The Royal College of Pathologists of Australasia
ABN 52 000 173 231

Durham Hall 207 Albion Street Surry Hills NSW 2010 Australia
Telephone 61 2 8356 5858 Facsimile 61 2 8356 5828

RCPA
The Royal College of Pathologists of Australasia

Guideline

Subject:	**Evidence Based Forensic Sampling Standards**
Approval Date:	February 2018
Review Date:	February 2022
Review By:	Faculty of Clinical Forensic Medicine Committee
Number:	2/2018

(This page is deliberately left blank)

Table of Contents

Please find a list of possible biological evidence collection methods that might be utilised in criminal cases to provide evidence of an assault.

Disclaimer: This is meant to be a guideline only for Australian New Zealand (ANZ) forensic examiners. Local evidence collection guidelines may proscribe different collection methods or different cut-off time periods. As these are often informed by analysis of forensic samples in your jurisdictional area, they should be consulted in the first instance.

ORAL SAMPLES

Name of Collections	**ORAL**
Name of Sample	**Buccal**
Methods of Collection	A swab is used to sample the mucosal cells from the inside of the cheeks
Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	Unlimited
Comments • Used for the collection of a baseline / reference DNA sample from either a complainant or person of interest. • Unpublished review of these collections (NSW) shows that success rate is high for the collection of sufficient DNA for creating full profiles. • DNA profiles can also be collected via a blood sample. No ANZ jurisdiction currently uses blood for reference sampling routinely.	
Relevant References	

Name of Collections	**ORAL**
Name of Sample	**Oral Swab**
Methods of Collection	A swab is used to sample the upper and lower mouth, around the teeth and gingivial recesses, under the tongue and back of the throat. Some services will use a single swab to sample both the upper mouth and the lower mouth. Other services may use two swabs: one for the upper and one for the lower mouth.

Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	Range from 12 hours to 48 hours after penile oral assault
Comments • Poor pickup rate for DNA. No longer used as a sampling method in NSW.	
Relevant References 1, 2, 3, 4, 5, 6, 7, 8, 9	

Name of Collections	**ORAL**
Name of Sample	**Oral Rinse**
Methods of Collection	Sterile water is used (amount is variable). Patient is asked to swish water around the mouth, back of throat (gargle) and through teeth. The water is then spit into a collection jar for transfer to the laboratory.
Suggested Storage	Liquid samples should be refrigerated unless there is likely to be early transport to the forensic laboratory i.e. within 5 days.
Collection Time Periods	Range from 12 hours to 48 hours after penile oral assault.
Comments • Oral rinses are often available as part of an Early Evidence Kit.	
Relevant References 5	

Name of Collections	**ORAL**
Name of Sample	**Oral Saliva**
Methods of Collection	Saliva is spit into a collection jar. If saliva is being collected it is generally collected prior to oral swabbing or rinsing.
Suggested Storage	Liquid samples should be refrigerated unless there is likely to be early transport to the forensic laboratory i.e. within 5 days.
Collection Time Periods	Range from 12 hours to 48 hours after penile oral assault

Comments • Not frequently collected as part of ANZ oral collections
Relevant References 1,2

Name of Collections	**ORAL**
Name of Sample	**Peri-oral swab**
Methods of Collection	A moistened swab is used to sample the outer aspect of the lips and adjacent skin. A second dry swab can be used after this. There is some suggestion that the second swab may increase DNA pickup rates from skin.
Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	Up to 72 hours after penile oral assault or kissing
Comments • A positive perioral swab does not prove penetration but may support the history of ejaculation and may help provide a profile of the alleged assailant. • Wet and Dry swabs are no longer used in NSW. This has been replaced by a single moistened swab.	
Relevant References 5	

Name of Collections	**ORAL**
Name of Sample	**Chewing gum**
Methods of Collection	Complainant is asked to chew gum which is then collected for analysis.
Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	
Comments	

• Need to ensure your local laboratory has the ability to test this type of sample.
Relevant References

Name of Collections	**ORAL**
Name of Sample	**Dental floss**
Comments: • The use of dental floss may result in bleeding from the gum, thereby providing a potential portal of entry for sexually transmitted infections. It is therefore not recommended.	
Relevant References 9	

