Differential Extraction from Semen Stains or Swabs utilizing the QIAcube and EZ1

Sample size for the extraction should be approximately 1/3 of a swab or a 3x3 mm cutting of a stain. This extraction is applicable for casework samples suspected of containing semen.

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1 LIMS Pre-Processing

1.1 In the **Analytical Testing » Test Batches** tram stop, select the appropriate extraction assay and Click **Edit**

Note: If you are creating a new extraction test batch use the **New Test Batch** tram stop followed by the create new test batch wizard. In that wizard include the following information: description, functional group, analysis, batch configuration, and test batch type (case test batch).

1.2 If necessary, click **Add Unknowns** and select any samples that need to be included on the test batch. Controls are present in the batch configuration. If additional controls are needed, click **Add QC Samples**

1.3 Select All Input Samples » click Add Output Sample » 🔍 » Auto Diff » click Select and Return » click Ok» click Create

1.4 Review the batch setup and complete the Batch Set Up Review performed by tab.

2 Tube setup

2.1 Obtain labeled cuttings in 1.5 mL Eppendorf tubes. Compare your sample labels and tube tops to the input sample list in LIMS and confirm that you have the correct samples.

2.2 Fill out the performed by tab for Incubation. This will add the date and time to the extraction negatives for the batch.

2.3 Obtain two 1.5 mL Eppendorf tubes for your extraction negatives, and label them with the associated **input** LIMS labels and tube tops labels.

2.4 Retrieve reagents for epithelial cell digestion and record the lot numbers in LIMS. Select the ATL buffer aliquot and QiaProK reagents and click “Calculate amount.” Use the volumes listed in the “Needed Amount” column to prepare the master mix (LIMS will calculate 1.2 x the number of samples). The amount of each reagent for one sample is listed below.

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Epithelial Cell Digestion master mix (per sample)		
Diluted ATL (µl)	Proteinase K (Qiagen) (µl)	Total
480	20	500

2.5 Add 500µl master mix to each of the samples and negative controls.

2.6 Incubate at 56°C for 1.5 hours (and no longer than 1.75 hours) at 900rpm in a thermomixer. Record the thermomixer temperature and usage log for the instrument in LIMS.

NOTE: The "epithelial cell" fraction will be denoted as F1 and the "sperm cell" fraction will be denoted as F2

2.7 Prepare your sample tubes:

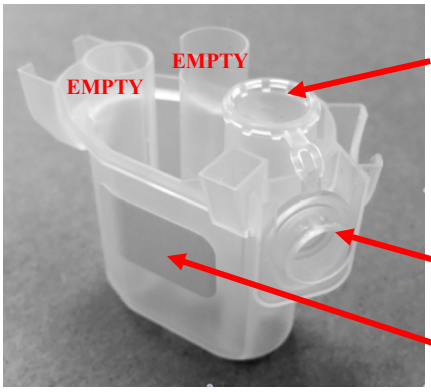
2.7.1 Print your labels. For the entire protocol you will need:

- One copy of the **input** sample labels
- Two copies of the **output F1** sample labels
- Three copies of the **output F2**sample labels

2.7.2 Obtain your tubes and label them:

- Spin baskets and 1.5 mL collection tubes – **input** labels
- Rotor adapters – **output F2** labels
- 1.5 mL Qiagen snap cap tubes – **output F2**labels
- 2.0 mL Qiagen screw cap tubes – **output F1**labels (place the label below the ridges on the side of the tube)

2.7.3 The 1.5 mL Qiagen snap cap tubes with the F2 labels will go inside of the rotor adapters. Bend the cap of each tube back and insert it into the cap holder in position L3 (see diagram below).



