened to be used by the defendant; or whether the complainant was

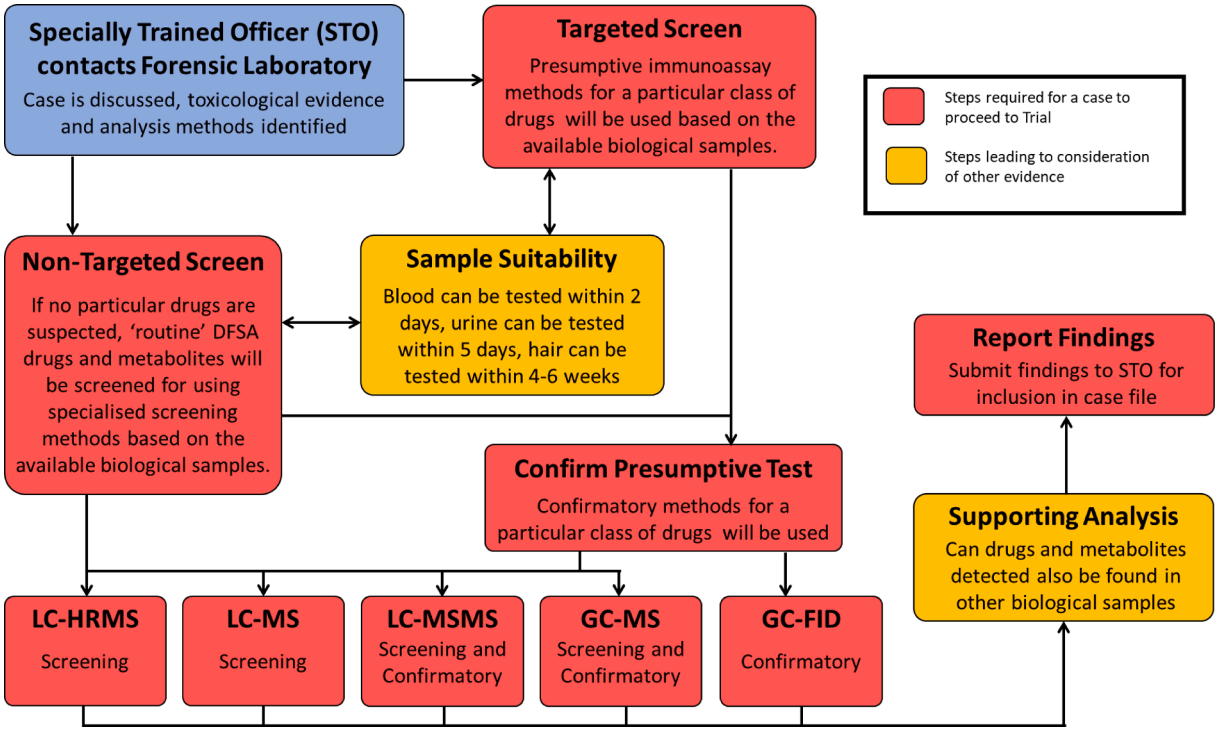


**Fig. 7.** Forensic Toxicology workflow .
adapted from [159,160]

**Table 5**
Table of common drug classes, associated screening methods (targeted and non-targeted), confirmatory testing methods and detection limits.

