



DNA APPENDIX J – DIRECT TO DNA WORKFLOW

Instructions

1. Important elements of each case, the relevance of the evidence submitted, and the investigative questions shall be evaluated through careful review of the cover letter, previous communication with the submitting agency (if applicable), and the sexual assault kit paperwork to ensure that each item of evidence is handled appropriately.
 - a. Analysts should contact the investigating agency via phone or email if additional information is needed or if there is ambiguity in the information provided (e.g. date of assault) that is relied upon when making analytical decisions. This communication must be recorded in the communication log and/or case object repository in accordance with the CBI-FS Evidence Manual EP4 and EP5.
 - b. The sexual assault kit paperwork (Colorado Sexual Assault Consent and Information Form and Sexual Assault Incident Form) will be reviewed prior to the initiation of analysis.
 - i. If the incident form is missing, the submitting agency must be contacted and this request must be documented in the case record to ensure proper analysis of evidence.
 - ii. If the Colorado Sexual Assault Consent and Information Form is missing or filled out incorrectly:
 1. If the patient was between 18-69 years old at the time of the assault, the analyst must contact the submitting agency PRIOR to initiating any examinations. The analyst must ensure that the victim consented to the analysis of evidence in accordance with CRS 24-33.5-113.
 2. If the patient was a minor (younger than 18) at the time of the assault or an at risk adult (70 or older), mandatory reporting laws require processing.
 3. If the consent form line regarding withdrawal of consent is not initialed, analysis of the kit may proceed. If the analyst receives communication during the analysis of the kit that the victim has withdrawn consent for testing, contact a technical leader for guidance.
 - c. The victim's reporting decisions have the following impact on analysis in the laboratory:
 - i. Law enforcement report: victim chose to report the crime to law enforcement. Case WILL be analyzed if the victim also consented to analysis.
 - ii. Medical report: victim chose not to report to law enforcement or participate in any investigation at the time of kit collection. Case WILL be analyzed if the victim also consented to analysis. Case will NOT be analyzed if the victim did not consent to analysis.
 - iii. Anonymous report: victim chose not to disclose their identity to law enforcement and for storage of their kit only. Case WILL NOT be analyzed unless the victim later changes their reporting option and signs a new consent form.
2. Sexual assault cases with SAEKs submitted will be analyzed according to the applicable scenario (or combination of scenarios) present within this workflow, provided the victim gave consent for such analysis in the Sexual Assault Consent and Information Form.
 - a. SAEKs will be processed initially, along with any reference samples submitted, following the appropriate scenario (or combination of scenarios) present within this workflow. The analyst(s) may choose to analyze other items of evidence in the first round of testing due to case circumstances, nature of the additional evidentiary items submitted, rush status of the case, or exigent circumstances.
 - i. Each page of the workflow depicts a different scenario (underline indicates the individual the SAEK was collected from). Navigate to the appropriate page based on case circumstances using information from the cover letter, RFLE, sexual assault incident form, notes on the kit protocol (if included), and any applicable notes on items within the kit. Follow the applicable workflow. Contact a technical leader with any questions about which workflow should be followed for a particular case.
 - ii. If the sexual assault kit was collected outside of NIJ recommended time frames (as depicted in each scenario), contact the technical leaders and your supervisor to discuss how to proceed with analysis in the case.
 - b. If comparable foreign DNA is not detected on items within the SAEK, additional items submitted along with the kit will be analyzed. This analysis can be included in the original lab record or it can be assigned to and performed in a new lab record. If multiple analysts are involved in the case, the analysts are encouraged to coordinate with one another to perform the additional serology and DNA analysis (if applicable) expeditiously and in a manner that minimizes the number of reports authored.
3. Additional information:
 - a. If a date or time range is provided for the sexual assault and this information cannot be clarified by the submitting agency (see 1.a above), the most conservative day in the range will be used for analysis. For example, if the alleged assault occurred 12/01/21-12/03/21 and the kit was collected 12/03/21, the kit will be analyzed as if the assault could have occurred within 24 hours of kit collection.
 - b. Neck swabs that were collected due to potential/suspected strangulation (based on information in the cover letter, sexual assault incident form, and/or kit envelope) will be analyzed if collected within 5 days (up to 119:59 hours).
 - c. If foreign genetic material is detected in any sample in the kit, the report will reflect the direct to DNA remark, "Additional serological examinations and/or further DNA analysis may be performed on suitable items by request. Please contact the reporting scientist if necessary."
 - d. If the victim reports consensual sexual activity **within 5 days** of the collection of the sexual assault kit:



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- i. The consensual partner must be considered when selecting sample for DNA and making amplification decisions. If the consensual partner is a different individual than the alleged assailant, all eligible samples (based on quant) must be amplified.
- ii. A request for appropriate elimination standards must be documented in the case communication log or the case object repository before the lab record is assigned for technical review if a profile is being entered into the CODIS DNA database.



