Sexual Assault Examination

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Learning Objectives

Knowledge of the types of injuries that may occur following sexual assault.

Management of the forensic assessment, including the taking of a forensic history and obtaining consent.

Demonstrate the importance of contamination reduction.

Ability to select the appropriate evidence-based samples based on the information provided by the complainant, witnesses, and police.

Knowledge of the evidence that underpins forensic collections and the timing of those collections.

Ability to provide appropriate aftercare including emergency contraception, post exposure prophylaxis.

Definitions

Sexual Assault has a legal definition which can vary across different jurisdictions. It is important that an examiner is very familiar with the legal definition in their area.

In the U.K., for example, "Sexual Assault" occurs if a person (A) intentionally touches another person (B), the touching is sexual and (B) does not consent. "Rape" occurs if person (A) penetrates the vagina, anus or mouth of another person (B) with his penis and (B) does not consent. There is another category called "Assault by penetration" which occurs if person (A) intentionally penetrates the vagina or anus of another person (B) with a part of his body or anything else, the penetration is sexual and "B" does not consent [1].

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M. M. Stark (ed.), *Clinical Forensic Medicine*,
https://doi.org/10.1007/978-3-030-29462-5_3

In the Republic of Ireland, "Rape" is defined as having occurred if a man has unlawful sexual intercourse with a woman who does not consent [2]. "Rape" is defined as the penetration (however slight) of the anus or mouth by the penis or penetration (however slight) of the vagina by any object held or manipulated by another person [3]. "Sexual Assault" is reserved for non-penetrative events (as above) and is defined as any indecent assault perpetrated on a man or a woman.

In Scotland a "Sexual Assault" occurs if a person (A), without consent of person (B), does any of the following [4]:

- Penetrates sexually by any means and to any extent the vagina, anus or mouth of (B)
- Intentionally or recklessly touches (B) sexually
- Engages in any other form of sexual activity in which (A) has physical contact with (B) (whether bodily contact or contact by means of an implement and whether or not through clothing)
- Intentionally or recklessly ejaculates semen onto (B)
- Intentionally or recklessly emits urine or saliva onto (B) sexually

The definition of "Rape" is when a person (A) penetrates to any extent, with their penis and without the consent of (B), the vagina, mouth or anus of (B) [5].

In NSW, Australia, the term "Sexual Assault" is equivalent to "Rape" in the above legislations (even if penetration is by an object) while all other assaults of a sexual nature, without penetration, are termed "Sexual Touching." Sexual touching replaced the term "Indecent Assault" in December 2018 [6].

Introduction

Sexual Assault Services may be independent, stand-alone services that provide dedicated assessment, care and forensic sampling for complainants of sexual assault. The services may cater for males and females; pediatric and adult cases. They may have dedicated space in which to conduct these examinations. They may see all complainants, even those who do not wish to disclose the assault to police. They may supply additional services such as counselling, psychology, advocacy and / or support people to assist the complainant. The unit may provide ongoing sexual health screening as required. Or they may not. If you are an examiner, or just wishing to know how sexual assault services might work, this chapter aims to provide an overview of such facilities and aims to assist the examiner, working in the field of sexual assault, in providing an evidence-based forensic medical assessment.

Sexual assaults create significant health and legislative problems for every society. All health professionals who have the potential to encounter victims of sexual assault should have some understanding of the acute and chronic health problems that may ensue from an assault. An examiner must have knowledge of the relevant sexual assault legislation in the jurisdiction within which they work, understand the admission and exclusion criteria which operates and be familiar with any cut off time periods for forensic sampling.

This chapter will not cover the epidemiology of sexual assault, as this has been covered extensively elsewhere, other than to state that it is a prevalent crime affecting more people than reports made to police. It affects men and women, girls and boys as well as those who identify as trans and intersex. It affects homosexuals, heterosexuals and bisexuals. It is seen in every culture, every community and across all religious denominations. "Despite its destructiveness, violence is ubiquitous in society" [7]. It has the ability to impact a person's life for the rest of his/her life. The aim of most sexual assault services is to reduce the psychological impact, restore a sense of power, control and choice, ensure all medical needs are met, provide forensic assessment and collection of evidence to the best standard possible and to assist the complainant to navigate their way through the process.

The following sections will include an overview of the relevant anatomy, development, and physiology. Injury and injury documentation will be covered. The practical aspects—which samples to obtain, when and how to obtain them, will also be addressed.

Throughout all the stages of the clinical forensic assessment, the forensic practitioner must avoid partisanship while remaining sensitive to the immense psychological and physical trauma that a complainant may have incurred. The continuing care of the complainant is essentially an ongoing process throughout and beyond the primary clinical forensic assessment.

While reference is made to the sexual assault "complainant" other terms that might equally apply include victim, patient, client or complainer.

While this chapter hopes to support an examiner tasked with performing sexual assault assessments it does not replace the specialist knowledge, skills, and attitudes that can only be acquired through more formalized theoretical and practical training and ongoing peer review and mentorship.

Neuroscience

The neuroscience of fear and fear reactions is still not completely understood. The brain is a complex and inter-related system which is affected by individual past experience as well as primary genetics. The following information is a very general outline of some of the current neuroscientific principles associated with fear and the fear response. It is hoped that a sexual assault examiner, by understanding these basic principles, might better understand the variability of presentations by complainants at time of examination (e.g. traumatized, distressed, absent, unperturbed etc.) and why there may be difficulties in trying to gain a cogent version of events.

It is thought that sensory information (sight, touch, smell, sound, taste) is transmitted to the thalamus. This information is then transmitted to other relevant areas of the brain for processing. When the thalamus does not function as it should (as may occur as a result of a flooding of the thalamus with stress hormones e.g. as a result of trauma), there can be interference with the way a person remembers the trauma i.e. they may not recall an event in an integrated or chronological way but,

rather, remember it as a series of images, sounds and sensations associated with intense emotions [8].

The thalamus is thought to be responsible for sending information to the amygdala (which lies in the limbic, unconscious brain) and to the frontal lobes (conscious brain). The road to the amygdala is extremely fast. If the amygdala interprets this information as a threat, this information is sent to the hypothalamus and brain stem resulting in the release of stress hormones such as cortisol and adrenaline. There is engagement of the autonomic nervous system (sympathetic pathway) which can result in an increase in blood pressure, heart rate, respiratory rate, body temperature, and slow down of both urination and defecation. There can be dilation of airways, increase in heart contraction, increased muscular action, sweating of palms, dilation of pupils and piloerection.

This reaction, however, can be modulated by the frontal lobe (particularly the medial prefrontal cortex) which allows for assessment of the situation [8]. In low to moderate stress levels, the prefrontal cortex can act to "calm down" the amygdala. In an "amygdala hijack", where there is an extreme stimulus, the amygdala shuts off the prefrontal cortex function (where conscious control and decisions are made). It is an evolutionary response to a risk environment where there is no time for thought.

The hippocampus attempts to regulate the fear response, lessening it when the danger has passed. Like the thalamus, it has a key role in the development of and in the processing of memory. If overwhelmed by stress hormones, it too can result in disorganized or fragmented memories.

Neuropsychology of Trauma

The phrase "fight or flight" was coined in 1929. For many who do not work with sexual assault complainants, it can be easy to assume that most victims of assault would seek to escape or offer some form of resistance. Much work has been done in the years since 1929 and it has been postulated that there are essentially four distinct fear responses. There is an initial freeze response (this has been referred to as the stop, look, listen response). It is a state of hyper vigilance. The next response is to flee or leave the situation. If this is deemed impossible the next response is to provide some form of resistance (or fight). Hence it might better be referred to as the flight or fight response (order reversed). The last response is tonic immobilization [9] (freeze, flight, fight, frozen?).

Möller et al. [10] reviewed 298 women who had presented to a sexual assault clinic. 70% of those women reported significant tonic immobility (using the tonic immobility scale-adult form–TIS-A) and 48% reported extreme tonic immobility during the assault. This has been described as an involuntary, temporary state of motor inhibition in response to extremely fearful situations that can, not only, prevent someone from being able to move but may cause them to shake, prevent them from screaming out, and lead to feelings of detachment from the ongoing event. Interestingly, alcohol intake within the previous 12 h before the assault reduced the risk of tonic immobility by half!

Tonic immobility was associated with the development of post-traumatic stress disorder and severe depression. Not surprisingly, previous trauma history and psychiatric treatment history were both associated with the tonic immobility response [10].

Whether a person has experienced freezing, chosen to attempt an escape or provide resistance, or whether they have found themselves traumatically immobile as a result, an examiner should be aware that these responses are quite individual, may not be the same if they were to be sexually assaulted on another occasion, and the complainant may have very little conscious control over any of these reactions. That is, what we think we might do, if we found ourselves in such a situation, may not bear any resemblance to what we actually do.

Complainants of sexual assault should be offered counselling to help them cope with the recognized immediate and long-term psychological sequelae of a sexual assault [11]. Some examination facilities have 24-h access to trained counsellors [12].

Illustrative Case *During a Sydney trial in October 2016, a jury heard details of how a NSW girl had suffered torture and sexual assaults, as she was growing up, by her father and assisted by her mother. Richard Guilliatt, journalist at The Australian, spoke to a woman who had known the family for 15 years. She is reported to have said, "I don't believe any of it…Honestly, I can't see it." Another mother told Richard, "I don't know anyone who knew them and trained with them who thinks this happened." The parents' middle daughter and son also deny the accusations. The middle daughter said that her youngest sister had been diagnosed with Dissociative Identity Disorder and "began to recollect memories that no one else in the family had…my sister is as much a victim as anyone. All I know is that she was admitted into the mental health system and we lost her." Her father was found guilty and sentenced to 48 years in prison. Her mother was sentenced to 16 years* [13].

Medical Examination

Basic Principles

A clear referral pathway should be identified for complainants of sexual assault. All complainants should have immediate triage assessment which includes recording of blood pressure, temperature, pulse rate and a Glasgow Coma scale ranking. Injuries should be identified and triaged. Issues with consent (decreased/altering consciousness, mental health, intellectual and developmental delay, age, intoxication etc.) should be ascertained and substitute consent obtained if required. The health of a patient takes priority. Working closely with treating staff can ensure that medical care is provided in a timely manner and evidence can be preserved where possible.

Examiners should aim to look at all body areas, when conducting a sexual assault assessment, in order to record injury or sites of pain and tenderness. Dependent upon the history given they should be able to competently use an auroscope, ophthalmoscope, speculum, proctoscope (anoscope), colposcope and camera or there should be a clinician available who can assist with these examinations if required.

Prior to any body part examination, the examiner should ensure they have the complainant's ongoing consent to proceed. An examiner should, at all times, be mindful to protect and preserve the dignity and privacy of the patient, exposing only those parts of the anatomy being examined at any one time.

Assessment for intoxication should be done when there is a history of recent drug or alcohol use.

Timing of the Examination

Although in general terms the clinical forensic assessment should occur as soon as possible, reference to the persistence data given under the relevant sections and the acute medical needs of the patient will help the forensic examiner determine whether the examination of a complainant should be conducted as soon as possible, may be deferred or should not be undertaken at all. Even when the nature of the assault suggests there is unlikely to be any forensic evidence, the timing of the examination should be influenced by the speed with which any clinical signs if present, such as superficial/minor injuries, might disappear.

Detaining police personnel should be consulted prior to making the decision regarding the timing of a suspect examination.

Place of the Examination

Specially designed facilities used exclusively for the examination of complainants / suspects of sexual offences are available in many countries. Furnishings in these units should be durable and suitable for cleaning between examinations [14]. The complainant may wish to have a support person present for all or part of the examination, and this wish should be accommodated if practical. The presence of this additional individual should be recorded in the event that a DNA elimination sample is needed from them at a later date. Suspects are usually examined in the clinical room of the police station and a same-sex chaperone should be present.

During the examinations of both complainants and suspects, the local ethical guidance regarding the conduct of intimate examinations should be followed [15].

Contamination Reduction

Advances in DNA analysis and trace evidence bring with them increased responsibility for examiners. Of course it is virtually impossible to eliminate all extraneous DNA but effort should be made, and these efforts should be documented where appropriate, to reduce the potential for contamination of evidence.

"In the examination process the principle is to minimize the inadvertent transfer of DNA material that could lead to the miscarriage of justice" [16].

Contamination has been an issue in many high-profile cases including Farah Jama (Australia) [17], the Avenger of Zuuk (Netherlands) [18] and Adam Scott

(Manchester) [19]. This has resulted, over the last several years, in an increasing number of cases where the question is not just "whose DNA is it" but "how or when did it get there" [20].

All Sexual Assault units should have protocols/guidelines which address the issues surrounding contamination reduction.

Sources of contamination may include:

- DNA from the examiner. This can occur by breathing over specimens, touching swab tips etc.
- DNA from previous complainant/s. This can occur if examination surfaces are not adequately cleaned and prepared.
- DNA from other people who have entered the room e.g. cleaners, complainant's support people, counsellors, police etc.
- DNA from previous samples
- DNA from other people the complainant has been in contact with or from contact with surfaces that have been touched by other people e.g. in ED waiting rooms, ambulances, police cars etc.
- In the laboratory

Washing provides a first and preliminary level of cleaning. The act of washing hands or equipment will remove some DNA simply by the mechanical process involved. The act of washing itself, however, will not provide a sterile environment and it does not provide a DNA free space.

Using "single use" equipment is preferential to multiuse equipment. Single use items may be both clean and, in some cases, sterile but they may not necessarily be DNA free. Equipment that must be re-used, for example a camera or computer, should be decontaminated between each examination [16].

Bleach (0.5% sodium hypochlorite) is one of the most effective agents for denaturing DNA. Many units are hesitant to use this contamination reduction agent because of the attendant occupational health and safety issues associated with it. It can potentially damage sensitive equipment (such as colposcopes or cameras). There are some alternate detergent/decontaminants available e.g. Virkon™ [21], Virachlor™, Actichlor™, that offer combined sterilization along with some DNA decontamination.

Ethylene oxide (a gas) is commonly used for the sterilization of medical equipment. It is a bactericidal, fungicidal and sporicidal disinfectant that is damaging to DNA. It is also used, therefore, to sterilize swabs and forensic evidence kits to reduce the incidence of background DNA contamination.

In the UK, the Forensic Science Regulator [22] provides guidance as to DNA free consumables, guidance for Sexual Assault referral centers as well as to cleaning and environmental monitoring (pending at time of writing).

Some forensic laboratories may request the voluntary submission of examiner DNA to help eliminate accidental upload to offender databases if contamination does occur.

Securing a dedicated area for seeing complainants of sexual assault will further reduce chances of cross contamination. Thought should be given to maintaining a

register to record anyone who accesses the area and those present during forensic examinations. Evidence kits should be sealed to prevent interference or reuse. Any unused items should be discarded, if open. Areas used for the storage and handling of consumables, samples and exhibits should be secure and access restricted to authorized personnel [16].

Powder free non-latex gloves should be easily accessible, worn by the examiner and replaced frequently during an examination. The powder in many types of gloves has been found to inhibit subsequent DNA analysis and therefore should be avoided [16]. Double gloving is recommended with regular changes of the top gloves when collecting different samples or examining different body areas. Some jurisdictions require that all used gloves should be retained and exhibited. Disposable plastic aprons and disposable sleeves are worn in some jurisdictions. In addition, the forensic practitioner should avoid talking, coughing, or sneezing over unsealed samples and should handle all samples as little as possible. Face masks further reduce the potential for this occurring. Choices for personal protection equipment (PPE) should be made keeping in mind patient acceptability versus potential risk.

It is suggested that any professional statements/expert statements/certificates prepared by forensic examiners should have a section that relates to the methods used to reduce potential contamination. Should these processes have been compromised in any way, for example a swab head was accidentally touched by the examiner or dropped on the floor, full disclosure should be made to the laboratory and to the court.

Any furniture, for example examination beds, chairs and lounges, in the sexual assault unit should be cleaned with bleach or a recommended cleaning agent that denatures DNA, before the examination. A disposable examination bed cover should be used and fresh paper roll may be used as a "sheet" if a DNA free examination bed cover is not available.

Decontamination (deep) cleaning of the whole forensic area should be carried out at least monthly to remove build-up of DNA contamination [16].

Consent

A person is assumed to have the capacity to provide consent unless there is reasonable evidence to support that it is lacking or may be impacted in a significant way.

It is a fundamental legal principle that:

> *Every human being of adult years and sound mind has a right to determine what will be done with his own body; and a surgeon who performs the operation without his patient's consent commits an assault, for which he is liable in damages* [23].

In someone who has capacity, consent must be freely given (not coerced) and it must be informed. They must have an understanding of the procedure proposed, consequences of providing and withholding consent (i.e. undergoing or not undergoing the procedure) and this must be presented to the complainant in a way they

can understand. Consent may be implied, verbal or written. Many sexual assault services, however, require consent for a forensic examination, releasing the information/forensic samples to third parties (e.g. police or forensic laboratories) and consent for imaging to be in writing. Despite having signed a consent form, the complainant can withdraw their consent for any part of the procedure at any time. Any withdrawal of consent should be documented.

Taking samples from suspects may require specific legislative authority [24]; the inspector's authority as well as the consent of the individual [25].

Consent should be reassessed and sought as the examination progresses and for each stage of the clinical forensic assessment, including the use of equipment (e.g. colposcope, camera, speculum, anoscope etc.).

Medical patients can rightfully expect that their personal health information will only be given to another person if this is important for their health care or can otherwise be justified legally and ethically. Health service providers owe patients a common law duty of confidentiality in relation to information obtained as part of the treating relationship. The duty, however, is not absolute. Confidentiality can be overridden when:

- The patient waives their right of confidentiality (or consents to its release)
- There is a valid court order or subpoena
- There are statutory provisions for mandated reporting e.g. in cases of child abuse and certain notifiable diseases
- There is an overriding "public interest" to do so (e.g. you have been informed that your patient intends to leave your office and murder someone)
- In some other emergency situations

Information obtained during a forensic assessment may not remain confidential if the complainant has waived this right of confidentiality by consenting for release of information/samples to a third party. The patient should be made aware that both the history and any other physical evidence collected, such as photographs, may eventually be made available to the court under these circumstances.

There may be examples of privileged information which are exempt, in legislation, from being disclosed in court. The examiner should be aware of these.

Obligations regarding confidentiality of information extend to the transfer of information. Steps must be taken to safeguard it and prevent inappropriate access. This includes transfer via mail, email, or fax. This is particularly relevant when you need to send statements or images to another person/agency. Do not discuss patient information in public areas where it is likely to be overheard.

Forensic photography is another aspect of forensic assessments that should not be done unless the proper consents have been obtained. Information that should be provided includes why the images are being taken, how they will be used (for what purposes), where they will be stored and for how long, who will have access (including the judicial system), and whether they will form part of a medical record [26].

Special consideration should be given to intimate images. These may include images (still or video) of the genitalia, breast, buttocks or anus. Some jurisdictions

have legislation which controls how these images are viewed within a judicial setting. In all cases, every endeavor should be made to ensure they are viewed only by those who have a legitimate reason for doing so.