| Drug Class | Major Representative | Targeted Immunoassay Screen | Non-Targeted Screen | Confirmatory Test | Proposed Detection Limit of Confirmatory Test | $T_{1/2}$ (Hrs) |
|---|---|---|---|---|---|---|
| Alcohols | Ethanol (ethyl glucuronide)<br>Ethanol (ethyl sulfate) | N/A | Solid Phase Extraction GC-MSMS | GC-FID, HS-GC-FID | 100 μg/l | 2–3 |
| Amphetamines | Amphetamine<br>PMMA<br>MBDB<br>MDA<br>MDEA<br>MDMA (ecstasy)<br>Methylamphetamine | Liquid Enzyme Immunoassay (EIA), Enzyme-Linked Immunosorbent (ELISA) | Liquid-Liquid Extraction followed by GC–MS or LC-MSMS, Extrahera extraction followed by UPLC-HRMS | Protein Precipitation, Solid-Phase Extraction followed by LC-MSMS | 10 μg/l<br>30 μg/l<br>10 μg/l<br>10 μg/l<br>10 μg/l<br>10 μg/l<br>10 μg/l | |
| Antidepressants | Amitriptyline<br>Nortriptyline<br>Citalopram<br>Desipramine<br>Imipramine<br>Fluoxetine<br>Paroxetine<br>Sertraline | NA | Liquid-Liquid Extraction followed by GC–MS or Extrahera extraction followed by UPLC-HRMS | Protein Precipitation, Solid-Phase Extraction followed by LC-MSMS | 10 μg/l<br>10 μg/l<br>10 μg/l<br>10 μg/l<br>10 μg/l<br>10 μg/l<br>10 μg/l<br>10 μg/l | |
| Antihistamines | Chlorpheniramine<br>Diphenhydramine<br>Hydroxyzine | N/A | Liquid-Liquid Extraction followed by GC–MS | Protein Precipitation, Solid-Phase Extraction followed by LC-MSMS | 10 μg/l<br>10 μg/l<br>10 μg/l | 13–27 |
| Barbiturates | Amobarbital<br>Pentobarbital<br>Phenobarbital<br>Secobarbital | N/A | Solid Phase Extraction followed by GC-MSMS, or UPLC-Q Exactive Orbitrap-MS | Solid-Phase Extraction followed by LC-MSMS | 25 μg/l<br>25 μg/l<br>25 μg/l<br>25 μg/l | |
| Benzodiazepines | Alprazolam<br>Bromazepam<br>Chlordiazepoxide<br>Clobazam<br>Clonazepam<br>Diazepam<br>Flunitrazepam<br>Lorazepam<br>Lormetazepam<br>Midazolam<br>Nitrazepam<br>Oxazepam<br>Phenazepam<br>Temazepam<br>Triazolam | Liquid Enzyme Immunoassay (EIA), Enzyme-Linked Immunosorbent (ELISA) | Liquid-Liquid Extraction followed by GC–MS or LC-MSMS or UPLC-Q Exactive Orbitrap-MS, Extrahera extraction followed by UPLC-HRMS | Protein Precipitation, Solid-Phase Extraction followed by LC-MSMS | 10 μg/l<br>10 μg/l<br>10 μg/l<br>10 μg/l<br>5 μg/l<br>10 μg/l<br>5 μg/l<br>10 μg/l<br>10 μg/l<br>10 μg/l<br>5 μg/l<br>10 μg/l<br>5 μg/l<br>10 μg/l<br>5 μg/l | 12–15<br>8–19<br>20–40<br>10–20<br>19–40<br>20–30<br>20<br>12<br>10<br>2–3<br>20–25<br>8<br><br>5–8<br>1.5–3 |
| Cannabinoids | $\Delta^9$-tetrahydrocannabinol tetrahydrocannabinolic acid | Liquid Enzyme Immunoassay (EIA), Enzyme-Linked Immunosorbent (ELISA) | Solid Phase Extraction followed by GC-MSMS, Extrahera extraction followed by UPLC-HRMS | Protein Precipitation, Solid-Phase Extraction followed by GC-MSMS or LC-MSMS | 10 μg/l | |
| Cocaine | Cocaine<br>Benzoylecgonine<br>Cocaethylene | Liquid Enzyme Immunoassay (EIA), Enzyme-Linked Immunosorbent (ELISA) | Liquid-Liquid Extraction followed by GC–MS or LC-MSMS, Extrahera extraction followed by UPLC-HRMS | Protein Precipitation, Solid-Phase Extraction followed by LC-MSMS | 50 μg/l<br>50 μg/l<br>50 μg/l | |
| Dissociative anesthetics | Ketamine<br>Phencyclidine | Liquid Enzyme Immunoassay (EIA), Enzyme-Linked Immunosorbent (ELISA) | Liquid-Liquid Extraction followed by GC–MS or LC-MSMS | Protein Precipitation, Solid-Phase Extraction followed by LC-MSMS | 1 μg/l<br>10 μg/l | |
| γ-hydroxybutyrate and related substances | γ-hydroxybutyrate (GHB) | N/A | N/A | LC-MSMS | 10 mg/l | |

(*continued on next page*)

