Department of Forensic Biology

Quality Assurance Manual

Version 3.0

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Introduction

As of this date, Quality Manual version 3.0 supersedes all previous Quality Assurance (QA) and/or Quality Control (QC) Manuals in the Department of Forensic Biology at the New York City Office Of Chief Medical Examiner (OCME). Where appropriate, references have been made to the Department of Forensic Biology Administrative Manual, Case Management Manual, Forensic Biochemistry Methods Manual, and Protocols for Forensic STR Analysis Manual.

References to specific quality manual guidelines (Standard 1.4.2.1) of the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB) are addressed below:

- **A quality policy statement including objectives and commitments by management.**
  This is listed in Section II.A - Goals and Mission, Section II.B - QA Objectives, and Section II.C - Authority and Accountability for the QA Program in the Administrative Manual.

- **The organization and management structure of the laboratory, its place in any parent organization, and relevant organizational charts.**
  This is diagrammed and discussed in Section II.D - Organizational Structure, in the Administrative Manual.

- **The relationships and responsibilities of management, technical operations, and support services in implementing the quality system.**
  This is presented in Section II.C - Authority and Accountability for the QA Program, and Section II.D - Organizational Structure, in the Administrative Manual.

- **Job descriptions, education, and up-to-date training records of laboratory staff.**
  Job descriptions for all laboratory personnel are described in Section II.D - Organizational Structure in the Administrative Manual. In addition, Civil Service job specifications for each job title are located in a filing cabinet containing ASCLD/LAB and DAB criterion files (see DAB Standard 5.1.1). Training records of laboratory staff are kept in a filing cabinet located near the departmental office.

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- **Control and maintenance of documentation of case records and procedure manuals.**
  The control and maintenance of documentation of case records is discussed in Section III.C - Data Analysis and Reporting, in the Administrative Manual.

  The Laboratory Director, or his/her designee, has the ultimate responsibility for all procedural manuals and assigns the writing and editing of manuals to the Deputy Director, Assistant Directors, QA Manager and/or Criminalist IVs on a regular basis. Minor revisions to each manual are made when necessary. The finalization of each revision occurs when (i) the Director, and if necessary, the Technical Leader, Assistant Directors, QA Manager or other laboratory members have reviewed the change(s), and (ii) the Director, or his/her designee, initials and dates each replacement page containing the revision(s) or signs each page of a newly revised manual. The Laboratory Director maintains the original signed copies of each procedural manual and keeps track of all changes that have been made. While every effort will be made to distribute copies of the edited pages to all, it is the responsibility of each analyst to ensure that their personal (unofficial) copy of a manual is up-to-date.

- **The laboratory's procedures for ensuring that measurements are traceable to appropriate standards, where available.**
  These are listed in Section VIII.D - NIST Standards and Section IX - Equipment Calibration and Maintenance in this manual.

- **The type and extent of examinations conducted by the laboratory.**
  These are listed and described in detail in the Forensic Biochemistry, Protocols for Forensic STR Analysis, and Crime Scene Investigation and Reconstruction Manuals.

- **Validation and verification of test procedures used.**
  This is described in Section III.I - Method Validation Procedures and Records, in the Administrative Manual.

- **Handling evidence items.**
  This is described in Section III.E - Evidence Handling Protocols, in the Administrative Manual, and Section III - Evidence Examination - Notetaking, Evidence Examination, and Packaging, in the Case Management Manual.

- **Major equipment and reference measurement standards used.**
  These are discussed in Section VIII.D - Reference Standards, and Section IX - Equipment Calibration and Maintenance, in this Quality Manual.

- **Calibration and maintenance of equipment.**
  This is presented in Section III.F - Equipment Calibration and Maintenance Logs in the Administrative Manual, and Section IX - Equipment Calibration and Maintenance, in this manual.

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- **Verification practices for ensuring continuing competence of examiners including interlaboratory comparisons, proficiency testing programs, and internal quality control schemes (e.g., technical peer review).**
  Proficiency testing and sample re-analysis are discussed in Section III.G - Proficiency Testing in the Administrative Manual. External proficiency testing for DNA methodologies is done in the laboratory according to DAB guidelines and the National DNA Index System (NDIS) standards for the operation of the Combined DNA Index System (CODIS). The technical peer review is conducted as described in III.C, Data Analysis and Reporting, in the Administrative Manual.

- **Gaining feedback and taking corrective action whenever analytical discrepancies are detected.**
  This is discussed in Section III.O.1 - Problems affecting the Laboratory's Mission, in the Administrative manual.

- **Monitoring court testimony to ensure the reporting of scientific findings in an unbiased and effective manner.**
  This is discussed in Section III.D - Court Testimony, in the Administrative Manual. All documents monitoring the court testimony of Criminalists, Assistant Directors, and Director are filed in a binder located in a designated area of the Forensic Biology Laboratory.

- **Laboratory protocol permitting departures from documented policies and procedures.**
  The specific procedures for analytical techniques done in this laboratory are thoroughly presented in the Forensic Biochemistry Methods Manual and Protocols for Forensic STR Analysis Manual. Any deviations from the printed procedure must be clearly documented on the data sheets (eg. worksheets, electropherograms, etc.) that are generated.

- **Dealing with complaints.**
  This is discussed in Section VIII - Complaints, in the Administrative Manual.

- **Disclosure of information.**
  This is discussed in Section III.C.6 - Dissemination of Disclosure of Results, in the Administrative Manual.

- **Audits and quality system review.**
  The Department of Forensic Biology Laboratory conducts audits annually in accordance to the standards dictated by ASCLD/LAB, DAB, and CODIS; this is further discussed in Section III.N - Quality Audit, in the Administrative Manual.

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I. Quality Manual Organization

The Quality Manual consists of various sections that address the current DAB standards. Its appendices contain reagent sheets (Appendix A), Quality Control procedures (Appendix B), and a list of usage and maintenance logs (Appendix C) that are currently being used in the laboratory.

A. Section II through Section XVI

These sections address the current DAB and ASCLD/LAB Standards and specifies the policies and procedures followed by the Department of Forensic Biology. These sections are controlled and must be approved by the Director (or a designee) prior to being implemented and/or changed.

B. Reagent sheets (Appendix A)

The Department of Forensic Biology documents the preparation of all internal critical reagents. This documentation is in the form of a reagent sheet that lists the chemical makeup and procedures necessary for the preparation of a given reagent. All current reagent sheets are filed in a series of **Reagent Sheet Binders**. A copy of each reagent sheet has also been included in this manual as Appendix A. Reagent sheets are worksheets, and do not require the Director's approval prior to being implemented and/or changed, but must be reviewed by the Quality Assurance Manager.

C. Quality Control Procedures (Appendix B)

The purpose of a QA program is to ensure that the laboratory meets a specified standard of quality. The QA program does this through the monitoring, verifying, and documenting of the performance of the laboratory. To accomplish these tasks, the Forensic Biology QA program has established a series of QC procedures that are designed to monitor critical aspects of forensic sample analysis in order to ensure that the resulting product conforms to the current standards set forth by ASCLD/LAB, DAB, and the Scientific Working Group for DNA Analysis Methods (SWGDAM). These QC procedures are contained in Appendix B and are identified by specific QC numbers. As an appendix, QC Procedures do not require the Director's approval prior to being implemented and/or changed, but must be reviewed by the Quality Assurance Manager.

D. Usage and Maintenance Logs (Appendix C)

Usage and Maintenance Logs are used by the laboratory to provide documentation of equipment use, calibration and maintenance. This documentation aids the QA program in identifying trends in equipment operation and analyst performance. This information can also assist the QA program in identifying potential or existing problems of quality. A list of the Usage and Maintenance Logs that are used in the laboratory for this purpose are located in Appendix C for the user's information.

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II. Goals and Objectives

The goals and objectives of the Department of Forensic Biology are listed in the Department of Forensic Biology Administrative Manual (Section II.A - Goals and Mission).

III. Organization and Management

The organization and management structure of the laboratory are diagramed and described in the Administrative Manual (see Section II.D - OCME and Department of Forensic Biology Organizational Structure and Figure 1 within).

IV. Personnel Qualifications and Training

Job descriptions for all laboratory personnel are described in the Administrative Manual (Section II.D - OCME and Department of Forensic Biology Organizational Structure). In addition, the Civil Service specifications for each job title are kept in the laboratory along with personnel transcripts, resumes, and documentation of continuing education and training.

V. Facilities

A. Security

Laboratory and building security are discussed in the Administrative Manual (Section III.E.3 - Security).

B. Contamination

1. Prevention

Several measures have been taken to prevent contamination within the Department of Forensic Biology. The laboratory is divided into physically isolated areas for evidence examination, DNA extraction, pre-amplification (amplification setup) and post-amplification (amplification and DNA typing). Each area has its own dedicated equipment. Once samples are accepted into the laboratory, they move through these areas in one direction only. Samples are first processed in the evidence examination area. They are then moved to the DNA extraction area. Following DNA extraction, aliquots of each sample are quantitated in the DNA quantitation area. Following DNA quantitation, aliquots of each sample are moved into the pre-amplification area. Here fresh kit reagents are stored and samples are prepared for amplification. Finally, the samples are amplified and typed in the post-amplification area. This laboratory setup helps eliminate cross contamination from amplified DNA areas back into non-amplified DNA areas.

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To avoid cross contamination between specimens, exemplar samples are processed separately from evidence samples. Also, only one sample is processed at a time using single-use disposable supplies whenever possible (eg. pipet tips), and scissors/tweezers are thoroughly cleaned between each sample (see Protocols for Forensic STR Analysis and Case Management Manuals for additional procedures to avoid cross contamination).

By far, the best defense against contamination is training for the analysts. The analysts must understand what is happening to the DNA at every step of the procedure. They must understand the rationale behind the laboratory setup and the methods of sample handling, so they are able to prevent problems before they arise. In this way, they are equipped to assess and to modify their individual habits as they practice each test of the training program.

2. Identification

Contamination can be identified as 1) the presence of signal in QuantiBlot, P30, and Amylase negatives (reagent blanks), 2) presence of ***alleles*** in extraction negatives or amplification negatives (reagent blanks), 3) presence of extraneous alleles in positive controls, or 4) presence of extraneous alleles in case samples. Contamination problems reflect a system failure or contamination of the samples by an outside source. The source may be equipment, reagents, the working environment, or an analytical error. Contamination can either be a single isolated event such as cross contamination between two samples or it can be persistent, such as contamination of a reagent or equipment. To remedy contamination caused by a single isolated event, the appropriate extraction, quantitation, amplification and/or STR analysis is repeated (also see the STR Results Interpretation section in the Protocols for Forensic STR Analysis Manual).

If the contamination persists or if several laboratory members are experiencing the same contamination, the QA Manager must be notified. The source of contamination should be identified, if possible, and eliminated. To demonstrate the elimination of the persistent contamination, a clean run (see QC155) may be performed. During a clean run, control samples are processed along with a series of negative controls. Negative controls are run at the extraction, amplification, and typing steps. The results from these samples will indicate the area in which contamination appears. By focusing attention on one area at a time, the source or sources of contamination can be systematically eliminated. In addition, recent casework may be reviewed and selected samples may be repeated later to verify the results. The analysts will be informed of any corrective action adopted to prevent the recurrence of the problem.

