Purchasing, Preparation, and Records for DNA Reagents

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Purchasing, Preparation, and Records for DNA Reagents

1 INTRODUCTION

This procedure supplements the FBI Laboratory requirements for the purchasing and receipt, preparation, and labeling of laboratory reagents and consumables used in the DNA discipline.

2 SCOPE

This document applies to DNA personnel responsible for the purchasing and receipt, preparation, and labeling of laboratory reagents and consumables used for the serological and/or DNA analysis of forensic evidence and/or databasing samples in the DNA discipline.

3 PROCEDURES

A. The DNA units comply with the FBI Laboratory level 1 documents (i.e., LAB-100 and LAB-200) and the *Quality Assurance Standards* (QAS) *for Forensic DNA Testing Laboratories* and *for DNA Databasing Laboratories* with regard to the quality control (QC) of reagents.
B. Reagent records will be maintained, generally via the DNA units' applicable Sample Tracking and Control Software (STACS).
C. Refer to DNA Procedures Introduction (i.e., BIO-100) for additional laboratory quality assurance, cleaning, and reagent storage instructions.

3.1 Commercial Reagent Records

A. Purchase requests will be prepared by appropriate personnel for all commercial reagents and consumables. These requests will describe the types of supplies and/or services requested and may be maintained in a written or electronic format. The appropriate manager will approve purchase requests prior to ordering.
B. Various companies may supply one or more chemicals, reagents, or DNA analysis kits used in the testing of forensic evidence and/or database samples. Final selection of suppliers will be in accordance with Federal Procurement Regulations - Simplified Acquisition Procedures.
    1. Suppliers of critical reagents are typically evaluated during validation, but the evaluation may be based on previous purchasing history and/or the results of QC testing.
    2. When a vendor, reagent specification (e.g., concentration), or consumable specification (e.g., Vivicon filter molecular weight cut-off) affects the laboratory activities, the pertinent information will be listed in the appropriate DNA procedure.
    3. Current supplier and purchasing information for reagents and consumables is generally maintained in STACS.
C. DNA personnel will ensure that quality affecting supplies, reagents, and consumables comply with specifications defined in the appropriate technical procedure and/or the purchase request. Any discrepancies will be brought to the attention of the personnel responsible for ordering DNA supplies, reagents, and

consumables. Quality affecting supplies, reagents, and consumables that conform to the expected specifications will be received into STACS.

D. The following information will be recorded for the receipt of commercial reagents and kits:
    - Name of material
    - Manufacturer lot number
    - Date received
    - Expiration date, when provided

E. Commercial reagents must be labeled with the identity of the reagent and will be directly labeled and/or use a barcode system to electronically track the expiration date.
    1. Commercial reagents are generally prepared and stored as recommended by the manufacturer. Additional guidance may be found in STACS or the appropriate DNA procedure.
    2. The expiration date of commercial reagents is determined by the manufacturer or utilizing the respective Safety Data Sheets.
        i. If no expiration date is provided by or recommended by the manufacturer, generally a 10-year expiration date will be assigned.
        ii. If the expiration date provided by the manufacturer only specifies a month and year, the recorded expiration date will be the last day of the month specified. The commercial reagent container will then be labeled with the newly established expiration date.
        iii. If an expiration date beyond that provided by the manufacturer is assigned, records to establish the extension of the expiration date will be maintained.
        iv. If an expiration date is exceeded, the QC procedure or use of the reagent on a known sample (e.g., positive control) may be used to demonstrate continued efficacy beyond the assigned expiration date. Records demonstrating the continued efficacy of the reagent will be maintained.

F. If a non-reagent consumable (e.g., swabs, tubes, microcons, Edge strips) has an expiration date assigned by the manufacturer, that expiration date is not applicable to DNA units' operations and may be extended as deemed appropriate by the Technical Leader (TL). The new expiration date will be recorded in STACS.

G. Water must be of suitable purity so that it does not interfere with the specificity, accuracy, and precision of the procedure.
    1. For water that will contact DNA samples or be used to make reagents for DNA testing, the water should be nuclease free. This is typically referred to as reagent grade, molecular grade, or nuclease free in the procedures and notes.
    2. The purified water available via faucets at the laboratory sinks is used for Tecan operation or general laboratory uses that do not involve processing of samples for DNA.