If a person is unable to consent, a substitute consenter or person with lasting power of attorney is often necessary. Legislation and policy in the different jurisdictions should outline who can provide consent in place of the patient and under what circumstances this can apply. Each examiner should be aware of the local guidelines relating to consent especially as it relates to the following category of complainants/suspects:

- Aged under 18 years
- Intoxicated
- Unconscious/impaired-consciousness
- Acute and significant mental health illness
- An involuntary mental health patient
- Significant intellectual or cognitive delay

If a person lacks capacity, consent from another person or organization may be required. Lack of capacity may be temporary (e.g. unconscious, intoxicated) or longer term (e.g. mental illness, significant intellectual impairment, dementia, brain damage, or underage). Where the impairment of capacity is likely to be short term, consideration should be given to delaying an examination until the person regains full capacity [27]. In such cases, the collection of early evidence (if possible) might be considered.

Even if a substitute consenter is required, available and provides their consent for examination or a procedure, it should not be undertaken if the complainant/suspect refuses the examination.

Chaperones

"A chaperone is an independent and impartial third person who is present during a physical examination in order to witness the conduct of the examination." A chaperone is not the same as a support person. As family and friends are often not independent they should not be used as chaperones [28].

A chaperone can serve several purposes. These include reducing the vulnerability (or feeling thereof) of a complainant and offering a measure of protection for the complainant and the examiner. When an intimate examination is conducted in the absence of a chaperone this should be documented in the medical records, inclusive of the reasons for the absence of a chaperone. Consideration should be given to postponing intimate examinations, if there will be no impact on the complainant's health, when the offer of a chaperone is declined [28].

An auditory chaperone, where the chaperone is not in the same room but in an adjacent space where they can hear, may be a viable alternative to having someone in the room itself. When examining a person of a different sex, it is recommended that the chaperone actually be present in the room.

Medical and Sexual History

A past medical history, current medications and any known allergies should be documented, as would be the normal practice for any medical examination. This information should not be included within a medical statement unless there is an obvious reason to do so. For example, some medical conditions can increase bruising e.g. liver failure. Some medications can do the same e.g. aspirin, anticoagulants, antidepressants as well as certain herbal medications. This information could be relevant when attempting to interpret mechanisms of causation for injuries seen.

Likewise, obtaining a complainant's recent sexual history may be relevant. This information may be used to exclude the DNA of anyone with whom the complainant had recent consensual intercourse. It may be relevant to interpretation of ano-genital injury. Once disclosed, the privacy of this information cannot be guaranteed and so caution should be used when seeking to obtain sensitive information.

Forensic History

The following aspects of an allegation should be ascertained:

- Time and date of assault,
- Time and date of forensic examination,
- Number of suspects,
- A list of all assaults (i.e. the assaults that are sexual, indecent or physical),
- Whether a condom was worn and whether ejaculation occurred (and where),
- Alcohol/drugs used (or suspected of having been used) that might affect consent for intercourse,
- Injuries or symptoms thought to have been sustained/occurred as a result of the assault/s,
- Any suspected memory loss,
- Whether any physical resistance was provided and
- Any other actions that might result in deposition of DNA from the offender onto the complainant's body (e.g. licking, biting, kissing, sucking, spitting, bleeding).

The relationship between the complainant and suspect might have significance for the forensic laboratory, for example if they lived together or are in a current relationship.

In some cases, when it is not immediately obvious to the examiner that a sexual assault (intercourse without consent) has occurred from the history given, an examiner might respectfully ask the complainant how the offender might have known that there was no consent. This can give a complainant the chance to explain what might have been said, what non-verbal clues might have been given or to describe why they did not feel able to verbalize the lack of consent if, in fact, that was the case.

There are arguments for taking short histories that simply direct the examiner to the sites that need forensic sampling and enable the provision of proper medical care and prophylactic medications. These types of histories will rarely provide fodder for Defense counsel who wish to highlight inconsistencies in a complainant's history. They also fit nicely within those judicial systems that do not seek to elicit the best history, but simply one version of events. There are converse arguments for taking more in-depth histories. These can be richer in detail, might provide information that suggest further forensic evidence collection possibilities and are often more useful for those who are seeking to defend themselves against false allegations. Whichever style you use, there will be strengths and weaknesses associated with the approach. It is useful for examiners to be aware of these.

Some examiners choose to use disclaimers, in statements to police, elucidating the purpose of the sexual assault history collected by the forensic examiner. The history might have been taken to direct the examination. It might not be a full or detailed record of events. The history given by the complainant to the examiner will probably not appear in its entirety in any subsequent statement. Most examiners will remove extraneous information not thought relevant for the case at hand. Of course, without all the facts pertaining to the case, it can sometimes be difficult to assume what may or may not be relevant. For this reason it is important to keep all contemporaneous notes.

Clarify all colloquial statements. Do not assume that you understand what the complainant means. Use quotation marks when directly quoting the patient.

Most sexual assault units will record relevant post assault activity conducted by the complainant, after the assault and prior to examination. Activities such as changing clothes, bathing, swimming, douching, urinating, defaecating, vomiting, drinking, eating, cleaning teeth can all impact, to varying degrees, the retention of offender DNA on the body of the complainant.

All complainants should be asked about alcohol consumption in the preceding 24 h, drug consumption (illicit, over the counter and prescribed) in the preceding 3–5 days, and if there is any suspicion that a drug was given to them without their consent or knowledge. Complainants who have urine collected for toxicological assessment should understand that drugs taken weeks previously (especially if a heavy or regular user) may also show up in analysis. In many jurisdictions, police are not interested and will not prosecute complainants who have illicit drugs in their system found in this way. An examiner should be aware of local police practices and advise complainants accordingly, prior to offering the collection of either blood or urine samples.

Forensic examiners should not ask suspects about the alleged incident.

Substance Use

Drugs and alcohol are covered in Chap. 12.

A forensic examiner should make every effort to have the results of toxicology analysis returned to them. These results can then be reviewed in conjunction with

the clinical presentation of the complainant/suspect at time of forensic examination. This is one of the few methods for an examiner to improve future assessments and increase their clinical experience with drugs and alcohol.

Injury Documentation

Part of any forensic assessment, for sexual assault, is the documentation of injury. As complainants may not be aware of all injuries that have been sustained as part of an assault, it is important to include a full body review to ensure no injury is overlooked.

Injuries can be documented in three ways: in writing (verbally), by drawing them on a diagram and by imaging them (photograph or video). High quality documentation requires all three.

When measuring an injury the examiner should always keep in mind any risk of contamination. Give consideration to the type of scale used e.g. DNA free, sterile, single use or reusable and take steps to reduce any risk of DNA contamination, as required. A good rule of thumb is to collect any forensic biological evidence from the body of the complainant prior to documenting the injury or imaging it.

It is good practice to ask the patient, for every injury observed, whether they know how the injury occurred. If they are unaware how the injury occurred, you might ask whether they had noted the injury prior to the assault. It is not a good idea to record, for example, a linear scar at the bottom of the abdomen as a "Caesarean scar" unless you have actually seen the medical notes confirming this. When recording a patient's response you might record that the injury was "stated to have occurred as a result of having been punched to the eye" or "stated to have occurred as the result of a fall in childhood" etc. Alternatively, you might directly quote the patient, "That happened when I fell onto the coffee table when I was six." This enables you to confirm in Court, if asked, that it was the patient who told you that the injury occurred in this manner.

Occasionally, there may be factors that impinge on the quality of your examination. These should be noted. They may include:

- Seeing a patient in an unusual site e.g. in a bed in a ward rather than in a dedicated forensic examination suite
- Poor lighting
- Multiple interruptions
- The complainant's emotional status
- The actions of relatives/friends
- Use of a translator

Whether a forensic examiner documents non-medical findings, such a tattoos or piercings, is a matter of personal preference. Sometimes a simple disclaimer acknowledging that tattoos or piercings were seen but not itemized, might be sufficient.

Whenever there is a clear account of the alleged incident, the anogenital examination can be tailored to the individual case (e.g. if an adult complainant only describes being made to perform fellatio, there is usually no indication to examine the external genitalia).

Be aware that a complainant may not always give an accurate account of the offence, for a variety of reasons. A New Zealand study in 1992 described 16 cases where penile vaginal penetration took place and the complainants denied ejaculation having occurred. Of the 16, seminal fluid was detected in 7, including 3 people who reported that a condom had been used [29].

Furthermore, children and some adults may not have the language skills or may feel unable to provide a detailed account of the sexual acts at the initial interview. In such cases, a comprehensive anogenital examination should be undertaken if the patient, or the person with legal authority to consent on behalf of the patient, gives his or her consent.

Physiology

Female Physiology

The female hypothalamic–pituitary–gonadal axis is developed at the time of birth. During the first 5 days of life, the level of gonadotrophin-releasing hormone (GnRH) rises, with a consequent transient rise in gonadal estrogen, attributable to the withdrawal of placental estrogen [30]. The estrogen causes prominence of the labia and clitoris and thickening and redundancy of the hymen. The neonatal vagina is purported to measure 4 cm in length [31]. Although after 3 months the GnRH levels gradually fall, the estrogenized appearance of the genitalia may persist for the first 2–4 years of life [32, 33]. During this period, the external genitalia gradually becomes less prominent; eventually, the hymen becomes thin and translucent and the tissues appear atrophic; occasionally, the hymen remains thick and fimbriated throughout childhood. The non-estrogenized vagina has relatively few rugae and lengthens by only 0.5–1.0 cm in early childhood [30, 31].

The hypothalamic–pituitary–gonadal axis is reactivated in late childhood, and the breasts and external genitalia alter accordingly. These changes are classically described in terms of their Tanner stage [34]. Under the influence of estrogens, the vagina lengthens to 7.0–8.5 cm in late childhood, eventually reaching its adult length of 10–12 cm [30, 31].

The estrogenized vagina is moist because of physiological secretions. This endogenous lubrication is enhanced with ovulation and with sexual stimulation [35]. The endogenous estrogen levels fall at the time of the menopause and as a consequence of this the vulva and vagina atrophy.

In times past, the physiology of the human sexual response was not particularly well understood. It had been assumed that injury during non-consensual intercourse was likely to occur more frequently than during consensual intercourse because of several factors, one of which was the lack of vaginal lubrication of the complainant (who was not wanting intercourse).

Mr. Scott Volkers was the head coach of the Australian women's swimming team when 3 swimmers, aged between 12 and 14 years, disclosed sexual abuse during their time under his care. It went to a committal hearing in 2002 and advice had been sought by the then-deputy director of the NSW Department of Public Prosecutions. The Deputy-Director gave advice that girls this age were unlikely to have fully developed breasts and hence allegations of groping (of the breasts) would be difficult to prove. One girl gave evidence of Mr. Volkers having rubbed her vagina through a pair of shorts and a swimming costume, resulting in an orgasm. The deputy director found this difficult to believe stating that it would be hard to accept that the girl could have been sufficiently relaxed for orgasm to occur. These were amongst the reasons for charges being dropped against him. Mr. Volkers was eventually re-arrested and charged with multiple child sex offences in 2017 [36].

Levin and van Berlo (2004) examined whether non-consensual sexual stimulation could lead to unwanted sexual arousal [37]. They opined that arousal was both a mental state and a physical state. They could occur independent and without the other, they could occur either before or after the other.

Physical changes that might occur in a woman who is aroused sexually include:

- Increased heart rate, blood pressure and respiration
- Increased blood flow to the breasts, nipple erection
- Clitoral engorgement of blood
- Increased vaginal lubrication
- Irregular contractions of pelvic muscles around the vagina
- Orgasm

In other words, arousal can occur even in the absence of consent.

Male Physiology

Semen is not produced until the male reaches puberty, which usually begins between 9 and 14 years of age [38]. Semen consists of seminal fluid (produced by the prostate) and spermatozoa. The normal volume of a single ejaculate is between 2 and 7 mL, and it will contain approximately 50–120 million spermatozoa per milliliter. There are numerous congenital and acquired causes for impaired spermatogenesis [39], resulting in either decreased numbers (oligospermia) or absence of (azoospermia) spermatozoa. Both conditions may be permanent or transitory depending on the underlying cause.

Forensic practitioners may be asked to comment on a person's ability to achieve a penile erection, particularly if the male is young or elderly. Masters and Johnson [40] note that during their research, "penile erection has been observed in males of all ages ranging from baby boys immediately after delivery to men in their late eighties"; they report that one 89-year-old study subject was able to achieve a full penile erection and ejaculate. Therefore, it is not possible to reach a conclusion regarding erectile efficiency based on age alone. When a defendant reports erectile dysfunction, the expert opinion of a urologist should be sought.

Penile erection may result from visual stimulation (including fantasy) or tactile stimulation. The penis, scrotum, and rectum are all sensitive to tactile stimulation [40], which may explain why involuntary penile erections can be experienced by males subjected to nonconsensual anal intercourse.

Physical changes that might occur in a man include:

- Increased heart rate, blood pressure and respiration
- Nipple erection
- Penis engorgement of blood
- Elevation of testicles
- Rhythmic contractions of pelvic muscles
- Orgasm
- Ejection of seminal fluid [37]

Levin and Berlo's conclusion was that, despite a limited amount of published literature, case and anecdotal reports suggest that the induction of arousal and even orgasm does not permit the conclusion that the subjects consented to the stimulation. "A perpetrator's defense against the alleged assault built solely on the evidence that genital arousal or orgasm in the victim proves consent has no intrinsic validity and should be disregarded" [37].

Anatomy

Female Genital Anatomy

The external female genitalia (vulva) includes the mons pubis, the labia majora, the labia minora, the clitoris, and the vestibule (which incorporates the openings of the urethra and the vagina).

The labia majora are the outer vaginal lips with skin on the external surface and mucosa internally. The labia minora are the inner vaginal lips and these are covered with mucosa. The posterior fourchette is the junction of both lower aspects of the labia minora. The fossa navicularis is the mucosal depression between the posterior fourchette and the vaginal wall/hymen/hymenal remnants. The anatomical vagina is a muscular canal that begins at the hymen and extends to the cervix. The hymen is the tissue that partially or completely surrounds the opening of the vagina.

All sexual assault examiners should confidently be able to identify these landmarks as they are often the site of injury in both consensual and non-consensual intercourse.

The skin of the labia majora and the outer aspects of the labia minora is keratinized squamous epithelium, but only the outer aspects of the labia majora are hair bearing. The inner aspects of the labia minora and the vestibule (including the hymen) is nonkeratinized. This area is usually pink but, in the non-estrogenized child, it may appear red because the skin is thinner and consequently the blood vessels beneath its surface are more apparent [31].

The vagina and cervix are covered by nonkeratinized squamous epithelium that normally appears pink in the estrogenized female. Occasionally, the columnar endocervical epithelium, which appears red, may be visible around the cervical os because of physiological or iatrogenic (e.g., exogenous estrogens) eversion of the endocervical canal; these are sometimes erroneously referred to as cervical erosions. The perineum is the area between the posterior fourchette and the perianal area (in a female). The perineal body is the central tendon located between the vestibule and the anus. It can occasionally be pigmented or white and is known as the median raphe [41].

Peri-urethral and peri-hymenal bands are small bands of tissue connecting two opposing surfaces, with the same colour and texture as the surrounding tissue [42].

Ano-rectal Anatomy

Most forensic examiners experience some difficulty with interpretation of anal and perianal injury. An understanding of the anatomy is a useful basis for considering issues that might be raised in relation to forensic injuries.

The rectum extends from the anal transitionary zone to the sigmoid colon and is 8–15 cm long. It is lined by typical intestinal mucosa and is red in the living. The rectum has only poorly defined dull sensation [43].

The anus is essentially divided into three sections. The uppermost section is called the proximal anus. This is a transition zone where the cells change from rectal cells to anal cells. It is usually located in the region of the anal columns and is purple [44].

The proximal anus is separated from the middle (or intermediate) zone by the pectinate (dentate) line. The middle zone is lined by cells called anoderm. The anal canal is lined by nonkeratinized squamous epithelium and is salmon pink in the living [45]. It is sensitive to touch, pain, heat, and cold to just above the dentate line [43].

The distal zone (closest to the exterior of the body) is lined by the same cells but has hair and sebaceous glands within it. The division between the middle and distal zone is called the anal verge and marks the point where anoderm becomes true skin. This is a histological demarcation, although occasionally it can be determined macroscopically.

The anal canal is narrow (in its non-distended state) measuring, on average, in adults (age range 18–90 years) 2.1 cm, with a range of 1.4–3.8 cm in males and 1.0–3.2 cm in females [46]. It is normally closed at one end by the internal and external anal sphincters.

The internal anal sphincter, smooth muscle under autonomic control, maintains approximately 80% of resting anal tone whereas the external sphincter, skeletal muscle, is responsible for the remainder. It has some voluntary control. This internal sphincter is a continuation of the circular muscle coat of the rectum and extends 8–12 mm below the dentate line.

This external sphincter encircles the internal sphincter but extends below it, ending subcutaneously. The lower edges of the external and internal sphincters can be distinguished on digital palpation. Although this sphincter is tonically contracted in

the resting state, this contraction can be overcome with firm pressure [44]. If the patient is asked to contract the anus during a digital assessment, the external sphincter can be felt to ensure contraction and closing of the anus tightly. However, because the muscle fibers are predominantly the slow-twitch type, a maximum contraction of the external sphincter can only be maintained for approximately 1 min [47].

Because the anal canal can evert and invert as the anal sphincters and pelvic floor muscles relax and contract, the anal verge/margin is not a fixed, identifiable landmark.

The anus and lumen of the anal canal usually appear as an asymmetric Y-shaped slit when viewed via a proctoscope (anoscope). The folds of mucosa and subcutaneous tissue (containing small convoluted blood vessels surrounded by connective tissue) between the indentations of the Y are referred to as the anal cushions. Although this appearance is usually obscured externally by the folds of skin on the perianal area, it may become apparent if the patient is anesthetized or as the anus dilates.

Distension of the rectum is the stimulus for the involuntary relaxation of the internal sphincter, inducing the desire to defaecate. This allows some stool or flatus into the upper anal canal for sampling and identification [48]. If appropriate, volitional Valsalva straining begins, leading to an increased intra-abdominal pressure, pelvic descent and the overcoming of the recto-anal inhibition reflex.

Manometry testing shows that the anal resting pressure is never zero in healthy individuals. It can decrease with age, some diseases and as a result of certain trauma [48]. It is unlikely then that semen deposited in the vagina or near the anus makes its way passively into the anal canal unless there was either some damage to the anal canal (as evidenced by incontinence) or had been moved there via secondary transfer. Secondary transfer can occur in many ways including wiping the area after toileting or iatrogenic contamination i.e. an examiner has contacted the swab with the outer perianal area in the process of inserting the swab.

The diastasis ani should not be confused with scarring or injury. It is a congenital midline depression which may appear in some people, either anterior or posterior to the anus. It marks a congenital absence of the superficial division of the corrugator ani muscle. It may be V-shaped or wedge shaped.

Adolescent Anatomy

Curtis and San Lazaro (1999)[49] stated that it had been their experience, in examining more than 1000 sexually active adolescents, the most common appearance of the hymen was of indeterminate disruption to the free edge. Complete clefting or significant gaps in hymeneal rim was thought to be unusual [50]. Very little is really known, however, about normal female adolescent genitalia and much is anecdotal, or studied without the use of magnification or photographically documented [51].

