4 DNA ISOLATION

4.1 GOALS:

4.1.1 To develop a basic understanding of the theory and procedure for DNA isolation from blood and buccal samples using the QIAGEN® BioRobot 9604.

4.1.2 To develop a basic understanding of the theory and procedure of DNA isolation from blood and buccal samples using the Manual QIAamp® extraction procedure.

4.1.3 To become acquainted with the sensitivity of the isolation procedure.

4.1.4 To become familiar with the limitations of the isolation procedure.

4.1.5 To become familiar with the use of controls incorporated at this stage of the procedure.

4.1.6 To become familiar with the reagents used for DNA isolation and the function of each.

4.1.7 To become familiar with the procedure for diluting the isolated DNA samples in preparation for PCR amplification.

4.1.8 To become familiar with the operation of the QIAGEN® BioRobot 9604.

4.1.9 To become familiar with proper documentation for DNA isolation and dilution in preparation for PCR amplification.

4.2 TASKS:

4.2.1 Prepare reagents necessary for DNA isolation.

4.2.2 Perform DNA isolation on a minimum of 20 dried blood stains provided by the training coordinator using the manual QIAamp® extraction procedure outlined in the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual Section III, Fluorescent Detection PCR-Based STR DNA Protocol PowerPlex® 1.1, 2.1, and 16 BIO Systems Manual. Continue on to Chapter 5, AMPLIFICATION. Complete the entire DNA analysis process for these 20 samples before proceeding to task 4.2.3 below.

4.2.3 Perform DNA isolation on a minimum of 20 buccal samples provided by the training coordinator using the manual QIAamp® extraction procedure outlined in the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual Section III, Fluorescent Detection PCR-Based STR DNA Protocol PowerPlex® 1.1, 2.1, and 16 BIO Systems Manual. Continue on to Chapter 5, AMPLIFICATION. Complete the entire DNA analysis process for these 20 samples before proceeding to task 4.2.4 below.

4.2.4 Set up the QIAGEN® BioRobot 9604 and reagents necessary for DNA isolation.

4.2.5 Perform DNA isolation on a minimum of 30 blood stains and 130 buccal samples provided by the training coordinator using the DNA extraction method outlined in the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual Section V, Forensic Biology Section Qiagen® BioRobt™ 9604 Procedures Manual. These samples will be

divided into four sets of 40 samples. Each set will be carried through the entire DNA analysis process before processing the next set of samples.

4.2.6 Read applicable literature and become familiar with the glossary terms. Refer to Appendices A and B.

4.3 TRAINING EVALUATION:

4.3.1 Knowledge

4.3.1.1 Review of notes and worksheets in training notebook by training coordinator.

4.3.1.2 Oral and practical examinations

4.3.2 Skills

4.3.2.1 The trainee should perform DNA isolation on a sufficient variety and number of samples to develop and exhibit an unquestionably sound technique for successfully isolating DNA using the QIAGEN® BioRobot 9604. This will be monitored by review of the documentation in the training notes and continual observation by the training coordinator.

4.3.3 Completion of the checklist by training coordinator.

STUDY QUESTIONS:

1. How do you isolate DNA from a blood or buccal sample manually using the QIAamp® procedure? Robotically using the QIAGEN® BioRobot 9604?

2. What is the function of the AL lysis buffer?

3. What are the components of the AL lysis buffer and what is the purpose of each component?

4. What is the function of the QIAGEN® protease in the isolation procedure?

5. What is the function of the AW1 wash buffer?

6. What are the two components of the AW1 wash buffer and what is the purpose of each component?

7. What is the function of the AW2 wash buffer?

8. What are the two components of the AW2 wash buffer and what is the purpose of each component?

9. What is the function of the AE elution buffer?

10. What is the major component of the AE elution buffer and what is the benefit of using this component?

11. How does the extraction procedure for dried bloodstains differ from the procedure used for DNA isolation from a liquid blood sample?

12. Is quantitation of the samples prior to amplification necessary? Please explain your answer.

13. Explain the operation of the QIAGEN® BioRobot 9604.

14. Why is it important to autoclave some reagents and certain supplies?

CHECKLIST FOR DNA ISOLATION

1. Trainee has prepared reagents necessary for DNA isolation.

   Date:_____ Training Coordinator:_____

   Comments:_____

2. Trainee has successfully set up the QIAGEN® BioRobot 9604.

   Date:_____ Training Coordinator:_____

   Comments:_____

3. Trainee has successfully and accurately completed all appropriate paperwork associated with the DNA isolation process.

   Date:_____ Training Coordinator:_____

   Comments:_____

4. Trainee has successfully isolated DNA from the following samples:

   Manually extracted a minimum of 20 bloodstains and 20 buccal samples using the QIAamp® extraction procedure.

   Date:_____ Training Coordinator:_____

   Comments:_____

   Extracted a minimum of 30 bloodstains and 130 buccal samples using the QIAGEN BioRobot™ extraction procedure.

   Date:_____ Training Coordinator:_____

   Comments:_____

5. Trainee has developed a basic understanding of the theory behind the manual QIAamp® and QIAGEN BioRobot™ extraction methods.

   Date:_____ Training Coordinator:_____

   Comments:_____

6. Notebook is organized and complete.

   Date:_____ Training Coordinator:_____

   Comments:_____

7. Trainee has read and understands all applicable literature.

   Date:_____ Training Coordinator:_____

   Comments:_____

8. Trainee has participated in question and answer sessions.

   Date:_____ Training Coordinator:_____

   Comments:_____

♦**END**