Oral References

1. Willott GM, Crosse MA. The detection of spermatozoa in the mouth. J Forensic Sci Soc. 1986 Mar-Apr;26(2):125-8. PubMed PMID: 3711852. Epub 1986/03/01. eng.
2. Allard JE. The collection of data from findings in cases of sexual assault and the significance of spermatozoa on vaginal, anal and oral swabs. Sci Justice. 1997 Apr-Jun;37(2):99-108. PubMed PMID: 9206315. Epub 1997/04/01. eng.
3. Tucker S, Ledray LE, Werner JS. Sexual Assault Evidence Collection. Wisconsin Medical Journal. 1990;89(7):407-11.
4. Janisch S, Meyer H, Germerott T, Albrecht UV, Schulz Y, Debertin AS. Analysis of clinical forensic examination reports on sexual assault. Int J Legal Med. 2010 May;124(3):227-35. PubMed PMID: 20182738. eng.
5. Nittis M, Franco M, Cochrane C. New oral cut-off time limits in NSW. Journal of forensic and legal medicine. 2016 Sep 22;44:92-7. PubMed PMID: 27697690.
6. Enos WF, Beyer JC. Spermatozoa in the anal canal and rectum and in the oral cavity of female rape victims. J Forensic Sci. 1978 Jan;23(1):231-3. PubMed PMID: 570593. Epub 1978/01/01. eng.
7. Faugno D, Girardin B, Radoff A. The Myths and Legends of Sperm, Sexual Assault, Sex and Contraception 2003. Available from: www.forensicnursing.com/articles/391clinical.html.
8. Nittis M, Stark M. Evidence based practice: laboratory feedback informs forensic specimen collection in NSW. Journal of forensic and legal medicine. 2014 Jul;25:38-44. PubMed PMID: 24931859.
9. Nittis M. Evidence Collection in Cases of Sexual Assault. In: Beran RG, editor. Legal and Forensic Medicine. Berlin: Springer-Verlag; 2013. p. 1335-58.

FEMALE GENITAL SAMPLES

Name of Collections	**FEMALE GENITAL**
Name of Sample	**External Labial**
Methods of Collection	Collect swab in location of contact as indicated by complainant.

	As a general rule, the swab is taken from the external aspects of both labia majora. If also sampling the mons, this should be clearly recorded. First swab is moistened with a few drops of sterile water. This may be followed with a second dry swab. Swabs may be labelled to indicate order of collection.
Suggested Storage	These samples generally do not need to be refrigerated if dry.
Collection Time Periods	1. **Penis vagina, ejaculation onto vulva/perineum, anal intercourse:** Ranges from 48 hours (2 days) to 168 hours (7 days) Up to 72 hours if anal intercourse (UK recommendations) 2. **Digital penetration**: Up to 12 hours (not washed) to 48 hours (UK). N.B: UK may change to 72 hours based on new research [1]. 3. **Cunnilingus:** 24 to 72 hours Should swab even when client has showered [2].
Comments • There appears to be less research available for this type of forensic collection than for other female genital collections.	
Relevant References 1,2, 5	

Name of Collections	**FEMALE GENITAL**
Name of Sample	**Vulval**
Methods of Collection	Swabs are taken of the vulval area, generally agreed to include both the inner surface of the labia minora and the fossa navicularis. Some

	jursidictions will swab only the lower half of the labia minora, others may include the entire inner surfaces. The swab may be moistened if the area is thought to be dry. Swabs for aromatic oils/lubricant: Collect the swab from the area of contact indicated by the client. Snap the swab shaft to fit in the small glass vial which is used specifically for this purpose. Replace the airtight lid on the vial. Collect after swabs for semen (if indicated) and contact DNA.
Suggested Storage	These samples generally do not need to be refrigerated if dry.
Collection Time Periods	**1. Penis Vagina:** From 24 to 72 hours pre-pubertal 120 (5 days) to 168 hours (7 days) for post pubertal 72 hours if anal intercourse (UK) **1. Digital penetration:** 12 hours (not washed) to 72 hours (regardless of whether or not washing has occurred). UK may change from 48 to 72 hours based on new research [1]. **2. Cunnilingus:** 24 hours to 72 hours Should swab even when client has showered [2]. **3. Swabs for aromatic oils/lubricant:** Nil evidence re timing.
Comments • Ensure you are clear as to the suggested limits of a vulval swab in your jurisdiction as these may not be consistent across Australia New Zealand.	
Relevant References **1, 2, 5**	

Name of Collections	FEMALE GENITAL
Name of Sample	**Low Vaginal**
Methods of Collection	Part labia majora and minora to view the opening of the vaginal canal. Insert the swab 2 to 3cm, past the hymen, being careful to avoid touching labia and vestibule. This same swab may be used to view the edges of the hymen. The swab may be moistened if the area being sampled might be dry. Swabs may be labelled to indicate order.
Suggested Storage	These samples generally do not need to be refrigerated if dry.
Collection Time Periods	**1. Penis Vagina:** From 24 to 72 hours pre-pubertal 120 (5 days) to 168 hours (7 days) for post pubertal 72 ours if anal intercourse (UK) **2. Digital penetration:** Ranges from not collected, in some jurisdictions, up to 48 hours (UK). UK may change to 72 hours based on new research [1]. **3. Cunnilingus:** Low vaginal swabs are not recommended for cunnilingus
Comments • Some jursidictions do not collect a separate low vaginal swab	
Relevant References 1	