3.2 Laboratory Prepared Reagent Records

A. Laboratory prepared reagents will be made in accordance with the information contained within STACS and/or the relevant DNA procedure(s). A list of reagent recipes and control preparation guidelines generally used in the DNA units are listed in Appendix A for reference.

B. Reagent preparation will be recorded, generally within STACS, to include:
   - Name of reagent
   - Date prepared
   - Lot number (e.g., barcode, batch identifier) assigned to the reagent
   - Lot number (e.g., barcode, batch identifier) of each component
   - Identity of the preparer
   - Identity of the individual performing the QC check (if applicable)
   - Lot/batch # of the QC controls (if applicable)
   - QC results (pass/fail) (if applicable)

   1. The information recorded for reagent preparation and use must be sufficient to provide a documented audit trail.
   2. Unless otherwise specified, reagents that are made internally will expire one year from the date prepared.
      i. If an expiration date is extended, records demonstrating the continued efficacy of the reagent will be maintained.
      ii. If an expiration date is exceeded, the use of the reagent on a known sample may be used to demonstrate continued efficacy beyond the assigned expiration date. Records demonstrating the continued efficacy of the reagent will be maintained.

C. Laboratory prepared reagents will be labeled with the identity of the reagent and will be directly labeled and/or use a barcode system to electronically track the following:
   - Preparer's identity
   - Date prepared
   - Expiration date
   - Lot number (e.g., barcode, batch identifier)

   1. Reagents stored in limited use quantities (i.e., single use aliquots) may be in a storage container (e.g., bag, box) labeled with the identity of the reagent with the associated barcode(s) readily available.
   2. Once dispensed for use (e.g., on a robotic workstation, on another instrument), the trough or tube will be labeled with the identity of the contained reagent with the necessary barcode available for scanning.

D. Laboratory prepared reagents will be stored at an appropriate temperature to prevent degradation or deactivation of the active ingredients. Recommended storage conditions may be listed in STACS and/or Appendix A.

E. Laboratory prepared reagents will be tested for reliability prior to or concurrent with use in casework examinations or DNA databasing. QC procedures for reliability testing may be contained in the appropriate DNA procedure or in STACS or reliability

testing may be accomplished by testing appropriate positive and/or negative controls. Dilutions of a stock commercial reagent (e.g., 1X CE buffer) will not require reliability testing. Multiple reagents may be simultaneously tested for reliability.

3.3 Critical Reagents

A. The following are identified as QAS critical reagents and will be evaluated prior to use in casework or for database sample processing:
   - Nuclear DNA Quantification Kits (i.e., Quantifiler TRIO)
   - Mitochondrial DNA Quantitative PCR (qPCR) system:
     - Double stranded synthetic standard (dsT8sig)
     - TaqMan® Fast Advanced Master Mix
     - Amplification primers (Qfor8, Qrev8, L, M, G, B)
     - Probes (QRL8 [FAM], C [VIC], and U [NED])
     - Double stranded Internal Positive Control DNA (C/E)
   - Mitochondrial DNA Quantification Kits (i.e., PowerSeq Quant MS System)
   - STR Amplification Kits (i.e., Globalfiler, Globalfiler Express)
   - Y-STR Amplification Kits (i.e., Y23)
   - Mitochondrial DNA amplification and sequencing Kits (i.e., PowerSeq CRM Nested System, Verogen MiSeq FGx Reagent kit)
   - Amplification and Sequencing components (if not within a test kit or system):
     - Thermostable DNA polymerase (i.e., AmpliTaq Gold)
     - Primers
     - Allelic Ladders
   - Rapid DNA Cartridge/Chip

B. The QC procedures used to ensure the reliability of critical reagents are contained in the appropriate DNA procedure or in STACS.

C. The results of the QC testing, as well as the reagent's acceptance or rejection for use, will be recorded. The reagent will be available for use once the necessary acceptance is recorded, generally in STACS.

4 SAFETY

A. Refer to the FBI Laboratory Safety Manual for information on personal protection, the proper disposal of the chemicals used in these procedures, as well as the biohazardous wastes generated.

B. Refer to the DNA Procedures Introduction (i.e., BIO-100) and/or the relevant DNA procedure for additional applicable safety information.

5 RECORDS

Records pertaining to the receipt, preparation, and/or QC of laboratory reagents will be kept in the STACS or an equivalent log or storage location. These records will be maintained by the DSU QA/QC Group and/or in the applicable DNA Unit. Records for commercial orders will be maintained by appropriate personnel.