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3. **Troubleshooting**

Often, the source of a contamination problem can be identified on the basis of experience. For example, in a Quantiblot run, a persistent appearance of a light signal in the extraction negative control or the standard negative control (lane 1H) indicates (i) contamination of the regents used during the extraction procedure, (ii) contamination of the solutions used during the Quantiblot run, or (iii) consistent contamination by the analyst during extraction. In the former case, this contamination may represent a build up of DNA in the reagents over the course of many extractions. The weak signal appears when the concentration of DNA in the extraction negative is greater than the threshold of detectability for the hybridization. Generally, fresh reagents will eliminate this problem. In the latter case, if necessary, corrective action in the form of discussion and/or retraining will be given to the identified analyst(s).

Electrophoresis runs which appear to have the same mixture of DNA types across all the samples, indicate a more serious contamination problem at the level of the instrument or amplification step. If tubes or reagents are contaminated during the pre-amplification set up, the contaminant DNA will be amplified along with the sample. The sample signals may even be overwhelmed by the contaminant. To solve this problem, the pre-amplification room must be cleaned out and the bench washed with a 10% bleach solution. All of the kit reagents must be changed and new reaction tubes must be aliquoted.

Documentation resulting from troubleshooting experiments are kept in the **QA/QC Troubleshooting/Issues** binder.

4. **QC Procedures**

In addition to proper technique on the part of the analyst, care must also be taken in the preparation of all in-house reagents and in keeping all apparatus that come in contact with forensic samples free of contamination. To this end, various QC procedures have been developed and are part of routine laboratory operation (see Appendix B).

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a. Reagent Preparation

Clean laboratory glassware is an essential in reagent preparation (see QC175). Furthermore, all aliquots of deionized water and Tris-EDTA (TE^{-4}) buffer are first sterilized using an autoclave (see QC115) prior to distribution throughout the laboratory. This procedure protects these reagents from possible bacterial contamination that could later result in the degradation of sample DNA. In addition, autoclaving conditions help to keep these solutions DNA-free. Other working reagents that are kept in the laboratory for long periods of time (eg. 0.5 M EDTA) may also be autoclaved to increase their shelf life.

b. Equipment Decontamination

Various QC procedures have also been developed to help maintain a DNA-free environment at the points of sample contact with the various apparatus used in DNA analysis. A 10% bleach solution is extremely effective in degrading DNA and is thus used for general cleanup procedures of equipment and the laboratory environment (eg. laboratory desks and benches). Regular decontamination procedures with 10% bleach are used for the disinfection of the P30 ELISA Plate Washer (QC235), micropipetman (QC215), microcentrifuges (QC140), thermocyclers (QC290), and biosafety/fume hoods (QC125). Documentation of these various decontamination procedures is kept in the Plate Washer Maintenance Log Binder, Micropipette Calibration Log Binder, Centrifuge Maintenance Log Binder, Thermocycler Calibration and Maintenance Log Binder and Biosafety/Fume Hood Maintenance Log Binder, respectively.

VI. Evidence Control

Evidence control, handling and documentation procedures are discussed in Section III.E - Evidence Handling Protocols of the Administrative Manual, and Section III - Evidence Examination - Notetaking, Evidence Examination, and Packaging, in the Case Management Manual. These procedures have been designed to ensure the integrity of all physical evidence that enters the laboratory.

VII. Validation

Validation procedures are according to the DAB guidelines that are listed in Section III.I - Method Validation Records of the Administrative Manual.

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VIII. Analytical Procedures

A. Introduction

Analytical procedures that are used by the Forensic Biology Laboratory are described in the Crime Scene Investigation and Reconstruction Manual, Biochemistry Methods Manual and Protocols for Forensic STR Analysis Manual. These manuals also include general guidelines for the interpretation of data. References to scientific literature on which these procedures are based are also included in these manuals.

B. Reagents

Reagents used to perform various analytical procedures in the laboratory are purchased from commercial vendors or prepared in the laboratory. Reagents that are purchased from commercial vendors (eg. calibrator standards for quantitation of human DNA, 30% hydrogen peroxide, sodium dodecyl sulfate, sodium hydroxide, etc.) are used either directly in a given analytical procedure (eg. calibrator standards for quantitation of human DNA, 30% hydrogen peroxide) or in the preparation of in-house reagents (eg. sodium dodecyl sulfate, sodium hydroxide).

Each reagent has a corresponding **reagent sheet** which may include the identity and application of the reagent, date of preparation, identity of individual preparing the reagent, reagent lot number (if critical reagent), standard batch size, ingredients of the reagent, procedure to follow when preparing the reagent, data log section, and the quality control procedures to be performed before the reagent is released for use into the laboratory (see Appendices A and B). Working copies of the reagent sheets are kept in the **Reagent Binders**.

At a minimum, every reagent (or its container) that is prepared by the Department of Forensic Biology is labeled with the identity of the reagent, the date of preparation or expiration, and the identity of the individual preparing the reagent. The reagent sheets may further dictate what, in addition, must be indicated on the label.

1. Lot Numbers

All critical reagents are assigned a lot number. Subsequent lots increase in numerical order (eg. 51, 52, 53, etc.). Some reagents that are usually made fresh for a given procedure and/or are not critical reagents, are not assigned lot numbers. Where applicable, the reagent sheet indicates the lot number of that reagent and the lot numbers of the ingredients that were used for making the reagent. The reagent sheets for each lot are also filed in the Reagent Binders along with any supporting quality control documentation.

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2. **Standard Batch Size**
Each reagent sheet indicates the standard batch size routinely prepared for each lot. The quantities listed in the ingredients section have been calculated for this standard batch. Occasionally, it may be convenient to prepare a batch larger or smaller than the standard batch size. In such cases, the preparer must note the adjusted amount of each ingredient added for preparation of the reagent. If changes in demand persist over time, the reagent sheet may be modified to reflect the new batch size.

3. **Ingredients**
An ingredient may be either purchased from an outside vendor or prepared in-house. The ingredients required for the preparation of the reagent and the amounts of each ingredient required for the standard batch size are listed at the top of the reagent sheet. When suitable, final concentrations, and/or a tolerance of measurement are also listed next to the amount of a given ingredient. The tolerance of measurement is calculated to define an acceptable range of variation that will not significantly change the final concentration of a given reagent. Also, certain ranges have been adopted based upon recommendations for optimum performance. Volume measurements which are made in the appropriate size graduated cylinders and which appear to the eye to be exact, fall well within the range of tolerance listed in the ingredients section.

4. **Procedure**
The procedure describes how to prepare the solution step by step and includes important notes regarding the safe handling of hazardous chemicals. The completed sheets must document exactly how the solution was prepared. Any deviation from the printed procedure must be clearly documented on the reagent sheet.

5. **Data Log**
The **Data Log** records information regarding the ingredients used in the preparation of reagents. This information includes the source of the ingredient, lot number of the ingredient, amount of ingredient used, date of preparation, and the identity of the individual preparing the reagent. Reagents prepared in the laboratory may also be listed as ingredients (eg. 20X SSPE which is used in the preparation of Quantiblot Hybridization Solution). In those cases, the source is listed as FB (Forensic Biology) and the laboratory lot number is recorded.

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6. Quality Control

The quality control section lists the appropriate QC tests to be performed, if any, before the solution is released for use in the laboratory. These QC test procedures have been assigned QC numbers and names (eg. QC145 Chelex Extraction).

The type and number of quality procedures required to be done on a given reagent is dictated by the nature of that reagent. For example, QC250 Quantiblot Hybridization, is listed in the quality control section for Quantiblot Wash Solution (see Quantiblot Wash Solution reagent sheet in Appendix B). To evaluate the performance of this component, it is not necessary to amplify and type test samples. Only the quantiblot hybridization procedure is necessary to establish quality of the Quantiblot Wash Solution. On the other hand, the QC procedure for 5% Chelex (QC145) requires an extraction, human DNA quantitation, amplification, and STR analysis of the appropriate controls. The newly prepared 5% Chelex solution is released into the laboratory when all the tests have been passed.

More than one solution may be tested with a given QC procedure. In this case, the quality test must be sufficient for all of the components. For example, if a single run is to be performed for 5% Chelex and Quantiblot Wash Solution, the quality test must begin with the extraction. QC145 Chelex Extraction is the appropriate test for the Chelex, and the procedure encompasses the hybridization necessary for the wash solution.

7. Documentation

After a quality test has been performed, the supporting documentation is attached to the original solution sheet and submitted for review. If the reagent performance is satisfactory, it will be released for general use in the laboratory. If the reagent fails to meet the standards set forth in the QC procedure, it may be submitted for further testing or discarded.

After a reagent has passed quality control and been released, the reagent sheet and quality control documentation are filed in the appropriate QC reagent binder. If more than one reagent has been tested for quality control in a single test run, the original quality control documents will be filed with one solution sheet and cross referenced on the reagent sheet of the other.

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C. Critical Reagents

By definition, "critical reagents are determined by empirical studies or routine practice to require testing on established samples before use on evidentiary samples in order to prevent unnecessary loss of sample." (FBI, 1998). Thus, all critical reagents in the Forensic Biology Laboratory have a QC test procedure listed on each respective reagent sheet. This QC test procedure must be performed in order for the reagent to be released for use in routine casework analysis.

D. Reference Standards

PCR standard reference materials (SRM) for STR analysis are obtained from the National Institute of Standards and Technology (NIST) and tested annually as a quality check on the equipment and procedures that are used by the lab for STR typing. The laboratory quantitates and determines the DNA profiles of the given SRM samples. The results of these experiments are compared to the allele identification results that are also provided by NIST. This information is filed in the **PCR NIST Standards Binder**.

Positive and negative controls are run for every analytical procedure that is done in the laboratory. A discussion of the purpose for various types of negative controls used in the laboratory is presented in the Protocols for Forensic STR Analysis Manual (see subsection Extraction Negative, Amplification Negative and Substrate Controls, in section STR Results Intepretation). A list of the correct DNA profiles for various positive controls used in STR typing is presented in the same section of the Protocols for Forensic STR Analysis Manual (see subsection Amplification Positive Control).

IX. Equipment Calibration and Maintenance

A. Introduction

Equipment calibration and maintenance is essential for establishing confidence in the results that are generated during routine testing of forensic DNA samples. Equipment calibration and maintenance procedures can be subdivided into three (3) separate categories:

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1. Weights and Measures

a. Temperature

The Department of Forensic Biology monitors the temperatures of all freezers, refrigerators, heat blocks, incubators, and water baths that are used for storage of evidence and all types of casework samples on a daily basis, when the laboratory is open. Temperature and humidity readings are taken from several spread out areas in the laboratory. Temperature readings are documented in the **Temperature Control Log (F190)**. Acceptable temperature readings for each specific apparatus are noted below.

Equipment	Set Temperature	Acceptable Temperature Range
freezers	-20°C	-2 to -25°C
	-80°C	-60 to -85°C
refrigerators	4°C	1 to 13°C
56°C heat block	56°C	56 ± 3°C
65°C heat block	65°C	65 ± 3°C
95°C heat block	95°C	95 ± 3°C
100°C heat block	100°C	100 ± 3°C
37°C incubator	37°C	37 ± 3°C
Quantiblot H_2O bath	50°C	50 ± 1°C

Digital thermometers that are used to monitor the temperature of laboratory refrigerators, freezers, cold rooms, incubators, heat blocks, water baths, and air temperature are calibrated or are replaced by new units according to the vendor specifications (eg., recalibration date; see QC270 in Appendix B.2). Digital thermometers and dedicated RTD probes used in calibrating thermal cyclers are calibrated annually to National Institute of Standards and Technology (NIST) traceable standards. Each of these measuring instruments or probes (eg., thermocouples with the

exception of the Type T-brown[1]) are calibrated yearly to National Institute of Standards and Technology (NIST) traceable standards (see QC270 and QC280 methods in Appendix B.2). The date of calibration is documented on the appropriate log sheet (see F165) and filed in the **Temperature Equipment Maintenance Log Binder**. All new temperature measuring instruments/probes must have proof of calibration (eg. documentation of traceability to NIST standards) prior to being used in the laboratory.