Rotor

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3 QIAcube setup and Sample Preparation:

3.1 Fill out the instrument usage log for the QIAcube in LIMS. Fill out the performed by tab for Separation and Lysis.

3.2 Check that the waste drawer is empty, and the liner is clean (can be wiped with 70% ethanol if needed).

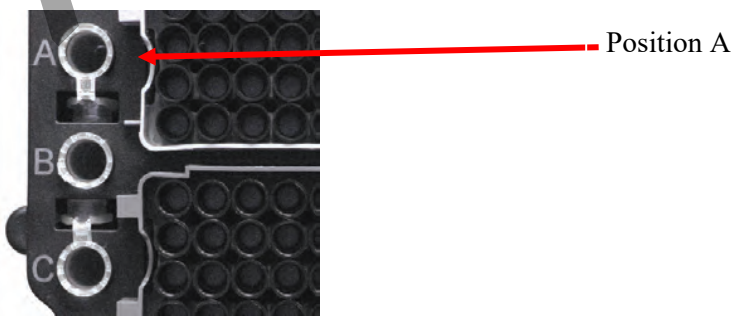
3.3 Fill both tip racks with Disposable Filter-Tips, 1000ul, wide bore.

3.4 Retrieve reagents for the sperm lysis buffer and record lot numbers in LIMS. Prepare buffer in a 2 mL screw cap tube – see the table below for minimum volumes required for this protocol:

NOTE: G2 buffer_AutoDiff reagent comes in 2 sizes: 11mL and 260mL bottles. Either size may be used for the sperm lysis buffer and the 30mL reagent bottle as described below.

Sperm Lysis Buffer reagents			
# samples	**G2 buffer_AutoDiff (µL)**	**Qiagen ProK (µL)**	**1M DTT (µL)**
3	398	27	106
4	506	34	135
5	623	42	166
6	750	50	200
7	863	58	230
8	975	65	260
9	1106	74	295
10	1219	81	325
(11 or) 12	1470	98	392

3.5 Once the sperm lysis buffer is prepared, seat the tube on the QIAcube worktable in Position A.



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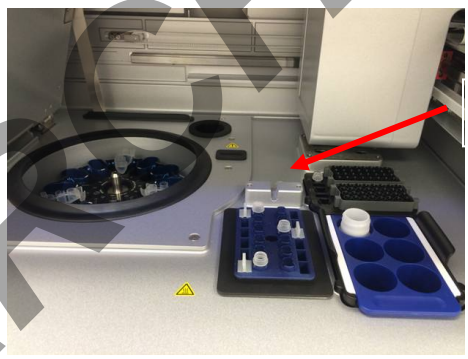
3.6 Retrieve and record lot number for G2 buffer_AutoDiff. Aliquot buffer G2 into a 30ml reagent bottle and place in Reagent Bottle Rack position 1. The minimum amount of buffer needed based on the number of samples is listed in the table below. Do not fill the bottle above the fill line on the side of the bottle. Make sure to pop any bubbles that may have formed covering the mouth of the reagent bottle using a clean pipette tip.

G2 minimum buffer amounts	
# samples	**G2 buffer (mL)**
3	8.68
4	10.74
5	12.8
6	14.86
7	16.92
8	18.98
9	21.04
10	23.1
(11 or) 12	27.22



Reagent bottle rack position 1

3.7 Referring to the "QIAcube Loading chart," (Appendix A) place sample plugs into the QIAcube shaker for each Extraction Negative and F1 fraction. **ONLY PLACE PLUGS IN APPROPRIATE LOCATIONS BASED ON THE NUMBER OF SAMPLES.**

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sample plugs placed in these slots

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3.8 After the incubation from step **2.6** is done, spin down the original samples briefly to remove condensation from the tube caps.