In Emans et al. study of 100 sexually active adolescent girls 21% were found to have myrtiform caruncles, defined as rounded bumps of hymen separated on both sides by a complete cleft. Interestingly, there is very little literature about myrtiform caruncles. Emans et al. failed to find myrtiform caruncles in 200 sexually inactive post menarchal girls [52].

Reliable information about the appearance of the adolescent/adult hymen remains scarce. This is not surprising, given the infrequency with which it is reviewed. A survey of 126 consultants (predominantly pediatricians and gynecologists) in 1997 found that 91 examined the genitalia of adolescents less than 5 times a year. Only 28 out of 75 assessed the hymen when doing a genital examination [50].

Pre-pubertal Anatomy

Hymen

Kinsey wrote: "I think any creator who claims that he had a purpose in creating the hymen certainly shows himself incapable of having done a good job" [53].

Interest in the human hymen has been predominantly cultural and spiritual. Medical interest, especially forensic, is relatively new. As late as 1987, a survey of 129 physicians, primarily pediatricians and family practitioners, asked to label anatomic parts on a picture of the genitalia of a young girl, showed that only 59% correctly identified the hymen [54].

Perhaps the simplest explanation for this anatomical chasm is that the genital examination is not a routine part of medical assessments in pre-pubertal girls. To date there is a dearth of longitudinal studies detailing normal hymeneal anatomy in any age group and none have been performed that follow girls aged over 9 years.

The hymen is a membrane which partially, or rarely completely, covers the external vaginal orifice. It is located at the junction of the vestibular floor and the vaginal canal.

The hymeneal membrane is of endodermal origin and consists of fibrous connective tissue attached to the vaginal wall (partly elastic and partly collagenous). It is comprised of squamous, stratified epithelium. The hymen is not richly supplied with nerve fibers. The vascular supply is rich in the lower border but scarce close to the edges. The amount of elastic fibers (and the ability of the hymen to stretch) is highly variable [55], dependent upon both age and hormonal status. Nowhere is this demonstrated more eloquently than in the case report of a 21 year old woman who was found to possess a micro-perforate hymen (an opening in the hymen, 2 mm in diameter) in the 27th gestational week of pregnancy. Her hymen was relatively elastic, and penile penetration was possible without damage or causing the patient discomfort. It was also easily stretched by the examiner's fingers, reverting back to its original shape afterwards [56]. How the histology alters with advancing years has yet to be documented.

Perhaps the greatest mystery of the hymen relates to its function. According to many sources, human females are the only species to possess a hymen but hymens have, in fact, been reported in African elephants [57], dogs and horses. The anatomy of these animals differs significantly from that of their human counterpart by way of retention of an uro-genital canal, where both the urethra and uterine body terminate. In domestic animals the hymen disappears during the fetal period or after birth, except in the rare case of persistence of an imperforate hymen [58].

In human fetuses, the lumen of the vagina also remains separated from the cavity of the uro-genital sinus by the hymen but, unlike the animal model, it ruptures during the perinatal period and remains as a membrane around the entrance to the vagina, and is penetrated by the act of intercourse [59].

Hypotheses for the persistence of the hymeneal membrane have included:

1. Evolutionary predominance in societies where virginity of wives was demanded
2. A protection mechanism dating back to an aquatic past with the hymen evolving to protect the vagina from marine "pollution"
3. A structure designed to increase the retention of sperm and hence raise fertilization success and
4. An embryological remnant designed to keep the surrounding area protected from fecal and other material

The hymen has proved a fascinating piece of human anatomy if only by virtue of the fact that:

"This delicate membrane has no known physiological function, but its psychological and cultural significance as a sign of virginity has been enormous" [59].

Tanner Staging of the Hymen

In 1992 Tanner staging of the hymen was attempted for the first time. Patients in this study ranged from Tanner Stage 1 to 5 based on hymen development [60].

Tanner staging of the hymen seems to be rarely attempted in current practice, however.

Tanner Stage One	Hymen has very thin rim. Fossa navicularis characterized by a network of fine blood vessels extending to the edge of the hymeneal rim.
Tanner Stage Two	Thin hymeneal rims with less dramatic vascular patterns. Reduction in superficial vascular prominence noted at the hymeneal rim, fossa navicularis, and vestibule.
Tanner Stage Three	Hymen thicker with beginnings of redundant folds; although slight vascularity seen in some patients, previous widespread superficial vascular network generally absent; close inspection revealed first evidence of clear vaginal secretions. This stage thought to reflect beginnings of true estrogen effect.
Tanner Stage Four	Hymens dominated by thick projections and redundant folds. Neither hymen nor vestibule had visible blood vessels.
Tanner Stage Five	Morphologic qualities of Tanner stage 4 patients' hymens expressed to a greater degree by Tanner stage five patients.

Pediatric Hymeneal Morphology

There have been 3 longitudinal studies of the developing hymen, ranging from birth to 9 years of age. A summary of the morphology at each age group is summarized below. In the final study the author has added 2 new categories (folded, micro perforate) and removed 2 (fimbriated, sleeve like/ventral). It is not known whether the new terminology was chosen to replace the old or whether these were, in fact, new categories of their own [61–63]. Annular is the predominant morphology at birth being steadily replaced, with age, by crescentic.

Age	Annular	Crescentic	Fimbriated	Sleeve like/Ventral	Septated	Folded	Micro-perforate/Small orifice	Reference
Birth	70%	0%	21%	7%	2%			[62]
1 year	54%	28%	7%	11%	0%			[62]
1 year	52%	29%	10%	10%	0%			[61]
3 years	41%	50%	2%	7%	0%			[61]
3 years	39%	61%			1%	1%	2%	[63]
5 years	23%	77%			1%	0%	2%	[63]
7 years	18%	82%			1%	0%	3%	[63]
9 years	10%	90%			0%	0%	3%	[63]

Importantly, no study has ever documented a newborn without a hymen [61, 62, 64] and hymeneal tissue appears redundant in all neonates.

Clearly, morphology of the hymen is not constant. Berenson, for the first time, demonstrated changes seen over time [62]. In this study, 4 different hymenal types were observed in newborns: Annular (n = 40/57; 70.2%); Fimbriated (n = 12/57; 21.1%); Ventral (n = 4/57; 7%) and Septate (n = 1/57; 1.8%). None of the children in this study were noted to have had a crescentic hymen at birth.

At age 1 year it was noted that of those who had been observed to have an annular hymen at birth, only 62.5% had the same morphology 12 months later. 32.5% had changed to a crescentic hymen and 5% had a ventral hymen.

Of those who had been born with a fimbriated hymen, only 33% remain fimbriated at 1 year, 41.7% became annular and 25% became crescentic. All ventral hymens noted at birth remained ventral hymens at 1 year. The single septate hymen had converted to an annular hymen (i.e. lost the septum).

External Ridge

Definition: A midline, longitudinal ridge of tissue on the external surface of the hymen usually anterior or posterior, extending to the edge of the hymen.

The frequency of external ridges decreases with age. They become infrequent after 4 years of age and are unlikely to form de novo [65]. Berenson's results with regard to the presence and location of external ridges, from 2 studies, are summarized below:

	0 years	1 year	3 years
[62]			
6 o'clock position	45	5	
12 o'clock position	7	2	
[61]			
6 o'clock position	106		8
12 o'clock position	12		1

Intravaginal Longitudinal Ridges (ILR)

Definition: Narrow, mucosa-covered ridges of tissue on the vaginal wall that may be attached to the inner surface of the hymen.

ILRs that extended to the hymeneal rim have been observed with almost the same frequency at birth and at 1 year of age [62].

In a review of the genital anatomy of preschool children (1 month – 6 years of age) ILRs appeared in 25%, one child having up to five appearing evenly around the rim [65].

Tags

Definition: An elongated projection of tissue arising from any location on the hymeneal rim.

Tags can appear or disappear. They are not an uncommon finding at birth, the majority of which will disappear by 3 years of age. New tags, in this time period, appear to result from the extension of an intravaginal or external ridge beyond the

rim or from fimbriated hymens that were noted to have had similar protrusions at birth. They can also form as the result of the disruption of a hymeneal septum [61].

Bumps

Definition: A solid, localized, rounded and thickened area of tissue on the edge of the hymen [66]. They are generally thought to be a normal variant.

Bumps were noted to originate from longitudinal intravaginal ridges, from external ridges and can occur independently of ridges [65].

Notches

Definition: A cleft or notch is an indentation in the rim of the hymen.

A deep notch (cleft) is defined as a V shaped defect extending through more than 50% of the width of the hymen [51] and a superficial notch (cleft) is one that extends through less than 50%.

A study of the hymens of 468 newborn girls [67] found that notches were a frequent anatomical variation and were found in 35% of the neonates with annular hymens [65].

Notches were observed significantly less often at 3 years than near birth. One reason for this is the evolution of most annular hymens with superior notches at birth into crescentic hymens. It has been noted that the majority of lateral notches at birth resolved by 3 years of age [61].

Superficial notches have been noted in the anterior and posterior rim of the hymen in the non-abused pre-pubertal population. Deep notches in the posterior half of a non-fimbriated hymen have only been reported in pre-pubertal girls with a history of vaginal penetration [68]. Joyce Adams et al., however, have relegated the deep notch to the category of findings where there is no expert consensus regarding degree of significance stating that a notch or cleft, at or below the 3 or 9 o'clock location, which extends nearly to the base is a "very rare finding" that should be interpreted "with caution" unless an acute injury was documented at the same location [69].

Further confounding this problem is the fact that notches may be dependent upon examination position. A study of 93 pre-pubertal girls (ages 10 months to 10 years) found more clefts when a traction method was used (6.6%) than when the separation (4.1%) or knee chest methods (2.2%) were used [42].

The interest in the posterior edge of the hymen is heightened by the finding that injury from sexual trauma predominantly occurs in this region, between five and seven o'clock [51, 64]. The mechanism for rupture is thought to occur as a result of the symphysis pubis preventing any anterior movement, forcing the penis posteriorly, causing trauma at the midline position to the posterior fourchette. Conversely, it has been suggested that in digital penetration, in children, the force is more likely to be directed to the sides rather than the midline [64].

In summary, notches may be congenital, arise de novo or disappear. They are seen at all ages. Partial tears of the hymeneal rim may resolve with the formation of a notch but not all notches are the result of injury, superficial notches having been documented in normal studies around the entire rim, although less common in the lower half.

Transections

Definition: An acute tear or laceration through the entire width of the hymeneal membrane extending from its edge to the vaginal wall attachment. Transections to the hymen suggest a prior penetrative event. Straddle accidents (without penetration) have not been known to cause acute hymenal injury.

Findings Caused by Trauma

Any examiner who will be conducting pediatric sexual assault examinations should be familiar with all the normal variants of anatomy in both sexes. Despite the number of variations possible there are only a few hard and fast signs of trauma. These include:

- Acute lacerations to any part of the genital anatomy
- Bruising, petechiae or abrasions to the hymen
- Perianal, Posterior Fourchette, Fossa Navicularis scars ("A very rare finding that is difficult to diagnose unless an acute injury was previously documented at the same location")
- Complete cleft below the 3 to 9 o'clock location that extends to the base with no discernible hymenal tissue at the location
- Signs of Female Genital Mutilation (FGM) cutting e.g. loss of part or all of the clitoris, clitoral hood, labia minora or majora [69]

Findings Diagnostic of Sexual Contact

- Pregnancy
- Semen identified from forensic swabs (taken directly from the child's body) [69]

Male Genital Anatomy

The male penis can be subdivided into the glans (head of the penis), coronal sulcus (neck) and shaft. There may or may not be a foreskin (prepuce). On the under surface of the penis there is a frenulum. There is generally two testicles contained within a scrotal sac.

During examination of the male genitalia, the forensic practitioner is expected to document any features that could assist with subsequent identification of the suspect, to note any acquired or congenital conditions that could make an alleged sexual act impossible, to describe in detail any injuries that could relate to a sexual act, and to retrieve any forensic evidence. Although the specifics of the medicolegal assessment of the male genitalia are case dependent, the principles of the examination, whether of the complainant or of the defendant, are the same.

Forensic practitioners may be asked to provide evidence on the size of a defendant's penis in the flaccid state to support a hypothesis that a certain sexual act could not have occurred because of inter-genital disproportion between the complainant and the defendant. However, such measurements are unhelpful because it is not possible to predict the maximum erectile size from the flaccid length, and

there is "no statistical support for the 'phallic fallacy' that the larger penis increases in size with full erection to a significantly greater degree than does the smaller penis" [40]. Furthermore, even when the erect penis is measured during auto-manipulation or active coitus, the measurements are recognized to be unreliable [40].

Forensic Examination

Examination of Adolescents

The Foley catheter technique, referenced by Carol Jenny MD in 1992, is used primarily to view post-menarchal hymens and requires the insertion of an indwelling catheter midway into the vaginal vault with inflation of the balloon with 40–50 mL of air (this can be modified dependent upon patient comfort and examiner requirement). The balloon is guided outwards to the hymen edge, allowing the hymen to drape over the balloon so that the hymenal edges can be readily visualized [70].

Jones et al. used the Foley catheter technique to examine the hymen of 20 adolescents aged 13–16 years. Use of the Foley catheter balloon technique was virtually painless and allowed identification of more hymenal abnormalities than labial traction alone. The results are summarized in the table below [71].

	Labial Traction Only	Foley Catheter Method
Laceration	2	7
Abrasion	0	3
Ecchymosis	1	4

Alternate methods for examination of the hymenal edge include the use of a swab. The swab is run around the internal edge of the hymen so that all edges can be adequately visualized. Occasionally, normal saline can be used in an attempt to "float" the hymen, separating the edges to better view the hymeneal rim.

Examination of Pre-pubertal Girls

Currently there are two positions that are used for the examination of pre-pubertal girls.

The supine separation technique conducted in the frog leg position (patient on their back, knees bent and flopped outwards) and the knee-chest approach (patient on knees, facing away from examiner, with chest to table and bottom in the air). The latter moves the perineal body and posterior fourchette dorsally, exposing the introitus, and the anterior two thirds of the vaginal canal can often be visualized. It can aid in opening the hymeneal orifice [72]. This latter method is primarily used to support or counter abnormal findings found in the supine frog leg position.

Hymeneal Measurements

Until as late as 1995 it was suggested that a hymeneal opening diameter could be used to determine (or support) a history of sexual abuse. It had been suggested that hymenal openings more than 4mm, in the pre-pubertal child, were associated with sexual abuse [73, 74].

Both specificity and sensitivity of this finding has since been questioned.

Berenson found that approximately one third of abused pre-pubertal children had a horizontal measurement of greater than 6.5 mm in the knee-chest position, whereas two thirds of abused children did not (a specificity of 86% but a sensitivity of only 29%). When the child was examined in the supine position, horizontal hymeneal diameters of greater than 6.5 mm were noted to have a specificity of 73% but a sensitivity of only 32%. They opined that the specificity and sensitivity were not sufficient to warrant its use in either confirming abuse in those who provided a history of such or detecting undisclosed abuse [75].

Berenson also demonstrated that less than 1.0 mm of hymeneal tissue at 6 o'clock had a specificity and a positive predictive value of sexual abuse of 100%. However, the sensitivity of this test was extremely low (1–2%) as almost all of the abused children had greater than or equal to 1 mm of tissue visualized at six o'clock in both the supine and knee-chest positions [75]. It has now been widely accepted that posterior hymenal width cannot be measured accurately and that there is insufficient evidence to determine the significance of a "narrow" posterior hymeneal width in pre-pubertal girls [76].

Lateral measurements of less than 1 mm were observed in the supine position at both 3 o'clock and 9 o'clock and at three o'clock in the knee chest position among abused and non-abused children [75].

It has been suggested that instead of worrying about measurements, more attention should be paid to finding a clear rim of posterior hymeneal tissue (supine position), and a free hymeneal edge from the nine o'clock to three o'clock positions, as this is likely to represent a normal finding [77].

Injury

Female Genitalia Injury

Whether there is injury after vaginal penetration can depend on the presence or absence of a number of variables including, but not limited to, the amount of force used, level of "enthusiasm" of the participants, period over which the penetration continues, the size of the penis (or object), use of or presence of lubrication, associated drug or alcohol use, angle of penetration, experience of the participants, use of objects/toys, extremes of age (e.g. the very young and the old) etc.

Lacerations and ruptures (full-thickness lacerations) of the vagina have also been described in the medical literature after consensual sexual acts [78–80]. They are most commonly located in the right fornix or extending across the posterior fornix; this configuration is attributed to the normal vaginal asymmetry whereby the cervix

lies toward the left fornix, causing the penis to enter the right fornix during vaginal penetration [80]. Factors that predispose to such injuries include previous vaginal surgery, pregnancy, and the puerperium, post-menopause, intoxication of the female, first act of sexual intercourse, and congenital genital abnormalities (e.g. septate vagina) [78]. Although most vaginal lacerations are associated with penile penetration, they have also been documented after brachiovaginal intercourse (fisting) [80], vaginal instrumentation during the process of a medical assessment [81], and the use of plastic tampon inserters [82]. Vaginal lacerations have been documented without any direct intravaginal trauma after a fall or a sudden increase of intra-abdominal pressure (e.g., lifting a heavy object) [80].

Healing of lacerations of the external genitalia is predominantly by first intention, with no residual scarring being detected at follow-up assessments [83, 84]. Nonetheless, scarring may occur occasionally in these areas, but it is important not to mistake a linear vestibularis, a congenital white line identified in the fossa navicularis (present in 25% of neonates), for a scar [85].

When a vaginal laceration may have been caused by an object that has the potential to fragment or splinter, a careful search should be made for foreign bodies in the wound [78] (this may necessitate a general anesthetic), and X-rays should be taken of the pelvis (anteroposterior and lateral), including the vagina, to help localize foreign particles [86]. Any retrieved foreign bodies should be appropriately packaged and submitted for forensic analysis.

Consensual V Non-consensual Injuries

The issue of determining the likelihood, or otherwise, of consent having been given based on the injury found has been studied extensively.

In 2003 Jones et al. concluded, after reviewing ano-genital injuries in adolescents after consensual intercourse, "clearly, the presence of ano-genital trauma suggests that penetration has occurred and implies nothing about consent" [87].

In 2006 Anderson et al. opined that "Currently many experts and laypersons alike believe that if women do not consent to intercourse, they are more likely to have injuries to their genital area. Based on the findings of this study and several other studies, there is evidence to suggest that injuries can be identified on examination after both non-consensual and consensual intercourse" [88].

Lincoln et al., in a 2013 journal paper, compared two groups of women. One group had sexual intercourse with consent and one group were complainants of sexual assault. It was noted that, of the 8 women in the study who had vaginal penetration with fingers only (and all were sexual assault complainants), 6 had observable injuries. Their opinion was that "Penetration exclusively with finger/s was more likely than any other scenario to result in an injury [Odds Ratio 11.25, $p<0.005$]" [89].

Dr. M. O'Keefe in 2008 perhaps best summarizes the current thought on the matter:

"On the basis of current research, it has not been found possible to identify clinical signs which might reliably distinguish non-consensual from consensual sexual intercourse" [90].