Any additional maintenance performed on refrigerators and freezers is documented in the **Temperature Equipment Maintenance Log Binder**.

b. Balances

The Mettler PJ6000 and AE260 (analytical) balances are used to weigh chemicals in the ranges of 1 to 200 g and <10 g, respectively, for the preparation of all laboratory reagents. At a minimum, balances must be calibrated annually to NIST traceable standards (see QC120 in Appendix B.2). Documentation of each calibration is kept in the **General Equipment Maintenance Binder**.

c. pH Meter

The pH meter is used to measure the pH of reagents. A two-point calibration and verification of the pH meter is performed weekly (see QC245 in Appendix B.2) and is documented in the **pH Log & Water System Binder**.

[1] Type T-brown thermocouples are used in the measurement of -80°C low temperature freezers. A verification of these thermocouples is done yearly (see QC285) since an exact low temperature for the storage of DNA extracts, tissue samples, etc., is not critical, and NIST traceable thermometers are not made for this low temperature range.

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d. Micropipettes

Micropipettes are used routinely in the laboratory to measure and dispense accurate volumes of reagents used for a given protocol. All micropipettes are calibrated twice each year by an outside vendor (see QC215 in Appendix B.2). In addition, if at any time, there is reason to suspect that a micropipette may not be performing to its specifications, a quick gravimetric check may be done by weighing specific volumes of water on the Mettler AE260 analytical balance. If the micropipette differs significantly from specifications, the QA Manager must be notified and the micropipette under question will be removed from laboratory operations and will be sent for calibration with the next outgoing shipment. When possible, spare calibrated micropipettes will be used as temporary replacements for any micropipettes that have been removed by this manner from regular operation. Micropipette calibration is documented in the **Micropipette Calibration QC Log Binder.**

2. Analytical Methods

Equipment that is used for specific analytical methods in the laboratory is also calibrated on a regular basis according to the specific QC procedure indicated below. Documentation of each calibration and maintenance procedure for each equipment is done on specific equipment log sheets (see Appendix C) that are filed in each specific equipment log book. Each log book is located near the equipment under consideration.

Equipment	Analytical Procedure	Calibration/ Maintenance Protocol
ABI 310 Genetic Analyzer	STR Capillary Electrophoresis	QC135
ABI 377 DNA Sequencer	STR Gel Electrophoresis	QC165
BioRad Benchmark Microplate Reader	P30 ELISA	QC230
GeneAmp PCR System 9600	STR PCR	QC300
GeneAmp PCR System 9700	STR PCR	QC300

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3. **Lab Personnel Safety**
The laboratory has chemical fume hoods and biological containment hoods that are inspected annually by an outside vendor (see QC125 in Appendix B.2). Documentation of inspections are kept in the **Chemical Fume Hood & Biological Cabinet Maintenance Log Book**.

X. Proficiency Testing

Proficiency testing is done in the laboratory according to ASCLD/LAB and DAB guidelines. These procedures are discussed further in the Administrative Manual (see Section III.G - Proficiency Testing).

XI. Corrective Action

Corrective action is discussed in the Administrative Manual (Section III.O - Non Conformity and Corrective Action).

XII. Reports

Written procedures for writing and issuing reports are presented in the Case Management Manual. In addition, see Section III.C - Data Analysis and Reporting, in the Administrative Manual and the Protocols for Forensic STR Analysis Manual.

XIII. Review

Case review and related issues are discussed in the Administrative Manual (Section III.C - Data Analysis and Reporting) and Case Management Manual (Section V - Report Writing).

XIV. Safety

he Department of Forensic Biology has a documented environmental health and safety program as listed in the Administrative Manual (Section III.L - Safety). This documentation is kept in the **Safety Binder**. The OCME building safety officer conducts at least three inspections each year of the laboratory. Documentation of these inspections is also kept in the Safety Binder.

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XV. Audits

The Department of Forensic Biology Laboratory conducts audits annually in accordance to the ASCLD/LAB, DAB, and CODIS guidelines (see Section III.N - Quality Audit in the Administrative Manual). Documentation generated are kept in a central filing system in the laboratory.

XVI. Subcontractor of Analytical Testing

Any laboratory that has been subcontracted must also comply to all of the ASCLD/LAB and DAB guidelines. In addition, an appropriate and documented review process will be established by the Department of Forensic Biology to verify the integrity of the data received from the subcontractor (see Section III.P - Subcontracting in the Administrative Manual).

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Appendix A

Reagent sheets that are used for the documentation of reagents used for Forensic Biochemistry Methods and STR Analysis are listed below in sections 1 and 2, respectively, and are presented in alphabetical order. All of these reagent sheets are included in this appendix.

1. Forensic Biochemistry Methods: Reagent Sheets

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2. Forensic STR Analysis: Reagent Sheets

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ACID PHOSPHATASE TEST REAGENT

Standard batch size: 2 x 500 ml Lot Number: _____

Application
Acid Phosphatase presumptive test for semen (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
Sodium Acetate, 0.1 M (pH 5.5)	0.1 M	1000 ml
Alpha-Naphthyl Phosphate (disodium)	0.1%	0.5 g
o-Dianisidine Tetrazotized Fast Blue Salt BN	0.1%	0.5 g

Procedure

1) Prepare two separate 500 ml flasks, each containing 500 ml of 0.1 M sodium acetate. Add sodium alpha-naphthyl phosphate to one bottle. Mix well. Add fast blue B salt to the other flask and wrap with aluminum foil to minimize its exposure to light. Mix well.

2) Aliquot 10 ml of each reagent into 15 ml conical tubes. Wrap fast blue B salt tubes with aluminum foil.

3) Store at -20°C.

Data Log

	source	lot	amount
Sodium Acetate, 0.1 M	_____	_____	_____
Sodium Alpha-Naphthyl Phosphate	_____	_____	_____
Fast Blue B Salt	_____	_____	_____

Quality Control Test
QC100 - Acid Phosphatase Spot Test Reagent

semen dilution	result
N	____
1/2	____
1/4	____
1/8	____
1/16	____
1/32	____
1/64	____
Negative	____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

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ALKALINE SUBSTRATE BUFFER

Standard batch size: 4 L Lot Number: _____

Application
p30 ELISA (see Forensic Biology Methods Manual)

Ingredients

	final concentration	amount
Diethanolamine	1.0 M	388 ml
Sodium Azide	0.02%	0.8 g
Magnesium Chloride ($MgCl_2 \cdot 6H_2O$)	0.5 mM	0.4 g
Hydrochloric Acid (concentrated)	N/A	As needed

Procedure

1) Dissolve the diethanolamine, sodium azide, and magnesium chloride in 3200 ml deionized water.

2) Adjust to pH 9.8 (+/- 0.1) with hydrochloric acid (approximately 20-40 ml)

3) Bring to 4 L volume with deionized water.

4) Store between 2-8°C in brown bottle or wrap clear bottle with aluminum foil.

Data Log

	source	lot	amount
Diethanolamine	_____	_____	_____
Sodium Azide	_____	_____	_____
Magnesium Chloride	_____	_____	_____
Hydrochloric Acid	_____	_____	_____

Quality Control
QC225 - p30 ELISA

Final pH value: _____ (9.8 +/- 0.1)

Results: ☐ Pass ☐ Fail Initials: _____

Cross-reference (date): _____

Made By: _____ Date: _____

Initials: **Date:** 6/12/2003

AMYLASE GEL BUFFER

Standard batch size: 4 L Lot Number: _____

Application
Amylase diffusion presumptive test for saliva (see Forensic Biology Methods Manual)

Ingredients	final concentration	amount
Sodium Phosphate, anhydrous, monobasic (NaH_2PO_4)	0.05 M	6.2 g
Sodium Phosphate, monohydrate, dibasic ($Na_2HPO_4 \cdot H_2O$)	0.05 M	7.8 g
Sodium Chloride	7 mM	0.4 g
10 N NaOH	N/A	As needed
Hydrochloric Acid (concentrated)	N/A	As needed

Procedure

1) Add the ingredients to 4 L of deionized water.

2) Adjust pH to 6.9 (+/- 0.1), if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

3) Store between 2-8°C.

Data Log	source	lot	amount
NaH_2PO_4, anhydrous	_____	_____	_____
Na_2HPO_4, anhydrous	_____	_____	_____
Sodium Chloride	_____	_____	_____
NaOH, 10 N	_____	_____	_____
Hydrochloric Acid (concentrated)	_____	_____	_____

Quality Control
QC105 - Alpha-amylase gel radial diffusion

Standard	Diameter
20 units	_____
2 units	_____
0.2 units	_____
0.02 units	_____
0.002 units	_____
Negative	_____

Final pH value: _____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

ANODE SOLUTION (IEF FOCUSING)

Standard batch size: 250 ml

Application
Hemoglobin (Hb) by IEF (see Forensic Biology Methods Manual)

Ingredients

	final concentration	amount
Glacial Acetic Acid	1%	2.5 ml

Procedure

1) Add the acetic acid to 247.5 ml deionized water.

2) Store at room temperature.

3) Make fresh as needed.

4) Write your initials and date of make (DOM) on reagent label.

Initials: **Date:** 4/30/2003

CASEIN STOCK SOLUTION

Standard batch size:2 L Lot Number: _____

Application
p30 ELISA (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
Hammerstein Casein	1%	20 g
NaOH, 10 N	N/A	as needed
Phosphate Buffered Saline	50%	1 L
Sodium Azide	0.1%	0.2 g

Procedure

1) Thoroughly dissolve the Hammerstein casein in 1 L deionized water. Adjust the pH to 8.0 (+/- 0.1) by adding NaOH (drop by drop) to help casein go into solution. Take care to ensure that the solution does not go over pH 8.0. **Do not add acid to lower pH!**

2) Add the PBS and sodium azide.

3) Store at -20°C in 40 ml aliquots.

Data Log

	source	lot	amount
Hammerstein Casein	_____	_____	_____
Sodium Hydroxide	_____	_____	_____
Phosphate Buffered Saline	_____	_____	_____
Sodium Azide	_____	_____	_____

Quality Control
QC225 - p30 ELISA

Final pH value: _____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

CATHODE SOLUTION (IEF FOCUSING)

Standard batch size: 250 ml

Application
Hemoglobin (Hb) by IEF (see Forensic Biology Methods Manual)

Ingredients

	final concentration	amount
Ethanolamine	1%	2.5 ml

Procedure

1) Add the ethanolamine to 247.5 ml deionized water.

2) Store at room temperature.

3) Write your initials and date of make (DOM) on reagent label.

Initials: **Date:** 4/30/2003

COOMASSIE BLUE STAIN

Standard batch size: 1 L

Applications

Ouchterlony radial diffusion-species determination and cross-over electrophoresis-species determination (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
Methanol	50%	500 ml
Glacial Acetic Acid	10%	100 ml
Brilliant Blue R	0.1% (w/v)	1.0 g

Procedure

1) Mix together methanol, glacial acetic acid, and 400 ml deionized water.