3.9 **Tube Transfer WITNESS:** Have a witness verify three sets of tubes:

- Original incubation tube (**input** sample labels and tube top)
- Spin basket tube (**input** sample labels)
- Rotor setup (**output F2** sample labels on outside of rotor adapter)

3.10 Prepare 10% bleach, distilled water, and 70% alcohol in three 50ml conical tubes. Clean a pair of forceps by dipping the forceps in each of the three tubes briefly and then drying with a fresh Lint free wipe. For each sample, transfer the substrate using the forceps to its associated spin basket tube. Pipette mix the sample lysate within the incubation tube a few times to disturb any sperm pellet that may have formed, and then pipette the entire lysate volume (~500ul) over the top of the substrate in the spin basket. Close the tube top over the spin basket. Repeat this step for each sample, cleaning and drying the forceps between each sample.

3.11 Spin the substrates in spin baskets at 13,200 rpm to 15,000 rpm for 2 minutes.

3.12 Using clean and dry forceps, remove and discard the spin baskets (including the swab remains – see NOTE) taking care to avoid bubbles at the rim of the open tube. **NOTE: Refer to Appendix B if it is necessary for a particular case or sample to retain the substrate remains for potential future testing.** Clean and dry forceps between each sample.

3.13 Transfer the lysate from the spin basket collection tube, pipette mixing as needed, to its corresponding F2 sample tube within the QIAcube rotor adapter.

3.14 **QIAcube Setup WITNESS**: Obtain a witness to verify samples and loading pattern on the QIAcube. Refer to “QIAcube Loading Chart” (Appendix A) for correct tube positioning based on sample number.

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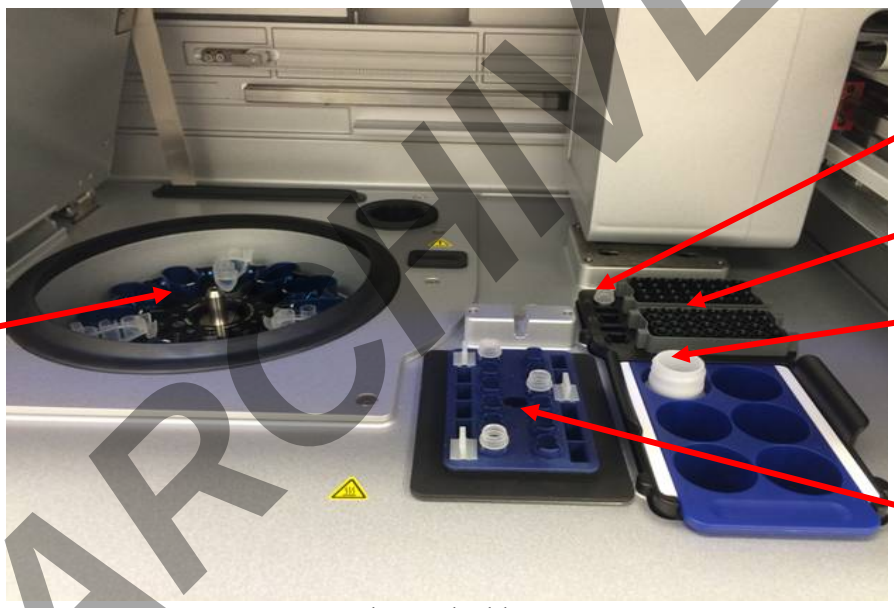
3.14.1 Load the rotor adapters containing the F2 samples into the QIAcube centrifuge, reading the F2 LIMS label and rotor position for that sample. If the batch size is 11 samples, an additional blank tube containing water must be placed in the rotor adapter in the 12th slot.

3.14.2 Load the empty F1 sample tubes into the QIAcube shaker, reading the F1 LIMS label and shaker position for each sample. Check to make sure that tubes are pressed all the way down. If the batch size is 11 samples, and additional blank tubes must be placed in the shaker in the 12th slot.

3.14.3 The witness should also check that all sample and reagent bottle caps are removed, tips are full, sample plugs are in place in the shaker, and robot setup is correct for the corresponding sample number.