Name of Collections	FEMALE GENITAL
Name of Sample	**High Vaginal**
Methods of Collection	**1. For semen**

	Part labia majora and minora to view the opening of the vaginal canal. A speculum may be inserted un-lubricated, lubricated with water or lubricant*, being careful to avoid contact with labia and vestibule. Open the bills and insert swab through the bills into the posterior fornix, being careful to avoid touching the speculum blades. Sweep the swab around the fornix and include the pool of secretions in the fornix. **2. Swabs for aromatic oils/lubricant:** Collect a whole vagina swab. Snap the swab shaft to fit in the small glass vial. Replace the airtight lid on the vial.
Suggested Storage	These samples generally do not need to be refrigerated if dry.
Collection Time Periods	**1. Penis Vagina:** 120 days) to 168 hours (7 days) for post pubertal 72 hours if anal intercourse (UK) **2. Digital penetration:** Ranges from not collected, in some jurisdictions, up to 48 hours (UK). UK may change to 72 hours based on new research [1]. **3. Cunnilingus:** Low vaginal swabs are not recommended for cunnilingus
Comments • A speculum must be used to collect a high vaginal swab. • A blind swab, without a speculum, can be used but this can only collect a "transvaginal" sample (see next table).	
Relevant References 1	

Name of Collections	**FEMALE GENITAL**
Name of Sample	**Transvaginal (blind)**

Methods of Collection	If the woman declines the offer of a speculum examination, carefully insert the swab through the vaginal opening up to the high vagina and sweep around the upper vagina and include the pool of secretions in the posterior fornix. At least one Australian jurisdiction recommends the collection of a transvaginal swab before insertion of a speculum. Swabs for aromatic oils/lubricant: Collect a whole vagina swab. Snap the swab shaft to fit in the small glass vial. Replace the airtight lid on the vial. Swabs may be labelled to indicate order.
Suggested Storage	These samples generally do not need to be refrigerated if dry.
Collection Time Periods	1. **Penis Vagina:** From 24 to 72 hours pre-pubertal 120 (5 days) to 168 hours (7 days) for post pubertal 121 ours if anal intercourse (UK) **2. Digital penetration:** 12 hours (not washed) to 48 hours (UK). UK may change to 72 hours based on new research [1]. **4. Cunnilingus:** Low vaginal swabs are not recommended for cunnilingus
Comments • A speculum must be used to collect a high vaginal swab. • A blind swab, without a speculum, can be used but this can only collect a "transvaginal" sample.	
Relevant References 1	

Name of Collections	**FEMALE GENITAL**

Name of Sample	**Endocervical**
Methods of Collection	Part labia majora and minora to view the opening of the vaginal canal. A speculum may be inserted un-lubricated, lubricated with water or lubricant*, being careful to avoid contact with labia and vestibule. Open the bills and insert swab through the bills into the posterior fornix, being careful to avoid touching the speculum blades. Sweep the swab around the fornix and include the pool of secretions in the fornix. Swabs for aromatic oils/lubricant: Collect a whole vagina swab. Snap the swab shaft to fit in the small glass vial. Replace the airtight lid on the vial.
Suggested Storage	These samples generally do not need to be refrigerated if dry.
Collection Time Periods	**1. Penile Vaginal** Up to 120 hours (5days) to 168 hours (7 days) or post pubertal Some suggestion that this should be extended to 10 days post penetration or to the end of next menses [3]. **2. For penile anal intercourse:** 72 hours if anal intercourse (UK) – particularly important if intercourse has occurred more than 24 hours previously.

Comments

- A speculum must be used to collect a high vaginal swab. A blind swab, without a speculum, can be used but this can only collect a "transvaginal" sample (see next table).
- As there is a tendency to forget to collect endocervical samples, on occasion, there has been argument to include it as part of the routine genital sample collection, allowing the laboratories to determine which samples are analysed i.e. taking an endocervical sample with the understanding it is only likely to be tested if more than 24 (or 48) hours has passed since assault.

Relevant References

3

Female Genital References

1. Sween KRMS, Quarino LAP, Kishbaugh JMMS. Detection of Male DNA in the Vaginal Cavity After Digital Penetration Using Y-Chromosome Short Tandem Repeats. *Journal of Forensic Nursing*. 2015;11(1):33(18)
2. Williams S, Panacek E, Green W, Kanthaswamy S, Hopkins C, Calloway C. Recovery of salivary DNA from the skin after showering. *Forensic Science Medicine and Pathology.* 2015; 11: 29-34
3. Speck P, Ballantyne J. Post-coital DNA Recovery Study. *US Department of Justice Document No. 248682*, March 2015
4. Loeve A, Bilo R, Emirdag E, Sharify M, Jansen FW, Dankelman J. In vitro validation of vaginal sampling in rape victims: the problem of Locard's principle *Forensic Science Medicine and Pathology.* 2013 9: 154-162
5. 27. Keating, S.M. The laboratory's approach to sexual assault cases. Part 1: sources of information and acts of intercourse. J Forensic Sci Soc. 1988; 28:35-7