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SCENARIOS: female victim SAEK processing with alleged male assailant(s) OR male victim with alleged female assailant kit

SAEK Inventory

1. Label each item in the SAEK.
2. Create each sub-item in FA.
3. Add “Not Analyzed” items to the serology worksheet.

Microscopic Exam for Sperm Time frames for analysis, per NIJ and SWGDAM recommendations, will be followed for smear slide examination (for DPD kits and kits where slides weren’t prepared at the hospital, slides will be created in serology for samples within these time frames):

Sample	Days (and Hours) Since Alleged Assault		
	1 (0-23:59)	2-3 (24-71:59)	4-5+ (72-120+)
Oral	Yes	No	No
Anal	Yes	Yes	No
Vaginal	Yes	Yes	Yes
Cervical	Yes	Yes	Yes

Note: The analyst may use his or her discretion in analyzing probative samples in cases exceeding these time frames. Slides may be prepared and examined in lieu of/in addition to smear slides if there are potential quality issues with the smear slides present in the kit. Slides may also be prepared from external samples and examined at the analyst’s discretion.

Sample Preparation for DNA

1. Internal samples (oral, anal, vaginal, and cervical):
 - a. If the corresponding slide is positive, half of the total number of swabs (up to 2 swabs) will be cut into a differential extraction tube.

Note: If one or more slide is positive, the analyst should select 1-2 samples for differential extraction based on case scenario and proceed to extraction. If there are multiple alleged assailants and/or a consensual partner, the number of samples selected for DNA extraction cannot be limited.
 - b. If the corresponding slide is negative, half of the total number of swabs (up to 2 swabs) will be cut into a PrepFiler column/tube assembly.
 - c. Speculum swabs are additional collections from the vaginal cavity. If case circumstances dictate (e.g. multiple assailants alleged or no vaginal swabs present in the kit), speculum swabs will be analyzed according to the vaginal slide results.
2. Using the information provided about the time elapsed between the alleged assault and kit collection as well as the location of sample collection, analysts will select external swabs for analysis. Half of the total number of swabs (up to 2 swabs) from each external sample selected will be cut into a PrepFiler column/tube assembly.
 - a. If multiple external genitalia collections are present, one from this anatomical area will typically be selected for analysis.
 - b. External samples may be suitable for analysis if collected within 5 days (up to 119:59 hours).

Note: If case circumstances/allegations indicate that an external ejaculation occurred, the analyst may choose to perform a differential extraction on the applicable external samples. The analyst may prepare a slide for a sperm search during the differential extraction process.
3. Additional items contained within the kit may be subsequently processed if warranted (and if proper consumptive permissions are obtained) for applicable samples, including pubic hair combings, fingernail collections, tampons, and drapes.

Extraction Microscope slides are typically not prepared during differential extraction.

Quantitation

1. Each extract will be assessed for the presence or absence of male DNA.
 - a. If no male DNA is detected, the sample will not be analyzed further.
 - b. If the F:M ratio is greater than 40:1 AND an insufficient quantity of male DNA is detected (<0.0006 ng/uL), the sample will not be analyzed further.
 - c. If the F:M ratio is less than 40:1, the sample is eligible for GF amplification. The sample may be subsequently amplified with YFP if unsuitable GF results are obtained. See point d below.
 - d. If the F:M ratio is greater than 40:1 and a sufficient quantity of male DNA (≥0.0006 ng/uL) is detected, the sample may be amplified with YFP (if appropriate references are present).
2. If processed separately, reference samples do not need to be extracted, quanted, or amplified if no evidentiary samples will be taken past quant.