QIAcube worktable

3.15 Close the instrument door and start the separation protocol on the QIAcube:

3.15.1 From the main menu, select “Pipetting Separation and Lysis 12A”

3.15.1.1 This protocol may be chosen from “Last”, if available. Otherwise, choose “DNA” → “Pipetting” -→ “Epithelial and Sperm Cell” → “Separation and Lysis 12A”

3.15.2 Press “Start”

3.15.3 The screen will display instructions to ensure the QIAcube has been loaded properly. After checking each step, press “Next” until the final step, and then press “Start.”

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3.15.4 Run time for protocol 12A is ~52 minutes for a full set of 12 samples.

3.16 During the QIAcube protocol 12A, set up the EZ1 for the F1s.

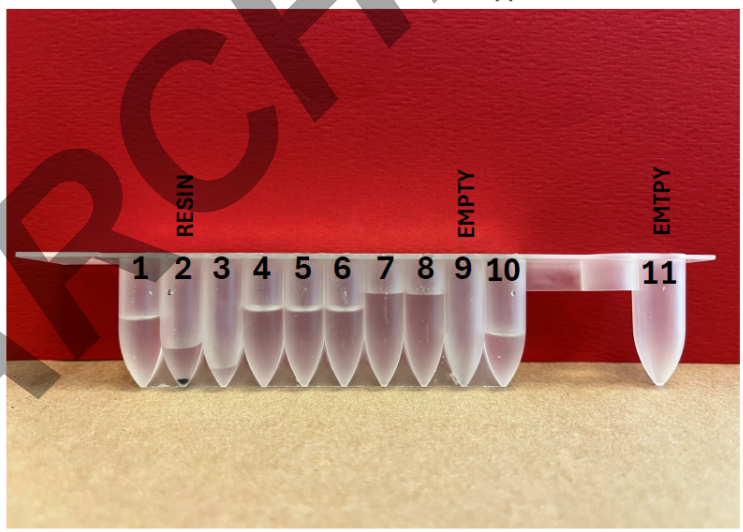
4 EZ1 setup for F1s:

4.1 Complete the usage log in LIMS for the instrument. Fill out the performed by tab for F1Purification.

4.2 Remove both the tube rack and cartridge rack from the EZ1.

4.3 Obtain reagent cartridges and record the lot number in LIMS. (Note – more than one lot may be used if there are not enough individual cartridges available for your full batch. Please indicate in the batch comments the additional lot number and for which samples it was used). Invert reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottoms of their wells. Check that the magnetic particles are re-suspended. Also, visually inspect each reagent well. All wells except wells 9 and 11 must contain liquid.

EZ1/2 CARTRIDGES: Well volumes should appear as below:



Set aside any cartridges that do not appear to be filled like the above and alert QA.

4.4 Slide EZ1 reagent cartridges into the cartridge rack, one cartridge for each sample being run. The cartridge label side should be at the blunt end of the rack, closest to the tube rack when loaded into the instrument. Place rack in EZ1.

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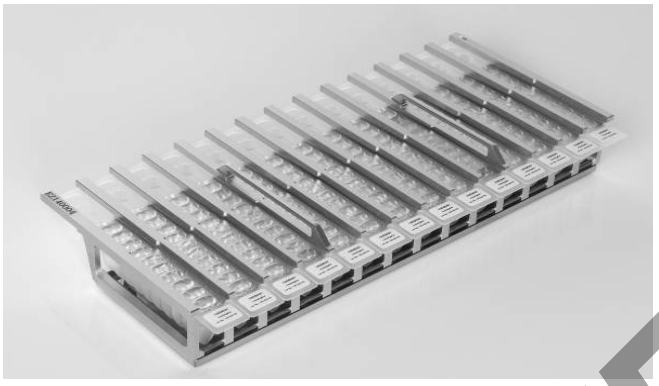

Reagent cartridge rack with cartridges loaded

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4.5 Label Qiagen 1.5ml screw cap elution tubes with **output** F1sample LIMS labels and labeled tube tops.