PENILE SAMPLES

Name of Collections	**PENILE**
Name of Sample	**Penile Shaft**
Methods of Collection	A swab moistened with water is rolled along the shaft of the penis on the upper and lower sides and then replaced into its sheath. The process may be repeated with a dry swab.
Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	1. **Saliva, vaginal fluids, rectal fluids, blood or semen** Up to 72 hours for all biological fluids: Some jurisdictions will sample the penis for biological fluids after washing and others will not. 2. **For penile contact with skin** Some jurisdictions will sample up to 72 hours and others up to 12 hours. No jurisdiction will sample the penis for skin contact after washing. 3. **Non-biological trace evidence and lubricant** May also be detected on the penis and collection may be considered up to the point of washing or timeframe determined by the relevant interpersonal contact.
Comments • Semen is not usually a relevant specimen to collect from the penis in cases of sexual assault and therefore in jurisdictions where the laboratory requires the practitioner	

to prepare a slide at the time of collection this is generally not required for penile samples. However each case should be considered on its own merits. • The laboratory may not identify cell types or the anatomical area of origin of any foreign DNA found. • This sample should be collected in all cases of reported or suspected sexual assault in a male including in victims where memory is deficient or absent. • Comment should be made as to whether the penis is circumcised or not
Relevant References **1, 2, 3, 4, 5, 6, 7, 8, 9**

Name of Collections	**PENILE**
Name of Sample	**Penile Glans / Sulcus**
Methods of Collection	A swab moistened with water is rolled around the tip (glans) of the penis (including the sulcus and meatus and any piercing). The glans penis is swabbed around the coronal sulcus and onto the glans and urethral meatus. This can be done using one swab or a swab each for the glans and coronal sulcus. The foreskin (if present) should be retracted and the internal surface swabbed (this should be done in conjunction with the coronal swab). Special attention should be paid to collecting specimens from a folded area or an area of attachment such as the frenulum. The swab is then replaced into its sheath. The process may be repeated with a dry swab.
Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	1. **Saliva, vaginal fluids, rectal fluids, blood or semen** Up to 72 hours for all biological fluids: Some jurisdictions will sample the penis for biological fluids after washing and others will not. 2. **Penile contact with skin** Some jurisdictions will sample up to 72 hours and others up to 12 hours. No jurisdiction will sample the penis for skin contact after washing.

	3. **Non-biological trace evidence and lubricant** May also be detected on the penis and collection may be considered up to the point of washing or timeframe determined by the relevant interpersonal contact.

Comments

- Biological fluids may collect under the foreskin (if present), sulcus, meatus and in recesses around any piercings or around any folded areas of attachment such as the frenulum.
- Semen may not be a relevant specimen to collect from the penis in cases of sexual assault and therefore in jurisdictions where the laboratory requires the practitioner to prepare a slide at the time of collection this may not be required for penile samples. However each case should be considered on its own merits.
- The laboratory may not identify cell types or the anatomical area of origin of any foreign DNA found.
- This sample should be collected in all cases of reported or suspected sexual assault in a male including in victims where memory is deficient or absent.

Relevant References

1-9

Penile References

1. Collins KA, Cina SJ, Pettanati MJ, Fitts M. Identification of Female Cells in Postcoital Penile Swabs using Fluorescence In Situ Hybridization. Archives of Pathology & Laboratory Medicine. 2000 July; 124(7): 1080.
2. Cina SJ et al. Isolation and identification of female DNA on postcoital penile swabs. American Journal of Forensic Medicine and Pathology 2000; 21(2): 97-100.
3. Farmen RKB, Haukeli I, Ruoff P, Froyland ES. Assessing the presence of female DNA on post-coital penile swabs: relevance to the investigation of sexual assault. Journal of forensic and legal medicine. 2012 Oct; 19(7): 386-9.
4. Janisch S, Meyer H, Germerott T, Albrecht UV, Schulz Y, Debertin AS. Analysis of clinical forensic examination reports on sexual assault. Int J Legal Med. 2010 May; 124(3):227-35. PubMed PMID: 20182738. eng.
5. Kaarstad K, Rohde M, Larsen J, Eriksen B, Thomsen JL. The detection of female DNA from the penis in sexual assault cases. 2007; 14: 159-160.
6. Keating SM. Inforamtion from penile swabs in sexual assault cases. Forensic Science Inernational. 1989; 43: 63-81.
7. Kennedy KM. Letter to the editor: Response to "Assessing the presence of female DNA on post-coital penile swabs: relevance to the investigation of sexual assault" published in the October 2012 edition of the Journal of Forensic and Legal Medicine. Journal of forensic and legal medicine. 2013 July.; 20(5) 559
8. Nittis M, Stark M. Evidence based practice: laboratory feedback informs forensic specimen collection in NSW. Journal of forensic and legal medicine 2014 July 25: 38-44
9. Tucker S, Ledray LE, Werner JS. Sexual Assault Evidence Collection. Wisconsin Medical Journal. 1990;89(7):407-11.