**Table 5** (*continued*)

| Drug Class | Major Representative | Targeted Immunoassay Screen | Non-Targeted Screen | Confirmatory Test | Proposed Detection Limit of Confirmatory Test | $T_{1/2}$ (Hrs) |
|---|---|---|---|---|---|---|
| H1-antihistamines | Diphenhydramine | N/A | Liquid-Liquid Extraction followed by GC–MS | Protein Precipitation, Solid-Phase Extraction followed by LC-MSMS | | |
| Opiates and opioids (licit narcotic analgesics) | Morphine<br>Codeine<br>Dihydrocodeine<br>Fentanyl<br>Methadone<br>Oxycodone | Liquid Enzyme Immunoassay (EIA), Enzyme-Linked Immunosorbent (ELISA) | Liquid-Liquid Extraction followed by GC–MS or LC-MSMS, Extrahera extraction followed by UPLC-HRMS | Protein Precipitation, Solid-Phase Extraction folowed by LC-MSMS | 10 μg/l<br>10 μg/l<br>10 μg/l<br>1 μg/l<br>10 μg/l<br>10 μg/l | |
| Z-drugs (hypnotics) | Zaleplon<br>Zolpidem<br>Zopiclone | N/A<br>N/A<br>N/A | Liquid-Liquid Extraction followed by GC–MS or Solid Phase Extraction followed by UPLC-Q Exactive Orbitrap-MS or Extrahera extraction followed by UPLC-HRMS | Protein Precipitation, Solid-Phase Extraction followed by LC-MSMS | 10 μg/l<br>10 μg/l<br>10 μg/l | <br>1.5–4.5<br>3.5–6.5 |

Common drugs found in cases of DFSA. List of drugs and proposed detection limits compiled from [160,173,174]. List of analytical approaches compiled from UK Acreditation Services website for the following companies; Eurofins Forenisc Services LTD, Key Forensic Services LTD, Orchid Cellmark LTD, Scottish Police Authority Forensic Services, Forensic Science Northern Ireland.

misled or deceived (covered in sections 75 and 76 of the Sexual Offences Act 2003). In such situations other forms of forensic evidence become supportive, specifically injury marks and trauma to the victim (taken during the forensic medical examination), toxicology reports from blood, urine and hair (processed by the forensic laboratory), and electronic data in the form of phone record, social media chat and past behaviour.

### 5.1. Marks and trauma

It is commonly observed that victims of rape and serious sexual assault can sustain bodily injuries, and clinicians and pathologists are often required to offer their expert interpretation about the injuries [136]. Injuries can occur all over the body and can vary in severity. Various attempts to categorise and classify injuries have been made in an effort to standardise the reporting of injuries sustained during sexual assault [136-139] although considerable variation exist [140]. Methods used in the UK follow guidance laid out by the Faculty of Forensic & Legal Medicine of the Royal College of Physicians [27] by recording the presence/absence of injuries using a standardised 'pro-forma for adult female and male forensic sexual assault examination [141] submitted together with a standardised forensic medical examination form for both complainant [29] and suspect [30]. While notes on injuries should take account of key characteristics (Table 4), the exact service provided by the forensic physician in attendance may vary depending on their level of expertise, the facilities they use, and whether the examination is performed in a SARC, hospital or police victim examination suite [142]. All areas of the body may be examined and injuries reported first