Amplification and Typing

1. Single alleged assailant and no consensual partner:
 - a. 1-2 samples suitable for amplification (see quantification section) with the best F:M ratio and/or male quant will be amplified. Analysts may use their discretion to amplify additional samples.
 - b. Non-sperm fractions are considered non-probative and do not need to be amplified. Analysts may use their discretion to amplify these samples if they are suitable for amplification and have more male DNA and/or a better F:M ratio than the corresponding fraction.
2. Multiple alleged assailants and/or a consensual partner:
 - a. Samples suitable for amplification (see quantification section) will be amplified.
 - b. Non-sperm fractions that are suitable for amplification (see quantification section) are considered probative and must be amplified.



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Serology

Conduct semen, saliva, and/or blood testing on remaining swab(s) from samples *with foreign DNA detected, when necessary/requested*. Use the overlay method, if possible/applicable, to prevent unnecessary sample consumption.

1. AP can be conducted on internal samples if the corresponding microscopic examination is negative.
2. p30 testing will only be conducted on internal samples if the corresponding microscopic examination is negative and proper consumptive permission is obtained.
3. Slides will only be prepared from external samples after DNA analysis if proper consumptive permission is obtained.
4. SALigAE® testing will only be conducted as an overlay, unless proper consumptive permission is obtained.



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SCENARIO: female victim SAEK processing with one suspect of unknown sex and no male consensual partner

SAEK Inventory

1. Label each sub-item in the SAEK.
2. Create each sub-item in FA.
3. Add “Not Analyzed” items to the serology worksheet

Microscopic Exam for Sperm Time frames for analysis, per NIJ and SWGDAM recommendations, will be followed for smear slide examination (for DPD kits and kits where slides weren’t prepared at the hospital, slides will be created in serology for samples within these time frames):

Sample	Days (and Hours) Since Alleged Assault		
	1 (0-23:59)	2-3 (24-71:59)	4-5+ (72-120+)
Oral	Yes	No	No
Anal	Yes	Yes	No
Vaginal	Yes	Yes	Yes
Cervical	Yes	Yes	Yes

Note: The analyst may use his or her discretion in analyzing probative samples in cases exceeding these time frames. Slides may be prepared and examined in lieu of/in addition to smear slides if there are potential quality issues with the smear slides present in the kit.

Sample Preparation for DNA

1. Internal samples (oral, anal, vaginal, and cervical):
 - a. If the corresponding slide is positive, half of the total number of swabs (up to 2 swabs) will be cut into a differential extraction tube.

Note: If one or more slide is positive, the analyst may assume the assailant is male and select 1-2 samples for differential extraction based on case scenario and proceed to extraction.
 - b. If the corresponding slide is negative, half of the total number of swabs (up to 2 swabs) will be cut into a PrepFiler column/tube assembly.
 - c. Speculum swabs are additional collections from the vaginal cavity. If case circumstances dictate (e.g. no vaginal swabs present in the kit), speculum swabs will be analyzed according to the vaginal slide results.
2. Using the information provided about the time elapsed between the alleged assault and kit collection as well as the location of sample collection, analysts will select external swabs for analysis. Half of the total number of swabs (up to 2 swabs) from each external sample selected will be cut into a PrepFiler column/tube assembly.
 - a. If multiple external genitalia collections are present, one will typically be selected for analysis.
 - b. External samples may be suitable for analysis if collected within 5 days (up to 119:59 hours).
3. Additional items contained within the kit may be subsequently processed if warranted (and if proper consumptive permissions are obtained) for applicable samples, including pubic hair combings, fingernail collections, tampons, and drapes.

Extraction Microscope slides are typically not prepared during differential extraction.

Quantitation Each extract will be assessed for the presence or absence of male DNA. If male DNA is detected in any sample, the analyst may assume the assailant in the case is male and not amplify samples to look for foreign female DNA.

1. If no male DNA is detected in any samples in the case, external samples, and oral, anal, and vaginal samples (as applicable) will be amplified since the suspect could be female. See the “Amplification and Typing” section for additional information.
2. If no male DNA is detected in a sample, but is detected in other samples in the case, the sample will not be analyzed further.
3. If the F:M ratio is greater than 40:1 AND an insufficient quantity of male DNA is detected (<0.0006 ng/uL), the sample will not be analyzed further.
4. If the F:M ratio is less than 40:1, the sample is eligible for GF amplification. The sample may be subsequently amplified with YFP if unsuitable GF results are obtained. See point 5 below.
5. If the F:M ratio is greater than 40:1 and a sufficient quantity of male DNA is detected (≥0.0006 ng/uL), the sample may be amplified with YFP (if appropriate references are present).