ANAL / RECTAL SAMPLES

Name of Collections	ANAL / RECTAL SAMPLES
Name of Sample	**Perianal swab**
Methods of Collection	A swab is used to sample the perianal area i.e. the area outside the anal verge. This should be done prior to collection of an anal or rectal swab. The swab may need to be moistened if the area is dry.
Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	If washing or wiping after defaecation has **not** occurred, there may be a yield of useful DNA from spermatozoa for some days. 1. **Penile Anal** Generally not collected after 72 hours (ANZ). 2. **Digital Anal** Varies from 12 hours (with no history of defaecation) to 72 hours. 3. **As an adjunct to penile vaginal penetration** May be taken up to 168 hours (7 days) (UK recommendations)
Comments A perianal swab may detect spermatozoa leakage from deposition in the vagina or the anal area.	
Research:	

Name of Collections	ANAL / RECTAL
Name of Sample	**Anal Swab**
Methods of Collection	A swab is used to obtain a sample from the anal canal
Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	1. **Penile Anal** Generally not collected after 72 hours (ANZ). 2. **Digital Anal**

	Varies from 12 hours (with no history of defaecation) to 72 hours. **3. As an adjunct to penile vaginal penetration** If taken after complaint of penile vaginal assault, may be taken up to 168 hours (7 days) (UK recommendations)

Comments

- The yield of useful DNA falls off rapidly after 24 hours and the expectation of finding spermatozoa after 48 hours is very low. The chance of successfully obtaining sufficient DNA to produce a profile might be reduced if defaecation has occurred.
- The amount of trace DNA residual from digital penetration is low and the chances of retrieval extremely low. If there has been washing, or wiping the specimen will not be useful.
- In some jurisdictions, advice for the timelines for these specimens has been extrapolated from studies of vaginal Y DNA retrieved following digital penetration. There are no published studies to assist forensic practitioners in this area.
- It is not difficult to introduce external DNA from the perianal area into the anus and beyond. Special care, therefore, must be taken when collecting both anal and rectal samples to ensure this is not done, if at all possible.

Relevant References

5.

Name of Collections	**ANAL / RECTAL**
Name of Sample	**Rectal swab**
Methods of Collection	A swab is used via a proctoscope to sample the rectal wall
Suggested Storage	The sample needs to be aerated but not refrigerated.
Collection Time Periods	Range from 12 hours to 72 hours after penile anal penetration

Comments

- The yield of DNA falls off rapidly after 24 hours. It is possible that Y-filer analysis may find useful DNA profiles at extended time intervals. The chance of successfully obtaining sufficient DNA to produce a profile might be reduced if defaecation has occurred.

Relevant References

Anal / Rectal References

1. Gingras F, Paquet C, Bazinet M, Granger D, Marcoux-Legault K, Fiorillo M, Séguin D, Baltzer F, Chamberland C, Jolicoeur C. Biological and DNA evidence in 1000 sexual assault cases. Forensic Science International: Genetics Supplement Series. 2009 Dec 31;2(1):138-40.
2. Nittis M, Stark M. Evidence based practice: Laboratory feedback informs forensic specimen collection in NSW. Journal of forensic and legal medicine. 2014 Jul 31;25:38-44.
3. Ingemann-Hansen O, Charles AV. Forensic medical examination of adolescent and adult victims of sexual violence. Best Practice & Research Clinical Obstetrics & Gynaecology. 2013 Feb 28;27(1):91-102.
4. Casey, David G., et al. "The persistence of sperm and the development of time since intercourse (TSI) guidelines in sexual assault cases at forensic science ireland, Dublin, Ireland." *Journal of forensic sciences* (2016).
5. Nittis M. Chapter: Evidence Collection in Cases of Sexual Assault in Beran RG. Ed. *Legal and Forensic Medicine, Springer-Verlag Berlin Heidelberg, 2013. Pp1335-1358*

SKIN

Name of Collections	**SKIN**
Name of Sample	**Skin for epithelial cells**
Methods of Collection	A moistened DNA free swab is used to sample the area indicated in the assault. The swab is moistened ideally with sterile water (not saline). Sufficient pressure should be applied to remove any foreign cells from the surface of the skin. Some jurisdictions recommend a moistened swab followed by a second dry swab.
Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	Ranges from up to 12 hours to 72 hours after assault and only if the person has not washed.
Comments: • NSW no longer recommends a two swab method.	
Relevant References	