It should also be recognized that not only can consensual intercourse result in injury but it can also result in significant injury.

Ahmed et al. reviewed patients admitted to a surgical and gynecological unit over a 7-year period. There was 1 labial, 9 posterior fourchette and 16 vaginal wall lacerations following consensual intercourse that required suturing [91].

Frioux et al. presented a case series of four female adolescent patients over a period of 6 months, each of which had developed significant vaginal bleeding after intercourse, 3 of which presented to the Emergency Department with vital signs consistent with compensated shock. Three of them described consensual intercourse prior to the injury (presenting with lacerations in the fornix, at the top of the vaginal vault) and one described the injury occurring during a sexual assault (presenting with vaginal wall laceration) [92].

Jones and O'Connor reviewed presentations to the Royal Brisbane (Australia) between 2007 and 2011. Vulval non-obstetric trauma was found in 19 of 519 cases. Injuries were due to, amongst other things, consensual (n = 7) and non-consensual (n = 3) intercourse. The seven post-consensual injuries comprised of a 7 cm labia minora tear, a 4 cm labia minora tear (requiring six sutures), a 2 cm labial minora tear, a 1.5 cm posterior fourchette tear, a 4 cm labia minora tear (requiring seven sutures), an unspecified size labia minora tear (requiring four sutures) and a 4 cm mid-labial sulcus tear and bruise [93].

Fisting

Cappelletti et al. [94] did a systematic review of fisting (brachiovaginal or brachioproctic insertion) in the forensic literature in 2016. They identified it as a "potentially dangerous sexual practice". When it involves insertion into the anus the fist may continue past the upper rectum, reaching the sigmoid colon or, less commonly, the descending colon.

The fourteen studies they selected had case numbers ranging from 1 to 11 people. They were able to determine consent status in 27 cases (consensual in 18/non-consensual in 9). Vaginal fisting is represented far less commonly than anal fisting. There was only 1 vaginal fisting case out of the 18 consensual and 3 of the 9 non-consensual cases studied. Two of the non-consensual cases involved fisting of both the vagina and the anus. Of the 18 consensual cases, 2 ended in death.

Of all the cases studied, eight were fatal.

Internal injuries of the rectum generally originated above the dentate line and extended to between 6 and 18 cm beyond the anal verge. When vaginal injuries occurred they were generally lacerations of the posterior part of both the cervix and vagina [94].

First Sexual Intercourse

Nearly 30 years ago, 100 women were interviewed at random and asked about their first coital experience. Results are summarized in the table below [95]:

Incidence of Bleeding		Incidence of Pain	
No Bleeding	44	No Pain	32
Slight Bleeding	35	Slight Pain	22
Moderate Bleeding	9	Moderate Pain	15
Heavy Bleeding	12	Severe Pain	31

While this study has apparent limitations such as requiring memory of events in the past; inability to exclude other reasons for bleeding other than coitus; and the inclusion of seventeen women who reported some other kind of vaginal penetration prior to first coitus (e.g. examination by physician, tampons, douching, diaphragm, masturbation, foreplay with digital penetration) it eloquently debunks the myth that bleeding on the "wedding night" is confirmation of virginity [95].

Adolescent Injury

While no child has ever had a documented congenital transection [61], Emans et al. documented complete transections in 3% of 200 non sexually active post-menarchal girls, between the four and eight o'clock positions. This was attributed to undisclosed sexual abuse or prior sexual experience [96]. Adams et al. have since reproduced these findings, noting the same frequency of girls in the "no previous sexual intercourse" group with either a deep notch or transection. These girls had described painful insertion of a tampon [51].

Goodyear-Smith et al., after re-analysis of Emans' data on tampon users versus pad users and the subsequent development of complete clefts, came to the conclusion, contrary to Emans, that there is a "definite possibility" that tampon use can be associated with an increased percentage of complete hymenal clefts [97].

Equally as interesting was the fact that 26% of the girls in the Emans study, who admitted past sexual intercourse, had no evidence of a complete cleft between the 3 and 9 o'clock position [96]. Likewise, another study of pregnant teenagers (definitive evidence of sexual contact) revealed genital changes in only 2 of 36 that were diagnostic of penetrating trauma [98]. In this study (at that time), findings that were interpreted as clear evidence of penetrating trauma included hymeneal transections / lacerations, laceration of the posterior fourchette, scar of the posterior fourchette associated with loss of hymeneal tissue between 5 and 7 o'clock as well as an absent hymen in the posterior half of the ring.

Pre-pubertal Injury

Heger et al. evaluated 2384 children for possible sexual abuse. A total of 96.3% of all children referred for evaluation had a normal medical examination. Only 4% of all children presented with medical findings diagnostic of abuse. These findings were primarily acute injuries, sexually transmitted diseases, positive forensics, or genital scarring (such as complete hymeneal transections) and included one child with an anal scar [99].

"Both the nature of the abuse and the process of disclosure impacts on the medical examination. Most children are not abused in a way to leave permanent physical findings. Children are usually abused by an individual known to them who wants continued access to them" [99].

The only conclusions that can be reached is that penetrative intercourse (through the hymen) is a rare occurrence in the pre-pubertal age group or that penetrative intercourse can occur, in some cases, without enduring symptoms or signs. It is possible that penetration more commonly occurs superficial to the hymen e.g. simulated intercourse. As yet unanswered is the role of grooming in preparing the pediatric vagina for penetration/sexual intercourse.

Sequelae of Transections

There are few papers that document the healing of ano-genital trauma in children. Heppenstall-Heger demonstrated that, of the 17 transections of the hymen that her team followed, 15 persisted (2 being repaired successfully at surgery), including the 6 followed to puberty [100].

Although there is anecdotal evidence to the contrary, there is no published study that disproves the notion that transections do not heal spontaneously, without residua, unless they are surgically repaired [100].

Male Genital Injury

After consensual sexual intercourse, lacerations of the foreskin and frenulum, meatitis, traumatic urethritis, penile edema, traumatic lymphangitis, paraphimosis, and penile "fractures" have all been described [101–104]. Accidental trauma is more common when there is a pre-existing abnormality, such as phimosis [101], or when the penis is erect. "Fracture" of the penis occurs when the erect penis is forcefully bent (or struck) rupturing the tunica albuginea of one or both corpora cavernosa. Patients sometimes hearing a cracking noise, loss of erection and pain [105].

Skin injury may be incurred if the genitals are deliberately bitten during fellatio [101]. Although the precise incidence of male genital trauma after sexual activity is unknown, anecdotal accounts suggest that it is rare to find any genital injuries when examining suspects of serious sexual assaults [106].

In children the genitalia may be accidentally or deliberately injured, and the latter may be associated with sexual abuse [107]. Bruises, abrasions, lacerations, swelling, and burns of the genitalia of prepubescent males have all been described [107, 108].

When obtaining the relevant forensic samples, the forensic practitioner should inspect the male genitalia with particular reference to the following points:

1. Congenital abnormalities, such as micro phallus and cryptorchidism. Penile length in the flaccid state is said to vary from 8.5 to 10.5 cm (measured from the anterior border of the symphysis along the dorsal surface to the distal tip of the penis), with a documented range of 6–14 cm [40].
2. Acquired abnormalities, such as circumcision, Peyronie's disease, balanitis xerotica obliterans, vasectomy scars, phimosis, tattoos, and piercing.
3. Signs of infection such as warts, discharge, erythema, and vesicles.

Foreign bodies may be worn around the base of the penis, sometimes also encircling the scrotum, in an attempt to increase and sustain penile tumescence. Such devices may result in local and distal genital trauma (penile tourniquet syndrome) [109]. In several case reports, children have had human hairs wrapped around the penis; these hairs may be virtually invisible because of edema or epithelialization [110]. Kerry and Chapman [111] have described the deliberate application of such a ligature by parents who were attempting to prevent enuresis.

Ano-rectal Injury

A lack of perianal or rectal injury does not mean that penetration did not take place or that the complainant consented to the sexual acts as it is generally accepted that with gradual dilatation and lubrication, consensual penile anal intercourse can be performed without any resultant injury [112, 113].

Tears (anal fissures and lacerations), abrasions, redness, and swelling of the "anus" have been described following consensual and nonconsensual acts [113–115]. However, the lack of detail regarding the nature of the consensual sexual acts makes it difficult to determine whether the findings were coincidental, i.e., due to nonsexual causes or directly related to a sexual act. For example, anal fissures may result from numerous other means that are unrelated to penetrative trauma, including passage of hard stools, diarrhea, inflammatory bowel disease, sexually transmitted diseases, and skin diseases [116, 117]. Bruises appear to be infrequent findings following consensual sexual acts. However, the lack of data on perianal injuries make it impossible to determine whether this is a significant observation.

Injury to the Mouth

If injury follows fellatio it is most commonly erythema of the palate, petechiae or purpura. Areas of petechial hemorrhage and confluent bruising have been described

on the soft palate and at the junction between the hard and soft palates after consensual fellatio [118–120]. These areas of bruising vary from discrete single or bilateral lesions of 1.0–1.5 cm in diameter, located on or either side of the midline [119], to larger bands of bruising that cross the midline [118, 120]. The bruises are painless and resolve in 7–10 days [118, 119], although they may reappear with repeated fellatio [119]. The uvula is usually spared and the hard palate is infrequently involved [121].

A forensic practitioner may be asked to explain to the court why these bruises occur. Although the precise mechanism is unknown, the following hypotheses have been proffered:

- *Repeated contraction of the palatal muscles*: As the penis touches the palatal mucosa, the gag reflex is activated, with resultant contraction of the soft palate and other constrictor muscles of the pharynx. It is suggested that the combination of retching and repeated palatal movements causes rupture of the blood vessels in the highly vascular palatal mucosa [118].
- *Sucking*: Sucking on the penis produces a negative intraoral pressure, which is postulated to cause rupture of the blood vessels in the palatal mucosa. This theory is supported by the anecdotal accounts of oral surgeons who found petechial hemorrhages on the palates of children who "made a habit of forceful sucking into a drinking glass" [119].
- *Blunt trauma*: Case reports describe palatal bruises subsequent to sexual assaults wherein a digit or digits have been forced into the mouth [122]. There is no specific evidence to support the hypothesis that direct blunt trauma from a penis can cause palatal bruising, however.

Differential diagnosis of palatal petechiae and purpura include:

- Blood dyscrasias e.g. disseminated intravascular coagulation, hemophilia, idiopathic thrombocytopenic purpura, leukemia
- Paroxysm of violent coughing, sneezing or vomiting
- Suction (negative intra-oral pressure)
- Infections e.g. infectious mononucleosis, measles, streptococcal infections
- Medications e.g. anticoagulants
- Systemic illnesses e.g. Hereditary hemorrhagic telangiectasias, scurvy
- Trauma e.g. fellatio, intubation, nasogastric tube, chemical and thermal injury
- Tumors e.g. nasopharyngeal carcinoma [121]

Whenever a complaint of nonconsensual fellatio is made, the head and face must be carefully examined because there may be other injuries around the oral cavity that support the allegation, such as bruises on the face and neck or lacerations of the frenula [123].

Basic Principles of the Forensic Analysis

Purpose and Value of Forensic Collections

The purpose of forensic sample collections, in cases of alleged or suspected sexual assault, is two-fold. The first is to collect sufficient evidence that can provide the identity of the alleged suspect; the second is to support a claim that sexual penetration occurred. Penetrative events can include the penetration of the anus or vagina by a penis, finger/s, tongue or other object or the penetration of the mouth by a penis. The forensic laboratory can also identify potential links with other offences.

Neither of these provide any evidence as to the fundamental issue of whether or not valid consent was present.

The value of forensic sampling is difficult to measure. If DNA is not recovered from the body samples it does not necessarily preclude an assault from having occurred. As time progresses, the likelihood of positive profiling from these samples is reduced. If the identity of the suspect is known and a claim is made by the accused that intercourse was consensual, it is likely that other evidence might be required to support a prosecution. It is therefore important for complainants to understand that body sampling for evidence collection is only one piece in the evidence puzzle. Other evidence that might be collected, dependent upon the individual circumstances of a case, include CCTV (close circuit television), discarded tissues/condoms/sanitary items, clothing worn immediately after the event by both the complainant and suspect, witness statements, complainant statements, forensic evidence from the body of the suspect, text messages, social media postings, mobile phone records, computer records etc. This evidence collection requires police involvement. The earlier a police investigation begins, the less chance important evidence will be lost, degraded or otherwise compromised.

In a review of data [124] collected from 257 cases of alleged sexual assault between 2005 and 2011, with victims aged 12 years or older, only 16.3% of cases had the biological samples needed and had DNA from a potential suspect sufficient to enable checking for a match. 31.6% of the samples without a "DNA match to suspect" did not have evidence kits taken, 29.9% had kits taken but no biological evidence was derived from them, 8% had biological evidence but no DNA analysis (possibly because the quality of the evidence was not adequate for DNA testing), 3.4% had DNA analysis that did not yield a DNA profile, and 10.3% had a DNA profile with no suspect for comparison. A suspect sample was not obtained in 30.3% of cases with a DNA profile [124].

Prosecutors opined that the value of biological evidence was for the establishment that a sexual act occurred, identifying suspects in stranger cases or when the complainant's ability to identify the suspect was compromised, and for confirming the correct person was being prosecuted even if there was additional evidence linking the defendant to the complainant [124].

The primary defense was usually that intercourse was consensual, especially when the defendant and the complainant knew each other. Less commonly used was a challenge of the integrity of the process [124].

In 2017 the Federal Bureau of Investigation (FBI) expanded the number of unique DNA markers searched in CODIS (The Combined DNA Index System—US national database) from 13 to 20, dramatically increasing the power of forensic DNA testing [125]. It also increased the chance of contamination via secondary and tertiary transfer evidence. This has presumably led to another common defense i.e. providing alternate explanations for how the DNA of the defendant was found on the complainant or crime scene.

Cale et al. [126] suggested that individuals can have their DNA deposited on an item, in sufficient quantities, to be the only contributor or the major contributor without ever coming into contact with that object i.e. through secondary transfer. They defined secondary transfer as the transfer of DNA from one object (or person) to another via an intermediate object (or person). Tertiary transfer has been defined as the transfer of DNA from skin to object to vector to object. For example, person A touched object 1 and person B (the vector) touched object 1 and then touched object 2. It is tertiary transfer if DNA from person A is then found on object 2 [127]. In most cases, the number of indirect steps is unknown and so some prefer the term "indirect transfer" in preference to "secondary", "tertiary" etc. [20].

Indirect transfer should be a concern because it could falsely link someone to a crime, it could link extraneous DNA onto a forensic sample and it could lead to the false conclusion that DNA recovered from an object was a result of direct contact [126].

Preliminary work carried out by the Body Fluid Forum of the UK and Ireland [128] indicates that female DNA may sometimes transfer to the man's underpants following non-intimate social contact. In one experiment, vigorous contact took place between both parties' hands and between the male's hands and the female's face; the male went to the toilet and simulated urination. Subsequent analysis showed that in a small number of cases female DNA was transferred to the male's underpants [129].

Lowe et al. [130] conducted experiments which showed that caution should be exercised in interpretation of results where trace DNA is involved. The experiment involved two people, one a good DNA shedder and another a poor shedder holding hands for 1 min after which the poor shedder immediately held a plastic tube. In one case, only the good shedder on the tube was detected even though it was the poor shedder who touched the tube. No evidence of a mixed profile was observed. Therefore, the vector was not always the one with the most dominant profile on an object.

Kanokwongnuwat et al. [131] observed that males shed more DNA than females. They explained that the larger area of fingerprint left by a male might explain some of the difference. It was also observed that a thumb print, on average, generated more alleles than a print from the little finger. It now seems likely that people are rarely either a consistently good or consistently bad shedder. DNA shedding, on any particular day is likely to be due to a complexity of factors [132].

Illustrative Case *The murderer, dubbed the Phantom of Heilbronn, had baffled German investigators for 2 years. Police had DNA which linked a female assailant to 40 crimes including 6 homicides stretching into Germany, Austria and France. She was linked to the murder of a policewoman in Heilbronn, Germany. After police had accumulated over 16,000 h of overtime investigating the cases they discovered that the cotton swabs they had been using for evidence collection had been contaminated by the same worker at a factory in Austria and that the "Phantom of Heilbronn never existed". The swabs being used had been sterilised and double packaged. This highlights the point that sterilisation alone does not necessarily denature DNA and that caution should be taken when basing an investigation solely on DNA evidence* [133].

Guidelines for Evidence Collection from Complainants and Suspects

Body evidence collection guidelines should be, wherever possible, evidence based. This evidence can come from published research. As DNA analysis methods have changed considerably over the last 4 decades this should be kept in mind when analyzing any recommendations made in older research publications. DNA extraction methods and the equipment used for this can differ between laboratories and so the best evidence would originate from a collation of results from the local forensic laboratory. Unfortunately, most examiners are not given access to this information. The reasons for this are often multifactorial.

In a recent commentary by Tully [134], Forensic Science Regulator for England and Wales, it was observed that the provision of forensic science was currently a "complex landscape", fragmented across public and private sectors. Some police forces exercised considerable control over the number of samples that could be analyzed in sexual assault cases, making the interpretation of results potentially more difficult. The cuts to forensic science services that have occurred presented an "almost existential threat to the profession".

Siloing of responsibilities i.e. police are responsible for the history of the assault, forensic examiners are responsible for body evidence collection and forensic scientists are responsible for analyzing only a predetermined number of selected specimens is an approach that may result in a court presentation less likely to be challenged. It is likely, however, that it will also not represent the full facts in the case.

Persistence Versus Success

When looking at different evidence collection methods it is worth considering two factors. Persistence relates to the longest time period recorded in which a substance lasted or could be identified. This may relate to complete spermatozoa, enzymes such as amylase, alkaline phosphatase or prostate specific antigen, autosomal profiles from DNA,

mitochondrial profiles from DNA (inherited through the maternal line), Y-STR profiles from DNA (inherited through the paternal line) etc. Success relates to the number of positive results from a given number of collection attempts. Both can be impacted by various things e.g. age of the complainant, time since intercourse and various post coital activities such as washing, eating and drinking. Up to 70% of swabs can be negative for semen. Results may be negative when evidence is collected from complainants who did not engage in a penetrative sexual assault (as may occur if there was no memory of an actual assault), where ejaculation did not occur (which would decrease the likelihood of DNA collection) or where there had been a significant lapse of time between assault and assessment. A negative swab therefore may not be a reflection of inefficient sampling.

Forensic examiners should be aware of any local guidelines for forensic sample collections. For example, in the U.K., the Faculty of Forensic and Legal Medicine [135] publishes and regularly updates Recommendations for the Collection of Forensic Specimens from Complainants and Suspects. The method chosen to collect various samples is likely to differ between sites and will often depend upon local laboratory capabilities to analyze those samples. The evidence underpinning current time periods for collection have been included in the section below. Cut off time periods in practice, however, should be determined regionally and an examiner should have a basic understanding of how those time limits were derived and the research data that underpins them. Guidelines should be reviewed regularly and should rely upon the experience of local forensic laboratories (statistically and not anecdotally) as well as published research.

