Examination and Direct Lysis Procedure for Cartridge Casings

1 Examination and Incubation

1.1 Follow the guidelines for note taking and evidence examination when examining any cartridge casing.

1.2 Prepare the bench for examination and prepare 10% bleach, distilled water, and 70% alcohol in three 50mL conical tubes. Clean a pair of reverse action forceps by dipping the forceps in each of the three tubes briefly and then drying with a fresh lint free wipe.

1.3 Complete the General Packaging Examination Worksheet for the outer packaging only. Label with the associated package label.

1.4 Gather the necessary number of appropriately sized tubes required to accommodate all cartridges and one extraction negative. Use the police paperwork to determine the size of your cartridges. One cartridge casing batch is limited to 13 total cartridge casings per case.

1.4.1 For cartridge casings that are **.22 caliber**, use a sterile 5mL tube, and a sterile 1.5mL tube for all items. Only use a sterile 1.5mL tube for the extraction negative.

1.4.2 For cartridge casings **greater than .22 caliber**, use a sterile 5mL tube for all items and extraction negative.

1.4.3 For a **mixture** of cartridge casings of different sizes, use a sterile 5mL tube for the extraction negative and those cartridge casings that are greater than .22 caliber and use a sterile 5mL tube and a sterile 1.5mL tube for the cartridge casings that are .22 caliber.

Note: Caution should be used to avoid touching the exterior surface of the cartridge casing. Use reverse-action forceps when possible. Do not place evidence and items stickers on the cartridge casing.

Note: For commingled items or unspent cartridges, consult the examination supervisor.

1.5 Complete the examination of the cartridge casings using the Cartridge Casing Item Examination Worksheet.

1.6 Describe the general condition of the item, such as staining, material and caliber.

1.6.1 If a distinct possible bloodstain is present, the entire stain should be collected and submitted as a separate sample than the cartridge casing. A portion of the swab used to collect the stain should be cut and KM tested. Sometimes this may not be possible

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due to the size and nature of the staining. If the stain is presumptively positive for blood, an additional cutting should be made and submitted for casework sample extraction on the EZ1. If the stain is negative for blood, the swab can be retained with the evidence. The entire cartridge casing should then be submitted for the Direct Lysis procedure.

1.6.2 If the staining covers the entire cartridge casing to the extent that there is no physical distinction between the cartridge casing and the possible blood stain, swab a small portion of the staining and test for KM. If the stain is presumptively positive for blood, an additional cutting of the swab should be submitted for casework sample extraction on the EZ1. The cartridge casing should then be packaged without further testing. If the staining is negative for blood, the entire cartridge casing should then be submitted for the Direct Lysis procedure.

1.7 Create the cutting and select the'EZ1CCEXT' Extraction Type in LIMS for the item and label a 5mL sized tube.

1.7.1 Cuttings associated with the direct lysis of cartridge casings should utilize the _T suffix.

1.7.2 Additionally, place the evidence item label, and cutting label on its respective original coin envelope.

1.8 Add the cartridge casing into its labeled 5mL tube with the opening of the cartridge casing facing up.

1.9 Repeat steps 1.5– 1.8 for all cartridge casings to be examined, using a clean pair of reverse action forceps between each cartridge casing.

1.10 Create an EZ1 Cartridge Casings Extraction Worksheet.

1.11 Fill out the Setup Reviewed By review task.

1.12 Prepare the Master Mix According to the table below, scan the reagent lot numbers in LIMS. Determine the amount needed based on the number of cartridge casings plus one extraction negative.

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	>.22 Caliber/mixture		.22 Caliber	
# of samples + extraction negative	Teknova Organic Sperm Wash buffer (uL)	QiaProK (uL)	Teknova Organic Sperm Wash buffer (uL)	QiaProK (uL)
2	1958.4	38.4	1224	24
3	2937.6	57.6	1836	36
4	3916.8	76.8	2448	48
5	4896	96	3060	60
6	5875.2	115.2	3672	72
7	6854.4	134.4	4284	84
8	7833.6	153.6	4896	96
9	8812.8	172.8	5508	108
10	9792	192	6120	120
11	10771.2	211.2	6732	132
12	11750.4	230.4	7344	144
13	12729.6	249.6	7956	156
14	13708.8	268.8	8568	168

1.13 Vortex the Master Mix briefly to mix.

1.14 Add Master Mix to all tubes.

1.14.1 For **.22 caliber**, using a pipette, add 520 uL of master mix to a labeled 1.5mL tube for the extraction negative and sample tubes. Transfer the .22 caliber cartridge casing using a pair of clean reverse action forceps from the 5mL tube into the 1.5mL tube with Master Mix with the opening of the cartridge casing facing up. **Be sure to avoid getting Master Mix inside the opening of the cartridge casings.**

1.14.2 For cartridge casings **greater than .22 caliber**, using a pipette, add 832 uL of Master Mix to the 5 mL extraction negative and sample tubes with the cartridge casings. **Be sure to avoid getting Master Mix inside the opening of the cartridge casings.**

1.14.3 For a **mixture** of cartridge casings of different sizes, using a pipette, add 520uL into the 1.5 mL tubes and 832uL into the 5 mL tubes and the extraction negative. Transfer the .22 caliber cartridge casing(s) using a pair of clean reverse action forceps from the 5mL tube into the 1.5mL tube with Master Mix with the opening of the cartridge casing facing up. **Be sure to avoid getting Master Mix inside the opening of the cartridge casings.**

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1.15 Once the Master Mix has been added, incubate at 56°C for 30 minutes. Record the incubation temperature in the 'Additional Information' section and add the incubator to the 'Instrument' box at the top of the worksheet.