Amplification and Typing

1. If the analyst was able to assume the assailant is male (based on slide or male quant results), the analyst will typically use their discretion to select the 2 samples with the best F:M ratio or best male quant for amplification.
2. If the analyst was not able to assume the assailant is male (based on slide or male quant results):
 - a. If the victim is <18 years old, >69 years old, OR medically reported, external samples must be amplified. If any of these external samples yield foreign genetic material, internal oral, anal, and vaginal samples (if present) must be amplified.
 - b. If the victim is 18-69 years old AND reported the alleged assault to law enforcement, no further analysis will typically be performed on items in the kit.
 - c. The report will reflect that additional amplifications may be performed if a female suspect is developed if not all samples were amplified.
3. Non-sperm fractions are considered non-probative and do not need to be amplified. Analysts may choose to amplify these samples if they are suitable for amplification and have more male DNA and/or a better F:M ratio than the corresponding sperm fraction.
4. If processed separately, reference samples do not need to be extracted, quanted, or amplified if no evidentiary samples will be taken past quant.

Serology Conduct semen, saliva, and/or blood testing on remaining swab(s) from samples *with foreign DNA detected, when necessary/requested*. Use the overlay method, if possible/applicable, to prevent unnecessary sample consumption.



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1. AP can be conducted on internal samples if the corresponding microscopic examination is negative.
2. p30 testing will only be conducted on internal samples if the corresponding microscopic examination is negative and proper consumptive permission is obtained.
3. Slides will only be prepared from external samples after DNA analysis if proper consumptive permission is obtained.
4. SALigAE® testing will only be conducted as an overlay, unless proper consumptive permission is obtained.



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SCENARIOS: female victim SAEK processing with unknown number and sex of assailants OR female victim SAEK processing with unknown sex of assailant(s) and a male consensual partner

SAEK Inventory

1. Label each sub-item in the SAEK.
2. Create each sub-item in FA.
3. Add “Not Analyzed” items to the serology worksheet

Microscopic Exam for Sperm Time frames for analysis, per NIJ and SWGDAM recommendations, will be followed for smear slide examination (for DPD kits and kits where slides weren’t prepared at the hospital, slides will be created in serology for samples within these time frames):

Sample	Days (and Hours) Since Alleged Assault		
	1 (0-23:59)	2-3 (24-71:59)	4-5+ (72-120+)
Oral	Yes	No	No
Anal	Yes	Yes	No
Vaginal	Yes	Yes	Yes
Cervical	Yes	Yes	Yes

Note: The analyst may use his or her discretion in analyzing probative samples in cases exceeding these time frames. Slides may be prepared and examined in lieu of/in addition to smear slides if there are potential quality issues with the smear slides present in the kit.

Sample Preparation for DNA

1. Internal samples (oral, anal, vaginal, and cervical):
 - a. If the corresponding slide is positive, half of the total number of swabs (up to 2 swabs) will be cut into a differential extraction tube.

Note: Since there are multiple assailants potentially involved and/or a consensual partner, the number of samples selected for DNA extraction cannot be limited based on a positive slide result.
 - b. If the corresponding slide is negative, half of the total number of swabs (up to 2 swabs) will be cut into a PrepFiler column/tube assembly.
 - c. Speculum swabs are additional collections from the vaginal cavity. If case circumstances dictate (e.g. no vaginal swabs present in the kit), speculum swabs will be analyzed according to the vaginal slide results.
2. Using the information provided about the time elapsed between the alleged assault and kit collection as well as the location of sample collection, analysts will select external swabs for analysis. Half of the total number of swabs (up to 2 swabs) from each external sample selected will be cut into a PrepFiler column/tube assembly.
 - a. If multiple external genitalia collections are present, one will typically be selected for analysis.
 - b. External samples may be suitable for analysis if collected within 5 days (up to 119:59 hours).
3. Additional items contained within the kit may be subsequently processed if warranted (and if proper consumptive permissions are obtained) for applicable samples, including pubic hair combings, fingernail collections, tampons, and drapes.