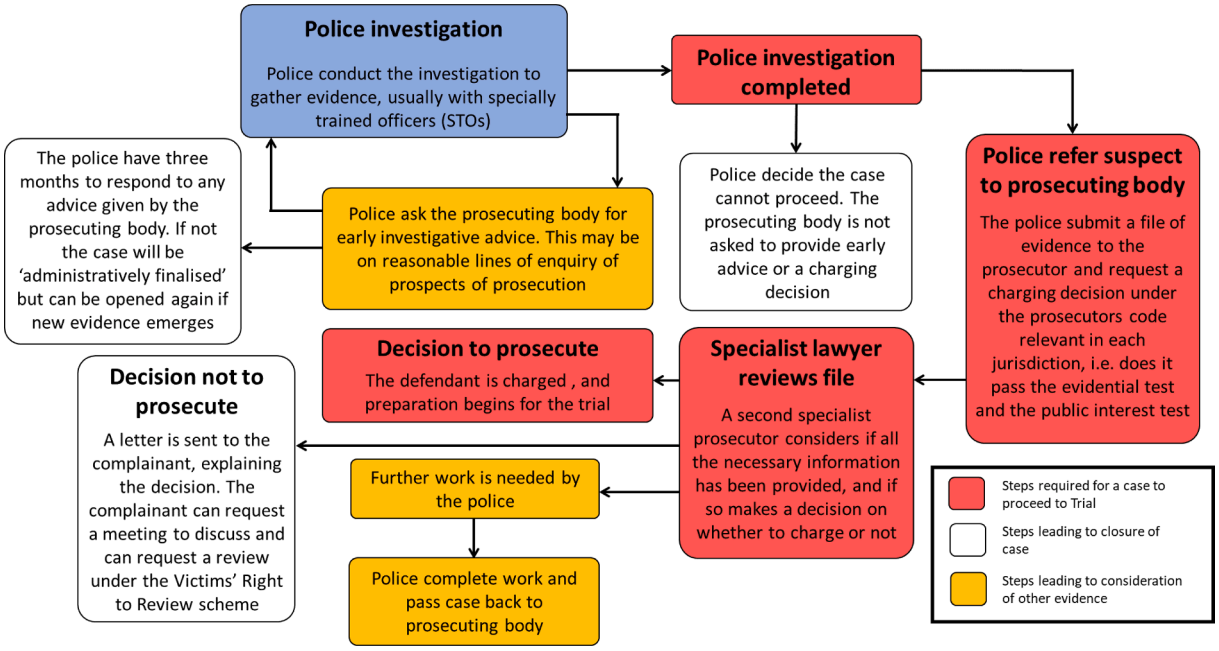


**Fig. 8.** Flow chart of a RASSO case. Modified from [182].

visually with findings recorded on a body chart [143], then secondarily using staining techniques to highlight injury and thirdly using a colposcope (magnifying instrument with light source and digital image capture) [142,144,145]. Photographs may be taken if deemed necessary and non-intrusive [146]. Particular attention is paid to the anogential area in instances of rape or penetrative assault and injury location can be classified as external (labia majora, labia minora, periurethral area, perineum, and posterior fourchette), internal (fossanavicularis, hymen, vaginal wall, cervix), and anal (anus and rectum) [28,147]. The most common locations of genetic injury in female teenagers and women are the posterior fourchette, labia minora, hymen, and fossa navicularis [145].

Documenting the location, type and severity of injury is important during an investigation as research has shown that the presence of acute injury can affect the decision to report an attack, record a crime, and prosecute [140,148]. However, literature shows that many victims do not show any evidence of injury post assault, leading to the deprioritisation of cases where no injury is observed [140]. The severity of the injury can vary with the type of assault with injuries sustained after penetrative assault with a foreign object more severe [149] while only minor injuries recorded in instances of oral rape [150]. Furthermore, injury can also occur following consensual sexual intercourse making it difficult to directly attribute the presence of trauma as an indicator of assault [151,152]. Such evidence is important to capture as data suggests 58% of victims in England and Wales reported physical force was used by the offender with 1 in 10 reporting choking or strangulation as a method of restraint used by the offender [153]. Recently, there have been high profile trials where defence lawyers have argued that the presence of injuries were simply sustained during consensual 'rough sex' and in extreme instances argue that fatal injuries are simply 'sex games gone wrong' [154]. Consequently, while the recording of injury sustained during rape and sexual assault undoubtedly forms a key part of the investigative process, their presence does not automatically infer a criminal act, nor does the absence of genital injury translate to the absence of sexual violence [155].

### 5.2. Toxicology analysis

While not strictly biological or molecular, toxicology analysis looks to detect the presence of chemical agents within biological matrices. All analytical methods used in the toxicological assessment of sexual assault samples must be validated according to guidelines developed by the United Kingdom and Ireland association of forensic toxicologists [156] which have been adopted by the Forensic Science Regulator [157]. A list of all the analytical methods used by each forensic provider in the UK and what samples they are validated for use on is available from the United Kingdom Accreditation Services (UKAS) [44]. Most laboratories in the UK tasked with detecting and identifying drugs in biological samples (blood, urine and hair) follow a similar process flow (Fig. 7), whereby a presumptive screen is first performed followed by more targeted confirmatory method [156,158]. The timeframes in which toxicological analysis can be performed vary between the different biological matrices collected. Blood samples need to be tested within two days, urine needs to be tested within five days and hair needs to be tested within 2 weeks [28]. This is largely due to the different chemical retation times of different drugs in each tissue type, meaning that those present in the blood and urine are broken down and/or excreted more quickly compared to those present in hair.