1, 2, 3, 4

Name of Collections	SKIN
Name of Sample	**Skin for semen**
Methods of Collection	A moistened DNA free swab is used to sample the area indicated in the assault. The swab is moistened ideally with sterile water (not saline). Tap water can be used, if sterile water is not available. Sufficient pressure should be applied to remove any foreign cells from the surface of the skin. Some jurisdictions recommend a moistened swab followed by a second dry swab. If you are working in a jurisdiction where a slide is made for semen samples, you would also need to make a slide from the swab collected.
Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	Up to 72 hours following an assault. This time might be extended if the complainant has not washed or the history warrants a prolonged collection period.
Comments: • NSW no longer recommends a two swab method.	
Relevant References 5, 6	

Name of Collections	SKIN
Name of Sample	**SKIN for blood and other stains**
Methods of Collection	A moistened DNA free swab is used to sample the area indicated in the assault. The skin should be sampled for blood if blood (or what is thought to be blood) is still visible on the skin.

	The swab is moistened ideally with sterile water (not saline). Sufficient pressure should be applied to remove any foreign cells from the surface of the skin. Some jurisdictions recommend a moistened swab followed by a second dry swab.
Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	Skin can be sampled for blood for as long as blood / blood stain / what is thought to be blood is still visible. Otherwise consider sampling up to 48 hours and possibly longer if not washed and indicated by the history.
Comments: • NSW no longer recommends a two swab method.	
Relevant References 7	

Name of Collections	**SKIN**
Name of Sample	**SKIN for saliva**
Methods of Collection	A moistened DNA free swab is used to sample the area indicated in the assault. The swab is moistened ideally with sterile water (not saline). Sufficient pressure should be applied to remove any foreign cells from the surface of the skin. Some jurisdictions recommend a moistened swab followed by a second dry swab.
Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	Up to 48 hours after assault
Comments: • Consider sampling for amylase / DNA if there has been a history of licking, biting, sucking • NSW no longer recommends a two swab method.	

Relevant References
6, 8, 9, 10

Skin References

1. Wiegand, P. and M. Kleiber, *DNA typing of epithelial cells after strangulation.* Int J Legal Med, 1997. **110**(4): p. 181-3.
2. Rutty, G.N., *An investigation into the transference and survivability of human DNA following simulated manual strangulation with consideration of the problem of third party contamination.* Int J Legal Med, 2002. **116**(3): p. 170-3.
3. Maguire, S., et al., *Retrieval of DNA from the faces of children aged 0-5 years: a technical note.* Journal of forensic nursing, 2008. **4**: p. 40-44.
4. Graham, E.A.M. and G.N. Rutty, *Investigation into Normal Background DNA on Adult Necks: Implications for DNA profiling of Manual Strangulation Victims.* Journal of Forensic Science, 2008. **53**(5): p. 1074 - 1082.
5. Gabby, T., et al., *Sexual Abuse of Children: The detection of Semen on Skin.* AJDC, 1992. **146**: p. 700-703.
6. Nittis, M. and M. Stark, *Evidence based practice: laboratory feedback informs forensic specimen collection in NSW.* J Forensic Leg Med, 2014. **25**: p. 38-44.
7. Tonkin, M., et al., *The effect of environmental conditions on the persistence of common lubricants on skin for cases of sexual assault investigation.* J Forensic Sci, 2013. **58 Suppl 1**: p. S26-33.
8. Sweet, D. and G. Shutler, *Analysis of Salivary DNA Evidnece from a Bite Mark on a Body Submerged in Water.* Journal of Forensic Science, 1999. **44**(5): p. 1069-1072.
9. Kenna, J., et al., *The recovery and persistence of salivary DNA on human skin.* J Forensic Sci, 2011. **56**(1): p. 170-5.
10. Williams, S., et al., *Recovery of salivary DNA from the skin after showering.* Forensic Sci Med Pathol, 2015. **11**(1): p. 29-34.

FINGERNAIL

Name of Collections	**FINGERNAIL**
Name of Sample	**Subungual and fingernail**
Methods of Collection	**1. Fingernail Cuttings** If fingernails are to be cut, a DNA free pair of scissors / nail clippers should be used, if available. Failing this, consideration should be given to a single use pair of scissors / clippers or sterilised scissors / clippers. Clipping should occur over a DNA free sheet, to prevent losing the nails. Generally the nails of one hand are collected and combined in a single transport pot. The nails of the other hand are likewise combined and put in a second pot. **2. Fingernail Swabbings** A fine / small moistened DNA free swab is used to sample the area beneath the nails. Different jurisdictions have different