2) Add brilliant blue R to the solution and stir for several minutes.

3) Filter the solution directly into a storage bottle.

4) Store at room temperature

5) Write your initials and date of make (DOM) on reagent label.

6) Make fresh as needed.

Initials: **Date:** 4/30/2003

DESTAIN SOLUTION

Standard batch size: 4 L

Applications

Ouchterlony radial diffusion-species determination and cross-over electrophoresis-species determination (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
Methanol	45.5%	1820 ml
Glacial Acetic Acid	9%	360 ml

Procedure

1) Mix together methanol, glacial acetic acid, and 1820 ml deionized water.

2) Transfer to a 4 L storage bottle.

3) Store at room temperature.

4) Write your initials and date of make (DOM) on reagent label.

5) Make fresh as needed.

Initials: **Date:** 4/30/2003

IODINE SOLUTION, 0.01 N

Standard batch size: 500 ml

Application

Amylase diffusion presumptive test for saliva (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
1 N Iodine (Iodine-Iodide Solution)	0.01 N	5 ml

Procedure

1) Mix 5 ml of 1 N iodine with 495 ml deionized water.

2) Store at room temperature in a brown bottle or aluminum foiled glass bottle.

3) Write your initials and date of make (DOM) on reagent label.

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

ISOELECTRIC FOCUSING HEMOGLOBIN (HB) PLATES

Standard batch size: 21 ml (5 plates)

Application
Hemoglobin by IEF (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Sucrose	11.9%	2.5 g
3% Acrylamide Premix	4.8%	1.0 g
Ammonium Persulfate (10% in H_20)	0.7%	150 uL
TEMED (neat)	0.07%	15 uL
Ampholyte pH 3-10	0.95%	0.2 ml
Ampholyte pH 6-8	2.4%	0.5 ml
Ampholyte pH 7-9	2.4%	0.5 ml

Procedure

1) Dissolve the sucrose and 3% acrylamide premix in 20 ml of deionized water.
2) Add the ampholytes.
3) Add the ammonium persulfate (APS) and TEMED (Make fresh stock of APS daily).
4) Allow 30-60 min for polymerization.
5) Can be used immediately or stored wrapped in wet paper towels and sealed in a Kapak bag at 2-8°C.

Data Log	source	lot	amount
Sucrose	_____	_____	_____
3% Acrylamide Premix	_____	_____	_____
Ammonium Persulfate	_____	_____	_____
TEMED	_____	_____	_____
Ampholyte pH 3-10	_____	_____	_____
Ampholyte pH 6-8	_____	_____	_____
Ampholyte pH 7-9	_____	_____	_____

Quality Control
QC190 - Isoelectric Focusing: Hb is done for new vendor shipment of ampholytes.

Bands	Allowable Separation	Actual Separation
A to F	>2 mm	_____
F to S	>3 mm	_____
S to C	>6 mm	_____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

KASTLE-MEYER (KM) REAGENT

Standard batch size: 1 L Lot Number: _____

Application
Kastle-Meyer (KM) presumptive test for blood (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Phenolphthalin	0.2%	2.0 g
Potassium Hydroxide	0.18 M	10.0 g
Absolute Ethanol (100%)	80%	800 ml
Zinc Dust	N/A	variable

Procedure
1) In an aluminum-foiled flask, dissolve the phenolphthalin in 200 ml deionized water.
2) Add potassium hydroxide. The phenolphthalin will dissolve.
3) Stir until clear (very light pink is OK)
4) Add the ethanol.
5) Add enough zinc dust to cover the bottom of bottle.
6) Store between 2-8°C in a dark or foiled bottle.

Data Log	source	lot	amount
Phenolphthalin	_____	_____	_____
Potassium Hydroxide	_____	_____	_____
Ethanol	_____	_____	_____
Zinc Dust	_____	_____	_____

Quality Control Test
QC200 - Kastle-Meyer presumptive test for blood (reagent does not have to be sensitive to 1/1,000,000)

Reagent Sensitivity

whole blood dilution	Before 3% H_2O_2	After 3% H_2O_2
N	_____	_____
1/10	_____	_____
1/100	_____	_____
1/1,000	_____	_____
1/10,000	_____	_____
1/100,000	_____	_____
1/1,000,000	_____	_____
Negative	_____	_____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

LEUCOMALACHITE GREEN (LMG) REAGENT

Standard batch size: 250 ml Lot Number: _____

Application

Leucomalachite Green (LMG) presumptive test for blood (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Leucomalachite Green (Oxalate Salt)	0.4%	1 g
Glacial Acetic Acid	40%	100 ml
Zinc Dust	N/A	5 g

Procedure

CAUTION: HYDROGEN GAS IS GENERATED! DO NOT SEAL BOTTLE TOO TIGHT!

1) Mix together leucomalachite green, glacial acetic acid, 150 ml deionized water, and zinc dust.
2) Heat solution (keep covered with foil for reflux to occur) by mixing on stir plate until solution is a clear light yellow color. This may take several hours.
3) Allow to cool and then filter.
4) Add enough zinc dust to cover the bottom of the bottle.
5) Store in a dark glass bottle refrigerated at 2-8°C.

Data Log

	source	lot	amount
Leucomalachite Green	_____	_____	_____
Glacial Acetic Acid	_____	_____	_____
Zinc Dust	_____	_____	_____

Quality Control

QC205 - Leucomalachite Green presumptive test for blood

Reagent Sensitivity

whole blood dilution	Before 3% H_2O_2	After 3% H_2O_2
N	_____	_____
1/10	_____	_____
1/100	_____	_____
1/1,000	_____	_____
1/10,000	_____	_____
1/100,000	_____	_____
1/1,000,000	_____	_____
Negative	_____	_____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

NUCLEAR FAST RED (RED CHRISTMAS TREE STAIN)

Standard batch size: 4 L Lot Number: _____

Application
Christmas Tree stain for spermatoazoa (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Aluminum Sulfate	0.07 M	100.0 g
Nuclear Fast Red	0.05%	2.0 g

Procedure

1) Dissolve the aluminum sulfate in 4 L of warm deionized water and add the nuclear fast red. Stir over low heat overnight. Allow to cool, then filter.

2) Label with a nine (9) month expiration date.

3) Store between 2-8°C.

Data Log	source	lot	amount
Aluminum Sulfate	_____	_____	_____
Nuclear Fast Red	_____	_____	_____

Quality Control
QC150 - Christmas Tree stain for spermatozoa

Results: ☐ Pass ☐ Fail Initials: _____

EXPIRATION DATE:

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PBS SOLUTION

Standard batch size: 1 L

Application
P30 ELISA (see Forensic Biochemistry Methods Manual)

Ingredients

	amount
Phosphate Buffered Saline (PBS) Tablets	5

Procedure

1) Dissolve the tablets in 1 L of deionized water.

2) Store between 2-8°C.

Data Log

	source	lot	amount
PBS Tablets	_____	_____	_____

Quality Control

QC225 - P30 ELISA done only on new shipments of tablets. Subsequent preparations are made at the bench by analysts and do not require completing this sheet.

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PBS-BSA SOLUTION

Standard batch size: 100 ml

Application
P30 ELISA (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
Phosphate Buffered Saline (PBS)	99.99%	100 ml
Bovine Serum Albumin (BSA, Molecular Biology Grade)	0.01%	0.01 g
	OR	
Phosphate Buffered Saline (PBS)	99.99%	100 ml
Bovine Serum Albumin, 5mg/ml	0.01%	0.5 ml

Procedure

1) Dissolve the BSA in PBS.

2) Use immediately to prepare stock solution of P30 antigen.

Initials: **Date:** 4/30/2003

PICRIC INDIGO CARMINE (GREEN CHRISTMAS TREE STAIN)

Standard batch size: 2 L Lot Number: _____

Application
Christmas Tree stain for spermatoazoa (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Picric Acid	0.06 M	26 g
Indigo Carmine	0.34%	6.8g

Procedure

CAUTION: PICRIC ACID IS EXPLOSIVE WHEN DRY AND SHOULD BE MAINTAINED WITH MORE THAN 10% dH_2O. WEIGH OUT PICRIC ACID WITH NEGLIGIBLE AMOUNT OF WATER IN WEIGH BOAT.

1) Dissolve the picric acid in 2 L of warm deionized water; add the indigo carmine and stir overnight, then filter.

2) Label with a nine (9) month expiration date.

3) Store between 2-8˚C.

Data Log	source	lot	amount
Picric Acid, Saturated	_____	_____	_____
Indigo Carmine	_____	_____	_____

Quality Control
QC150 - Christmas Tree stain for spermatazoa

Results: ☐ Pass ☐ Fail Initials: _____

EXPIRATION DATE:

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

POTASSIUM CYANIDE SOLUTION (KCN), 0.05%

Standard batch size: 200 ml

Application
Hemoglobin (Hb) by IEF (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Potassium Cyanide	0.05%	0.1 g

Procedure

CAUTION: **POTASSIUM CYANIDE IS A TOXIC COMPOUND THAT CAN BE ABSORBED BY CONTACT WITH SKIN OR BY INHALATION. USE ADEQUATE PROTECTION, INCLUDING LAB COAT, GLOVES, AND EYE PROTECTION, WHEN HANDLING THIS COMPOUND.**

1) Dissolve the potassium cyanide in 200 ml of deionized water.

2) Store at room temperature.

3) Make fresh as needed.

4) Write your initials and date of make (DOM) on reagent label.

Initials: **Date:** 4/30/2003

SALINE (0.85% NaCl)

Standard batch size: 10 L

Application

Ouchterlony radial diffusion-species determination and cross-over electrophoresis-species determination (See Forensic Biolochemistry Methods Manual).

Ingredients	final concentration	amount
Sodium Chloride	0.85%	85.0 g

Procedure

1) Dissolve the sodium chloride in 10 L of deionized water in a carboy.

2) Store at room temperature.

3) Make fresh as needed.

4) Write your initials and date of make (DOM) on reagent label.

Initials: **Date:** 4/23/2004

SODIUM ACETATE, 0.1M (pH 5.5)

Standard batch size: 1 L Lot Number: _____

Application
Acid Phosphatase presumptive test for semen (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Sodium Acetate, Anhydrous	0.1 M	8.21 g
Glacial Acetic Acid	-----	as needed

Procedure

1) Dissolve the sodium acetate in 900 ml of deionized water.

2) Adjust pH to 5.5 (+/- 0.1) with glacial acetic acid.

3) Transfer solution to a graduated cylinder and bring the volume up to 1 L. Mix well.

4) Store at room temperature.

Data Log	source	lot	amount
Sodium Acetate, Anhydrous	_____	_____	_____
Glacial Acetic Acid	_____	_____	_____
Final pH Value	_____		(5.5 +/- 0.1)

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

SPECIES AGAROSE GEL

Standard batch size: 150 ml (variable number of aliquots)

Application

Ouchterlony radial diffusion-species determination and cross-over electrophoresis-species determination (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Species Tank Buffer	50%	150 ml
Sigma Type I Agarose (or equivalent)	1%	3 g

Procedure

1) Mix species tank buffer with 150 ml deionized water.

2) Dissolve Sigma type I agarose (or equivalent) in the solution by heating on a stir plate.

3) Once solution is clear, dispense 7 ml aliquots into 20 x 150 mm test tubes.