6 REVISION HISTORY

Revision	Issued	Changes
00	09/15/2022	Reformatted DNA 609-9 into new template and assigned new Doc ID. Minor edits throughout. Deleted obsolete reagent recipes.
01	04/01/2024	Added or recommended when no expiration date provided by manufacturer. Revised 3% Bleach preparation to at least weekly. Removed Takyama reagents from Appendix A.
02	12/16/2024	Removed Yfiler, 3130, and mito Sanger Sequencing information. Added NGS reagents. Revised known semen slide preparation. Changed bleach for cleaning evidence to molecular grade. Added SOP references to App A.

7 APPENDIX A: REAGENT RECIPES AND CONTROL PREPARATION

7.1 Reagent Preparation Guidance:

A. Final volumes may be adjusted by proportionally adjusting the components.
    1. Listed storage volumes are based on operational use but may be adjusted as needed.
B. Graduated cylinders and/or pipettes closest in capacity to the volume of liquid being measured should be used.
C. If the pH meter is used, the performance will be verified prior to use.
D. Any reagent in which microbial growth is observed must be discarded.
E. Store all reagents in sterile containers unless otherwise noted.
F. When available, purchased ready to use reagents of equivalent or higher quality may be substituted for the reagents listed below.
G. Relevant Level 2 Documents listed below may not be all inclusive.

7.1.1 Acid Phosphatase Spot Test Solution, 100 mL

Refer to BIO-420 for QC information.

- Add 2.6 g Acid Phosphatase Spot Test powder to 100 mL reagent grade water and stir until dissolved.
- Store frozen for up to one month.

7.1.2 3% Bleach Solution, 100 mL (for TECAN use)

- Dilute 3 mL molecular grade bleach (or equivalent) to 100 mL with reagent grade water.
- Store at room temperature.
- Prepare at least weekly.

7.1.3 10% Bleach Solution, 50 mL (for use on evidentiary items)

- Dilute 5 mL molecular grade bleach (or equivalent) to 50 mL with reagent grade water.
- Store at room temperature.
- Prepare daily.

7.1.4 10% Bleach Solution, 10 L (for cleaning purposes)

- Dilute 1 L household bleach (or equivalent) to 10 L with water.
- Store at room temperature.
- Prepare at least weekly.

7.1.5 Buffer EB + 0.1% Tween 20

As used in BIO-552. Buffer EB may be substituted with 10mM Tris-HCl pH 8.5.

- Add 5 µL of Tween 20 to a 50 mL conical tube

- Bring volume up to 50 mL with Buffer EB
- Store at room temperature

7.1.6 Demineralization/Extraction Buffer, 1L

As used in BIO-511.

- Add 900 mL of 0.5M EDTA solution to a container and stir on medium.
- While stirring, add 10g of N-Lauroylsarcosine sodium salt.
- Allow mixture to go into solution.
- Adjust to pH 8.0 with hydrochloric acid (HCl) or Sodium Hydroxide (NaOH).
- Bring final volume to 1 L with 0.5M EDTA solution.
- Store at room temperature.

7.1.7 1M DTT (Dithiothreitol), 10 mL

As used in BIO-510 and BIO-512.

- Dissolve 1.54 g of DTT in 10 mL of reagent grade water.
- Store frozen.

7.1.8 5M DTT (Dithiothreitol), 2 mL

As used in BIO-513.

- Dissolve 1.54 g of DTT in 2 mL of reagent grade water.
- Store frozen.
-

7.1.9 3% Hydrogen Peroxide Solution, 1 L

Refer to BIO-410 for QC information.

- Dilute 100 mL of 30% hydrogen peroxide solution to 1 L with reagent grade water.
- Store refrigerated.

7.1.10 mtDNA Pre-Amplification Quantitative PCR (qPCR) System

Refer to BIO-521 for QC information.

7.1.10.1 mtDNA qPCR Double Stranded Internal Positive Control (IPC) DNA

- Reconstitute forward (C) and reverse (E) oligonucleotides in TE^{-4} buffer.
- Prepare 100 µM solutions of the forward and reverse oligonucleotides using information from vendor certificate of analysis.
- Combine these in equal volumes and tightly cap, mix, and quick spin the tube.
- This generates the **primary (1°) stock** of the double-stranded IPC DNA at 50 µM (3 x 10^{13} copies/µL). Store frozen.
- Prepare a dilution series using TE^{-4} as follows:
    - 2° stock: Transfer 10 µL of primary stock into 1,542 µL TE^{-4} (1.94 x 10^{11} copies/µL).