	methodologies which range from using a single swab for each nail sampled, to using a single swab for each hand. The swab is moistened ideally with sterile water (not saline). It can be used to sample beneath the nail and, if required, can also sample the outer surface of the nail itself and adjacent skin. Some jurisdictions will collect a moist and dry swab for each collection. **3. Fingernail Scrapings** Ideally a blunt, DNA free instrument (e.g. plastic spatula) is used to dislodge any collected DNA from beneath the nail, without dislodging cells from the donor's nails.
Suggested Storage	These samples generally do not need to be refrigerated.
Collection Time Periods	DNA has been recovered up to 48 hours after assault, even after handwashing. [1.] It is proposed that useful DNA might be retrieved via nail swabbing of the nails of alleged perpetrators who digitally assault their female victims up to at least 18 hours later.[4.]
Comments: • If people cohabitate it is important to recognise that a low level of foreign DNA may already be present beneath the fingernails. [2, 3] • There is no research showing the preferable method of collecting subungual evidence or, if more than one method was to be used (e.g. swabbing and cutting) which should go first. [5.]	
Relevant References **1, 2, 3, 4, 5**	

Nail References

1. Lederer, T; Betz, P; Seidl, S. DNA analsysis of fingernail debris using different multiplex systems: a case report. Int J Legal Med. 2001; 114(4-5):263-6
2. Cook, O; Dixon, L. The prevalence of mixed DNA profiles in fingernail samples taken from individuals in the general population. Forensic Sci. Int. Genet. 2007; 1(1):62-8
3. Malsom, S; Flanagan, N; McAlister, C; Dixon, L. The precalence of mixed DNA profiles in fingernail samples taken from couples who co-habit using autosomal and Y-STRs. Forensic Sci Int Genet. 2009; 3(2):57-62
4. Flanagan, N; McAlister, C. The transfer and persistence of DNA under the fingernails following digital penetration of the vagina. Forensic Science International: Genetics [in press] 2010.
5. Nittis M. Chapter: Evidence Collection in Cases of Sexual Assault in Beran RG. Ed. *Legal and Forensic Medicine, Springer-Verlag Berlin Heidelberg, 2013. Pp1335-1358*

EARLY EVIDENCE KIT COLLECTIONS

Name of Collections	EARLY EVIDENCE KIT – OVERVIEW OF ALL SPECIMENS
Types of potential EEK samples	**Oral rinse, urine for both forensic biology and for toxicology, vulval gauze wipe, perianal gauze wipe, penile gauze wipe, blood for toxicology**
Methods of Collection	1. **Urine for Forensic Biology (for detection of sperm, DNA)** **Indication** Females: Following penetration / attempted penetration of vagina by penis (even if no ejaculation), finger Males – Following the patient's penis being in contact with a mouth, body part or cavity. **Method** Collect the first portion when passing urine, it does not have to be the first time that the patient passes urine after the alleged assault. Self-collected by patient in bathroom using equipment from the EEK kit. **2. Vulval Gauze** **Indication** Following penetration / attempted penetration of vagina by penis (even if no ejaculation), finger **Method** Self-collected by patient in bathroom using equipment from the EEK kit. Patient puts on gloves. Patient then uses gauze to very gently wipe/dab vulva and places gauze in the collection pot and replaces the top. **3. Perianal Gauze**

	Indication If patient needs to open bowels following penetration / attempted penetration of anus by penis (even if no ejaculation), finger, object **Method** Patient puts on gloves, moistens gauze with water and wipes gently around the outside of the anus prior to defecation and places this gauze in the collection pot and replaces the top.
Suggested Storage	Specimens placed into tamper-evident bag, signed and sealed. 1) If EEK "collected" by police then the EEK is handed over directly to police. 2) If EEK "collected" by ED nurse / doctor then: - If police involved and present in ED then handover immediately to police with patient informed consent - If no police involved yet or police not present or patient unable to consent e.g. intoxicated, then the bag is stored until it is able to be handed over. It may be stored securely in a variety of places including: with the patient, within the ED or within a sexual assault unit. Please consult local guidelines to ascertain secure options. Best to freeze or refrigerate if there is a delay in handover.
Collection Time Periods	Whenever there is likely to be a delay in having a full forensic assessment and evidence is likely to be compromised by the delay.
Comments: • Prevents loss of potentially forensically significant material if there is a delay before full forensic exam e.g. if essential medical procedures required before a formal sexual assault examination or if patient has to travel large distance to exam. • Also allows for patient comfort as they can drink, pass urine and open bowels with less concern for loss of forensic evidence.	

- Can be performed by non-forensically trained staff, i.e. those who have had an in-service training.
- EEK is an adjunct to the full forensic kit and should not be used instead of or to delay full forensic collection.

  With the exception of the oral rinse the EEK specimens cannot indicate penetration because they are external samples, but they may be useful in determining that sexual activity took place and for identification (DNA profile).
- Consent is **very** important. If the patient is intoxicated (alcohol or drugs) and thus incapable of consenting at that moment and there is an allegation or strong suspicion of sexual assault, specimens can be collected with their assent (if conscious) or to prevent loss of evidence (e.g. prior to catheterisation if unconscious). Specimens are NOT handed to police until formal, written, informed consent has been obtained from the patient.
- Anecdotally the EEK samples, and in particular the urine and vulval gauze samples, have continued to yield good results in Western Australia. EEK samples and forensic results appear to have been accepted by WA courts. WA is unaware of any challenges to date.