4) Gel can be used immediately or may be stored covered with Parafilm at 2-8°C.

Initials: **Date:** 4/30/2003

SPECIES TANK BUFFER

Standard batch size: 1 L

Application

Ouchterlony radial diffusion-species determination and cross-over electrophoresis-species determination (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Barbital (sodium salt)	0.05 M	8.76 g
Barbital (free acid)	7 mM	1.28 g
Calcium Lactate	0.07 M	0.38 g
10 N NaOH	----	as needed
Hydrochloric Acid (concentrated)	----	as needed

Procedure

1) Dissolve barbital (sodium salt and free acid), and calcium lactate in 800 ml deionized water.

2) Adjust the pH to 8.6, if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

3) Dilute to 1 L with deionized water.

4) Store at room temperature.

5) Make fresh as needed.

6) Write your initials and date of make (DOM) on reagent label.

Initials: **Date:** 4/30/2003

TAKAYAMA REAGENT

Standard batch size: 100 ml Lot Number: _____

Application
Takayama Hemoglobin Test

Ingredients	final concentration	amount
Dextrose (Glucose)	0.5%	0.5 g
Sodium Hydroxide	0.25 M	1.0 g
Pyridine	20%	20 ml

Procedure

1) Dissolve dextrose in 5 ml deionized water.

2) Dissolve sodium hydroxide in 10 ml deionized water.

3) Transfer both the dextrose and sodium hydroxide solutions to a flask and add the pyridine.

4) Dilute solution to 100 ml with deionized water.

5) Store between 2-8°C in a brown glass bottle.

Data Log	source	lot	amount
Dextrose (Glucose)	_____	_____	_____
Sodium Hydroxide	_____	_____	_____
Pyridine	_____	_____	_____

Quality Control
QC265 - Takayama Hemoglobin Test

Positive Control _____

Negative Control _____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

UREA DIFFUSION TEST AND BLANK PLATES

Standard batch size: 613.5 ml (10 plates)

Application
Urea diffusion presumptive test for urine (see Forensic Biochemistry Methods Manual)

Ingredients	final concentration	amount
Agarose, type 1	1%	6 g
Bromothymol Blue, 1.5%	1%	6 ml
Urease (3 U/ml)	1.2%	7.5 ml

Procedure
1) Dissolve the agarose into 600 ml of boiling deionized water.
2) Add the bromothymol blue solution to the dissolved agarose.
3) Allow the solution to cool to 50°C.
4) Separate the solution into two 300 ml portions.
5) To one portion, add the urease solution.
6) Dispense 30 ml aliquots of both solutions into 10 cm^2 petri dishes and allow to solidify.
7) Store between 2-8°C.

Data Log	source	lot	amount
Agarose, Type 1	_____	_____	_____
Bromothymol Blue	_____	_____	_____
Urease	_____	_____	_____

Quality Control
QC305 - Urea Gel Diffusion is done on new shipments of urease.

Standard	Diameter	Concentration
urea, 5%	_____	
urea, 0.5%	_____	
urea, 0.05%	_____	
urea, 0.005%	_____	
negative	_____	
urine stain, N	_____	_____
urine stain, 1/10 dilution	_____	_____

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

UREASE, 3 U/ml

Standard batch size: 100 ml Lot Number: _____

Application
Urea diffusion presumptive test for urine (see Forensic Biochemistry Methods Manual)

Ingredients

	final concentration	amount
Urease	3 U/ml	~10 mg (see calculation)
Deionized Water	----	100 ml

Procedure

1) Add the Urease to 100 ml of deionized water.
2) Mix so that all of the Urease is dissolved into solution.
3) Make fresh for each batch of urea diffusion plates.

Calculation
300 U (units) x concentration of vendor urease (g/U) = amount of Urease to add.

Quality Control
QC305 - Urea Gel Diffusion (done only on new vendor lot/shipment of urease)

Note: Use "Urea Diffusion Test and Blank Plates" reagent sheet for documentation.

Initials: **Date:** 4/30/2003

AMMONIUM PERSULFATE (0.1g ALIQUOT)

Standard batch size: ~50 tubes x 0.1g Lot Number: _____

Application
Gel casting for the ABI 377 sequencer (see Protocols for Forensic STR Analysis) and Isoelectric Focusing Hemoglobin Plates (see Quality Assurance Manual)

Ingredients	Aliquot	Total Amount
Ammonium Persulfate (Electrophoresis Grade)	0.1 ± 0.01 g	5 ± 0.5 g

Procedure

CAUTION: WHEN WORKING WITH POWDERED AMMONIUM PERSULFATE WEAR GLOVES, EYE PROTECTION, AND LAB COAT FOR SAFETY.

1) Using weigh paper, weigh 0.1 ± 0.01g aliquots of ammonium persulfate.

2) Transfer the aliquots to 1.5 ml microcentrifuge tubes.

3) Cap all tubes tightly and label rack containing tubes with the lot number.

4) Store at room temperature.

Data Log

	source	lot	amount
Ammonium Persulfate	_____	_____	_____

Quality Control

QC165 - STR Gel Electrophoresis (done on new vendor lots/shipments of APS)

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

BSA SOLUTION, 5 mg/ml

Standard batch size: ~50 tubes x 0.1g Lot Number: _____

Application
YM1 and Quad STR Reaction Mixes (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Bovine Serum Albumin (BSA, molecular biology grade)	2.5% (w/v)	125 mg
Sterile Deionized Water	N/A	25 ml (guideline)

Procedure

1) Autoclave a 50 ml glass beaker with a stir bar in it.
2) Add the BSA to 20 ml of sterile water in the glass beaker.
3) Stir gently over **very low** heat until the BSA is completely dissolved.
4) Add the solution to a 50 ml disposable conical tube.
5) Add sterile water to a final volume of 25 ml.
6) Aliquot approximately 0.5 ml of BSA solution into 1.5 ml microcentrifuge tubes.
7) Label each tube with "BSA" and the lot number.
8) Store at -20°C.

Data Log	source	lot	amount
BSA	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control

QC250 - QuantiBlot Hybridization - (test 20 µL of solution)

Results: ☐ Pass ☐ Fail Initials: _____

QC240 - YM1 STR/PCR Amplification
QC165 - STR gel electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

CELL LYSIS BUFFER (CLB)

Standard batch size: 2L Lot Number: _____

Ingredients

	final concentration	amount
Sucrose	320mM	219 ± 3g
TRIS	10mM	2.4 ± 0.1g
Magnesium Chloride, Hexahydrate	5mM	2.0 ± 0.1g
Triton X-100	1.0%	20 ± ml
Hydrochloric Acid	----	as needed

Procedure

1) Dissolve the sucrose, TRIS, and magnesium chloride in approximately 1.5 L deionized water.
2) Add the Triton to the solution.
3) Adjust the pH to 7.6 (± 0.1) with hydrochloric acid
4) Mix well.
5) Adjust the volume to 2L with deionized water.
6) Filter sterilize.
7) Dispense into sterile 50ml centrifuge tubes.
8) Store at 2-8°C.

Data Log

	source	lot	amount
Sucrose	_____	_____	_____
TRIS	_____	_____	_____
Magnesium Chloride, Hexahydrate	_____	_____	_____
Triton X-100	_____	_____	_____
Hydrochloric Acid	_____	_____	_____

Quality Control

QC250 - QuantiBlot Hybridization (test 20 µL of solution)

Results: ☐ Pass ☐ Fail Initials: _____

Final pH: _____ (7.6 ± 0.1)

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

CHELEX, 5%

Standard batch size: 800ml Lot Number: _____

Application
DNA Extraction (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Chelex 100	5%	40 g
Sterile Deionized Water	----	800 ml

Procedure

1) Filter sterilize approximately 900 ml deionized water. The water will evaporate off in step 4.
2) Pour the water into a 1L bottle.
3) Save the bottom container from the disposable filter unit.
4) Autoclave the water at 250°F for 30 minutes.
5) Add 40g of the Chelex 100 to the bottom container of the filter unit.
6) Allow the water to cool after autoclaving.
7) Add sterile water to the Chelex 100 to a volume of 800 ml using the graduation markings on the disposable filter container.
8) Mix on a magnetic stir plate.
9) While the stock solution is mixing, aliquot 10 ml each into 50 ml conical tubes.
10) Label each tube with its contents, date of make (DOM), your initials, and date.
11) Store at 2-8°C.

Data Log	source	lot	amount
Chelex 100	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control

QC145 - Chelex Extraction

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

CHELEX, 20%

Standard batch size: 500ml Lot Number: _____

Application
DNA Extraction (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Chelex 100	20%	100 ± 2 g
Sterile Deionized Water	----	450 ± 50 ml (guideline)

Procedure

1) Filter sterilize approximately 600 ml deionized water. The water will evaporate off in step 4.
2) Pour the water into a 500 ml bottle.
3) Save the bottom container from the disposable filter unit.
4) Autoclave the water at 250°F for 30 minutes.
5) Add the Chelex to the bottom container of the filter unit.
6) Allow the water to cool after autoclaving.
7) Add sterile water to the Chelex to a volume of 500 ml using the graduation markings on the disposable filter container.
8) Mix on a magnetic stir plate.
9) While the stock solution is mixing, aliquot 10 ml each into 50 ml conical tubes.
10) Label each tube with its contents, date of make (DOM), your initials, and date.
11) Store at 2-8°C.

Data Log	source	lot	amount
Chelex 100	_____	_____	_____

Quality Control
QC160 - Differential Extraction

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

CHROMOGEN SOLUTION

Application
QuantiBlot Hybridization (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Chromogen:TMB	0.2%	60 mg
Ethanol, 100% Reagent Grade	--------	30 ml

Procedure

CAUTION: DO NOT USE ETHANOL STORED IN A METAL CONTAINER; ONLY USE 100% REAGENT GRADE ETHANOL.

1) Bring bottle of Chromogen:TMB to room temperature.
2) Before opening, lightly tap the bottle on the counter to bring its contents to the bottom.
3) Carefully remove the stopper and reconstitute the chromogen:TMB with the room temperature ethanol.
4) Recap the bottle and seal with Parafilm.
5) Tilt the bottle several times to ensure that all the powder is removed from within the rubber cap.
6) Shake on an orbital shaker for 30 minutes or longer.
7) Write your name and Date Of Make (DOM) on the reagent label.
8) Store at 2-8°C and away from rust.
9) The solution is stable for six months.

Quality Control
QC250 - QuantiBlot Hybridization is done on new lots/shipment of Chromogen and is documented on F183 Raw Material Quality Control Test Form.

Initials: _____ **Date:** 4/30/2003

DEOXYNUCLEOTIDE TRIPHOSPHATES, 2.5 mM (dNTPs)

Standard batch size: ~32 tubes x 1000 µL Lot Number: _____

Application
Quad and YM1 STR reaction mixes.

Ingredients	final concentration	amount
dATP, 10 mM, 320 µL/tube	2.5 mM	8000 µL (25 tubes)
dCTP, 10 mM, 320 µL/tube	2.5 mM	8000 µL (25 tubes)
dGTP, 10 mM, 320 µL/tube	2.5 mM	8000 µL (25 tubes)
dTTP, 10 mM, 320 µL/tube	2.5 mM	8000 µL (25 tubes)
Autoclaved, microcentrifuge tubes		~32 tubes

Procedure

NOTE: ALIQUOT ALL TUBES AT ONE TIME AND IN A ROOM FREE FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USE ONLY FILTER PIPET TIPS OR A REPEAT PIPETTOR FOR ALL PIPETTING.

1) Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.
2) Pool together the manufacturers' shipment of all dNTPs into a 50 ml falcon tube. Mix well.
3) While wearing clean gloves, remove all 1.5 ml microcentrifuge tubes from the bag and place them in a clean rack designated for the PCR preparation room only.
4) Aliquot 1000 µL of dNTP mix into each tube.
5) Once aliquotting is complete, cap all tubes and store in a labeled rack away from all sources of DNA. Label each tube with lot number and item description.
6) Store frozen at -20°C.

Data Log	source	lot	amount
dATP	_____	_____	_____
dCTP	_____	_____	_____
dGTP	_____	_____	_____
dTTP	_____	_____	_____

Quality Control
QC240 - PCR amplification (YM1 or Quad analysis)
QC165 - Gel Electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Cross reference (date) _____

Made By: _____ Date: _____

Initials:  **Date:** 4/30/2003

DIGEST BUFFER

Standard batch size: 4L Lot Number: _____

Application
Organic Extraction procedure (see Protocols for Forensic STR Analysis)

Ingredients	final concentration	amount
EDTA, 0.5 M	10 mM	80 ± 4 ml
TRIS	10 mM	4.8 ± 0.4 g
Sodium Chloride	50 mM	11.6 ± 0.8 g
SDS, 20%	2.0%	400 ± 4 ml
Hydrochloric Acid	-----	As needed

Procedure

1) Add the EDTA, TRIS, sodium chloride, and SDS to approximately 1.5 L deionized water.
2) Adjust the pH to 7.5 (± 0.1) with hydrochloric acid.
3) Bring up to the final volume with deionized water and mix well.
4) Measure and record the final pH.
5) Aliquot into 50 ml conical tubes.
6) Label each tube with its contents, date of make (DOM), your initials, and date.
7) Store at room temperature.

Data Log	source	lot	amount
EDTA, 0.5 M	_____	_____	_____
TRIS	_____	_____	_____
Sodium Chloride	_____	_____	_____
SDS, 20%	_____	_____	_____
Hydrochloric Acid	_____	_____	_____

Quality Control
QC250 - QuantiBlot Hybridization (Test 20 µL of solution)

Results: ☐ Pass ☐ Fail Initials: _____

Final pH: _____ (7.5 ± 0.1)

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

DITHIOTHREITOL (DTT), 1M

Standard batch size: 20 ml Lot Number: _____

Application
Differential Extraction (see Protocols for Forensic STR Analysis)

Ingredients	final concentration	amount
Dithiothreitol	1.0 M	3.06 ± 0.05 g
Sterile Deionized Water	----	19 ml

Procedure

1) Add the DTT to approximately 19 ml sterile deionized water in a 50 ml conical tube.
2) Mix well by vortex agitation.
3) When the DTT is dissolved, bring up to volume with sterile deionized water.
4) Filter sterilize.
5) Dispense 250 µL aliquots into sterile 1.5 ml microcentrifuge tubes.
6) Label with a four month expiration date, contents, and the lot number.
7) Store at -20°C.

Data Log	source	lot	amount
Dithiothreitol	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control

QC250 - QuantiBlot Hybridization (Test 20 µL of solution)

Results: ☐ Pass ☐ Fail Initials: _____

EXPIRATION DATE:

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

ETHLYENE-DIAMINE-TETRA-ACETIC ACID (EDTA), 0.5M

Standard batch size: 1L Lot Number: _____

Application
Preparation of Tris-EDTA, 1X and Digest Buffer (see Quality Manual)

Ingredients	final concentration	amount
EDTA	0.50 M	186 ± 1 g
Sodium Hydroxide, 10 N	------	variable

Procedure

1) Add the EDTA to approximately 500 ml deionized water.
2) Adjust the pH to 8.0 (± 0.1) with sodium hydroxide solution.
3) Mix well.
4) The EDTA will dissolve as the pH reaches 8.0.
5) Bring up to volume with deionized water.
6) Check and record the final pH.
7) Dispense into 125, 500, or 1000 ml bottles.
8) Autoclave at 250°F for 20 minutes.
9) Store at room temperature.

Data Log	source	lot	amount
EDTA	_____	_____	_____
Sodium Hydroxide, 10 N	_____	_____	_____

Quality Control

Final pH: _____ (8.0 ± 0.1)

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

FORMAMIDE, DEIONIZED

Standard batch size: ~36 tubes x 1400 µL Lot Number: _____

Application
STR Analysis on the ABI Prism 310 Capillary Electrophoresis Genetic Analyzer (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Formamide (super pure grade)	N/A	50 ml

Procedure

CAUTION: THIS PROCEDURE MUST BE PERFORMED UNDER THE CHEMICAL FUME HOOD! FORMAMIDE IS HARMFUL BY INHALATION, INGESTION, AND SKIN ABSORPTION. WEAR GLOVES, EYE GLASSES, AND LAB COAT.

1) Make sure that you are using a super pure grade of formamide. Super pure grade formamide has been pre-treated with a mixed-bed resin (available from commercial supplier).

2) Dispense the deionized formamide into 1.5 ml reaction tubes in aliquots of 1400 µL and store at -15 to -20°C.

3) Label the tube rack with the lot number, the date of make (DOM), and initials.

Data Log	source	lot	amount
Formamide	_____	_____	_____

Quality Control
QC130 - Capillary electrophoresis (ABI 310) performed on new vendor lots/shipments of reagent.

Results: ☐ Pass ☐ Fail Initials: _____

Cross reference (date) _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

FORMAMIDE AND LOADING BUFFER (5:1)

Standard batch size: 48 ml (40 tubes) Lot Number: _____

Application
Gel Electrophoresis on the ABI 377 Sequencer (see Protocols for Forensic STR Analysis)

Ingredients	final concentration	amount
Formamide	83%	40 ml ± 0.8 ml
Sequencing Loading Buffer	17%	8 ml ± 0.4 ml

Procedure

1) Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper. Label 40 1.5ml reaction tubes in preparation for step 4.

2) Add formamide and sequencing loading buffer to a 50 ml conical tube. Mix well.

3) Perform the Quality Control test listed below and document the results.

4) Aliquot approximately 1.2 ml of the solution into each of the 40 1.5 ml microcentrifuge tubes.

5) Store at 2-8°C.

Data Log	source	lot	amount
Formamide	_____	_____	_____
Sequencing Loading Buffer	_____	_____	_____

Quality Control
QC165 - STR gel electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Cross reference (date) _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

HYDROGEN PEROXIDE, 3%

Standard batch size: ~90 X 0.2 ml Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Hydrogen Peroxide, 30%	3%	1.5 ml ± 0.1 ml

Procedure

1) Add hydrogen peroxide to a 15 ml disposable tube.

2) Add deionized water to a final volume of 15 ml.

3) Aliquot approximately 130 µl of hydrogen peroxide into 1.5 ml brown microcentrifuge tubes.

4) Label the rack with a one month expiration date. Enter the expiration date into the Quality Assurance Chemical/Reagent Database.

5) Store at 2-8°C in the dark.

Data Log

	source	lot	amount
Hydrogen Peroxide, 30%	_____	_____	_____

EXPIRATION DATE:

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

NEGATIVE FEMALE CONTROL DNA

Standard batch size: 10 ml Lot Number: _____

Application - YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Procedure

1) **Stock solution:**
For the stock solution, extract a 3cm x 3cm portion of the dried bloodstain or 1/3 of an oral swab following the organic extraction procedure in the Protocols for Forensic STR Analysis Manual. Adjust the final volume to 200µL. Submit a 1/100 and a 1/ 1000 dilution for QuantiBlot.

Data Log	Source	Date prepared	DNA concentration
Bloodstain or Oral Swab	_____	_____	_____

2) **Working solution:**

Based on the Quantiblot results prepare 10 tubes with 1ml of a dilution with a concentration of 2ng/20µL.

Use the following formula: **C1 x V1 = C2 x V2**
(1000 µL)(2 ng/20 µL) = (z)(DNA concentration)
z = required volume of DNA per ml

Prepare ten (10) 1.5 ml microcentrifuge tubes with TE^{-4} (1000 µL - the req. DNA vol.). Add the DNA to each tube. Mix well.

Submit 25µL of each tube for QuantiBlot. The tubes should come back with a reading of 2.5ng. Discard tubes that have readings <1.25. Tubes with readings of 1.25 or 5 ng should be amplified and checked if the expected peak heights can be achieved.

Data Log	source	lot	amount
DNA stock	_____	_____	_____
TE^{-4}	_____	_____	_____

Quality control
QC240 - Y STR amplification for 4 of the 10 tubes.

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

ORGANIC EXTRACTION BUFFER

Standard batch size: 1 L Lot Number: _____

Application
Organic Extraction (see Protocols for Forensic STR Analysis)

Ingredients	final concentration	amount
Tris	10 mM, pH 8.0	1.2 g
EDTA, Disodium Salt, Dihydrate	50 mM, pH 8.0	18.6 g
NaCl	100 mM	5.8 g

Procedure

5. Measure 500 ml deionized water into a beaker.
6. Add 1.2 g Tris and dissolve with agitation and mild heat.
7. Check the pH and adjust to approximately 7.5 (+/- 0.1) by adding either HCl or NaOH dropwise.
8. Add 18.6 g EDTA and allow it to dissolve.
9. Again, adjust the pH to approximately 7.5 (+/- 0.1) by adding either HCl or NaOH dropwise.
10. Add 5.8 g NaCl to the solution and allow it to dissolve.
11. Adjust the volume to 1 L with ddH_2O.
12. Autoclave the solution for 25 minutes.
13. Transfer into labeled 50 mL conical vials.

Data Log	source	lot	amount
Tris	_____	_____	_____
EDTA	_____	_____	_____
NaCl	_____	_____	_____

Quality Control

Final pH: _____ (7.5 ± 0.1)

QC250 - QuantiBlot Hybridization - (test 20 µL of solution)

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PCR REACTION MIXTURE (COFILER AND PROFILER PLUS)

Standard batch size: 100-800 tubes x 20 µL Lot Number: _____

Application
Cofiler and Profiler PCR Amplification (see Protocols for Forensic STR Analysis)

Ingredients	Final Conc	1 Tube Amount	100 Tubes	200 Tubes	400 Tubes	800 Tubes
PCR Reaction Mix	1x	20µL	2000µL	4000µL	8000µL	16000µL
AmpliTaq Gold	5U	1µL	100µL	200µL	400µL	800µL

Procedure

NOTE: **ALIQUOT ALL TUBES AT ONE TIME AND IN A ROOM FREE FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.**

1) Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.
2) Add the ingredients to either a 1.5 ml microcentrifuge tube or a 15 ml conical tube using pipetmen dedicated to PCR preparation area only. Vortex and spin the reaction mixture briefly.
3) While wearing clean gloves, remove a sufficient amount of 0.2 ml PCR reaction tubes from the bag and place them in a clean rack designated for the PCR prep room only.
4) Add 20 µL per tube using a designated repeat pipettor or tips with hydrophobic filters.
5) Cap all tubes and store in a labeled rack away from all sources of DNA.
6) Store at 2-8˚C.