***Presumptive screening*** may be considered either to be 'targeted', whereby when there is some prior information as to the suspected drug used in the DFSA, or 'non-targeted'. Targeted screening methods are typically immunoassay based, whereby an antibody that is selective for a drug (the antigen) form a complex that becomes detectable [161,162]. Not all drug classes can be detected using immunoassay approaches (Table 5) and different tests need to be developed for each analyte. Commonly used approaches include enzyme-linked immunosorbent assay (ELISA) [163]; homogeneous enzyme-immunoassay (HEIA); Enzyme Multiplied Immunoassay Technique (EMIT) [164,165]; Cloned Enzyme Donor Immunoassay (CEDIA) [166]; Fluorescence Polarization Immunoassay (FPIA) [167] and Kinetic Interaction of Micro-particles in Solution (KIMS) [168]. Samples are typically analysed in 96 or 384 plate format on a laboratory chemical analyser. Data can be qualitative (reported in the form of a positive signal compared to a blank control) or quantitative (reported as a concentration based on a standard curve calculation). Research shows that drug detecting immunoassays tend to overestimate the amount of analyte, cross react with other samples and produce false negatives [e.g. 163, 166]. In the absence of any prior information as to the suspected drug used in the DFSA, non-targeted screening methods may be preferred as they can detect a number of different drug classes simultaneously, but still only provide a presumptive identification. Common non-targeted screening methods use chromatographic methods to separate chemical analytes in a Gaseous (GC) or Liquid (LC) system [156] which are then paired with a detector for accurate identification such as Mass Spectrometry (MS), Flame Ionisation Detector (FID) or High Resolution Mass Spectrometry (HRMS). Data is observed as a chromatogram with relative abundance plotted on the Y-axis and Retention Time (RT) plotted on the X-axis. The retention times of the observed compounds are compared to theoretical and predicted reference data to provide a tentative identification [169]. ***Confirmatory Tests*** for illicit drugs of abuse use the same or similar methods as non-targeted screening (Table 5) but the retention times of specific peaks are compared to commercially obtained certified reference materials (CRM) [169].

Toxicological results may support the allegation of non-consensual sex with Drug Facilitated Sexual Assault (DFSA) defined as "offenses in which victims are subjected to non-consensual acts while they are incapacitated or unconscious due to the effects of alcohol and/or drugs, and are therefore prevented from resisting and/or are unable to consent" [168]. The UK government Advisory Council on the Misuse of Drugs (ACMD) [170] recognise two forms of DFSA; 1) Proactive DFSA, where the assault involves the covert or forcible administration of an incapacitating or disinhibiting substance by an assailant, and 2) Opportunistic DFSA, where an assault occurs by an assailant with a victim who is profoundly intoxicated by his or her own actions to the point of near or actual unconsciousness, and thus lacks the capacity to consent [171]. The two groups of drugs primarily used in DFSA are central nervous system depressants and central nervous system stimulants. The depressants most commonly recorded in DFSA investigations include alcohol, benzodiazepines, gamma-hydroxy butyrate (and related substances) and ketamine, with cocaine and MDMA (ecstasy) recorded as the most common stimulants. Data shows there are over 50 types of compound that have been used to commit DFSA (Table 5) but research shows that alcohol is the most common [172].

## 6. Progresing rape and serrious sexual Offences (RASSO) casework

Once the Officer In the Case (OIC) has received all relevant forensic data pertaining to the case, they will decide whether there is sufficient evidence to refer the case to the relevant prosecuting body (Fig. 8), either the Crown Prosecution Service (CPS) for England and Wales [4], the Public Prosecution Service for Northern Ireland (PPSNI) [5] or the Crown Office and Procurator Fiscal Service (COPFS) for Scotland [6]. In England and Wales, sexual offences are prosecuted as part of the CPS Violence Against Women and Girls (VAWG) Strategy and rape specialist prosecutors must be familiar with the CPS policy for prosecuting cases of rape [175], which explains how the CPS deal with cases in which an allegation of rape has been made. Published in 2012, it gives advice on what the CPS does, how rape cases are prosecuted, and what victims can expect from the CPS. In Northern Ireland, prosecutors working for the Serious Crime Unit [176] follow their own prosecution policy [177] which was published in 2010 and likewise provides guidance on how the

PPS make decisions on prosecuting rape cases. In Scotland, prosecutors working for the National Sexual Crimes Unit [178] take guidance from the COPFO prosecution code [179] which covers general prosecution policy and prosecution of serious crimes.