If you are a new forensic examiner or an examiner with only an intermittent or small caseload there is value in taking a "cookie cutter" approach to forensic collections e.g. if there is an allegation of penile vaginal penetration, guidelines might suggest that you collect vulval, low vaginal and high vaginal samples. If you are an examiner who works at an established unit with a generous caseload experience, you might consider a more "bespoke" approach to forensic collections e.g. you may choose to add additional swab sites or exclude swab sites dependent upon the particular circumstances of the case. Always be guided by local recommendations and if deviating from suggested practice, record the reasons for doing so.

Slides

The majority of samples collected for sexual assault cases will be in the form of swabs. In some jurisdictions, examiners are required to make a slide from those swabs in cases where the sample has been taken for the purpose of finding DNA from semen. In other jurisdictions, the forensic laboratory will make their own slides from the submitted swabs. A slide is used as a screening test. Generally, if spermatozoa are seen on the slide, the corresponding swab will be sent for DNA analysis as sperm are recognized as a good potential source of DNA. Slides are not required if a swab has been taken with the view of detecting DNA from other sources such as epithelial cells, blood or saliva.

Some laboratories will assess slides and record estimates of intact spermatozoa. This and sperm density are sometimes used to provide a time since intercourse (TSI) determination. Advice as to the value of this should be sought from the local forensic laboratory.

Some laboratories will test all swabs submitted, some will screen slides first and choose the best swabs to test, and some laboratories will only be financed to test one swab. An understanding of how your local laboratory works and makes these decisions can be useful.

The swabs and containers used to collect forensic evidence differ from those used in clinical tests. They should be DNA free. Most swabs used for this purpose are sterilized with Ethylene Oxide.

Forensic swabs should be placed in plastic sheaths that do not contain transport media or in specially designed boxes that allow the swabs to air-dry. Given the risk of contamination, swabs should not be allowed to air dry prior to packaging.

Blood and Urine samples

Blood samples for drug and alcohol analysis should be placed in containers with a preservative that prevents decomposition and fermentation (e.g. sodium fluoride), and an anticoagulant (e.g. potassium oxalate). All the containers should be shatterproof. Consideration should be given to placing the urine container in a plastic bag in case of leakage.

Lubricants

Water-based lubricants from a single-use sachet (Pedicat® or KY® Lubricating Jelly) may be used to moisten the proctoscope/speculum to facilitate its insertion into a body orifice. The empty sachet should be retained and packaged in a "tamper-evident bag". Sterile water may be used as a substitute to lubricants.

Packaging and Continuity

Any retrieved items must be packaged quickly and efficiently to prevent accidental loss of material and minimize decomposition of the sample. The use of bags with integral tamper-evident seals is recommended to prove that the sample has not been contaminated with exogenous substances since it was sealed.

It is the responsibility of the person who obtains the sample to ensure it is appropriately labelled and sealed. A crisis worker, police officer, or scene of crime officer may assist with the labelling process, but the forensic practitioner must check the labels before signing.

The information on each exhibit and tamper-evident bag should comply with local recommendations. It would generally include:

- Name of person (or reference number) from whom the sample was taken (examinee)
- The healthcare professional's name
- Description of exhibit or site sampled e.g. high vaginal swab
- Date on which the sample was taken
- Blood and urine only – time at which the sample was taken (24 h clock)

Where two swabs have been taken from the same site, it is *imperative* that there is a clear indication on the label regarding the order in which the swabs were obtained. This is most easily done by describing the first of the two samples as sample A and the second as sample B.

Tamper-Evident Exhibit Bags

The use of bags with integral labels will prevent accidental detachment of this vital information.

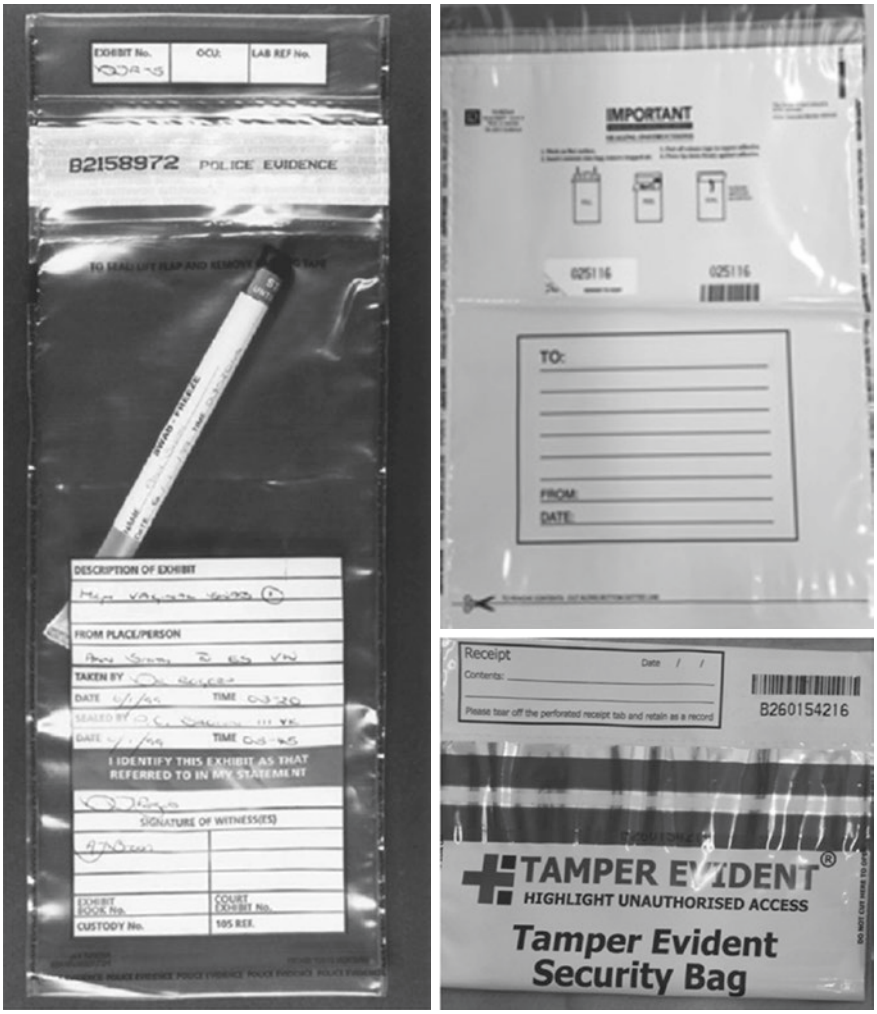

Figure above: Examples of tamper-evident bags.

Clothing

The clothing worn by the complainant during or after the incident may be an invaluable source of information in terms of the nature of the assault (e.g. damage to clothing and body fluid stains) and the identification of the suspect. Even stains on clothing that has been washed have been found to contain sufficient spermatozoa to produce a DNA profile [136, 137]. Ruan et al. showed that 74% of UV treated cotton swatch samples produced DNA profiles after laundry with household garments. Of the mixed profile samples, the majority were from two to three persons with one being a four person mix. They observed that whilst we assume that DNA transfer occurs within the washing machine, there are in fact other opportunities for transfer such as the mixing of clothing in the laundry basket and the varied method of drying clothes [138].

Kulstein et al. observed that a prolonged storage period between semen deposition and laundering resulted in increased DNA quantities and full profiles [139].

Clothing should be placed in paper bags, or into breathable plastic bags, that prevent the accumulation of condensation which could accelerate decomposition of body fluids. Submitted clothing should be sealed and labelled as described previously. When the clothing is overtly wet or possibly contaminated with accelerants, the forensic science laboratory should be asked for advice on packaging and storage.

The forensic scientist must be provided with salient information regarding the incident and subsequent actions of the complainant to determine the type of forensic analysis required. A useful means of transmitting this information is via a pro forma. One example is the Forensic Medical Examination proforma as produced by Faculty of Forensic and Legal Medicine [140].

Analysis

Identification microscopy (e.g. spermatozoa) and immunodiagnostic techniques are used in the identification of body fluids. Microscopy is also used for hairs and fibers, although less commonly since the advent of DNA analysis.

Discovery of the specificity of an individual's DNA profile has considerably enhanced the information that can be provided by a forensic science service (FSS) for connecting a person to an offence and linking offences to each other. Although a detailed consideration of current DNA techniques is beyond the scope of this chapter, a general understanding of the terms and techniques will benefit the forensic practitioner. A useful information source has been provided by the Royal Society of Edinburgh: Forensic DNA Analysis – A Primer for Courts [141].

Except for identical twins, each person's nuclear DNA is unique. An individual's gender and DNA profile may be obtained from any of his or her body fluids or tissues (e.g. blood, semen, and bones). The current technical process used for DNA profiling is termed short tandem repeat (STR) analysis. STR loci are a class of polymorphic markers consisting of simple repeated sequences of 1–6 base pairs in length. STRs are present throughout the human genome (DNA), occurring on

average every 6–10 kb along the DNA and may exhibit a high degree of length variation resulting from differences in the number of repeat units displayed by individuals. Their abundance and hypervariability make them ideal markers for the identification of an individual. When a DNA STR analysis is performed, the specific areas of interest on the molecule are initially targeted. Multiple copies of these areas are then produced using polymerase chain reaction (PCR) techniques, which amplify minute amounts of DNA. The DNA pieces are then sorted according to their size, producing the individual's DNA STR profile [142].

DNA STR analysis, including a DNA sex test [143], is part of the routine forensic assessment of biological samples in Europe. The formation of the European DNA Profiling Group has led to the standardization of DNA analysis procedures used in the European community and associated western European countries.

Standard DNA profiling systems used to be focused on the analysis of ten STR loci known as AMPlfSTR® SGMPlus™. This system has been superseded by multiplexes that utilize an increased number of STRs, containing the original 10 SGMPlus STRS plus six more and a gender marker in the case of DNA 17 multiplexes or an extra eleven STRs, two Y chromosome markers, and a gender marker in the case of AmpFISTRGlobalfiler. Increasing the number of loci provides a greater discriminating power but more importantly increases the sensitivity of DNA analysis. These systems contain all of the Interpol and European Networks Forensic Science International (ENFSI) recommended European loci, which provides points of comparison for DNA intelligence purposes outside of the UK [144–151].

When DNA profiling was first applied to forensic science, large amounts of nucleated material were required. However, the use of PCR technology has enabled much smaller amounts of material to be analyzed. Due to the increased sensitivity of the newer multiplexes, DNA profiles can be obtained from ever decreasing amounts of DNA or from poor quality DNA samples e.g. samples of DNA that have become degraded or are mixed with chemical inhibitors.

Y-Short tandem repeats (YSTRs) analysis is a well-established technique routinely used in casework that targets the STR regions located on the Y-chromosome, therefore male DNA only. Over 200 STR markers have been identified on the Y chromosome. Commercial kits are currently available for at least 23 of these [152]. Y-STR analysis has proven successful in detecting male DNA where there has been digital or penile penetration with no ejaculation and in cases of sexual assault where the male has been vasectomized [153]. In these cases, the absence of sperm mean only a small amount of male DNA may be present on intimate swabs and the female's DNA would swamp this. By specifically targeting the Y chromosome STR markers, it is possible to locate and amplify a small amount of male DNA present.

Y-STRs are inherited down the paternal line. As with routine STR testing, Y-STRs vary considerably between unrelated individuals however close paternal line male relatives are likely to share the same Y-STR profile.

Y-STR profiles cannot be loaded to the UK National DNA Database and Y-STR profiling has a lower discriminating power than autosomal STR profiling. Y-STR profiling can, however, provide results in sexual assault cases where autosomal STR profiling has been unsuccessful.

Fluorescence in situ hybridization (FISH) is a technology that uses a fluorescein-tagged Y-specific DNA probe to label male epithelial cells. Once identified, the cells may be separated from the rest of the sample and submitted for DNA profiling. Without separation, the profile may be dominated by female DNA from the examinee, making it difficult to interpret.

Mitochondrial DNA analysis has been used in forensic casework. This technique examines the DNA contained within mitochondria in the cell body and obviates the need for nuclear material [154]. As there are numerous mitochondria in a single cell and each contains multiple copies of the mitochondrial genome, it is possible to extract far more mitochondrial DNA than nuclear DNA. This is useful when cells have become degraded through decomposition or burning. The technique is also suited to discrete samples, such as hairs without roots and for fecal material. Mitochondrial DNA is only passed from mother to child (unlike nuclear DNA, there is no contribution from the father); therefore, all the descendants along the maternal line will have the same mitochondrial DNA. It also has a much lower discrimination power than nuclear DNA profiling. In sexual offences, therefore, the selection of material to be analyzed by this technique is limited and its use needs careful consideration.

The forensic science laboratory must be notified when it is alleged that people who are closely related have been involved in a sexual offence, because their profiles will have greater similarity than profiles from individuals picked at random, and further differentiating tests may need to be performed.

Increasingly in the past few years, messenger RNA (mRNA) profiling has been proposed as a new method of body fluid identification and a lot of research is being carried out in this area. Techniques to use mRNA profiling to distinguish between menstrual blood and traumatic bleeding and buccal epithelial cells from other epithelial cells are being investigated [155, 156].

In addition to the autosomal systems, some laboratories are using DNA quantifiers, e.g. Quantifiler ™ Trio DNA Quantification Kit. This enables detection of amounts of autosomal versus male DNA as well as the quality of the DNA (i.e. presence and extent of degradation). Knowing the quantity and quality of the DNA assists the laboratory in determining the most suitable way of progressing testing.

Skin

The comments in this section refer to non-genital skin.

Areas of skin that have been licked, kissed, sucked, bitten, ejaculated, bled or spat on by either the suspect or the complainant can be sampled. DNA from saliva may also be found with self-lubrication of the penis or fingers. Cellular material, amenable to DNA profiling techniques, has also been identified where there has been skin-to-skin contact (e.g. manual strangulation or gripping the arm) [157, 158]. In a simulated strangulation it was possible to detect victim only DNA in 12 of 29 samples but full DNA profiles of both victim and offender in 7, up to 6 h after touching occurred [158].

Trace DNA

Trace DNA can mean a variety of things:

- It is sometimes referred to as any substance collected for testing to aid an investigation [20]
- It can sometimes be used to refer to samples where the quantity of DNA available for testing is below a certain threshold. This has variously been described as "low template" and "low copy number DNA" [20]
- It can refer to any sample where there is uncertainty that it may be associated with the crime itself [159]
- It can refer, on occasion, to DNA that cannot be attributed to an identifiable body fluid [132]

The small number and nature of these transferred DNA-bearing cells, from skin, often make identification of the cellular source of origin (buccal, epithelial, etc.) either impractical or impossible [160].

Method of Sampling

The double-swab technique, first described by Sweet et al., is the recommended method to recover dried stains or possible cellular material from skin in many jurisdictions [161]. When using this technique, sterile water is used to moisten the cotton tip of the first swab. The tip of the swab is then rolled over the area of skin using circular motions while rotating the swab on its long axis to ensure maximum contact between the skin and the swab. Then, a second dry swab is rolled over the same area to absorb the water left on the skin by the initial swab and collect any remaining cells. Minimal pressure should be applied to prevent exfoliation of the patient's own epithelial cells. It may be important to understand that in the Sweet et al. experiment, only 5 subjects were used. A known quantity of saliva was placed on the skin and testing performed 10 minutes later. The difference between the single swab results vs the double swab results was determined by the percentage of DNA collected in relation to the theoretical amount of DNA deposited on the skin. The single swabs netted 35.3 ± 4.8% vs the double swabs which collected an estimated 44.6 ± 6.4% of the available DNA. This would indicate that, in some cases, the single swab may have had a better result!

Graham and Rutty tested two lines of varying amounts of DNA on a non-absorbent surface, swabbing 15 h later. One line was swabbed with a wet swab and the second was swabbed using both moist and dry swabs. They generated 16 samples and concluded that there was no additional benefit for adding a dry swab [158].

Pang and Cheung collected 20 wet and dry swabs from touched surfaces. Sixteen out of 20 wet swabs were positive for DNA as opposed to 12 out of 20 for the dry swabs. Of the 12 dry swabs, the corresponding wet swab was negative in 2 cases. 5 of the dry swabs returned greater quantities of DNA than the wet swab. Pang and Cheung opined that the second dry swab might collect more DNA if the first wet swab rehydrated the epithelial cells, making them easier to dislodge and collect with the second swab [162].

Hanson and Ballantyne, while comparing DNA profiles from cervico-vaginal swabs, reviewed results from 2 couples where 2 swabs were taken from the same area, expecting that the first swab was more likely to have greater amounts of DNA. Couple 1 demonstrated that more alleles were indeed found on the first swab but the second swab had one allele identified, which had not been present on the first. In couple 4, it was unclear which swab had been done first but alleles were found on each swab not present in the other [163]. It is possible, therefore, that any success noted with the double swab method might be related more to the fact that 2 swabs were collected, rather than the fact that a moist/dry formula is used.

The performance of various types of swabs was tested by NSW Police Forensic Services Branch scientists. They demonstrated that single cotton swabs were equivalent or better than double swabbing in gaining a reportable DNA profile from material on all relevant surface types [164]. It has also been postulated that one possible reason for this is that if the first swab is saturated with water, it might inhibit the amount of material it can subsequently pick up when swabbing the skin. It is therefore recommended that, if using a swab moistened with water, that only one drop of water (or as little as is practical) be used.

Alternative Light Source

Some authors comment that ultraviolet (UV) light causes fluorescence of semen and saliva and advocate its use in determining the areas of skin to be swabbed [165, 166]. This advice must be interpreted cautiously, because a study by Santucci and colleagues found that although many creams and ointments fluoresced when exposed to a Wood's lamp (wavelength 360 nm), none of the 28 semen samples examined did [167].

In addition, other authors have commented that detergents, lubricants (particularly those that contain petroleum jelly), and milk fluoresce [168].

When semen stains are exposed to a high-intensity light source of variable wavelengths (e.g. the Polilight®) and viewed using goggles to block the strong excitation light, semen may be detectable even when the background surface is fluorescent [169]. Furthermore, the location of the stain may be recorded using photography.

Nelson and Santucci have described training forensic physicians to use an alternative light source (the Bluemaxx BM500) to identify semen (100% sensitivity) and to differentiate it from other products [170].

Nolan et al. used an alternative light source (Polilight-Flare®II Plus) to detect seminal fluid on a range of fabrics which were laundered up to 6 times. All unwashed sample materials fluoresced strongly. Fabrics with less absorbency (such as satin, nylon and lace) did not fluoresce following one wash. Following the second wash cycle no fluorescence was visible in any of the fabrics. Conversely, microscopy was positive for cotton and terry toweling after the sixth wash [171].

A survey of several forensic science laboratories in the UK and Ireland [128] found that most felt that using alternative light sources to detect stains on clothing and inanimate materials was of limited value because of the number of false positives and false negatives.

Detection of Saliva

Saliva technically refers to the fluid that originates directly from the salivary glands. Oral fluid, however, relates to secretions from salivary glands, nasal secretions, bacteria and bacterial products, desquamated epithelial cells and food debris amongst other things [172]. DNA that is present in oral fluid is largely derived from the cellular material that is discarded from the mouth.