4.6 Assemble EZ1 tips and tip holders. Place them in row 2 of the EZ1 tube rack.

4.7 At the conclusion of protocol "separation and Lysis 12 A" on the QIAcube, the screen will then give instructions on how to clean the QIAcube. Press "Next" after each step until the main menu appears on the screen. Open the QIAcube instrument door and take out the F1 fraction samples from the QIAcube shaker, capping each sample with a tube cap as it is removed.

5 QIAcube set up for F2s:

5.1 Refill the tip racks on the QIAcube with disposable Filter-Tips and ensure buffers are in place and uncapped. Empty the waste drawer.

5.2 Close the instrument door and start the sperm washing protocol on the QIAcube:

5.2.1 From the main menu, select "Pipetting Separation and Lysis 12B"

5.2.1.1 This protocol may be chosen from "Last", if available. Otherwise, choose "DNA" → "Pipetting" -→ "Epithelial and Sperm Cell" → "Separation and Lysis 12B".

5.2.2 Press "Start"

5.2.3 The screen will display instructions to ensure the QIAcube has been loaded properly. After checking each step, press "Next" until the final step, and then press "Start."

5.2.4 Run time for this protocol is ~48 minutes for a set of 12 samples.

6 EZ1 F1 Sample preparation and purification:

6.1 While the QIAcube is running, prepare the F1 samples for the EZ1.

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6.2 Record lot numbers for MTL and carrier RNA into LIMS. Add 400µl of MTL buffer, and 1µl carrier RNA (1µg/µl) to each F1fraction Extraction Negative and sample obtained from the QIAcube. Precipitate may form in the tube with the addition of the MTL buffer.

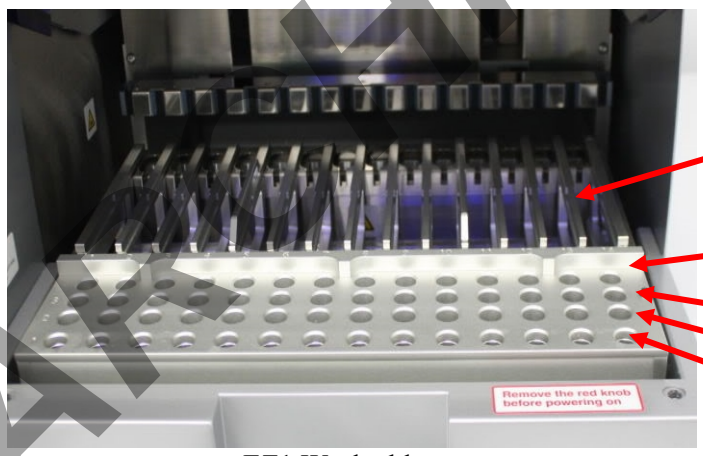
6.3 EZ1 F1 Setup WITNESS: Have a witness verify the samples and loading of the F1 Extraction Negatives and samples on to the EZ1.

6.3.1 Load the 2ml F1 sample tubes into row 4 of the EZ1 tube rack, reading the LIMS label and removing the screw cap as you load each sample.

6.3.2 Next, load the labeled 1.5mL F1 sample elution tubes into row 1 of the EZ1 tube rack, reading the LIMS label and tube top and removing the screw cap as you load each sample.

6.3.3 Place tube rack into the EZ1. Make sure that the tube rack is loaded onto the instrument after the cartridge rack.

6.3.4 Witness should verify that all samples, reagents, and racks are loaded appropriately on the instrument.



EZ1 Worktable

6.4 Run the EZ1 protocol for purification of the F1 fractions:

6.4.1 If needed, press “ESC” to get to the main menu.

6.4.2 From the main menu press “Start” to begin a run.

6.4.3 When asked if you would like to create a run report, press “ESC” to select no.

6.4.4 For F1 fractions, press “3” to select the large volume protocol

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6.4.5 Press “2” to select elution in TE.