### 6.1. Case assessment

Rape and sexual offence cases are first assigned a primary prosecutor specialising in sexual assault casework who will review the information and together with a secondary reviewing prosecutor determine whether there is sufficient evidence to charge the suspect or not. Under CPS guidance, prosecutors can only reach this decision when the case passes both stages of the *Full Code Test* [180]. Under PPI guidance, prosecutors can reach this decision when the case passes the *Test for Prosecution* [181]. Although not defined as a 'test' per se, the COPFS also provides a rationale allowing the Procurator Fiscal (Scottish prosecutor, hereafter fiscal) to determine whether they can initiate criminal proceedings [179]. Typically, there are two stages to this decision making:

#### 6.1.1. The evidential stage

Whereby the prosecutor/fiscal determines whether there is enough evidence to provide a realistic prospect of conviction against the defendant on each charge. Evidence supporting the claim that sexual intercourse occurred can include 'eye witness' evidence, medical, biological and DNA evidence as well as statements by the accused. For example, presence of spermatozoa on intimate swab swabs collected during the forensic medical examination are strongly indivcative that a sexual act has occurred, but not necessarily the sexual act under investigation. Further DNA testing can be used to identify or exclude the defendant. In some instances there is agreement over the fact that intercourse has occurred but the issue of consent is debated, as detailed in Section 5 above. An individual consents if they agree by choice, and have the freedom and capacity to make that choice. The law does not require the victim to have resisted physically to prove a lack of consent [175,177]. While the question of consent is a matter for the jury to decide, it is considered very carefully by the lead prosecutor. Often during this stage the prosecutor/fiscal may conduct pre-trial interviews with the victim to discuss the evidence. Such interviews may seek to further understand the sexual history and character of the victim which can be upsetting and is often interpreted as victim blaming.

#### 6.1.2. The public interest stage

Whereby the prosecutor must decide if a prosecution is needed in the public interest. A prosecution will usually take place unless: "*there are public interest factors tending against prosecution which clearly outweigh those tending in favour*" [180]. One of factors considered during the public interest stage is the consequences for the victim of the decision whether or not to prosecute. However, given that cases are prosecuted on behalf of the public at large a balance is struck between the interests of the victim and the interests of the public. Other factors that are considered during this stage include whether a weapon or violence was used or threatened, whether the suspect was in a position of authority or trust, whether there is evidence that the offence was planned, the level of harm caused, and whether the defendant has relevant, previous convictions [9].

If, after assessing the evidence and public interest it is decided that there is enough evidence to charge the suspect, the prosecutor/fiscal will make such a recommendation and inform the police reading any pre-trial conditions such as releasing the defendant on bail or remanding them in custody awaiting trial. If, the prosecutor/fiscal decides that the case should not proceed, a second reviewing prosecutor/fiscal must confirm the decision [175,177]. In such instances the victim has the right to a review of their case which can result in overturning the original decision not to prosecute [9]. The entire process from allegation to decision to prosecute is outlined in Fig. 8.

### 6.2. Trial

Trials involving sexual offences in England, Wales and Northern Ireland begin in the magistrates court with more serious cases being transferred to the Crown Court. The decision to transfer a case depends on the severity of the sexual assault as the sentencing powers of the magistrates court are limited to 6 months imprisonment for one offence and 12 months for more than one offence. Cases involving rape and assault by penetration are always heard in the Crown Court due to the seriousness of the offence, while cases involving sexual assault or sexual coercion may only be heard by the magistrates court [9]. If the trial is initially heard in the magistrates' court the defendant will enter their plea. If they plead guilty, the case may be adjourned while a sentencing date is determined. If they plead not guilty a trial date will be determined where a district judge and lay magistrates will preside. If the case is transferred to the Crown Court, the first hearing will be the Plea and Case Management Hearing whereby the defendant again enters their plea. Likewise, if they plead guilty, the case may be adjourned while a sentencing date is determined and if they plead not guilty a trial date will be determined. Trials at the Crown Court will be heard in front of a judge and jury. Trials involving sexual assault in Scotland proceed along similar lines with serious sexual offences such as rape and assault by penetration being heard in the High Court in front of a judge and jury, while other sexual offences that, upon conviction, carry a lesser sentence can be heard in the Sheriff Court where sentences up to five years can be passed [183]. In many cases, the defence will ask the court to be allowed to interview the victim before the trial and ask questions about the victims sexual history and character. Again, this can be a traumatic experience for the victim and is designed to cross examine the victim testimony and expose possible weaknesses in the narrative. Consequently, the adversarial nature of the criminal justice system can appear to perpetrate common rape myths and stereotypes such as rape occurs between strangers, victims provoke rape by dressing provocatively, drunk people are asking to be raped and that victims cry rape when they regret having sex [175,184].