The only means of identifying the presence of saliva on the skin is by detecting the enzyme amylase. The traditional presumptive test is the Phadebas® amylase test. The Rapid Stain Identification of Human Saliva (RSID™—saliva) can detect human salivary α amylase at lower concentrations [173]. However, amylase can be present in body fluids other than saliva, so in some instances this test is not suitable.

In humans, amylase is expressed on two genetic loci:

- AMY1—salivary amylase—which is abundantly present in saliva with lower quantities found in perspiration and breast milk.
- AMY2—pancreatic amylase—which is found in urine, semen, feces and vaginal fluids [174].

Care needs to be taken with interpretation. The presence of amylase may be a result of secondary, rather than primary transfer.

Cunnilingus and Anolingus

Cunnilingus is the sexual activity in which the female genitalia is licked, sucked, or rubbed by the lips and/or tongue. Anolingus ("rimming") is the sexual activity in which the anus is licked, sucked, or rubbed by the lips and/or tongue.

In some jurisdictions, penetration of the vagina or anus with the tongue, during nonconsensual cunnilingus or anolingus, is considered to be legally analogous to nonconsensual penile penetration of the vagina and anus. For example, in England the offence of "assault by penetration" is defined as nonconsensual penetration of the anus or genitalia by an object or a body part (See definitions at start of Chapter) [175].

Repeated thrusting of the tongue over the edges of the mandibular incisors during cunnilingus or anilingus may cause ulceration of the lingual frenulum, which completely heals within 7 days [176]. Such lesions should be specifically sought during the examination of the suspect's oral cavity when such an act has been described by the complainant or when the precise details of the assault are unknown.

Persistence and Success

In 1992, a study conducted at the Metropolitan Police Laboratory, London, using vaginal swabs from volunteer female donors who had not participated in cunnilingus, revealed high levels of endogenous amylase [177]. Furthermore, amylase has been

specifically isolated from cervical mucus [178]. DNA analysis is undertaken on the vulval and/or vaginal swabs. If the suspect's DNA profile is obtained, it can be used to support an allegation of cunnilingus although, obviously, the precise interpretation will depend on whether the complainant was subjected to other sexual acts that could account for the presence of the DNA (e.g. ejaculation, digital penetration etc.).

There is no published persistence data regarding the maximum time it is possible to obtain the assailant's DNA pattern from the female genitalia after cunnilingus/anolingus. It is worth noting that areas of clothing which were in contact with the genitals after such acts can be worth testing as saliva / DNA may have transferred to them.

Several studies have looked at persistence of DNA on skin, thought to have originated from oral fluids.

Graham and Rutty took five volunteers and had their partners deposit saliva on their neck. The following day, DNA profiles from the partners were obtained from the neck swabs of three of the five volunteers [158].

Sweet and colleagues have shown that it is possible to obtain a DNA profile from saliva stains (corresponding to a bite mark) on cadaver skin when the saliva was deposited up to 48 h earlier [179]. In a separate paper they showed the resilience of DNA which had been recovered from the right breast (bite mark) of the deceased body of a female who had been found 5.5 h after being in a river with a slow- moving current [180].

Kenna et al. in 2011 took samples of saliva from three male donors and plated it onto the legs of their three female volunteers. The women were instructed not to wash the affected area. Full male DNA profiles were obtained in all but one combination (n = 8 out of 9) at 96 h [181].

Hair

Hair is most commonly sampled to detect body fluids or retrieve foreign hairs or particles. It has been known for many decades that numerous ingested prescribed and illicit drugs (e.g. barbiturates, amphetamines, opiates, cocaine, benzodiazepines, γ-hydroxy butyrate, and cannabis) are deposited in the hair [182]. Although toxicology of hair was originally used to detect drugs that had been repeatedly ingested, recent advances in analytical techniques have meant that toxicology may be useful after single-dose ingestion as would occur in a substance-facilitated (sexual) assault [183, 184]. This is particularly pertinent because complainants of possible drug-facilitated (sexual) assaults frequently do not report the incident expeditiously because of amnesia and/or doubt about what might have happened, and drugs may be accessible to analysis for longer periods in hair compared to blood or urine [185].

Method of Hair Sampling

Cutting

Hairs should be sampled by cutting if they appear to be contaminated by material that has the potential to have forensic significance (e.g. semen). If the patient does not consent to having the contaminated hairs cut or if it is not practical to cut them

because of the extent of foreign material contamination, then the relevant areas can be swabbed.

For drug analysis, hair can be collected up to 6 months (and sometimes longer) following a relevant incident. Sampling should not occur less than 4 weeks after the incident, as it takes at least this long for hair to grow sufficiently from the scalp to enable collection. Examinees should be advised not to cut, dye, bleach or perm their hair in the intervening time. Hair toxicology testing is only done by certain laboratories. Prior to collection it is recommended that the appropriate laboratory is contacted. Who (or what organization) will be paying for the process should be determined (as it is often not cheap). Discussion about available kits and appropriate method for collection and delivery also need to be determined prior to sampling. Hair samples should not be refrigerated or frozen but stored dry at normal room temperature [135].

Foreign Hair

Shed hairs, if in the catagen or anagen growth phase are suitable for routine nuclear DNA testing. Provided a root is present, close to a 100% success rate should be achieved. Most shed hairs (95%) are, however, usually in the telogen phase and contain little nuclear DNA. Mitochondrial DNA can be examined instead. It is a more time consuming and expensive process than nuclear DNA analysis, requires specialist interpretation and has lower random match probabilities [186]. While there may still be a place for macroscopic/microscopic examination of hair, most forensic laboratories have abandoned the practice and available expertise is often limited.

Any foreign particles or foreign hairs identified on the head or pubic hair should be collected with forceps (preferably DNA free) and submitted for analysis [187, 188].

Pubic hairs may be transferred between individuals during sexual intercourse. Exline et al. [189] studied volunteer heterosexual couples who combed their pubic hairs immediately after sexual intercourse in the "missionary" position. Even under such optimal collection conditions, pubic hair transfers were only observed 17.3% of the time using macroscopic and microscopic comparisons. Pubic hair transfer to males (23.6%) was more common than transfer to females (10.9%).

Some studies on sexual offence case material have shown lower rates of pubic hair transfer between complainant and suspect. Mann [190] reported that only 4% of female complainants and no male complainants were identified as having pubic hairs consistent with the assailant hairs isolated from combings of the pubic hair, and Stone [191] identified foreign pubic hairs among the pubic hair combings of 2% of the complainants studied. A survey of sexual offence case material submitted to laboratories throughout the USA, however, found pubic hairs that associated the complainant and the suspect in 15% of cases [192].

Nails

Fingernails should be examined as part of the examination of the hands of a complainant/suspect and any staining/breaks should be noted and possibly photographed.

Sampling

Sampling of nails often occurs if there is a history of a complainant having scratched their offender or if there is an allegation of digital vaginal penetration. In the latter case it will be the suspect's nails that are sampled. Generally, there are two accepted methods used for the collection of fingernail/subungual evidence and these are swabbing and/or cutting of the nail. Most examiners who have attempted cutting someone else's fingernails can probably attest to the difficulty of the process. The current FFLM guidelines for the collection of evidence advise clipping only if visible material can be seen or if a broken nail needs to be matched with a recovered nail fragment [135]. Conversely, the Australian guidelines advocate either process or both if considered appropriate. They recommend a single swab to be used for each nail (ten in total).

When the nail is being swabbed as a result of a history of digital penetration, or if blood can be seen, additional swabs of the nail bed and nail surface are recommended. Nail clippings from each hand can be combined into two separate collection jars i.e. right hand and left hand [193].

Another consideration, when collecting nail evidence, is the type of nail of the complainant/suspect. The nails might be short or acrylic (fake) nails and cutting them might prove impossible. In these cases, the nails should be swabbed. When cutting longer nails it is important to ensure the implement (scissors or clippers) being used has been adequately prepared to reduce the risk of DNA contamination.

Available research looking at preferential sampling methods is limited.

What has been demonstrated:

- Sticks or scissors (sharp objects) should not be used to collect subungual evidence as they have the potential to remove too much of the donor DNA [194].
- The harder a complainant scratches the suspect, the more DNA that can be found on subsequent testing [194].
- The more nails that are clipped and sent for analysis, the better the success rate for finding offender DNA [195].
- Subungual evidence can be collected even if the examinee has washed their hands several times. It may persist up to 2 days following an allegation of digital vaginal penetration [196].
- Fingernail evidence can give rise to the question of background foreign DNA i.e. DNA that might be present and might not bare any relevance to the crime being investigated. In the Cook study the fingernails were swabbed from 100 volunteers and foreign DNA was detected in 13% with only 6% giving reportable mixed DNA profiles [197]. The presence of a mixed DNA profile in a fingernail sample may lead to reasonable doubt in court as to whether the DNA transfer occurred prior to, or during, an assault. Males, in this study, were more likely to provide a mixed profile from fingernail sampling, than women.
- Malsom et al. took 12 couples that co-habitated and swabbed all their fingernails on both hands on three separate occasions. The results demonstrated that as the couples spent increasing amounts of time together, the incidence of mixed DNA profiles increased. 61% of samples gave full or partial donor profiles. 37% exhibited DNA profiles additional to that of the donor [198].

- People who bite their nails were significantly less likely to give a mixed DNA profile, even when the fingernails were swabbed [198].
- A 2015 study suggested that only 4 mm^2 of fingernail sample is required for testing [199].

It is important to remember that foreign DNA found under the fingernails can have an innocent explanation, and care must be exercised when considering this type of testing if the victim and suspect have legitimate access to each other.

Oral Cavity

Buccal Swabs

In sexual assault cases an examiner is often required to collect a baseline sample of the complainant's DNA. This is done with a buccal swab (patient reference swab). Instructions are included in most buccal swab kits. A properly collected buccal swab will provide adequate material for DNA profiling. It is best to do this after any other oral collections have been done. Police will usually collect buccal swabs from suspects.

Oral (Mouth) Swabs

The oral cavity may be sampled when fellatio was performed during the sexual assault or in circumstances in which the details of the incident are unknown. Fellatio (also referred to as irrumation) is a sexual activity in which the penis is placed in the mouth; sexual stimulation is achieved by sucking on the penis while it moves in and out of the oral cavity. Ejaculation may or may not occur. It is common for the semen to be spat or vomited onto clothing where it will remain until washed. Any potentially contaminated clothing or scene samples, therefore, should be submitted for forensic examination.

There is no current worldwide consensus as to what is the best oral sampling method. The following is one suggested method:

Swab the gingival recesses (between lip and teeth), over teeth, back of throat, under the tongue including dentures, dental fixtures and any oral piercings [135].

Success and Persistence

Given the lack of research as to the best site or method of collection it is not surprising that the success rates for sperm detection are appallingly low. 554 analyzed oral swabs, collected in NSW from 2010 to 2015, showed sperm detection in 4.2% (n = 21). All sperm positive samples were collected within 18 h except for one case in which the complainant admitted to post assault consensual oral intercourse with a partner. Of those collected 24 h or more after the assault, all were negative for spermatozoa with the only positive result coming from a deceased person [200].

Lack of success is due to a number of factors which include the hostile environment of the mouth with oral bacteria, enzymes, salivation, eating and

drinking all potentially washing away evidence quickly. Although rinsing of the mouth, drinking, and brushing of teeth do not necessarily remove all traces of spermatozoa [201], such activities should be discouraged until the samples have been obtained.

Willott and Crosse [202] reported that spermatozoa are found more often in the saliva sample compared with mouth swabs, but also highlight several cases in which spermatozoa were recovered from swabs taken from specific areas of the oral cavity (e.g. under the tongue, the roof of the mouth, and the lips).

Willott and Allard found spermatozoa on only 9 oral swabs out of 74 tested (12%) [203].

Tucker et al. reviewed 369 cases of sexual assault where there was reported to have been oral involvement. Only 4 cases (1%) resulted in a sample positive for sperm and all of these were examined within 4 h of the assault [204].

A Norwegian study reviewed 22 oral samples and found that none were positive for sperm. All studies were done within 72 h; the majority having been conducted within 24 [205].

Perioral (Lip) Swabs

A moistened swab can be used to collect evidence from around the lips of a complainant or suspect. This might be considered if a history of kissing or licking has been given (DNA from saliva); if there is a history of oral sex (DNA from semen) or if a suspect has placed his hands over the complainant's mouth for a period of time (DNA from epithelial cells).

Analysis of 71 peri oral swabs was performed over a 5-year period in NSW. Sperm was detected on 13 of the samples (18.3%). None were positive after 24 h with the majority positive less than 18 h post assault [200].

Oral Rinses

One suggested method for the collection of oral rinses advises that the complainant places a small amount of sterile water into his/her mouth (up to 5 ml), swishes the fluid around the mouth and through the teeth, then "spits" the contents into a DNA free collection jar. This is then spun down using a centrifuge, usually by the forensic laboratory. The residual cells are examined for spermatozoa and analyzed for DNA.

In the Nittis et al. study, 60 cases were analyzed by the laboratory where there was a direct comparison between oral swabs (taken first) and oral rinses (taken as the second sample). The oral rinse was shown to be a more successful method to recover sperm (17 of 104 submitted samples – 16.3%) and has led to a decision in NSW to abandon oral swab collections in preference for oral rinses. Sperm, in all cases, was only detected when there was less than 18 h between assault and testing [200].

Chewing Gum

Interestingly, sufficient spermatozoa for a DNA profile have also been recovered using standard extraction techniques from chewing gum that was retained in the mouth during non-consensual fellatio [206].

Female Genitalia

In many jurisdictions, the legal interpretation of "vaginal penetration" refers to penetration of the labia and does not require that the penis actually enter the anatomical vagina (See definitions at start of Chapter).

The age at which a female can legally give consent for penile–vaginal intercourse varies from country to country e.g. in England and Australia the age of consent is 16 years.

Forensic science laboratories are frequently requested to determine whether semen is present on the swabs taken from the female genitalia because semen evidence can play a central role in the identification of the suspect. The presence of semen usually confirms that sexual activity has taken place, but the absence of semen on the swabs does not mean that penetration did not occur. The female genitalia should also be sampled if a condom was used during the sexual act and if cunnilingus is alleged to have occurred.

Method of Sampling

Macroscopic examination of the external genitalia should occur before the insertion of a speculum, because even gentle traction on the posterior fourchette or fossa navicularis during a medical examination can cause a superficial laceration at these sites. Whenever possible, the vagina and cervix should be inspected via an illuminated and transparent speculum after the high vaginal samples have been obtained. Colposcopy and the application of toluidine blue dye are two specialist techniques used by some forensic practitioners during female genitalia examinations.

When nonconsensual penile–vaginal penetration is alleged, the swab samples are plated onto a slide and examined microscopically by the forensic scientist to identify spermatozoa and DNA analysis is performed on any spermatozoa found if deemed necessary.

The scientist is able to provide objective evidence in terms of the quantity (determined crudely) and quality of the spermatozoa present and may be asked to interpret the results in the context of the case. When providing expert evidence regarding whether vaginal penetration has occurred, the scientist must be able to rely on the forensic practitioner to obtain the samples in a manner that will refute any later suggestions by the defense that significant quantities of spermatozoa, which were only deposited on the outside of the vulva, could have been accidentally transferred to the high vaginal area during the medical examination [207]. It is worth noting that there has been no research to support or refute this hypothesis.

Areas of the female genitalia that are sampled have been determined historically. Earlier reports document collections at the vulva, low and high vaginal areas. Later, an endocervical swab was seen as being useful if there was a delay in presentation. The original research that underpins this was based on 36 patients [208]. The value of sampling three internal genital areas (vulva, low and high vagina) is uncertain, other than to increase the chance of collecting DNA by collecting three samples. What can be inferred about depth or likelihood of penetration from DNA/sperm

only found at one of these sites, or assessments made based on the amount of semen seen at these sites, is uncertain and any opinions on such should be approached with caution until further research is available.

Other potential genital or nearby areas for swabbing include the external labial area, the mons or perineum. It should be clear in the examiner's mind what anatomical areas are being sampled when submitting a "vulval" swab or "external labial" swab as these may differ from examiner to examiner or from unit to unit.

External Labial

In cases where cunnilingus is alleged (licking of the female genital area), swabs of the external aspect of both labia majora can be collected using a moistened swab. The external area is considered to be the skin side (or potential hair bearing aspect) of the outer vaginal lips (labia majora).

Vulval

The vulval swab, in NSW for example, is sampled from the lower half of the vaginal entrance, between the inner aspects of the lower ends of both labia minora, including the fossa navicularis. UK FFLM recommendations suggest that the vulval swab will include both the vulva and perineum and that all genital swabs include both a moist and dry sample [135].

Low Vaginal

The vagina anatomically originates at the hymen. A low vaginal sample is taken by introducing the swab into the lower part of the vagina, past the hymen (or hymeneal remnant).

High Vaginal

This should be collected using a speculum. If lubricant is used, specify the type of lubricant for the forensic laboratory. In most cases the speculum can be inserted with no lubrication but insertion should be done slowly and carefully. The swab is inserted through the speculum into the vagina and a sample is taken from the top of the vaginal vault. There will be some circumstances when a speculum cannot be used and then the high vaginal sample is called a blind high vaginal swab. The swab is introduced into the vaginal vault and progressed carefully as far as it will go. It is, in fact, a trans-vaginal swab, sampling all areas of the vaginal vault. Whether or not a speculum has been used should be identified in the case notes.

Endocervical

The endocervical sample should be taken from the cervical os. It is not necessary to introduce the swab through the os, as done with Pap smears, but merely sample the cells at the os opening. There has been some suggestion in the literature that if the os cannot be visualized, the cervix might be sampled anywhere along its length. Joki-Erkkila et al. [209]. demonstrated that the combination of an endocervical swab and a cervical canal brush could extend the time period for Y-DNA positive samples (up to 144 h – 6 days). It should be noted that cervical brushing can result

in bleeding. The possibility of introducing a bleeding site (and thus a portal of entry for sexually transmitted diseases) should be weighed against the benefit likely to be obtained from a positive DNA sample.

Urine

Spermatozoa have been found in post assault urine samples. In some cases, where spermatozoa were not found via microscopy, Y-STR quantification (and, in theory, profiling) was still possible. Joki-Erkkila et al. [210] found the quantity of measurable male DNA was higher (median 0.68 ng/μl) in the post coital urine sample when compared to vaginal swabs (median 0.06 ng/μL), cervical swabs (median 0.02 ng/μl) and cervical brushings (median 0.02 ng/μL).

Speculum

In the process of sampling the vagina, the speculum may accumulate body fluids and trace evidence. Therefore, the used speculum can be retained, packaged separately, and stored in accordance with local policy. If the speculum is visibly wet on removal, swabbing may be undertaken to retrieve visible material. If storage space is restricted, swab the instrument and retain the swabs instead.

Seminal Fluid

Normal semen has an odor similar to bleach (sodium hypochlorite). 60% of the semen volume originates from the seminal vesicles, 20% from the prostate, 5% from spermatozoa and the remaining 15% is comprised of various gland secretions e.g. Cowper's and Littre's glands.

Sperm are created in the testes and stored in the epididymis, travelling to the urethra via the vas deferens during ejaculation. Semen has a volume in the range of 1.5–5 ml [211].