1.16 During the incubation, label a 2mL Collection Tube for each sample and extraction negative using the associated test batch input label.

1.17 Remove the samples from the incubator.

1.18 **Tube Setup WITNESS**: Have a witness verify the two sets of tubes:

1.18.1 Original incubation tube (LIMS sample label)

1.18.2 Collection Tube (LIMS sample label)

1.19 Remove the cartridge casing from the tube using a pair of clean reverse action forceps.

1.20 Holding the cartridge casing with a pair of clean reverse action forceps, use a dry cotton swab to swab the entire outside of the cartridge casing and place the swab upright in a tube rack.

1.21 Once the cartridge casing has been swabbed, moisten a brown paper towel with 70% ethanol and use it to wipe the cartridge casing. **Do NOT use a Lint Free Wipe.**

1.22 Place the cleaned cartridge casing back into its original packaging and seal the package.

1.23 Transfer lysate and cotton swab:

1.23.1 For **.22 caliber** cartridge casings, add a Lyse & Spin Column to the 2mL Collection Tube. Then, break the cotton portion of the swab into the Lyse & Spin Column in the 2mL Collection Tube. Using a pipette, transfer all the lysate to the Lyse & Spin Column, trying to minimize the production of bubbles.

1.23.2 For cartridge casings **greater than .22 caliber**, using a pipette, transfer 200uL of lysate from the incubation tube to its associated 2mL Collection Tube. Add a Lyse & Spin Column to the Collection Tube. Break the cotton portion of the swab into the Lyse & Spin Column. Using a pipette, transfer the remaining lysate to the Lyse and Spin Column, trying to minimize the production of bubbles.

1.24 Repeat steps 1.19 – 1.23 for all samples in the batch.

1.25 The extracts will continue with the EZ1 Cartridge Casing Extraction Protocol.

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2 EZ1 Cartridge Casing Extraction Protocol

2.1 Incubate samples at 56°C for 90 minutes on a thermomixer at 900rpm. Record the thermomixer temperature in the 'Additional Information' section and add the thermomixer to the 'Instrument' box at the top of the worksheet.

2.2 Fill out the 'Incubation Run By' and 'Response' dropdowns.

2.3 During the incubation:

2.3.1 Label Qiagen 1.5 screw cap elution tubes with the output label for each sample and extraction negative.

2.3.2 Add the EZ1 instrument to the 'Instrument' box at the top of the worksheet.

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

2.3.4 Obtain reagent cartridges and record the lot number.

Note: More than one reagent lot may be used if there are not enough individual cartridges available for your full batch. Please indicate in Extraction By review comments the additional lot number and for which samples it was used.

2.3.5 Invert the EZ1 reagent cartridges twice to mix the magnetic particles. Once resuspended, inspect each reagent well to ensure that all but the last two wells contain liquid.

2.3.6 Slide the EZ1 reagent cartridges into the cartridge rack ensuring that each sample to be extracted has an associated reagent cartridge. Place rack in EZ1.

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

2.3.8 Place tube rack into the EZ1.

2.4 Remove samples from the thermomixer and spin at 13,200rpm – 15,000rpm for one minute to recover the lysate.

2.4.1 If liquid is still present in the Lyse & Spin Column of any sample or the extraction negative, centrifuge only those samples for another minute to recover the remaining lysate.

2.4.2 After the second spin, any liquid remaining in the Lyse & Spin Column can be manually pipetted from the Lyse and Spin Column into the Collection Tube.

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2.5 Discard the Lyse & Spin Column containing the cotton swab using a pair of clean forceps or a lint free wipe.

2.6 Add 1uL of carrier RNA to each lysate. **DO NOT ADD MTL BUFFER**.

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

2.8 **EZ1 Setup WITNESS:** Have a witness verify the lysates, elution tubes, and loading of the Extraction Negative and samples on to the EZ1.

2.8.1 Remove and discard the cap and load the sample tubes into row 4 of the EZ1 tube rack, reading the LIMS label.

2.8.2 Next, load the labeled elution tubes to row 1 of the EZ1 tube rack, reading the LIMS label and removing the screw cap as you load each sample.

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

2.9 Run the EZ1 protocol for purification of the samples:

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

2.9.2 From the main menu, press “Start” to begin the run.

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

2.9.4 Press “3” to select the large volume protocol.

2.9.5 Press “2” to select elution in TE.

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

2.9.7 The screen will display instructions to ensure the EZ1 has been loaded properly.

2.9.8 After checking each step, press “ENT” until the final step and then press “Start.”

2.9.9 The protocol run time is ~ 18 minutes.

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

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

2.12 Open the instrument door and remove the tube rack. Remove the 1.5mL elution tubes, capping each one.

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

2.13.1 Samples with measured volumes below 36uL should be documented in the Lab Supervisor Issues Log and test batch comments.

2.13.2 Samples with measured volume below 27uL must not proceed for further testing. All other samples can proceed to quantitation.

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

2.15 Fill out the 'Extracted Run By' and 'Response' dropdown.

2.16 Create the QUANTTRIO submission for the samples.

2.17 Verify the samples listed on the Trio Submission Worksheet click [Save] and close the Submission worksheet.

3 Clean the EZ1

3.1 Note: Do not use bleach on the instrument, only 70% ethanol with lint free wipes. Never spray ethanol directly on the instrument

3.2 Wipe down the inside of the instrument using a lint free wipe and 70% ethanol.

3.3 Close the EZ1 door.

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

3.5 Note: The UV lamp needs a minimum switch-on time of 20 minutes. Do not interrupt a UV light cycle before 20 minutes have passed since it will reduce the lamp's lifetime. Do not touch UV lamp with your fingers. Call QA when a UV lamp needs to be replaced.

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