Data Log	source	lot	amount
Reaction Mix	_____	_____	_____
AmpliTaq Gold	_____	_____	_____

Quality Control
QC110 Amplification Kits - Only for the first kit of each shipment/lot

Results: ☐ Pass ☐ Fail Initials: _____

Cross reference (date) _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PHOSPHATE BUFFERED SALINE (PBS)

Standard batch size: 4L Lot Number: _____

Application
Chelex Extraction (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Phosphate Buffered Saline (PBS) Tablets	N/A	20

Procedure

1) Dissolve the tablets in 4 liters of deionized water.
2) Measure and record the final pH (should be 7.5 ± 0.1).
3) Autoclave at 250°F for 20 minutes. Allow to cool.
4) Dispense into 50 ml conical tubes. Label each tube with contents, DOM, and your initials.
5) Store at room temperature.

Data Log

	source	lot	amount
PBS Tablets	_____	_____	_____

Quality Control

Final pH: _____ (7.5 ± 0.1)

QC250 - Quantiblot Hybridization

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

POSITIVE CONTROL FOR QUAD ANALYSIS

Standard batch size: ~3 ml Lot Number: _____
DNA concentration: Approximately 1.25-2.5 ng/20 µL

Application

Quad STR Analysis (see Protocols for Forensic STR Analysis)

Procedure

1) Prepare bloodstain card(s) such that at least 20 to 30, 3x3 mm cuttings can be obtained. It is preferable that the donor is heterozygous at all four Quad loci.
2) Place each 3x3 mm cutting into a fresh microcentrifuge tube and perform Chelex extraction as described in Protocols for Forensic STR Analysis.
3) Pool the extracts into a 15 ml Falcon tube and keep refrigerated while determining the DNA concentration of this solution by Quantiblot analysis as described in Protocols For Forensic STR Analysis.
4) Amplify three samples of the current positive control so that one sample contains 0.5 ng, one sample contains 1 ng, and one sample contains 2 ng of DNA based on the Quantiblot results, as well as a sample of the most recent past lot of positive control. Electrophorese and analyze samples. All four samples must yield the correct type.
5) Determine the working dilution of the positive control by comparing the results of all three samples and determining which one produces peaks mostly in the range of 1000-3000 RFUs.
6) Prepare the working stock of the positive control in a 50 ml Falcon tube using the calculations shown below. **Take precaution to dispense these volumes accurately and vortex the resulting dilution!!!**
7) Dispense 27 µL aliquots into approximately one-hundred (100) 0.5 ml PCR reaction tubes for immediate use as positive control samples in casework. Freeze the remainder in 1.5ml microcentrifuge tubes to contain approximately 300 µL aliquots each. When necessary, thaw one tube and dispense another set of 100, 0.5 ml PCR reaction tubes to contain 27 µL each of the working stock of PE.

Calculations

$z = \frac{\text{total volume of positive control yielded by extraction}}{\text{volume of positive control that yielded best result}} =$ # of positive control tubes that can be made (round down to nearest whole number)

x = volume of positive control to add per tube (eg., 1.6 µL)
y = 27 -x = volume of TE^{-4} to add per tube (eg., 27 - 1.6 = 25.4 µL)

x(z) + y(z) = volumes of ingredients to add in a 50 ml conical tube for final dilution. Mix and dispense as discussed in step 7 above.

(Next Page)

Initials: **Date:** 4/30/2003

POSITIVE CONTROL FOR QUAD ANALYSIS

Lot Number: _____

Data Log

	Source	[Initial] (via Q-blot)	z	x(z)	y(z)
Bloodstain	_____	_____	_____	_____	_____
TE^{-4}	_____				

Quality Control

Results: ☐ Pass ☐ Fail Initials: _____

Cross reference (date) _____

(Attach Q-blot sheet, Amplification sheet and Electropherograms to the Reagent Sheet)

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

POSITIVE MALE CONTROL DNA

Standard batch size: 10 ml Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Procedure

1) **Stock solution:**
Extract a 3cm x 3cm portion of a dried bloodstain following the organic extraction procedure in the "Protocols for Forensic STR Analysis" manual. Adjust the final volume to 200µL. Submit a 1/100 and a 1/1000 dilution for QuantiBlot.

Data Log	Source	Date prepared	DNA concentration
Bloodstain	_____	_____	_____

2) **Working solution:**

Based on QuantiBlot results, prepare 10 tubes with 1ml of a dilution with a concentration of 2ng/20µL.

Use the following formula: **C1 x V1 = C2 x V2**

(1000 µL)(2 ng/20 µL) = (z)(DNA concentration)

z = required volume of DNA per ml

Prepare ten (10) microcentrifuge tubes with TE^{-4} (1000µL - the req. DNA vol.). Add the DNA to each tube. Mix well.

Submit 25µL of each tube for Quantiblot. The tubes should come back with a reading of 2.5ng. Discard tubes that have readings <1.25. Tubes with readings of 1.25 or 5 ng should be amplified and checked if the expected peak heights of 500-3000 RFUs can be achieved. Discard the tubes with peak heights outside of this range.

Data Log	source	lot	amount
DNA stock	_____	_____	_____
TE^{-4}	_____	_____	_____

Quality control
QC240 - Y STR amplification for 4 of the 10 tubes.

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PRIMER, DYS19/1 (50 pM/µL) Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence NED - 5' CTA CTG AGT TTC TGT TAT AGT 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
DYS19/1 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure
1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log

	source	lot	amount
Primer DYS19/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculations checked by _____

Quality Control
QC240 PCR Amplification (Y STR) and electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PRIMER, DYS19/2 (50 pM/µL) Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence 5' ATG GCA TGT AGT GAG GAC A 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
DYS19/2 primer		50 pM/µL	----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure
1) Add the sterile deionized water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log

	source	lot	amount
Primer DYS19/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (Y STR) and electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PRIMER, DYS389/1 (50 pM/µL) Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence NED - 5' CCA ACT CTC ATC TGT ATT ATC T 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
DYS389/1 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log	source	lot	amount
Primer DYS389/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (Y STR) and electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PRIMER, DYS389/2 (50 pM/µL) Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence 5' TCT TAT CTC CAC CCA CCA GA 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
DYS389/2 primer		50 pM/µL	----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log	source	lot	amount
Primer DYS389/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (Y STR) and electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PRIMER, DYS390/1 (50 pM/µL) Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence 6-FAM - 5' TAT ATT TTA CAC ATT TTT GGG CC 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
DYS390/1 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log

	source	lot	amount
Primer DYS390/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (Y STR) and electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PRIMER, DYS390/2 (50 pM/µL) Lot Number: _____

Application
YM1 STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence 5' TGA CAG TAA AAT GAA CAC ATT GC 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
DYS390/2 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log	source	lot	amount
Primer DYS390/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (Y STR) and electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PRIMER, F13A1/1 (50 pM/µL) Lot Number: _____

Application
QUAD STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence JOE - 5' AT GCC ATG CAG ATT AGA AA 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
F13A1/1 primer		50 pM/µL	----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log

	source	lot	amount
Primer F13A1/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (QUAD STR) and Electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PRIMER, F13A1/2 (50 pM/µL) Lot Number: _____

Application
QUAD STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence 5' GAG GTT GCA CTC CAG CCT TT 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
F13A1/2 primer		50 pM/µL	----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log	source	lot	amount
Primer F13A1/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (QUAD STR) and Electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PRIMER, FES/FPS/1 (50 pM/µL) Lot Number: _____

Application
QUAD STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence 5' GG GAT TTC CCT ATG GAT TGG 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
FES/FPS/1 primer		50 pM/µL	----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log	source	lot	amount
Primer FES/FPS/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (QUAD STR) and Electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PRIMER, FES/FPS/2 (50 pM/µL) Lot Number: _____

Application
QUAD STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence 6-FAM - 5' GCG AAA GAA TGA GAC TAC AT 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
FES/FPS/2 primer		50 pM/µL	----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log	source	lot	amount
Primer FES/FPS/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (QUAD STR) and Electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PRIMER, TH01/1 (50 pM/µL) Lot Number: _____

Application
QUAD STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence 6-FAM - 5' GT GGG CTG AAA AGC TCC CGA TTA T 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
TH01/1 primer		50 pM/µL	----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure
1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log

	source	lot	amount
Primer TH01/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (QUAD STR) and Electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: _____ **Date:** 4/30/2003

PRIMER, TH01/2 (50 pM/µL) Lot Number: _____

Application
QUAD STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence 5' GTG ATT CCC ATT GGC CTG TTC CTC 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
THO1/2 primer		50 pM/µL	----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure
1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log

	source	lot	amount
Primer THO1/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (QUAD STR) and Electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

PRIMER, VWA/1 (50 pM/µL) Lot Number: _____

Application
QUAD STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence JOE - 5' CC CTA GTG GAT GAT AAG AAT AAT CAG TAT 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
VWA/1 primer		50 pM/µL	----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log	source	lot	amount
Primer VWA/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (QUAD STR) and Electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: _____ **Date:** 4/30/2003

PRIMER, VWA/2 (50 pM/µL) Lot Number: _____

Application
QUAD STR Analysis (see Protocols for Forensic STR Analysis)

Physical data

Sequence 5' GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (µL)
VWA/2 primer		50 pM/µL	----
Sterile Deionized Water	-----	-----	

Calculations
Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

1) Add the sterile water to the original primer tube. Mix well.
2) Dispense 200 µL aliquots into 1.5 ml microcentrifuge tubes. Label each tube with its contents and lot number.
3) Store at -20°C.

Data Log	source	lot	amount
Primer VWA/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control
QC240 PCR Amplification (QUAD STR) and Electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

QUANTIBLOT CITRATE BUFFER

Standard batch size: 8 L Lot Number: _____

Ingredients

	final concentration	amount
Trisodium Citrate	0.06 M	147.2 ± 0.2 g
Citric Acid	0.025 M	43.4 ± 2 g (guideline)

Procedure

1) Dissolve the sodium citrate in approximately 6 L deionized water in a carboy.
2) Adjust the pH to 5.0 (± 0.2) by addition of citric acid (approximately 40 g).
3) Adjust the final volume to 8 liters with deionized water.
4) Mix well.
5) Measure and record the final pH (must be 5.0 ± 0.2).
6) Aliquot into amber bottles and store at room temperature.

Data Log

	source	lot	amount
Trisodium Citrate	_____	_____	_____
Citric Acid	_____	_____	_____

Quality Control

Final pH: _____ (5.0 ± 0.2)

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

QUAD STR/PCR REACTION MIXTURE

Standard batch size: 50-200 tubes Lot Number: _____

Ingredients:

	Final Concentration	1 Tube Amount	50 Tubes	100 Tubes	200 Tubes
10X PCR Buffer II	1X	5 µL	250 µL	500 µL	1000 µL
dNTP's (2.5 mM)	200 µM	4 µL	200 µL	400 µL	800 µL
sterile dH20	--------	6.6 µL	331 µL	662 µL	1324 µL
BSA (5 mg/ml)	160ug/ml	1.6 µL	80 µL	160 µL	320 µL
VWA/1 (50 pM/µL)	0.22 µM	0.22 µL	11 µL	22 µL	44 µL
VWA/2 (50 pM/µL)	0.22 µM	0.22 µL	11 µL	22 µL	44 µL
THO1/1(50 pM/µL)	0.22 µM	0.22 µL	11 µL	22 µL	44 µL
THO1/2 (50 pM/µL)	0.22 µM	0.22 µL	11 µL	22 µL	44 µL
F13A1/1 (50 pM/µL)	0.25 µM	0.25 µL	17 µL	25 µL	50 µL
F13A1/2 (50 pM/µL)	0.25 µM	0.25 µL	17 µL	25 µL	50 µL
FES/1 (50 pM/µL)	0.20 µM	0.20 µL	10 µL	20 µL	40 µL
FES/2 (50 pM/µL)	0.20 µM	0.20 µL	10 µL	20 µL	40 µL
AmpliTaq (5u/µL)	5 U.	1 µL	50 µL	100 µL	200 µL
TOTAL		20 µL	1 ml	2 ml	4 ml

Procedure

NOTE: **ALIQUOT ALL TUBES AT ONE TIME AND IN A ROOM FREE FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.**

1) Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper

2) Add the ingredients to either a 1.5 ml microcentrifuge tube or a 15 ml centrifuge tube using pipetmen designated to PCR preparation area only. Vortex and spin the reaction mixture briefly.