6.4.6 Press “1” to select the 40uL elution volume.

6.4.7 The screen will display instructions to ensure the EZ1 has been loaded properly. After checking each step, press “ENT” until the final step, and then press “Start.”

6.4.8 The protocol run time is ~18 minutes

6.5 Create a reference tube with 36µl of 0.1X TE^{-4}.

6.6 After the protocol is completed, press “ENT” to continue.

6.7 Open the instrument door and remove the tube rack. Remove the 1.5mL F1 elution tubes, capping each sample with the labeled tube top.

6.8 Compare each extract to the reference tube containing 36µl of 0.1X TE^{-4}. If the volume appears lower than 36µl, measure the extract.

6.8.1 Measured volumes below 36µl should be documented in the Lab Supervisor Issues Log and the test batch comments.

6.8.2 Samples with measured volumes below 27µl must not proceed for further testing.

6.9 Discard all the used cartridges, lysate tubes, tip-holders, and tips.

6.10 The EZ1 instrument can now be set up for the F2 purification.

7 EZ1 F2 Sample preparation and purification:

7.1 Complete the usage log in LIMS for the instrument. Fill out the performed by tab for F2 Purification.

7.2 Remove both the tube rack and cartridge rack from the EZ1.

7.3 Invert reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottoms of their wells. Check that the magnetic particles are re-suspended. Visually inspect each reagent well and ensure that all wells except wells 9 and 11 contain liquid.

7.4 Slide EZ1 reagent cartridges into the cartridge rack, one cartridge for each sample being run. The cartridge label side should be at the blunt end of the rack, closest to the tube rack when loaded into the instrument. Place rack in EZ1.

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7.5 Label Qiagen 1.5ml screw cap elution tubes and caps with **output** F2 sample LIMS labels and labeled tube tops.

7.6 Assemble EZ1 tips and tip holders. Place them in row 2 of the EZ1 tube rack.

7.7 After the "Separation and Lysis 12 B" protocol is complete on the QIAcube, the F2 fractions will be found in the 1.5ml tubes in the rotor adapters. Remove the tubes from each rotor adapter, closing the cap as you remove each sample. Discard the rotor adapters.

7.8 Vortex the F2 fraction samples for 5 seconds. Incubate at 70°C for 10 minutes at 900rpm shaking in a thermomixer. Complete the usage log for the instrument and record the thermomixer temperature in LIMS. (The QIAcube may be cleaned during this incubation, or after the F2 fractions are loaded onto the EZ1 – refer to step **7.14**.)

7.9 Vortex the samples vigorously for 10 seconds after the incubation. Briefly spin down the samples in a centrifuge.

7.10 Add 1µl of carrier RNA to each sample.

7.11 Holding the lid closed with your thumb, cut through the tube cap connector for each F2 tube.

7.12 **EZ1 F2 Setup WITNESS**: Have a witness verify the samples and loading of the F2 Extraction Negatives and samples on to the EZ1:

7.12.1 Read the F2 sample LIMS label and remove and discard the lid with a tube opener. Place the tubes with F2 fraction into row 4 of the EZ1 tube rack.

7.12.2 Next, load the empty labeled 1.5mL F2 sample elution tubes into row 1 of the EZ1 tube rack, reading the LIMS label and tube top, removing the screw cap as you load each sample.

7.12.3 Place tube rack into the EZ1. Make sure that tube rack is loaded onto the instrument after the cartridge rack.

7.12.4 Witness should verify that all samples, reagents, and racks are loaded appropriately on the instrument.

7.13 Close the instrument door and start the protocol for F2 purification.

7.13.1 From the main menu, press "Start" to begin a run.

7.13.2 When asked if you would like to create a run report, press "ESC" to select no.

7.13.3 For F2 fractions, press "1" to select the trace protocol.

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7.13.4 Press “2” to select elution in TE.