Extraction Microscope slides are typically not prepared during differential extraction.

Quantitation Each extract will be assessed for the presence or absence of male DNA. Male quantification results will be reported. All samples are suitable for amplification to look for foreign female DNA.

1. If no male DNA is detected OR If the F:M ratio is greater than 40:1 AND an insufficient quantity of male DNA is detected (<0.0006 ng/uL), the sample is not eligible for YFP amplification.
2. If a sufficient quantity of male DNA is detected (≥0.0006 ng/uL), the sample may be amplified with YFP (if appropriate references are present).

Amplification and Typing

1. If the victim is <18 years old, >69 years old, OR medically reported, external samples must be amplified. If any of these external samples yield foreign genetic material, oral, anal, and vaginal samples (if present) samples must be amplified.
2. If the victim is 18-69 years old AND reported the alleged assault to law enforcement, the analyst may proceed with amplification of (sperm and non-sperm fractions of) items with F:M ratio less than 40:1 only.
3. The report will reflect that additional amplifications may be performed if a female suspect is developed if not all samples were amplified.
4. If processed separately, reference samples do not need to be extracted, quanted, or amplified if no evidentiary samples will be taken past quant.

Serology Conduct semen, saliva, and/or blood testing on remaining swab(s) from samples *with foreign DNA detected, when necessary/requested*. Use the overlay method, if possible/applicable, to prevent unnecessary sample consumption.

1. AP can be conducted on internal samples if the corresponding microscopic examination is negative.
2. p30 testing will only be conducted on internal samples if the corresponding microscopic examination is negative and proper consumptive permission is obtained.
3. Slides will only be prepared from external samples after DNA analysis if proper consumptive permission is obtained.
4. SALIgAE® testing will only be conducted as an overlay, unless proper consumptive permission is obtained.



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SCENARIO: female victim SAEK processing with female suspect(s) and male consensual partner within 5 days of kit collection

SAEK Inventory
 1. Label each sub-item in the SAEK.
 2. Create each sub-item in FA.
 3. Add “Not Analyzed” items to the serology worksheet (including cervical swabs and slide).

Microscopic Exam for Sperm
 Time frames for analysis, per NIJ and SWGDAM recommendations, will be followed for smear slide examination (for DPD kits and kits where slides weren’t prepared at the hospital, slides will be created in serology for samples within these time frames):

Sample	Days (and Hours) Since Alleged Assault		
	1 (0-23:59)	2-3 (24-71:59)	4-5+ (72-120+)
Oral	Yes	No	No
Anal	Yes	Yes	No
Vaginal	Yes	Yes	Yes

Note: The analyst may use his or her discretion in analyzing probative samples in cases exceeding these time frames. Slides may be prepared and examined in lieu of/in addition to smear slides if there are potential quality issues with the smear slides present in the kit.

Sample Preparation for DNA

- Internal samples (oral, anal, and vaginal):
 - If the corresponding slide is positive, half of the total number of swabs (up to 2 swabs) will be cut into a differential extraction tube.
 - If the corresponding slide is negative, half of the total number of swabs (up to 2 swabs) will be cut into a PrepFiler column/tube assembly.
 - Speculum swabs are considered additional collections from the vaginal cavity. If case circumstances dictate (e.g. multiple assailants alleged or no vaginal swabs present in the kit), the speculum swabs will be analyzed according to the vaginal slide results.
- Using the information provided about the time elapsed between the alleged assault and kit collection as well as the location of sample collection, analysts will select external swabs for analysis. Half of the total number of swabs (up to 2 swabs) from each external sample selected will be cut into a PrepFiler column/tube assembly.
 - If multiple external genitalia collections are present, one will typically be selected for analysis.
 - External samples may be suitable for analysis if collected within 5 days (up to 119:59 hours).
- Additional items contained within the kit may be subsequently processed if warranted (and if proper consumptive permissions are obtained) for applicable samples, including pubic hair combings, fingernail collections, tampons, and drapes.

Extraction Microscope slides are typically not prepared during differential extraction.

Quantitation Each extract will be assessed for the presence or absence of human DNA. In this scenario, quant data does not otherwise inform amplification decisions.