Acid phosphatase is found abundantly in the prostate gland and, therefore, in seminal fluid. In most laboratories, the Brentamine test [212] is used to detect acid phosphatase. However, acid phosphatase is also found to a lesser extent in vaginal secretions, so further confirmatory testing is necessary to determine whether the fluid is semen. Usually this is microscopy to visualize spermatozoa but under some circumstances (e.g. vasectomy) spermatozoa may be absent from semen.

If no spermatozoa are detected, an attempt is made to confirm the presence of semen by other means. Some laboratories use immunological tests such as Seratec prostate-specific antigen (PSA) [213–215] or The Rapid Stain Identification (RSID™) test for semen [216]. The latter is an immunochromatographic strip test, which uses two monoclonal antibodies specific for human semenogelin.

Because the PCR DNA techniques are now so sensitive, it is often possible to obtain a DNA profile from cellular material present in the seminal fluid even when no spermatozoa are present.

Blood

Whenever bleeding is noted during the medical examination, the forensic practitioner should communicate to the scientist any possible source for the bleeding

(menstrual, trauma or unknown). When no explanation for the bleeding was given the presence of blood must be interpreted with caution, particularly if in small quantity, because traces of uterine blood may be present at any time of the cycle.

Even though work is still being undertaken to determine the source of DNA, Tozzo et al. (2018) [217] ascertain that tissue specific mRNA detection not only can provide these answers but provides additional benefits:

- It has high sensitivity due to the possibility of PCR amplification
- High specificity due to the pattern of gene expression
- Unique for the functional status of cells and organs
- Can obtain simultaneous DNA isolation without material loss
- mRNA is quite stable in forensic stains.

Success and Persistence: Adult Female

In a 3 year review of results obtained from sexual assault patients in NSW the following was observed:

- 368 high vaginal swabs were tested. 280 were positive and had been tested within 48 h of assault; 12 of the positive samples had been tested between 48 and 96 h with only 1 positive result being found between 4 and 7 days after the assault.
- 77 cases, where both a low vaginal and high vaginal swab was tested, the results matched in 64 cases but differed in 13.
- 84 cases, where both a low vaginal and vulval sample was collected, the results matched in 71 cases but differed in 13 [218].

Janisch revealed that 46.5% (53 out of 114) who had vaginal samples collected within 12 h of their alleged assault had samples that were positive for sperm. 43.1% (22 out of 51) were positive when examined between 12 and 24 h post assault. No sperm was detected in their study (213 people) 3 days post assault [205].

Owers [219] presented results from a large scale study of 2269 cases of penile–vaginal penetration sexual assault allegations. In this study spermatozoa were detected in 32% of the cases analyzed where the alleged offence had occurred 3–4 days previously, 16% where the alleged offence had occurred 4–5 days previously, 20% where the alleged offence had occurred 5–6 days previously, and 7% where the alleged offence had occurred 6–7 days previously, significantly above the level detected in other publications. This study highlights that TSI data is significantly affected by the sperm recovery method used during the initial examination of vaginal swabs for the identification of the presence of semen and that much of the published data in relation to time since intercourse (TSI) intervals and the expected persistence of semen in post-coital samples relates to extraction methods which are no longer routinely used in casework by many forensic laboratories. Although it is widely recognized that the expectation for obtaining a DNA profile increases as the level of recovered spermatozoa increases, this study also demonstrated that with enhanced sperm recovery techniques there is an increase in the number of single

source male DNA profiles and 'usable' mixed DNA profiles (i.e. mixed DNA profiles for which a clear major or more prominent male contributor could be determined) from seminal pellets with low/trace levels of spermatozoa and that with the increase in sensitivity of current DNA techniques that nearly all (95%) of the seminal pellet samples submitted for DNA analysis in this study provided usable DNA profiles.

While longer times for persistence are the exception rather than the rule, caution should be taken when these time periods are obtained from older research. Note that semen will persist in dead bodies for much longer time intervals.

The quantity of semen in the vagina will diminish progressively with time, usually as a result of drainage. The posture and activity of the complainant subsequent to the act are likely to affect this. Similarly, washing, douching, or bathing may accelerate the loss of semen. Drainage of semen from the vagina may also result in soiling of intimate clothing items worn at the time, and these can prove valuable sources of body fluids.

It has been observed that spermatozoa can be isolated for longer periods in the endocervix. Studies that compared paired swabs from the vagina and cervix have found that 2 days or more after vaginal ejaculation there is a larger quantity of spermatozoa on endocervical swabs compared with the vaginal swabs [220]. It is recommended that where possible, therefore, an endocervical swab be taken in addition to the swabs from the vagina. It has been shown, in some jurisdictions where endocervical swabs are only taken after 24 or 48 h post assault, that these samples can be accidentally forgotten if they do not form part of the routine female genital collections [221].

Time Since Intercourse

There is interest in the possibility of determining the timing of intercourse. Opinions have been formulated in the past by looking at a combination of the detection of prostatic acid phosphatase (AP), detection of prostate specific antigen (PSA/p30), the identification of spermatozoa using microscopy and the density of sperm and/or presence of intact sperm (spermatozoa with tails).

Dziak et al. have proposed that rough time estimates might be established by looking at vaginal smears. Many sperm, including intact sperm, has been estimated to indicate time since intercourse may be up to 72 h, but likely to be within 24 h. Few sperm (including intact sperm) or many sperm (with no intact sperm) are likely to be seen within 72 h of intercourse. Few sperm (none intact) is likely to represent intercourse that has occurred within the last 7 days [222]. These determinations are relatively subjective as they require forensic scientists to estimate whether few or many sperm are seen and whether few or many are intact. This method of scoring has been used for about 40 years. In 2015, Tobe et al. tried to assess the reliability of the scoring system by developing slides with random dilutions of seminal fluid and asking 37 examiners to assess the slides. Each slide was assessed by a minimum of 25 investigators. On no slide was there a consensus between all scores. Sperm were not seen 56 times (9.6%) and 27 investigators (73%) did not see sperm on at least one slide. Their opinion was that sperm scoring was highly subjective and there was room to improve the objectivity of the ratings [223].

Success and Persistence- Pediatric female

Given the anatomical development of the female genitalia with age, it is possible that different sampling approaches (with regards to time for cut off periods) should be considered especially for pre-pubertal girls. Persistence of spermatozoa in the vagina of young children is thought to be markedly reduced because they have decreased cervical mucus [224] and shorter (and hence smaller) vaginal cavities. Christian et al. reviewed the medical records of 273 children aged under 10 years. All children had been examined within 44 h of the alleged/suspected sexual assault. No swabs taken from the child's body were positive for sperm/semen after 9 h. The majority of forensic evidence was gathered from linen or clothing and it was recommended that these forms of evidence should be collected in every case where possible to do so [225].

Nittis and Stark (2014) reviewed NSW sample results. The pubertal status of the complainant was not known and an assumption was made that if the child was aged 11 years or younger and no attempt had been made to obtain a high vaginal sample that they were likely to have been pre-pubertal. 105 samples, fitting this criteria, had been tested. 15 of the positive samples for obtaining a DNA profile had come from skin (n = 5), underpants (n = 7), vulva (n = 1), the penis (n = 1) and the anus (n = 1). The positive vulval sample had been collected within 6–12 h following the alleged assault and the positive skin / penile samples were all collected within 12 h [221].

For the above reasons, female genital sampling of pre-pubertal children is undertaken, in NSW Australia, in cases where 24 h or less has passed since an alleged/suspected assault.

Colposcopy

A colposcopy has historically been performed using a binocular microscope. Many centers, particularly those in the USA, advocate the use of the colposcope for external and, where relevant, internal genital and / or anal assessments of complainants of sexual assault.

The colposcope can provide considerable advantages over gross visualization. First, it provides magnification (5–30 times) and greater illumination, enabling detection of more abnormalities. The abnormalities that are detected by magnification but missed by macroscopic examination are likely to be small injuries only, the significance of which might be controversial.

Second, with the attachment of a still or DVD video camera, the colposcope allows for a truly contemporaneous, permanent video/photographic record of the genital/anal findings. If a DVD video is used, it will document the entire genital examination and will show any dynamic changes, such as reflex anal dilatation and movement of the hymen. If appropriate, the medical findings can be demonstrated to the complainant and carer.

Finally, if a remote monitor is used, the whole examination can be viewed by another doctor for corroboration or teaching purposes without additional parties having to be present during the intimate examination. Ensure consent is obtained if this method is being used.

The use of colposcopes is not without issue. Some examiners have difficulty with binocular vision and find the equipment difficult to use. Colposcopes have,

historically, been expensive pieces of equipment that occupy large amounts of space and were often not portable. There are now many options available on the market, including cameras that project only onto a monitor (no binocular vision required) and can be packed into a bag and transported where needed. Prices have reduced substantially.

Obviously, it is important that in all cases the colposcopic evidence be interpreted in the context of the information that is currently available regarding colposcopic assessments after consensual sexual acts [88, 114, 115, 226–228].

Toluidine Blue and Fluorescein

Toluidine blue stains breaches of the keratinized squamous epithelium, binding to nuclear material of tissues, and can highlight lacerations of the posterior fourchette that are not apparent on gross visualization [229, 230]. Use of toluidine blue increased the detection rate of posterior fourchette lacerations from 4% to 58% in adult (older than 19 years) complainants of nonconsensual vaginal intercourse, from 4% to 28% in sexually abused adolescents (11–18 years old), and from 16.5% to 33% in pediatric sexually abused patients (0–10 years old) [230, 231].

In contrast, adult complainants of nonconsensual vaginal intercourse and sexually abused children had significantly more lacerations demonstrable by toluidine blue staining than control groups [230].

Vulval swabs for forensic analysis must be taken before the stain is applied. Toluidine blue (1%) is then painted on the posterior fourchette, using a swab, before any instrumentation. After a few seconds, the residual stain is removed with lubricating jelly and gauze [229]. This is potentially messy and can result in residual staining of underpants, if care is not taken. The patient may experience some stinging at the application site. The time parameters within which the use of toluidine blue is beneficial in highlighting injuries have not been identified.

In March 2017, Kathryn Laughon [232] postulated use of another dye to highlight injury. Toluidine blue was thought to be less efficient when used on darker skin individuals because of the lack of contrast between the dark blue stain and the dark skin. She has proposed the use of Fluorescein which has been widely used in ophthalmology. The dye enters the space between cells, becoming more concentrated in areas where cell membranes have been disrupted or cell death has occurred. In her research Fluorescein improved detection of injury from about 37% (when not used) to 99% (when used), which was similar to her findings for toluidine blue. She also found that it did not appear to interfere with DNA typing. As Fluorescein does not depend upon contrast to be seen, it should work effectively across all skin types.

Male Genitalia

Method of Sampling

After an allegation of fellatio, swabs from the complainant's penis can be examined for saliva, and an amylase test may be carried out. It is worth remembering that

amylase may also be present and detectable on underwear that was in contact with the penis after the deposition of the saliva [233]. DNA profiling can be carried out on the swabs or clothing. When an allegation of vaginal or anal intercourse is made, penile swabs from the suspect can be examined for cells, feces, hairs, fibers, blood and lubricants.

It should be noted that there is the potential for vaginal fluid from recent previous intercourse, unrelated to the allegation, to be detected by DNA analysis of swabs taken from the unwashed penis. In one reported case DNA, from the complainant's boyfriend, was found on the suspect's penis [234].

Data collected by the MPFSL between 1987 and 1995 [235] have shown that after vaginal intercourse, cellular material from the complainant can be recovered from the coronal sulcus (groove around the penis just below the glans) even if the suspect has washed or bathed since the offence. Swabs taken from the meatus and urethra are not suitable for microscopic assessment because some male urethral cells can be similar to vaginal cells [236].

Current Faculty of Forensic and Legal Medicine guidelines for the collection of penile samples suggest 2 sampling areas. The first area is the penile shaft (including the external foreskin covering the glans, if present) and the second area is the coronal sulcus (and internal foreskin, if present) [135]. Some jurisdictions advocate a further third sample area, the glans. The swabs must be labelled accordingly, and the order in which the samples were obtained must be relayed to the scientist. The same samples are also taken if it is believed that a lubricant or condom has been used during a sexual act or if the assault involved fellatio or anal intercourse.

DNA STR profiling of body fluids on the penis is the method of choice used to provide evidence of penile–vaginal/oral/anal contact. It has proved particularly useful when multiple suspects have had intercourse with a single complainant [234], because DNA STR profiles matching the other suspects may also be found on the penile swabs taken from one suspect.

Success and Persistence Male

Female DNA profiles have been obtained on penile swabs up to 24 h post-coitus [237].

Karrstadt et al. [238] reviewed results from 227 cases, over a 3-year period, and found that in 57% of cases, no suitable material was found from the penile swabs. 26 of the remaining 97 provided a DNA profile of the female. They found that success was more likely in those cases where the male had penetrated three body orifices i.e. mouth, anus and vagina. The finding of Lugol positive cells also resulted in a better rate of success for DNA profiling. No success was found after 15 h.

The above study may have had relatively poor success rates as, with all sexual assault cases, it is uncertain whether penetration, as described, has occurred. Farmen et al. (2012) used 11 volunteer couples who provided 14 post-coital penile swab samples between 5 and 24 h post intercourse. All males were asked not to wash prior to sampling. Samples were collected from the coronal sulcus. Between 5 and 12 h, 90% of samples recovered full female DNA profiles. Female DNA was recovered from all samples and a full female profile was discovered from one couple at 24 h [239].

Cina et al. looked at the potential for isolating male and female DNA from post-coital condoms. Swabs were taken from both the internal and external condom surfaces during one penile vaginal encounter where a condom had been used, 8 h previously. As expected, female DNA could be found on the external surface and male DNA from the internal surface [240].

Perianal Area and Anal Canal

Buggery is a lay term used to refer to penile penetration of the anus (anal intercourse) of a man, a woman, or an animal (also known as bestiality). Sodomy relates to anal intercourse between humans only.

Sampling

The presence of semen in the anus or rectum can be corroborative evidence of alleged anal intercourse in conjunction with the presented history and possible physical findings.

Be aware that sperm sampled from the anal canal in women may not necessarily be the result of penile anal penetration. It has been postulated that the presence of spermatozoa within the anal canal/rectum may be a result of drainage from sperm deposition into the vagina or surrounding area. The evidence base for this is limited and largely relies upon research from Davies [212], Enos and Beyer [241] and Willott [203], published between 1974 and 1982.

It might be more accurate to assert that a positive finding from the anal swab, especially when sperm numbers are low, may be a result of secondary transfer. It seems no more likely that semen leaks past the anal sphincters into the anal canal than water does, when swimming or having a bath. Contamination may be iatrogenic i.e. occurred during the forensic procedure or it may be a result of everyday activities e.g. toileting.

Swabs should also be taken if a condom or lubricant was used during the sexual assault and if anolingus is alleged.

Sampling should start from the most external point and subsequent samples should be taken in an internal direction. The first site sampled is usually, therefore, the perianal area using a moistened swab (or double swabbing method if recommended in your jurisdiction). This is followed by sampling of the anal canal, just distal to the external anal sphincter. As it is possible to introduce DNA from the perianal area into the anus when collecting forensic evidence it is suggested that an examiner use a method of sampling that reduces, as much as possible, the chance of this occurring. One option is to wash the perianal area, after sampling, with sterile water and gauze, and prior to anal sampling. In all cases, separation of the buttocks for several seconds will result in the relaxation of the external sphincter. A swab can be introduced about 1.5 cm into the anus (just past the sphincter) and removed using the same caution, decreasing the potential for the swab to touch the perianal skin.

Sampling from the lower rectal area, if required, should only be done using an anoscope/rectoscope/proctoscope. Measures should be taken to reduce the potential

for contaminating the swab during collection and during removal of the swab from the body. This should be the last sample collected and the swab should be taken just distal to the proctoscope, being careful not to touch the instrument when withdrawing the swab.

Water-based lubricant from a single-use sachet (Pedicat® or KY® Lubricating Jelly) may be used to moisten the proctoscope to facilitate its insertion into the anus. If lubricant is used, it should be noted on the form returned to the forensic scientist.

In the process of sampling the rectum/anal canal, the proctoscope may accumulate body fluids and trace evidence. The used proctoscope can be retained, packaged separately, and stored in accordance with local policy. Alternatively, if the proctoscope is visibly wet on removal, swabbing may be conducted to retrieve visible material.

Stool samples and toilet paper need not be collected routinely because the other samples described should be adequate for laboratory requirements.

Condoms and Lubricants

Traces of lubricant found on vaginal or internal anal swabs may provide confirmatory evidence of recent penetration of a body orifice. This has particular relevance if a condom is worn during a penetrative act.

While the wearing of a condom might reduce/eliminate DNA found from biological evidence, trace lubricant might be detected, the presence of which might help support whether a crime has occurred, corroborate the version of events provided as well as assist with determining whether penetration has occurred [242].

Consequently, if the forensic practitioner has used lubricant (other than sterile water) on specula, proctoscopes, it must be communicated to the forensic scientist. The most commonly encountered lubricants applied directly to the penis to aid penetration are Vaseline® (petroleum-based product) and KY® Jelly (water-based product) [243]. Various other substances have been used to facilitate penetration during a sexual assault including hand cream, cooking oil and margarine, the diversity of the products apparently reflecting what is immediately at hand. Saliva is also used as a lubricant.

The constituents of condom lubricant (e.g. polydimethylsiloxane (PDMS) and polyethylene glycol (PEG)) [244] are also found in numerous other skin care products and suppositories. Therefore, when relevant, the forensic practitioner should ask whether the complainant has applied anything to the genital/anal area in the preceding 2 days [245]. This information should be noted on the paperwork that is made available to the forensic scientist so that the scientist can source the relevant product to check what it contains. The same dusting agents are used on some clinical gloves. Therefore, the forensic practitioner should wear nonpowdered gloves when sampling the genital and anal area [246].

To maximize the possibility of lubricant detection, the necessary swabs should be obtained as soon as possible after the incident. If the practitioner is aware that condom lubricants may be an issue, lubricant should not be used on the speculum or proctoscope during the examination. The forensic science laboratory must then be told that lubricant analysis may be relevant, because this potentially requires scientists from more than one discipline to examine the same sample, e.g. when both

body fluids and lubricant analysis are requested. If the forensic science laboratory is not made aware of this requirement, potential evidence could be inadvertently destroyed during laboratory processes.

Many factors may affect the length of time that a lubricant will persist on skin or in a body orifice. Condom lubricant has been detected on a swab taken from an unwashed penis 50 h after intercourse and, in a different case, on a vaginal swab (also when the complainant had not washed or douched) taken 24 h after intercourse, but detection after such prolonged periods would appear to be exceptional. Water-based lubricants (e.g., those containing polyethylene glycol) have only been detected within 8 h of the sexual act [243, 247].

Success and Persistence Ano-rectal Samples

Under normal circumstances, semen is unlikely to persist in the anus for more than 24 h. The maximum recorded interval between the act of anal intercourse and the identification of spermatozoa on a rectal swab is 96 h [203]. In one exceptional case, however, where a female lay prone in the hospital for several days because of injuries sustained during a sexual assault, semen was detected on anal swabs taken 113 h after the act of anal intercourse [203]. These results were published in 1982. More recent research has not replicated these time periods.