7.13.5 Press “1” to select the 40uL elution volume.

7.13.6 The screen will display instructions to ensure the EZ1 has been loaded properly. After checking each step, press “ENT” until the final step, and then press “Start.”

7.13.7 The run time for this protocol is ~18 minutes.

7.14 Clean the QIAcube instrument:

7.14.1 DO NOT USE BLEACH ON THE INSTRUMENT, ONLY 70% ETOH WITH LINT FREE WIPES. NEVER SPRAY ETHANOL DIRECTLY ON THE INSTRUMENT.

7.14.2 Empty the waste drawer and check that the liner is clean.

7.14.3 Remove but DO NOT DISCARD THE SAMPLE PLUGS.

7.14.4 Discard the sperm lysis buffer tube. The G2 buffer may be capped and stored on the instrument deck. Remove pipette tip racks and consolidate any partial racks of tips for future use.

7.14.5 Wipe down the entire inside of the QIAcube instrument using a lint-free wipe with 70% EtOH, including the waste drawer.

7.15 After the F2 run is complete on the EZ1, press “ENT” to continue.

7.16 Open the instrument door and remove the tube rack. Remove 1.5mL F2 elution tubes, capping each sample with its associated labeled tube top.

7.17 Compare each extract to the reference tube containing 36µl of 0.1X TE^{-4}. If the volume appears lower than 36µl, measure the extract.

7.17.1 Measured volumes below 36µl should be documented in the Lab Supervisor Issues Log and the test batch comments.

7.17.2 Samples with measured volumes below 27µl must not proceed for further testing.

7.18 Discard all the used cartridges, lysate tubes, tip-holders, and tips.

8 Clean the EZ1 instrument:

8.1 DO NOT USE BLEACH ON THE INSTRUMENT, ONLY 70% ETOH WITH LINT FREE WIPES. NEVER SPRAY ETHANOL DIRECTLY ON THE INSTRUMENT.

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8.2 Remove reagent cartridges and any waste, discard appropriately.

8.3 Wipe down the inside of the instrument using a lint free wipe and 70% EtOH.

8.4 Close the EZ1 door.

8.5 Follow the prompts on the screen to start a UV run, setting the time to 20 minutes.

9 LIMS Post Processing

9.1 Select all output samples in the test batch in LIMS and click **Data Entry.** Complete the data entry for all samples within LIMS. Pass/Fail should be indicated for both the QIAcube and EZ1 runs for each sample.

9.2 In the data entry screen, all samples can be assigned a storage location in the next available storage box. Store the extracts at 2° to 8°C or frozen.

9.3 Select all output samples and click **Review.** Complete the review of each sample and schedule any additional tests as needed.