Amplification and Typing
 1. Samples lacking a male quant will be amplified to determine if foreign female DNA is present.
 2. Non-sperm fractions are considered probative and must be amplified.

Serology Conduct saliva, and/or blood testing on remaining swab(s) from samples *with foreign DNA detected, when necessary/requested*. SALigAE® testing will only be conducted as an overlay, unless proper consumptive permission is obtained.



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SCENARIO: female victim SAEK processing with alleged female assailant(s) and no male consensual partner

SAEK Inventory
 1. Label each sub-item in the SAEK.
 2. Create each sub-item in FA.
 3. Add “Not Analyzed” items to the serology worksheet (including cervical swabs and all slides).

Microscopic Exam for Sperm
 No microscopic examinations for sperm will be conducted on smear slides, if present. Microscope slides will not be prepared for DPD kits or kits where slides weren’t prepared at the hospital.

Sample Preparation for DNA
 1. Internal samples (oral, anal, and vaginal):
 a. Typical time frames for analysis:

Sample	Days (and Hours) Since Alleged Assault	
	1 (0-23:59)	2-5 (24-120+)
Oral	Yes	No
Anal	Yes	No
Vaginal	Yes	Yes

Note: The analyst may use his or her discretion in analyzing probative samples in cases exceeding these time frames.
 b. Half of the total number of swabs (up to 2 swabs) will be cut into a PrepFiler column/tube assembly.
 c. Speculum swabs are considered additional collections from the vaginal cavity. If case circumstances dictate (e.g. multiple assailants alleged or no vaginal swabs present in the kit), the speculum swabs will be analyzed.
 2. Using the information provided about the time elapsed between the alleged assault and kit collection as well as the location of sample collection, analysts will select external swabs for analysis. Half of the total number of swabs (up to 2 swabs) from each external sample selected will be cut into a PrepFiler column/tube assembly.
 a. If multiple external genitalia collections are present, one will typically be selected for analysis.
 b. External samples may be suitable for analysis if collected within 5 days (up to 119:59 hours).
 3. Additional items contained within the kit may be subsequently processed if warranted (and if proper consumptive permissions are obtained) for applicable samples, including pubic hair combings, fingernail collections, tampons, and drapes.

Extraction
 Differential extractions are not performed in this scenario.

Quantitation
 Each extract will be assessed for the presence or absence of human DNA. In this scenario, quant data does not otherwise inform amplification decisions.

Amplification and Typing
 Samples will be amplified to determine if foreign female DNA is present.

Serology
 Conduct saliva, and/or blood testing on remaining swab(s) from samples *with foreign DNA detected, when necessary/requested*. SALIgAE® testing will only be conducted as an overlay, unless proper consumptive permission is obtained.



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SCENARIOS: male victim SAEK processing with alleged male assailant(s) OR male victim SAEK processing with unknown sex assailant(s)

- SAEK Inventory**
1. Label each sub-item in the SAEK.
 2. Create each sub-item in FA.
 3. Add “Not Analyzed” items to the serology worksheet.

Microscopic Exam for Sperm Time frames for analysis, per NIJ and SWGDAM recommendations, will be followed for smear slide examination (for DPD kits and kits where slides weren’t prepared at the hospital, slides will be created in serology for samples within these time frames):

Sample	Days (and Hours) Since Alleged Assault		
	1 (0-23:59)	2-3 (24-71:59)	4-5+ (72-120+)
Oral	Yes	No	No
Anal	Yes	Yes	No

Note: The analyst may use his or her discretion in analyzing probative samples in cases exceeding these time frames. Slides may be prepared and examined in lieu of/in addition to smear slides if there are potential quality issues with the smear slides present in the kit. Slides may also be prepared from external samples and analyzed at the analyst’s discretion.

*If the victim is prepubescent (≤7 years old), a differential extraction is not necessary on his own penile swabs.

- Sample Preparation for DNA**
1. Intimate samples (oral, anal, and penile/scrotum):
 - a. For oral and anal swabs, if the corresponding slide is positive, half of the total number of swabs (up to 2 swabs) will be cut into a differential extraction tube.

