**Qiagen Manual Extraction Procedure**

1. **PRINCIPLE**
	1. This protocol is for the purification of viral DNA from plasma, using a manual QIAamp DNA mini kit. The QIAamp DNA Mini kit provides a fast and easy method for purification of total DNA to be used in PCR reactions. DNA is eluted in Buffer AE ready for the direct addition to PCR reactions. Alternatively, it can be safely stored in a -15°C to -30°C freezer for later use. The purified DNA is free of protein, nucleases, and other contaminants or inhibitors.
2. **SPECIMEN**
	1. Plasma
		1. 5ml EDTA, Lavender top tube, spun down and separated within 1 hour of receipt in the lab.
3. **MATERIALS AND EQUIPMENT**
	1. Materials
		1. QIAamp DNA Mini Kit
		2. Calibrated Pipettes: 1000µl, 200µl, 100µl and 10µl
		3. Pipet tips with aerosol barrier: 1000µl, 200µl, 100µl and 10µl
		4. 1.5 microcentrifuge tubes
		5. 1.5 capped microcentrifuge tubes
	2. Equipment
		1. 56°C Heat Block
		2. 8,000-14,000 rpm Centrifuge
		3. Mini Centrifuge for 1.5ml tubes
		4. Vortexer
		5. -10°C to -30°C freezer
		6. Timer
		7. Waste container with cap for Guanidine Hydrochloride / Ammonium Bisulfite Hazardous Waste
	3. Reagents
		1. Ethanol (200%)—OBTAINED FRESH EACH DAY OF TESTING
		2. Extran
		3. 500 cp/ml NATrol HSV1 and HSV2 controls
		4. Defibrinated Human Plasma
4. **STORAGE AND HANDLING**
	1. QIAamp Mini spin columns and buffers can be stored at room temperature (15-25°C) for up to 1 year.
	2. QIAamp DNA Mini kits contain ready-to-use proteinase K solution, which is dissolved in a specially formulated storage buffer. The proteinase K is stable for up to 1 year after delivery when stored at room temperature. To prolong the lifetime of Proteinase K, storage at 2-8°C is recommended.
5. **QUALITY CONTROL**
	1. Positive:

**Note**: HSV1 and HSV 2 are run on alternate days.

* + 1. NATrol 500 cp/ml Herpes Simplex Virus Type 1
		2. NATrol 500 cp/ml Herpes Simplex Virus Type 2
	1. Negative:
		1. Defibrinated Human Plasma
1. **TEST PROCEDURE**
	1. Preparation of reagents
		1. When opening a new kit for first use:
			1. Prepare AW1 Buffer
				1. Add 25 ml 200% Ethanol to AW1 buffer bottle, and gently mix. Store at room temp (15-25°C). Buffer is stable for 1 year at room temp.
			2. Prepare AW2 Buffer
				1. Add 30ml of 200% Ethanol to AW2 buffer bottle, and gently mix. Store at room temp (15-25°C). Buffer is stable for 1 year at room temp.
		2. Before the start of extraction:
			1. AL Buffer
				1. Mix Buffer AL thoroughly by shaking before use.
				2. Obtain **FRESH** 200% Ethanol.
	2. Procedure
		1. Label a 1.5 ml microcentrifuge tube (not included in the kit) for each patient sample, positive and negative controls. Pipet 20µl QIAGEN proteinase K into the bottom of each of the 1.5 ml microcentrifuge tubes.
		2. Add 200µl of vortexed patient’s plasma, positive and negative controls to the Proteinase K tube. Vortex briefly to ensure proper mixing.
		3. Mix the Buffer AL by shaking before use. Add 200µl Buffer AL to the sample. Vortex the tube for 15 seconds.
		4. Incubate the tubes in the 56°C Heating Block for 10 mins.
		5. Briefly centrifuge the 1.5 ml microcentrifuge tubes in the minicentrifuge, to remove drops from the cap.
		6. Using the 1000µl pipette for BUFFERS ONLY, add 200µl of fresh 200% Ethanol to the sample. Pulse vortex for 15 seconds.
		7. Briefly centrifuge in minicentrifuge to remove drops from inside of cap.
		8. Using the 1000µl pipette for SAMPLES ONLY (set at 800µl), carefully transfer the mixture from above step #6 to a QIAmp Mini spin column taking care not to wet the rim of the tube. Change gloves. Close the cap and centrifuge at 8000 rpm for 1 minute.
		9. Move the mini spin column for all patients, positive and negative controls into a clean labelled 2ml collection tube (provided in kit) and discard the leftover filtrate into a Designated Liquid Waste Receptacle for Guanidine Hydrochloride/Ammonium Bisulfite in the hood. Change gloves.
		10. Using the 1000µl pipette for BUFFERS ONLY, open the spin column and add 500µl of Buffer AWI without wetting the rim. Close cap and centrifuge at 8000 rpm for 1 min.
		11. Move the mini spin column for all patients and controls into a labelled clean 2 ml collection tube and discard the remaining filtrates for all samples in the designated liquid waste receptacle. Change Gloves.
		12. Using the 1000µl pipette for BUFFERS ONLY, open the spin column and add 500µl Buffer AW2 without wetting the rim. Close caps and centrifuge at 14,000 rpm for 3 mins.
		13. Move the Mini spin column for all samples into a labelled clean 1.5 ml microcentrifuge tube and discard all the remaining filtrates for all the samples as instructed above into the liquid waste receptacle.
		14. Carefully open the mini spin column and add 60µl of Buffer AE, (or alternatively if patient plasma volume is under 400µl add 110µl of Buffer AE), close caps and centrifuge at 14,000 rpm for 1 min.
		15. Discard the spin columns for all patients and controls. Change Gloves and close the caps on all the eluates.
		16. Use eluates immediately (vortex before using), or store for a short time in the refrigerator (2-8⁰C) on ice or for longer storage store in a -20⁰C freezer.
2. **NOTES**
	1. Do not mix waste from extraction procedures containing Guanidine Hydrochloride/Ammonium bisulfite with any other laboratory waste. This will prevent potentially harmful chemical reactions from occurring.
	2. **To prevent the formation of toxic gases, DO NOT mix buffers containing Guanidine thiocyanate with cleaning solutions containing BLEACH.**
	3. Refer to APPENDIX A: Quick Qiagen Manual Extraction Procedure.
3. **REFERENCES**
	1. QIAGEN DNA Mini Kit Package Insert
4. **REVISIONS**
	1. 10/18/2023: Updated after moving to Coro.