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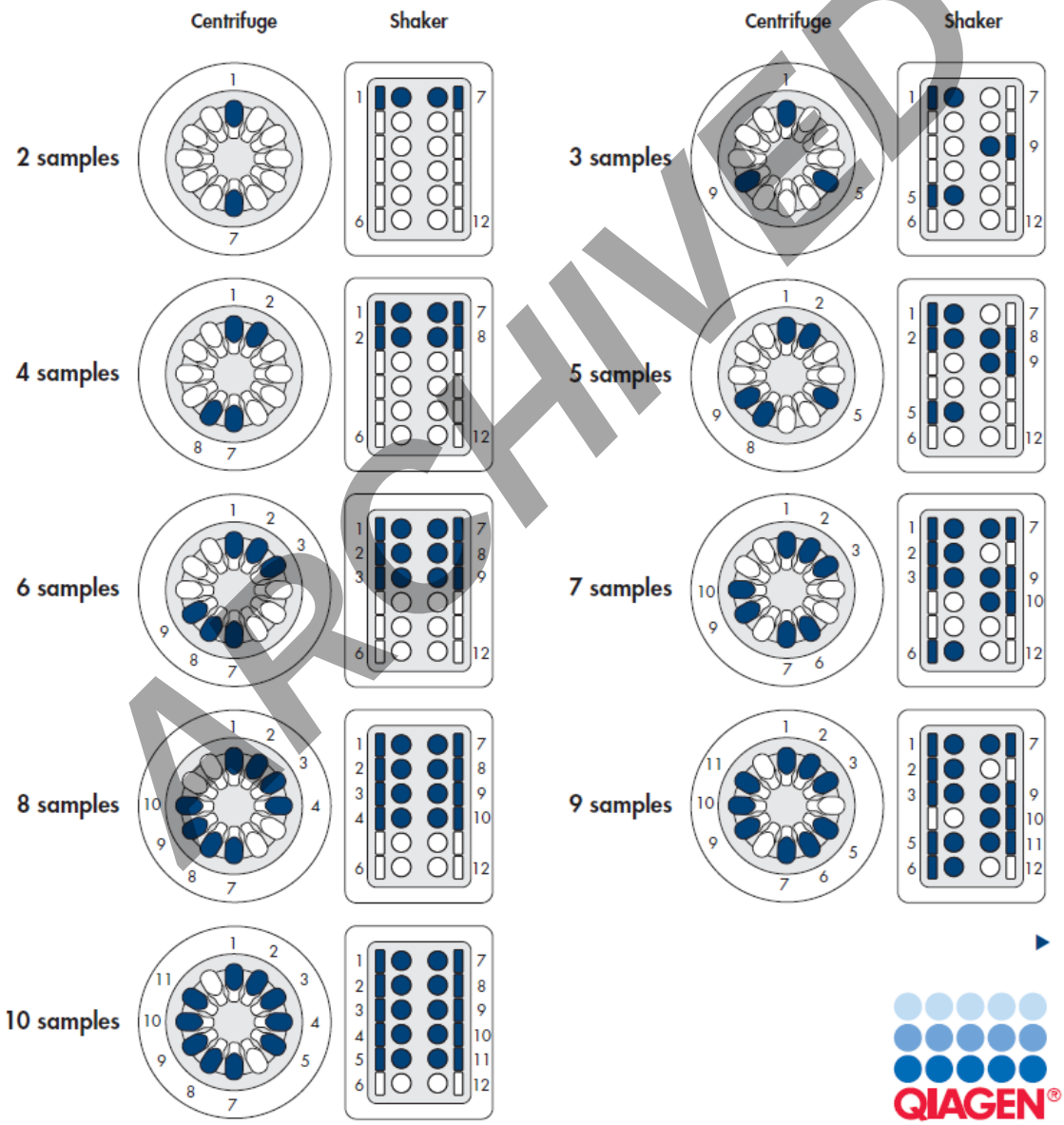
10 Appendix A: QIAcube Loading Chart

The chart below describes how to correctly load the QIAcube centrifuge and shaker if fewer than 12 samples are to be processed in one run. Please note, if only 11 samples are on the batch, a blank must be added to fill the 12th slot.



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11 Appendix B: Retaining a substrate remains

11.1 In the event that a substrate needs to be retained, follow these steps after Step **3.12** of the SOP:

11.1.1 Go to Analytical Testing >> Manage Sample.

11.1.2 On the Search by Query list click 'Test Batch Input Samples'.

11.1.3 Search using the test batch ID.

11.1.4 Select the sample which will be retained and click [Un-Dispose].

11.1.5 With the sample selected, click [Assign Storage].

11.1.6 Associate the sample with the next available extract storage cryobox and location.

11.1.7 Back in the Manage Sample page with the sample still selected click [Print Label] on the Side Bar.

11.1.8 Affix this label to a clean, 1.5 mL tube that the substrate will be stored in.

11.1.9 During the spin basket step (Step 3.13 of the SOP) place the substrate, using clean forceps, into the labeled 1.5 mL tube.

11.1.10 Store the substrate at 2° to 8°C, or frozen.

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