Note: If one or more slide is positive and the case has no complicating factors (consensual partner or multiple assailants), the analyst may select 1-2 samples for differential extraction based on case scenario and proceed to extraction.
 - b. For oral and anal swabs, if the corresponding slide is negative, half of the total number of swabs (up to 2 swabs) will be cut into a PrepFiler column/tube assembly.
 - c. For penile/scrotum swabs, half of the total number of swabs (up to 2 swabs) will be cut into a differential extraction tube, unless the victim is prepubescent (≤7 years old).
 2. Using the information provided about the time elapsed between the alleged assault and kit collection as well as the location of sample collection, analysts will select external swabs for analysis. Half of the total number of swabs (up to 2 swabs) from each external sample selected will be cut into a PrepFiler column/tube assembly. External samples may be suitable for analysis if collected within 5 days (up to 119:59 hours).

Note: If case circumstances/allegations indicate that an external ejaculation occurred, the analyst may choose to perform a differential extraction on the applicable external samples. The analyst may prepare a slide for a sperm search during the differential extraction process.
 3. Additional items contained within the kit may be subsequently processed if warranted (and if proper consumptive permissions are obtained) for applicable samples, including pubic hair combings, fingernail collections, tampons, and drapes.

Extraction Microscope slides are typically not prepared during differential extraction.

Quantitation Each extract will be assessed for the presence or absence of human DNA. In this scenario, quant data does not otherwise inform amplification decisions.

- Amplification and Typing**
1. Samples (including sperm and non-sperm fractions) will be amplified to determine if foreign DNA is present.
 2. Samples may be subsequently amplified with YFP if appropriate references are present and unsuitable GF results are obtained.

- Serology** Conduct semen, saliva, and/or blood testing on remaining swab(s) from samples *with foreign DNA detected, when necessary/requested*. Use the overlay method, if possible/applicable, to prevent unnecessary sample consumption.
1. AP can be conducted on internal samples if the corresponding microscopic examination is negative.
 2. p30 testing will only be conducted on internal samples if the corresponding microscopic examination is negative and proper consumptive permission is obtained.
 3. Slides will only be prepared from external samples after DNA analysis if proper consumptive permission is obtained.
 4. SAlIgAE® testing will only be conducted as an overlay, unless proper consumptive permission is obtained.



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SCENARIOS: male victim SAEK processing with alleged female assailant(s) OR female victim with male suspect kit processing

SAEK Inventory 1. Label each sub-item in the SAEK.
2. Create each sub-item in FA.
3. Add “Not Analyzed” items to the serology worksheet (including all slides).

Microscopic Exam for Sperm No microscopic examinations for sperm will be conducted on smear slides, if present. Microscope slides will not be prepared for DPD kits or kits where slides weren’t prepared at the hospital.

Sample Preparation for DNA 1. Intimate samples (oral, anal, and penile/scrotum):
a. Typical time frames for analysis:

Sample	Days (and Hours) Since Alleged Assault	
	1 (0-23:59)	2-5 (24-120+)
Oral	Yes	No
Anal	Yes	No

Note: The analyst may use his or her discretion in analyzing probative samples in cases exceeding these time frames.

- b. For oral and anal swabs, half of the total number of swabs (up to 2 swabs) will be cut into a PrepFiler column/tube assembly.
 - c. For penile/scrotum swabs, half of the total number of swabs (up to 2 swabs) will be cut into a differential extraction tube, unless the victim is prepubescent (≤7 years old).
2. Using the information provided about the time elapsed between the alleged assault and kit collection as well as the location of sample collection, analysts will select external swabs for analysis. Half of the total number of swabs (up to 2 swabs) from each external sample selected will be cut into a PrepFiler column/tube assembly. External samples may be suitable for analysis if collected within 5 days (up to 119:59 hours).
3. Additional items contained within the kit may be subsequently processed if warranted (and if proper consumptive permissions are obtained) for applicable samples, including pubic hair combings, fingernail collections, tampons, and drapes.

Extraction Microscope slides will not be prepared during differential extraction.

Quantitation Each extract will be assessed for the presence or absence of human DNA. In this scenario, quant data does not otherwise inform amplification decisions.

Amplification and Typing Samples (including sperm and non-sperm fractions) will be amplified to determine if foreign DNA is present.

Serology Conduct semen, saliva, and/or blood testing on remaining swab(s) from samples *with foreign DNA detected, when necessary/requested*. SALigAE® testing will only be conducted as an overlay, unless proper consumptive permission is obtained.