## 7. Summary

The investigative processes described in this review have remained largely unchanged for the last decade and have become embedded in the sexual assault casework workflow because they are well described, tested and provide results that are appropriate. However, despite having such well-developed bioanalytical forensic methods, convictions for rape and serious sexual assault have been steadily declining. The VAWG report published by the CPS for England and Wales [182] shows that 4,370 rape cases were referred to the CPS by the police in 2017–18 from a total of 41,186 recorded rapes, suggesting that only 10.6% of recorded rapes were referred. Furthermore, of these cases, only 2,635 cases resulted in conviction, only 6.4% of rapes initially recorded. The same pattern is also observed in the devolved nations, with the 2018–19 Statistical Bulletin for Cases Involving Sexual Offences published by the PPSNI showing that 556 rape cases were referred by the police in 2017–18 [185] from a total of 967 recorded rapes [176], suggesting that 57.5% of recorded rapes were referred. Furthermore, of these cases, only 51 cases resulted in conviction, only 5.3% of rapes initially recorded. In Scotland, the 2017–18 Criminal Proceedings in Scotland report published by National Statistics for Scotland suggests that 249 rape cases were referred to the COPFS by the police in 2017–18 [186] from a total of 2255 recorded rapes [187], suggesting that 11.0% of recorded rapes were referred. Furthermore, of these cases, only 107 cases resulted in conviction, only 4.7% of rapes initially recorded. The number of convictions as a proportion of the recorded offences are generally similar between each of the regions and continues to be among the lowest compared to other crimes. Cited reasons for the low conviction rates include; 1) a reduction in the number of police referrals to the CPS; 2) an increase in the use of digital evidence which takes time to analyse; 3) a

greater number of consultations between prosecutors and police to discuss case strategy leading to delays in charging; and 4) instances where police have not responded to early investigative advice from prosecutors [182]. The observed reduction in conviction rates has been highly reported in the media with journalist investigations suggesting that prosecutors have been asked to de-prioritise 'weak' cases to improve conviction rates and that specialised training courses designed to help prosecutors and police deal with rape cases have been axed [188,189]. More impactful perhaps is the claim that half of rape victims are actively seeking to drop their case due to invasive disclosure demands by police seeking to access mobile phone data and long delays in the investigative process [190]. Such data suggests that it is the manner in which an investigation proceeds and the interaction of the police and criminal justice system that plays a strong part in a victims decision to proceed to trial.

The next decade is set to bring some changes to both the methods used in the forensic analysis of body fluids and DNA and also the workflow used in the collection and processing of samples. Given that the success of an investigation is based on informed collaboration between multiple agencies, the impact such changes have on the wider investigative strategy is important to consider from both an evidential and quality assurance point of view as well as a consideration on whether such changes are likely to result in a higher number of successful prosecutions.

### 7.1. Improvements in the methods of body fluid detection

The last 20 years has seen the development of an alternative technique for confirming the identity of body fluids in sexual assaults, namely the use of messenger RNA (mRNA) and micro RNA (miRNA) based methods [191-193]. Early research looking into the expression patterns of different markers isolated from body fluids have helped develop a panel of bio-markers have been identified that show expression patterns specific to relevant body fluids [194-200]. The approach first uses commercial RNA extraction kits to recover purified RNA from the evidence or medical examiner swab before using Reverse Transcriptase (RT-) PCR to generate a complementary DNA (cDNA) copy of the RNA which is prior to detection. Given the RT-PCR step, these methods are likely to be much more sensitive than existing methods and therefore generate evidence of greater probative value. The process has multiple steps, requires specialised laboratory equipment and takes a number of hours to perform. Issues with RNA analysis are largely to do with marker stability and cross reactivity. Stability data shows that RNA is preserved better in dried samples than wet samples, although a sharp decline in RNA is observed even in dry samples over 25 days [201]. Freezing also slows down the rate of degradation [202]. Regardless of the sharp decline in quality, research data shows that body fluid detection is possible for blood after 13–16 years and saliva after 2–6 years [203] when using mRNA markers. Research has also shown that miRNA is more stable than mRNA as a biomarker, likely due to its smaller fragment size [204]. Cross reactivity can occur as RNA is expressed in a number of different tissues although much of the early research specifically looked at removing such markers and only suggesting markers for use that show little to no expression in other tissues. Despite the identification of these marker panels, there has yet to be a commercialised kit released that allows standardised testing between laboratories. Furthermore, each laboratory may use a different method for detecting the RNA biomarker with fragment analysis [194], high resolution melting [205], HyBeacon probe detection [206,207] and Massively Parallel Sequencing (MSP) [208] all demonstrating potential. Consequently, forensic laboratories in the United Kingdom are faced with optimising and validating their own in-house protocols and/or by gaining experience of RNA testing methods by collaborating in proficiency trials organised by the European DNA Profiling Group (EDNAP) [209-214]. Despite the research and development, the use of RNA analysis for body fluid identification is still not routinely applied to sexual assault casework, likely due to the methods seeming no more advantageous than the existing presumptive and confirmatory methods that are also cheaper and quicker to use. However, it is considered likely that the RNA analysis using MSP approaches will become the standardised method in the future as forensic providers in the United Kingdom migrate away from traditional capillary electrophoresis (CE) models to MSP based methods [215]. Further benefits from this shift in technology includes the existence of marker panels that allow the inference of ancestry and penotypic traits [216,217] suggesting that the culprit will become easier to identify in the absence of a specific suspect. Indeed the advancement of genomic technologies theoreticaly allows DNA testing of the entire human microbiome to answer a variety of forensic questions such as post-mortem interval estimation, individual identification, and tissue/body fluid identification, among others [218]. While these latter technologies are heavily researched, well described and accepted by the forensic community they are currently not routinely applied and are remain costly.