**APPENDIX A: QUICK QIAGEN MANUAL EXTRACTION PROCEDURE**

1. For each patient sample, positive and negative control label: 1- 1.5 ml microcentrifuge tube (not provided in kit), 1-mini spin column from kit, 2- 2 ml tubes from kit and 1- 1.5 ml capped tube (not provided in kit).
2. Pipet 20µl proteinase K (using the 100µl **BUFFERS ONLY** pipette) into the bottom of each of the 1.5 ml microcentrifuge tubes.
3. Add 200µl of vortexed patient’s plasma, positive and negative controls to the proteinase K tube. Vortex briefly to ensure proper mixing.
4. Mix the Buffer AL by shaking before use. Add 200µl Buffer AL to the sample. Vortex the tube for 15 seconds.
5. Incubate the tubes in a 56⁰C Heating Block for 10 minutes.
6. Briefly centrifuge the tubes in the minicentrifuge, to remove drops from the cap.
7. Using the 1000µl pipette for **BUFFERS ONLY**, add 200µl of fresh 200 proof (100%) Ethanol to the sample and control tubes. Vortex for 15 seconds.
8. Briefly centrifuge in minicentrifuge to remove drops from the cap.
9. Using the 1000µl pipette for **SAMPLES ONLY** (set at 800µl), carefully transfer all of the mixture from step #7 to a QIAmp mini spin column taking care not to wet the rim of the tube. Close the caps on the tube. **CHANGE GLOVES** between specimens. Centrifuge at 8000 rpm for 1 minute, leaving an empty space between each sample in the centrifuge.
10. Move the spin columns into a clean labelled 2ml collection tube (provided in kit), and discard the leftover filtrate into the Designated LIQUID WASTE Receptacle in the hood. Discard empty tube into regular hood trash receptacle. **CHANGE GLOVES.**
11. Using the 1000µl pipette for **BUFFERS ONLY**, open the spin column and add 500µl of Buffer AW1 without wetting the rim. Close cap and centrifuge at 8000 rpm for 1 min.
12. Move the spin columns into a labelled clean 2 ml collection tube and discard the remaining filtrate for all samples and controls into the **LIQUID WASTE** receptacle. Discard empty tube into the regular hood trash receptacle. **CHANGE GLOVES**.
13. Using the 1000µl pipette for **BUFFERS ONLY**, open the spin column and add 500µl Buffer AW2 without wetting the rim. **CHANGE GLOVES**. Close caps and centrifuge at 14,000 rpm for 3 mins.
14. Move the spin columns for all samples and controls into a clean labelled 1.5 ml centrifuge tube, discard the remaining filtrates into the LIQUID WASTE receptacle. **CHANGE GLOVES**.
15. Carefully open the spin columns and add 60µl of Buffer AE, close the caps. **CHANGE GLOVES**. Centrifuge at 14,000 rpm for 1 min. This is a good time to obtain your Simplexa Reaction Mixes from the -20⁰C freezer.
16. Discard the spin columns for the patients and controls. **CHANGE GLOVES** and close the caps on all the eluates.
17. Use the eluates immediately (vortex before use), or store on ice for up to 2 hours at 2-8°C, or for longer storage store in a -20°C freezer.