Relevant References:

1, 2, 3, 4, 5

EEK References:

1. Australian Standards on Forensic Kits
2. WA SARC EEK paper - Smith DA, Webb LG, Fennell AI, Nathan EA, Bassindale CA, Phillips MA. Early evidence kits in sexual assault: an observational study of spermatozoa detection in urine and other forensic specimens. Forensic Sci Med Pathol. 2014 Sep;10(3):336-43.
3. Finnish paper - Joki-Erkkilä M, Tuomisto S, Seppänen M, Huhtala H, Ahola A, Karhunen PJ. Urine specimen collection following consensual intercourse - A forensic evidence collection method for Y-DNA and spermatozoa. J Forensic Leg Med. 2016 Jan;37:50-4. doi: 10.1016/j.jflm.2015.10.008. Epub 2015 Nov 2.
4. Newton M. The forensic aspects of sexual violence. Best Prac Res Clin Obstet Gynaecol. 2013;27:77–90.
5. Anna-Marie Ashley, Scientist in Charge, Forensic Biology, PathWest LMWA. Personal communication

TOXICOLOGY

Name of Collections	OVERVIEW OF COLLECTION
Types of potential Toxicology samples	**Toxicology: Urine, Blood, Oral fluid (saliva), Hair, Other**

Methods of Collection (consent from the subject and consultation with the local toxicology laboratory is essential)	1. **Urine for Toxicology (for detection of drugs and metabolites)** **Indication** All patients: Following recent (up to 4-5 days) drug use or covert administration **Method** Collect a sample of urine into a clean unused jar. It does not have to be a first pass or midstream specimen. If the specimen is from a victim, it can be self-collected by patient (or parent) in bathroom. If the specimen is from a perpetrator, prisoner or suspect it should be collected using procedures defined in the appropriate Standard which is AS 4308:2008 **2. Blood** **Indication** All patients: Following recent (1-2 days) drug use or covert administration **Method** Consent from the patient is essential. Once consent is obtained approximately 5 ml of blood is collected by venepuncture into a sterile tube with fluoride/oxalate preservative. Suitable tubes that can be used in hospital if a dedicated toxicology kit is not available include blood sugar tubes or those from police traffic blood kits. **3. Oral fluid (saliva)** **Indication** All patients: Following recent (hours) drug use or covert administration **Method**

	Other than Police traffic screening when special devices are used, these specimens are rarely required for other forensic purposes. In the event that an oral fluid specimen might be required for other toxicological purposes, advice should be sought from the local toxicology laboratory. **4. Hair** **Indication** <u>All patients</u>: for investigation of historical cases of drug administration **Method** Local toxicology laboratories will have their own requirements for hair sampling and may have produced kits for this purpose. The general principle is that a tuft of hair about the diameter of a pencil is cut from an unobtrusive area such as the nape of the neck as close to the scalp as possible. Clean disposable scissors should be used and the collector should be gloved. The specimen is wrapped in aluminium foil with the cut end clearly identified and then packed in a suitable envelope. **5. Other** **Indication** A great many other body tissues and fluids may be useful for toxicological analysis in unusual circumstances. These include faeces, placental tissue, meconium, breast milk, finger and toe nails, deciduous or permanent teeth, vomitus, sweat, sebum or any other available substance. **Method** Local toxicology laboratories should be consulted regarding requirements for unusual types of samples.

Suggested Storage	Specimens must be labelled with the patient's name, DOB and time and date of collection, and placed into tamper-evident bag, signed and sealed. An information sheet or form should be included with details of the case to guide the laboratory. Important information for the laboratory includes: • Identification of the patient and other relevant people such as police and the collector • What specimens have been collected • Time of collection • Time of suspected drug use (if known) • Specific drugs that are suspected (if known) • Prior use of alcohol, medications and drugs Blood collected into fluoride oxalate tubes does not require refrigeration but should be transported to the laboratory as soon as possible. Urine or other body fluid samples should be frozen or refrigerated if there will be a delay of more than about a day in handover to the laboratory.
Collection Time Periods	Blood specimens are useful if collected within about 24 hours of an incident. Urine specimens can be collected up to several days after suspected drug use. These intervals may vary according to the specific drug and local laboratory requirements. Advice should be sought accordingly.
Comments: • Consent is very important. If the patient is intoxicated or otherwise incapable of consenting at that moment and there is an allegation or strong suspicion of drug involvement, specimens can be collected with their assent (if conscious) or to prevent loss of evidence (e.g. prior to catheterisation if unconscious). Specimens are NOT handed to police until formal, written, informed consent has been obtained from the patient.	