### 7.2. Changes to the forensic analysis process flow

Alongside the development and implementation of new technology in the forensic laboratory, another possible development is to move presumptive testing earlier in the workflow and thereby generate investigative intelligence sooner. One approach commercialised in the last few years has been the advent of RapidDNA analysis platforms which allow DNA analysis in the police station [219-221]. Currently optimised for reference samples, the continued development of these systems is likely to allow the analysis of evidence samples at extremely low levels such as degraded crime scene samples as well as mixed DNA samples. One of the main issues that is currently preventing the application of this technology in RASSO investigations is the inability of the platforms to perform differential extraction of male and female DNA fractions from a single swab. That said, however the technology can theoretically be optimised to work with Y-STR kits possibly allowing the sole amplification of the male component for suspect matching and may help screen and prioritise samples where there is a backlog [222].

Another possible rapid intervention would be the presumptive testing of samples by SARC staff during the forensic medical examination or by victims in their own homes. As a mechanisim for detecting seminal material the use of lateral flow tests as described in Section 3.2 could provide early investigative intelligence to both victim and police. Questionnaire data from the SARC community showed that that majority of SARC staff believe a rapid detection device would be broardly useful if deployed at a referral centre (39% - Extremely Useful; 37% - Useful; 18% - Slightly Useful; 3% - No Effect, 0% - Slightly Unhelpful; 3% - Unhelpful; 0% - Extremely Unhelpful) (authors unpublished data). Furthermore, this concept data also suggests that a 'positive' semen detection result from the test may give the patient more confidence to progress their case, while the opposite is true for a negative test, underlying the importance of providing guidance to victims if tests are to be taken at SARCs or by the victim themselves. The use of such tests outside the forensic laboratory would need to be supported by associated research including all stakeholders within the criminal justice system and partner with mental health professionals to advise on victim well-being. The merit of such rapid intelligence also needs to be weighed against the number of false negatives and possible cross-examination of the data by a defence barrister, who may question to validity of the result. As such it is likely that the result may need repeating again later by a trained forensic scientist. However, the widespread use of lateral flow tests in the home during the Coronavirus pandemic desmonatrated their suitability as a screening tool prior to confirmatory PCR testing and a similar model for sample triage could be useful in sexual assault casework.

### 7.3. Concluding remarks

The collection, recovery and analysis of forensic bioanalytical evidence in relation to sexual assault casework is well established, relying on a number of collaborating authorities and institutions all working together to support victims of sexual assault. Despite such efforts, the number of recorded offences resulting in prosecutions is very low compared to other crimes. Modification to these forensic methods and where they sit in the pipeline may have a small but measureable impact to the way the Criminal Justice System approaches sexual assault casework. Therefore the integration of non-laboratory based analytical methods earlier in the forensic pipeline is likely to be a fruitful area of research in the near future, especially in the United Kingdom where the existing infascrtucture and network of practioners may facilitate such adoption.

*CRediT authorship contribution statement*

**Nick Dawnay:** Conceptualization, Project administration, Visualization, Formal analysis, Writing – original draft. **Kayleigh Sheppard:** Investigation, Methodology, Resources, Supervision, Validation, Data curation, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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