Janisch found that only 7 anal swabs out of 37 (18.9%) were positive for sperm, when taken within 24 h of assault [205].

More recently, a review of NSW forensic results demonstrated that of the 105 anal/rectal samples collected and tested for DNA only 36 resulted in a usable profile. All were collected within 48 h of the alleged assault. Of the 63 perianal samples tested, 19 were positive for DNA and had all been collected within 24 h [221].

Swabs should be taken even if the complainant has defecated since the assault. An unpublished review of 36 MPFSL cases of alleged anal intercourse in which the complainant had defecated before the examination found that in six cases (four female and two male) the internal/external anal swabs were still positive for spermatozoa, although only a few were present; one of these subjects, a male, had a positive external anal swab 52 h after the anal intercourse (Allard J, personal communication, 1998). Anal swabs have produced a positive DNA STR analysis up to 48 h after the incident (Elliott K, personal communication, 2003). Obviously, it would be preferable to avoid defecation and urination before sampling but it is acknowledged this is not always practical.

Blood and Urine Analysis

When drugs or alcohol have been consumed or possibly administered before or during a sexual assault, consideration should be given to the need to obtain samples of blood and urine for toxicological analysis. The length of time that a drug or its metabolites remain detectable in blood or urine depends on several factors,

including the quantity taken, the individual's metabolism, and the sensitivity and specificity of the analytical methods used by the laboratory [248]. Although the metabolites of some substances may be excreted for up to 168 h in the urine [248], many are detectable for only few hours. In general, drugs and their metabolites will be identifiable for longer in urine than in blood.

The limit of detection is the lowest concentration of analyte that the analytical process can reliably differentiate from the background.

The limit of quantitation is the lowest concentration of analyte that can be reliably identified and quantitated with a certain degree of reliability.

Method of Sampling

Cut off periods for the collection of blood and urine may differ between jurisdictions and from case to case. Current FFLM recommendations suggest blood should be collected if the incident occurred within the last 3 days and suggest urine sampling up to 5 days [135]. Samples from complainants do not need to be witnessed.

Ideally, a forensic toxicology kit should be used. Wipes that contain alcohol should not be used to clean the skin before the blood sample is taken. If volatiles (e.g. amyl nitrate) are suspected, a portion of blood must be collected into a separate container.

Analysis

Forensic science laboratories have the capability of detecting a range of prescribed and illicit substances, but the persistence of different substances or their metabolites in the blood and urine of an individual depends on numerous factors.

Certain information may be required to assist the forensic scientist with interpretation of the toxicological results:

- Sex and body weight
- The time that any drugs/alcohol were consumed (start of consumption and when consumption ceased)
- Did the complainant have an alcoholic drink after the incident and prior to the medical examination?
- The exact time/date that the blood and urine samples were taken
- Details of any prescribed medication or other substances normally consumed by the individual, including quantity and the date and time of most recent use

Persistence Data

The detection windows depend on a few different factors, including the amount of substance used / administered and the frequency of use. Specialist advice is often available from the toxicology section of the local forensic laboratory.

Note that long acting drugs that may persist up to 5 days in urine include Methadone, long acting benzodiazepines e.g. clonazepam and nitrazepam (as well as diazepam), and possibly ketamine. The z-drugs (Zolpidem and Zopiclone) may be seen in urine up to 3 days (See Chap. 12).

Early Evidence

Some Sexual Assault units facilitate the collection of early evidence samples. The UK Forensic Science Service with Metropolitan Police developed one of the very first early evidence kits in 2001 [249]. Other services have developed their own variations in the two decades since. They are most useful for the collection of evidence that might otherwise disappear quickly, e.g. oral samples. Other samples that lend themselves to early collection include blood for toxicology (although this needs to be initiated by a health care practitioner), urine samples for biological evidence or toxicology, oral rinses, and "wipes"/swabs/equivalent for the early collection of vaginal and anal samples. All examiners that work for units that have access to these kits should be very familiar with their components and be able to provide necessary advice on techniques for their utilization. If considering early collections, and no current guidelines exist in your jurisdiction, please consult your local forensic laboratory for advice about the best sampling methods.

Early evidence collection, if available, may be the responsibility of police, hospital emergency department staff or forensic examiners. In all cases, chain of custody should be maintained and recorded.

At time of writing there was limited literature concerning the efficacy of early collection samples. Smith et al. 2014 [250] reviewed 88 alleged sexual assault cases that presented to their unit. Both early evidence and full forensic specimen collections were obtained for each. Early evidence samples included oral swabs, oral rinses, first void urine, vulval and peri-anal wipes and "other" (including sanitary items). Spermatozoa was detected in 35% (22/63) of early evidence kit first void urine specimens and 32% (18/57) of early evidence vulval wipes when penile vaginal penetration had been alleged. Spermatozoa was detected in 40% of cases where vaginal swabs were then taken by a forensic examiner as part of a full forensic assessment. These results appear to indicate that a urine sample more successfully results in offender DNA evidence than a wipe (although the difference may not be statistically valid). Gaining a urine specimen from a patient is relatively easy to do, most patients have experience with the process and this type of sample could be facilitated by non-medical personnel such as police. For these reasons, it may be a preferable choice over a "wipe" or self-collected swab.

When the allegation was penile anal penetration, spermatozoa were found in 33% (2/6) of early evidence anal wipes and in 67% (2/3) cases of early evidence urine samples (when there had been no allegation of successful penile vaginal penetration). Spermatozoa were found in 36% (4/11) of cases where a full forensic was conducted. Although case numbers are small, the suggestion is that

sperm recovered from a urine sample can't be assumed to have been deposited in the vagina.

When the allegation was penile oral penetration spermatozoa was only detected in one early evidence oral rinse (1/18) but in none of the full forensic oral samples [250]. The numbers were, however, quite low.

Joki-Erkkila et al. [210] opined that during voiding, vaginal secretion flow was increased secondary to the relaxation of the pelvic floor and the increased intra-abdominal pressure. This would be one reason for the apparent success in voided urine being analyzed not only for toxicology but for sperm/DNA. In their research 88 volunteers collected various post coital urine samples following consensual intercourse. There were 205 urine specimens in total. The samples were centrifuged and the resultant cell pellet was sent for Y STR testing. Y DNA was measurable in 84.4% of samples. This increased to 96.5% of samples when the urine was the first post coital collection. Most specimens were collected within 24 h of coitus. After 24 h Y DNA was measurable in 40.9% of samples (9/22). The researchers considered the cut-off limit for identification of the male at ≥0.01 ng/μL Y-DNA. 74.6% of all samples returned this amount of Y-DNA. Not surprisingly, the percentage of positive samples decreased in proportion to the void number i.e. less Y-DNA was found in the sixth to tenth voids when compared to the first post coital void. It should be noted these results were based on Y-STR quantification PCR as opposed to Y-STR profiling [210].

Early Evidence kits are self-collected in many cases. This self-collection may be facilitated by non-forensic medical and nursing staff or by other emergency workers such as police or ambulance officers. As potential forensic evidence it needs to survive challenges in court with regards to chain of evidence, collection technique and potential cross contamination. Despite this, there seems little doubt that this type of evidence has a valuable place in sexual assault assessments.

Products of Conception

Occasionally forensic examiners may be asked for advice, or to participate in, the collection of products of conception for the purpose of identifying an alleged sexual assault offender. It is recommended that guidelines around this type of sampling should be available if required. They should be designed in consultation with police and forensic laboratories and updated regularly. Some general principles have been outlined below:

Informed consent should be obtained, as per any forensic collection procedure. In some jurisdictions, the procedure will not be carried out unless a report has been made to police. The type of fetal sample will depend upon the type of termination procedure. Medical terminations can be done up to 9 weeks, suction curettes up to 12–14 weeks and dilatation and evacuations generally from about 14 weeks onwards.

Most laboratories will require an estimated date of conception and will accept a tissue sample, rather than the entire fetus. All equipment used should be sterile and

preferably disposable. Formalin should not be used and a chain of custody should occur, with documentation of every step in the process, including who handled the specimen. A medico-legal report documenting the receipt, sealing, storage and handover of the specimen may be required.

Prophylaxis

The risk of contracting a sexually transmitted infection following a sexual assault may differ vastly from city to city, state to state and country to country. Every attempt should be made to locate local guidelines that base their recommendations on local prevalence and incidence rates. Included below is some general information but forensic examiners should consult local pharmaceutical texts.

Ideally, guidelines should exist to assist a complainant's access to STI prophylaxis medication regardless of whether or not they consent to a full forensic examination. Complainants should be advised to practice "safe sex" until all results have been returned and they have been successfully treated for any infections.

Pregnancy

Female complainants at risk of pregnancy must understand that the efficacy of all emergency contraception decreases as the time since assault increases.

There are three options that might be considered for a complainant who, as a result of an assault, may be at risk of pregnancy. These include:

Copper IUD (Intrauterine Device)

Although not always considered first line, because training and experience are required to have them inserted and removed, they do offer some benefits over other choices. Once inserted they will work for up to 10 years. As Marie Stopes Australia has observed, it is the cheapest option around for contraception costing less than "a Netflix and Abstinence" approach [251]. It is the only long term, hormone free, reversible contraceptive on the market. It has a failure rate of less than 1 in 100 and doesn't require a woman to remember to take anything. The copper IUD can make tails separate from the body of sperm. As Marie Stopes has observed, "Makes it kind of hard to get to the egg when you can't swim." Lastly, it is the most effective form of contraception available, working for up to 120 h after unprotected sex. It should be considered as a first line option in those women who have a body mass index >25 kg/m^2 as there is a decline in efficacy of Ulipristal Acetate and Levonorgestrel as BMI increases [252].

Ulipristal Acetate 30 mg

This is a single dose treatment.

There is a demonstrated improved efficacy of Ulipristal, when compared to Levonorgestrel, for the prevention of unwanted pregnancies when taken within the 0–120 h post intercourse period. A reduction in pregnancies of about 2.15–1.36% (if taken within 72 h of intercourse) has been demonstrated [253].

If the woman also has been taking a medication (or herb) that induces cytochrome P450 CYP3A4, or had treatment with these medications in the previous 4 weeks, there may be a decrease in the efficacy of Ulipristal as Ulipristal is metabolised by CYP3A4. Patients should be offered an alternate form of contraception such as a non-hormonal emergency contraceptive e.g. copper intrauterine device or 3mg (double dose) Levonorgestrel [254, 255].

If vomiting occurs within 3 h after ingestion of the medication the dose will need to be repeated.

Ulipristal is thought to prevent ovulation by suppression of the LH (luteinising hormone) surge for at least 5 days, which is beyond the lifespan of sperm. The effects post-fertilisation are currently unknown. The timing of ovulation cannot be predicted and if ovulation has already occurred Ulipristal may not be effective.

If a woman wishes to initiate or resume regular hormonal contraception after using Ulipristal, they should be advised not to commence hormonal contraception sooner than 5 days after the intake of Ulipristal AND to use a reliable barrier method until the next menstrual bleed. Breast feeding should be avoided for 1 week after ingestion of Ulipristal.

Levonorgestrel 150 mg

This is a single dose treatment. It has limited evidence of efficacy after 3 days (72 h) [255, 256].

Women requiring post exposure pregnancy prophylaxis must be asked specifically about whether or not they may be taking any cytochrome P450 3A4 inducers as the dose may need to be doubled i.e. 3 mg stat, however bear in mind evidence for efficacy is lacking [254].

The forensic examiner must advise that, if vomiting occurs within 2 h after ingestion of the medication, the dose will need to be repeated. Vomiting is, however, uncommon.

The possible mechanisms of action of Levonorgestrel include:

- Prevention of ovulation
- Altering the transport of sperm and egg through the tube
- Changing the lining of the uterus in a way that may discourage implantation.

Because Levonorgestrel does not interrupt an established pregnancy, defined as beginning with implantation, it is not considered an abortifacient [256].

Hepatitis B Prophylaxis

Hepatitis B is covered in detail in Chap. 10.

The forensic examiner may assume a potential risk of Hepatitis B, following sexual assault/rape, if there has been a penetrative sexual event (excluding digital penetration) AND the assault was thought to have occurred within the previous 7 days AND there is no history of a complete Hepatitis B vaccination.

Booster doses may not be recommended for immune-competent persons who have completed a primary course of Hepatitis B immunisation. There is good evidence that a completed primary course provides long lasting protection even when antibody levels decline with time and become undetectable [257, 258].

Human Immunodeficiency Virus (HIV)

Generally there must be an occasion of receptive intercourse (i.e. penile anal or penile vaginal), there must be an offender who is either HIV +ve or from a group that is considered high risk e.g. men who have sex with men, from a high HIV prevalence country etc. and the penetrative event must have occurred within the last 72 h in order to be offered HIV prophylaxis.

In order to quantify the potential risk to a complainant an examiner needs to know the risk associated with the type of exposure that has occurred (e.g. for cases of receptive vaginal or anal intercourse, insertive vaginal or anal intercourse etc.). This is multiplied by the risk of the source being HIV +ve. These are very location specific statistics. Whether or not HIV post exposure prophylaxis should be offered is generally determined by the potential risk i.e. exposure risk x source risk.

Unaids website [259] provides up to date sero-prevalence of individual countries for HIV (use the key population atlas). A high prevalence country (HPC) is any country with sero-prevalence of the general population >1%. As an example, at time of writing, sub-Saharan African countries and sex worker contacts from South and South East Asian countries were considered to be high prevalence populations.

Note also that the sexual exposure risk in children is higher than in adults due to the increased risk of mucosal trauma, vaginal wall thinness and cervical ectopy [260].

Animal studies suggest that the sooner HIV Post Exposure Prophylaxis (PEP) is given, the greater the chance of preventing seroconversion. Therefore, it is currently recommended that HIV PEP is commenced no more than 72 h after the assault [261] and, like most prophylactic medication, the sooner it is commenced the more effective it is likely to be. Many sexual assault referral centers provide starter packs to ensure rapid access to the appropriate medicines. Patients considering HIV PEP should be advised of the unproven efficacy, potential side effects, and the fact that the long-term consequences are not fully understood [262]. If HIV PEP is given, baseline serological tests should be obtained. The recommended course of treatment is generally for 4 weeks, and patients should be monitored by a genitourinary physician during this period.

Chlamydia

There are two schools of thought with regards to Chlamydia prophylaxis. Those that recommend post exposure prophylaxis are likely to do so because sexual health follow up rates of sexual assault complainants is often poor, Chlamydia Trachomatis infection may be asymptomatic and can lead to serious morbidity if untreated

(infertility, ectopic pregnancy, chronic pelvic pain, arthritis, peri-hepatitis, proctitis, epididymo-orchitis), prophylactic treatment is easy to administer in a stat dose, is well tolerated and has few drug interactions, sexual assault victims are often young and therefore in the highest risk group for Chlamydia.

Some of the reasons to not recommend prophylaxis include the fact that the presence of any pre-existing Chlamydia infection is unknown, there may be inadequate contact tracing, Chlamydia is easily treated at follow up if STI check is positive and prophylactic treatment may discourage patients from seeking STI follow up if they believe they have already been treated.

Other Prophylactic Treatments

Gonorrhoea is not a particularly common infection seen in sexual assault victims in Australia and so routine prophylaxis is generally not prescribed, although this may depend upon the location of the sexual assault unit. There is an increased prevalence rate in some indigenous communities.

Prophylactic Treatment and Testing for Suspects

In cases of alleged sexual assault, all suspects should be advised to attend a Sexual Health / Genito Urinary Medicine (GUM) clinic for STI screening. A suspect has the same right to access post-exposure prophylaxis in a timely fashion [263].

Baseline STI Testing

Recommendations regarding this are likely to differ. It does offer an opportunity to target an at-risk population who may be unlikely to follow through with a sexual health appointment. Urine and swab sampling, if positive, may not however necessarily represent the infection status of the complainant but, rather, the infection status of the offender resulting in unnecessary anxiety or treatment i.e. if swabbing the vulva after non-consensual ejaculation, the sample might be a mix from both the complainant and the offender.

Expertise and Peer Review

> *The overriding objective of this procedural code is that criminal cases be dealt with justly. Dealing with a criminal case justly includes acquitting the innocent and convicting the guilty, dealing with the prosecution and the defense fairly, recognizing the rights of the defendant…* [264].

Forensic examiners, dealing with sexual assault complainants and suspects, should be aware of any potential conflicts of interest and instructing parties must be informed of this as soon as practicable [265].

Every examiner who works at the medicolegal interface should be aware of bias (over 20 different types have been identified) as they can influence decision making and opinions.

Examiners should be engaged in a process of continuing professional development in order to demonstrate continued competency in their area of expertise [265].

The expectation that there should be peer review of a statement, prior to its submission to court, is likely to increase. Peer review may occur in different ways. A reviewer may read a statement and offer comments about grammar, spelling and the opinion reached based on the evidence cited in the certificate/statement. A reviewer may, however, additionally review contemporaneous material collected at time of examination, for example original injury diagrams, patient histories and photographs. It is the latter, arguably, which should allow the reviewer to reach an independent assessment of the information that was available to the primary examiner. If a statement has been peer reviewed, should this be identified within the statement? Should a list of the materials reviewed be specified? Should difference of opinions be recorded for the court and when should this be done? Should reviewed drafts of statements be kept and presented to court upon demand? It is likely that examiners who work within sexual assault units will need to consider the pros and cons of each course of action and develop guidelines for staff to ensure a consistent and transparent approach.

Conclusion

Aiming for perfection is only likely to lead to disappointment. Aiming for improvement is much more achievable. It is important, therefore, to review each case after it has been performed in an attempt to identify what was done well and what might have been done better, where there were shortcomings in knowledge and how that information might be more readily accessed on the next occasion, where the system failed and where we failed. Replicating the things that worked well and improving upon the things that might have been done better will ensure competency in this area of clinical forensic medicine.

The UK Forensic Science Regulator provides updated advice on pertinent issues, including the Forensic Medical Examination Standard—Adult and Child Sexual Assault Complainants. This latter document contains a self-assessment table and is one way to ensure your service and practice meets acceptable standards [389].

Key Points

- It is essential that injuries are documented in detail in the clinical notes, describing the injury in writing, drawing on a diagram, and by imaging (photograph or video). High quality documentation requires all three.
- Examiners should be aware of the principles behind forensic sampling and local guidance should be regularly reviewed to ensure that the samples taken are appropriate and evidence based that is, based on the experience of local forensic laboratories (statistically and not anecdotally), as well as published research.

Self-Assessment Exercises

1. Which forensic samples would be required following an allegation of penile penetration of the vagina within the previous 48 h?

2. Outline the psychological response to rape and what support you could offer to a victim of sexual assault?
3. How would you manage the risk of pregnancy and sexually transmitted diseases in a complainant of sexual assault?

Acknowledgement Thanks to Karen Alexander & Carol Rogers - Forensic Scientists, the UK and Ireland Association of Forensic Science Providers' (AFSP) Body Fluid Forum (BFF)
With acknowledgement to the previous authors – Dr. Debbi Rogers & B. Martina Mc Bride.

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