- 3° stock: Transfer 10 µL of 2° stock into 1,542 µL TE^{-4} (1.25×10^9 copies/µL).
- 4° stock: Transfer 10 µL of 3° stock into 990 µL TE^{-4} (1.25×10^7 copies/µL).
- 5° stock: Transfer 10 µL of 4° stock into 990 µL TE^{-4} (1.25×10^5 copies/µL).
- 6° stock (working dilution): Transfer 10 µL of 5° stock into 990 µL TE^{-4} 1.25×10^3 copies/µL).

- Store Frozen

7.1.10.2 <u>mtDNA qPCR Double Stranded Synthetic Standard (dsT8sig)</u>

- Reconstitute Tfor8sig and Trev8sig oligonucleotides in TE^{-4} buffer.
- Prepare 2 µM solutions of the forward and reverse oligonucleotides based on their respective molecular weights of 34,960.7 g/mol and 35,969.3 g/mol.
- Combine these in equal volumes and tightly cap, mix, and quick spin the tube.
- This generates the **primary (1°) stock** of the double-stranded **dsT8sig** standard at 1 µM (6.023×10^{11} copies/µL).
- Store frozen.

- Prepare the **secondary (2°) stock** of **dsT8sig** from the **1° stock** by adding 1194.6 µL TE^{-4} buffer to a 10 µL aliquot of the primary (1°) stock. Tightly cap, mix, and quick spin the tube. The **2° stock of dsT8sig** should be at a final concentration of 5×10^9 copies/µL.
- Prepare 10 µL aliquots of the **2° stock** and store frozen.

- Prepare the mtDNA Quantitiative PCR Standard Dilution Series by adding 990 µL TE^{-4} buffer to a 10 µL aliquot of the **2° stock.** This can be stored refrigerated and used for up to two months.
  - Tube A: Transfer 20 µL from diluted **2° stock** and add 80 µL TE^{-4} buffer.
  - Tube B: Transfer 10 µL from tube A and add 90 µL TE^{-4} buffer.
  - Tubes C-G: Transfer 10 µL from previous tube and add 90 µL TE^{-4} buffer.

7.1.10.3 <u>mtDNA qPCR Primers</u>

The mtDNA qPCR Primers are Forward [Qfor8, L, G] and Reverse [Qrev8, M, B].

- Reconstitute all primers in TE^{-4} buffer.
- Prepare 100 µM stock solutions of each primer using information from vendor certificate of analysis.
- Store frozen.

- Prepare working dilutions of each primer in TE^{-4} buffer as follows:
  - For Qfor8 and G (1.25 µM) transfer 12.5 µL of 100 µM stock solution to a new tube and add 987.5 µL of TE^{-4}.
  - For Qrev8 and M (22.5 µM) transfer 225 µL of 100 µM stock solution to a new tube and add 775 µL of TE^{-4}.
  - For L and B (7.5 µM) transfer 75 µL of 100 µM stock solution to a new tube and add 925 µL of TE^{-4}.
- Store frozen.

7.1.10.4 mtDNA qPCR Probes

The mtDNA qPCR Probes are QRL8 [FAM], C [VIC], and U [NED].

- Prepare a 6.25 µM working dilution from each 100 µM probe stock.
    - Transfer 62.5 µL of 100 µM stock solution to a new tube and add 937.5 µL of TE^{-4}.
- Store frozen and protected from light as much as possible.

7.1.10.5 mtDNA qPCR Primer/Probe/IPC Mix (PPI Mix)

- Prepare working dilutions of all primers, probes, and IPC DNA.
- Add 80 µL of all primers, probes, and IPC DNA into each tube. Vortex, pulse spin.
- Store frozen.

7.1.11 Phenolphthalin Solution, 1 L

Refer to BIO-410 for QC information.

- Combine 4 g phenolphthalin, 40 g NaOH, and 200 mL reagent grade water.
- Add 800 mL of ethanol and mix.
- Store refrigerated in an amber bottle over zinc (generally enough to cover the bottom of the bottle).
- This solution may be used for up to 3 months.

7.1.12 PowerSeq CRM Nested System Index Plate

Refer to BIO-531 for QC information.

- Add 6 µl of the appropriate indexes to each well as depicted below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	D701 D501 Vol: 12	D702 D501 Vol: 12	D703 D501 Vol: 12	D704 D501 Vol: 12	D705 D501 Vol: 12	D706 D501 Vol: 12	D707 D501 Vol: 12	D708 D501 Vol: 12	D709 D501 Vol: 12	D710 D501 Vol: 12	D711 D501 Vol: 12	D712 D501 Vol: 12
B	D701 D502 Vol: 12	D702 D502 Vol: 12	D703 D502 Vol: 12	D704 D502 Vol: 12	D705 D502 Vol: 12	D706 D502 Vol: 12	D707 D502 Vol: 12	D708 D502 Vol: 12	D709 D502 Vol: 12	D710 D502 Vol: 12	D711 D502 Vol: 12	D712 D502 Vol: 12
C	D701 D503 Vol: 12	D702 D503 Vol: 12	D703 D503 Vol: 12	D704 D503 Vol: 12	D705 D503 Vol: 12	D706 D503 Vol: 12	D707 D503 Vol: 12	D708 D503 Vol: 12	D709 D503 Vol: 12	D710 D503 Vol: 12	D711 D503 Vol: 12	D712 D503 Vol: 12
D	D701 D504 Vol: 12	D702 D504 Vol: 12	D703 D504 Vol: 12	D704 D504 Vol: 12	D705 D504 Vol: 12	D706 D504 Vol: 12	D707 D504 Vol: 12	D708 D504 Vol: 12	D709 D504 Vol: 12	D710 D504 Vol: 12	D711 D504 Vol: 12	D712 D504 Vol: 12
E	D701 D505 Vol: 12	D702 D505 Vol: 12	D703 D505 Vol: 12	D704 D505 Vol: 12	D705 D505 Vol: 12	D706 D505 Vol: 12	D707 D505 Vol: 12	D708 D505 Vol: 12	D709 D505 Vol: 12	D710 D505 Vol: 12	D711 D505 Vol: 12	D712 D505 Vol: 12
F	D701 D506 Vol: 12	D702 D506 Vol: 12	D703 D506 Vol: 12	D704 D506 Vol: 12	D705 D506 Vol: 12	D706 D506 Vol: 12	D707 D506 Vol: 12	D708 D506 Vol: 12	D709 D506 Vol: 12	D710 D506 Vol: 12	D711 D506 Vol: 12	D712 D506 Vol: 12
G	D701 D507 Vol: 12	D702 D507 Vol: 12	D703 D507 Vol: 12	D704 D507 Vol: 12	D705 D507 Vol: 12	D706 D507 Vol: 12	D707 D507 Vol: 12	D708 D507 Vol: 12	D709 D507 Vol: 12	D710 D507 Vol: 12	D711 D507 Vol: 12	D712 D507 Vol: 12
H	D701 D508 Vol: 12	D702 D508 Vol: 12	D703 D508 Vol: 12	D704 D508 Vol: 12	D705 D508 Vol: 12	D706 D508 Vol: 12	D707 D508 Vol: 12	D708 D508 Vol: 12	D709 D508 Vol: 12	D710 D508 Vol: 12	D711 D508 Vol: 12	D712 D508 Vol: 12

7.1.13 Proteinase K, 360 µg/ml

As used in BIO-541.

- In a 15 mL conical tube add:
  - 5 ml of 1M Tris-HCl (pH 8.0)
  - 3.82 mL of molecular grade water
  - 1 mL of 1M $CaCl_2$
  - 180 µl of 20 mg/mL Proteinase K
- Prepare 200 µl aliquots in 1.5 mL tubes
- Store frozen

7.1.14 Quantifiler® TRIO DNA Calibrators

The calibrator samples will be prepared from the Quantifiler® Trio Standard Stock (~100 ng/µl). Refer to BIO-520 for QC information.

- Prepare a 1:10 dilution (~10 ng/µl):
  - Add 30 µl of the Quantifiler® Trio Standard Stock to 270 µl of TE^{-4}.
- Prepare a 1:50 dilution (~2 ng/µl):
  - Add 6 µl of the Quantifiler® Trio Standard Stock to 294 µl of TE^{-4}.
- Store frozen, until thawed, then may be stored refrigerated and used for up to two months.

7.1.15 Stain Extraction Buffer (SEB), 1 L

SEB is 10 mM Tris-HCl / 100 mM NaCl / 10 mM EDTA / 2% SDS, pH 8.0

- Dissolve 5.84 g NaCl in approximately 500 ml reagent grade water.
- Add 10 mL 1M Tris-HCl.
- Add 20 ml 0.5M EDTA.
- Add 100 mL 20% SDS.
- Adjust to pH 8.0 with HCl.
- Adjust the final volume to 1 L with reagent grade water.
- Store at room temperature.

7.1.16 SEB with Dithiothrietol (SEB w/DTT), 5 mL

As used in BIO-512. SEB w/DTT is 10 mM Tris-HCl / 100 mM NaCl / 10 mM EDTA / 2% SDS / 39mM DTT

- Add 30 mg of DTT to 5 mL of SEB and stir until dissolved.
- Store at room temperature for up to 1 month.

7.1.17 200mM Tris-HCl pH 7.0

As used in BIO-552.

- Add 10 mL 1M Tris-HCl pH 7.0 to 40 mL of molecular grade water

7.1.18 *Tween Wash Solution, 0.5%*

As used in BIO-552.

- Prepare a 10% Tween solution:
  - Add 5ml of 100% Tween 20 to 45 mL of 18 MegaOhm water.
- Prepare a 0.5% Tween solution:
  - Add 475 mL of 18 MegaOhm and 25 mL of 10% Tween solution to 1 liter bottle.
- Store refrigerated for up to one week.

7.2 Control Preparation Guidance:

7.2.1 Blood/Buccal Internal Standard (BIS)

An individual providing the BIS control must have a previously characterized and documented STR profile. Refer to BIO-301 and BIO-305 for use information.

- A blood BIS control is an FTA bloodstain card prepared with liquid blood or blood collected via a finger stick.
- A buccal BIS control is the FTA card from a Whatman EasiCollect™ device which is used to collect and transfer a buccal sample to the card, the collector from a Bode Collector device used for a buccal collection, or an equivalent card collected using an approved collection device.

7.2.2 Blood Known Positive Swab

Refer to BIO-410 for use information.

- Prepare a 1:20 dilution of whole blood.
- Add approximately 50uL of diluted blood to each clean swab.
- Allow the swabs to dry completely.
- Store refrigerated or at room temperature.

7.2.3 mtDNA Positive Control (2800M)

Refer to BIO-521 and BIO-532 for use information.

- Quant 2800M stock and a 1:50 dilution with the mtDNA pre-amplification qPCR assay [Refer to BIO-521].
- Prepare a 2000 cn/uL dilution with TE^{-4}:

$$[2000\ cn/uL \times 1000\ uL]/[Quant\ of\ Stock] = \text{Stock (uL) to add to make 1mL final volume}$$

- Prepared dilution may be quanted to confirm the concentration.
- Prepare aliquots in 1.5 mL tubes:
  - 50 µL for quant
  - 300 µL for amp
- Store frozen.

7.2.4 *20 pM PhiX control*

Refer to BIO-552 for use information.

- Combine:
    - 2 µL of PhiX control stock (10 nM),
    - 3 µL of Qiagen Buffer EB + 0.1% Tween, and
    - 5 µL of 0.2N NaOH.
- Incubate at room temperature for 5 minutes.
- Add 990 µl of chilled HT1 buffer.
- Store frozen for up to two weeks.

7.2.5 Semen Known Positive Slide

Semen slide preparation may vary based on the concentration of sperm in the available semen sample. Refer to BIO-421 for use information.

- Dip a swab into a semen sample and cut the swab into a tube for differential extraction. (Prepare ~4 tubes for a batch of slides.)
- Add female fraction master mix [Refer to BIO-510] and process on QiaCube with the 12A and 12B protocols.
- Remove tubes from QiaCube and combine the M fraction tubes.
- Pipette 4 µL of the M fraction onto a slide.
    - This prepared slide will be stained and viewed in accordance with BIO-421 to confirm the presence of semen cells.
    - The M fraction sample can be diluted, if necessary, to reduce cell clumping.
- Pipette 4 µL of the M fraction onto remaining slides in the batch.
    - The M fraction sample may be stored frozen for future use. If additional slides are made later, one slide must be stained and viewed in accordance with BIO-421 to ensure the presence of sperm cells in the new batch of slides.
- Allow slides to air dry (generally for 10 minutes).
- Store slides at room temperature.

7.2.6 Semen Known Positive Swab Preparation

Refer to BIO-420 for use information.

- Add 1.5 mL of human semen to 3.0 mL of water.
- Add approximately 100 µL of diluted semen to each swab.
- Allow the swabs to dry completely.
- Store frozen.