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3) While wearing clean gloves, remove sufficient amount of 0.5 ml tubes from the bag and place them in a clean rack designated for the PCR prep room only.

4) Add 20 µL per tube using a designated repeat pipettor or tips with hydrophobic filters.

5) Cap all tubes and store in a labeled rack away from all sources of DNA.

6) Store at 2-8˚C.

Data Log	source	lot	amount
10X PCR Buffer II			
dNTP's (2.5 mM)			
Sterile dH_20			
BSA (5 mg/ml)			
VWA/1 (50 pM/µL)			
VWA/2 (50 pM/µL)			
THO1/1 (50 pM/µL)			
THO1/2 (50 pM/µL)			
F13A1/1 (50 pM/µL)			
F13A1/2 (50 pM/µL)			
FES/1 (50 pM/µL)			
FES/2 (50 pM/µL)			
AmpliTaq (5u/µL)			

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

QUANTIBLOT DNA STANDARDS

Standard batch size: variable Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients	final concentration	amount
DNA Standard A	varies	1000 µl
TE^{-4}, 1X	1X	3000 µl

Procedure

1. Pool the contents of four DNA Standard A tubes (use all from one lot number). Each tube contains 250 µL of standard. Vortex to mix thoroughly and centrifuge briefly.
3. Label seven sterile1.5 ml microcentrifuge tubes, A - G.
4. Aliquot 500 µL of 1X TE^{-4} into the six tubes labeled B - G.
5. **Tube A:** Transfer 1000 µL of DNA Standard A into the tube labeled A. This is now DNA Standard A. **Tube B:** Add 500 µL of DNA Standard A to the 500 µL of 1X TE^{-4} in tube B. Vortex to mix thoroughly/centrifuge briefly. **Tube C:** Add 500 µL of DNA Standard B to the 500 µL of 1X TE^{-4} in tube C. Vortex to mix thoroughly/centrifuge briefly. Continue the serial dilution through tube 1G.
6. Store at 2° to 8°C. Label with an expiration date of 3 months.

Standard Tube	Concentration (ng/µL)	Quantity (ng/5µL)
1A	2	10
1B	1	5
1C	0.5	2.5
1D	0.25	1.25
1E	0.125	0.625
1F	0.0625	0.3125
1G	0.03125	0.15625

Data Log

	source	lot	amount
DNA Standard A			
TE^{-4}, 1X			

EXPIRATION DATE:

Quality Control
QC250 - QuantiBlot Hybridization

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

QUANTIBLOT HYBRIDIZATION SOLUTION

Standard batch size: 6 L Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
SSPE, 20X	5.0 X	1500 ± 10 ml
SDS, 20%	0.50 %	150 ± 1 ml

Procedure

1) Combine the SSPE and 4350 ml deionized water into a carboy.
2) Add the SDS.
3) Warm the solution until all solids are dissolved.
4) Mix well.
5) Dispense into 1 L pre-labeled bottles.
6) Store at room temperature.

Data Log	source	lot	amount
SSPE, 20X	_____	_____	_____
SDS, 20%	_____	_____	_____

Quality Control

QC250 Quantiblot Hybridization

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

QUANTIBLOT PRE-WETTING SOLUTION

Standard batch size: 4 L Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
NaOH, 10 N	0.4 N	160 ± 10 ml
EDTA, 0.5 M	25 mM	200 ± 10 ml

Procedure

1) Measure 3640 ml deionized water into a 4 L erlenmeyer flask.
2) Add 160 ml NaOH and 200 ml EDTA.
3) Mix well.
4) Dispense into 1 L pre-labeled bottles.
5) Store at room temperature.

Data Log	source	lot	amount
NaOH, 10 N	_____	_____	_____
EDTA, 0.5 M	_____	_____	_____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

QUANTIBLOT SPOTTING SOLUTION

Standard batch size: variable Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
Pre-Wetting Solution	-----	74.85 ml (± 1ml) per bottle
Bromothymol Blue, 0.04%	0.00008%	150 µL (± 1 µL) per bottle

Procedure

1) Measure 74.85 ml Pre-Wetting Solution into a graduated cylinder and pour into a pre-labeled 100 ml bottle.

2) Repeat for remaining bottles, if necessary.

3) Add 150 µL bromothymol blue to each bottles.

4) Cap and mix well by inverting.

5) Store at room temperature.

Data Log

Bottles made: _____

	source	lot	amount
Pre-Wetting Solution	_____	_____	_____
Bromothymol Blue, 0.04%	_____	_____	_____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

QUANTIBLOT WASH SOLUTION

Standard batch size: 20 L Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
SSPE, 20X	2.5 X	2500 ± 50 ml
SDS, 20%	0.10 %	100 ± 5 ml

Procedure

1) Add 2500 ml SSPE and 17.4 L deionized water into a carboy.
2) Add in 100 ml 20% SDS.
3) Mix well.
4) Aliquot into five 4L brown, pre-labeled bottles.
5) Store at room temperature

Data Log	source	lot	amount
SSPE, 20X	_____	_____	_____
SDS, 20%	_____	_____	_____

Quality Control

QC250 Quantiblot hybridization

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

SEQUENCING LOADING BUFFER

Standard batch size: 25 ml Lot Number: _____

Ingredients

	final concentration	amount
500 mM EDTA, pH8.0	25 mM	1.25 ± 0.05 ml
Blue Dextran	50 mg/ml	1250 mg ± 10 mg

Procedure

NOTE: PREPARE AWAY FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.

1) Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.
2) Pipette EDTA into a 25 ml cylinder. Fill up to 25 ml using deionized water.
3) Decant into an 100 ml Erlenmeyer flask. Add Blue Dextran. Stir at room temperature until dissolved.
4) Label 25 1.5 ml reaction tubes.
5) Add 1000 µL of the sequencing loading buffer to each tube. Close all tubes.
6) Store at 2-8°C.

Data Log

	source	lot	amount
0.5 M EDTA, pH 8.0	_____	_____	_____
Blue Dextran	_____	_____	_____

Quality Control

QC165 - STR gel electrophoresis

Results: ☐ Pass ☐ Fail Initials: _____

Cross Reference (date) _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

SODIUM DODECYL SULFATE (SDS), 0.1%

Standard batch size: 20 L Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients	final concentration	amount
Sodium Dodecyl Sulfate (SDS), 20%	0.1 %	100 ± 10 ml
	OR	
SDS (solid)	0.1%	20 ± 0.2 g

Procedure

NOTE: This solution is "made at the bench," no reagent sheet is required.

1) Add approximately 15 L of deionized water into a 20 L carboy.
2) Add 100 ml 20% SDS. Mix.
3) Bring up to a final volume of 20 L with deionized water. Mix.
4) Store at room temperature.

OR

1) Warm approximately 750 ml deionized water on a stirring hot plate.
2) Add the SDS (solid) and allow to dissolve.
3) When the solution is clear, bring up to a final volume of 20 L with deionized water.
4) Store at room temperature.

Initials: **Date:** 4/30/2003

SODIUM DODECYL SULFATE (SDS), 20%

Standard batch size: 1 L Lot Number: _____

Ingredients	final concentration	amount
Sodium Dodecyl Sulfate (solid)	20 %	200 ± 5 g

Procedure

CAUTION: **AN AEROSOL MASK OR FUME HOOD MUST BE USED WHEN MAKING THIS SOLUTION. EYE PROTECTION IS RECOMMENDED.**

1) Warm approximately 750 ml deionized water on a stirring hot plate.
2) Add a fraction of the SDS, allowing the solids to dissolve before adding more.
3) Add the SDS until it is all in solution.
4) When the solution is clear, bring up to volume with deionized water.
5) Filter sterilize the warm solution.
6) Store at room temperature.

Data Log	source	lot	amount
Sodium Dodecyl Sulfate	_____	_____	_____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

SSPE, 20X

Standard batch size: 8 L Lot Number: _____

Application
QuantiBlot Analysis (see Protocols for Forensic STR Analysis)

Ingredients

	final concentration	amount
EDTA, Disodium Salt	20 mM	59.6 ± 1.4 g
Sodium Hydroxide, 10 N	-----	80 ± 10 ml (guideline)
Sodium Phosphate, Monobasic	200 mM	220 ± 6 g
Sodium Chloride	3.6 M	1680 ± 20 g

Procedure

1) Dissolve the EDTA in approximately 6 liters deionized water (use SSPE carboy).
2) Adjust the pH to approximately 6.0 with 10N sodium hydroxide to help dissolve the EDTA.
3) Add the sodium phosphate first and then the sodium chloride.
4) Adjust the pH to 7.4 with 10N sodium hydroxide (about 80 ml).
5) Adjust the final volume to 8 liters with deionized water.
6) Measure and record the final pH.
7) Store at room temperature.

Data Log	source	lot	amount
EDTA, Disodium Salt	_____	_____	_____
Sodium Hydroxide, 10N	_____	_____	_____
Sodium Phosphate, Monobasic	_____	_____	_____
Sodium Chloride	_____	_____	_____

Quality Control

Final pH: _____ (7.4 ± 0.2)

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

STERILE DEIONIZED WATER

Standard batch size: 2L Lot Number: _____

Application
DNA Extraction (see Protocols for Forensic STR Analysis)

Procedure

1) Filter sterilize 2 L of deionized water.

2) Autoclave at 250°F for 20 minutes.

3) Aliquot 10 ml each into 15 ml centrifuge tubes (200 tubes). Label each tube with its contents, the date of make (DOM), and your initials.

4) Store at room temperature.

Quality Control

QC250 - Quantiblot Quality Control of Solutions (test 20 µL of Solution)

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

TRIS-EDTA (TE^{-4}), 1X

Standard batch size: 500 ml Lot Number: _____

	Ingredients	final concentration	amount
1)	TRIS-HCl, pH 8.0, 1 M	10 mM	5.0 ± 0.3 ml
2)	EDTA, 0.5 M	0.1 mM	100± 2µL

Procedure

1) Add the TRIS and EDTA to 495 ml deionized water. Mix well and filter.
2) Autoclave at 250°F for 20 minutes.
3) Dispense into 15 ml sterile centrifuge tubes. Label each tube with its contents, the date of make (DOM), and your initials.
4) Store at room temperature.

Data Log	source	lot	amount
TRIS-HCl, pH 8.0, 1 M	_____	_____	_____
EDTA, 0.5 M	_____	_____	_____

Quality Control

Final pH: _____ (7.4 ± 0.2)

QC250 - QuantiBlot Quality Control of Solutions (test 20 µL of solution)

Results: ☐ Pass ☐ Fail Initials: _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

TRIS-HCl, 1M - pH 8.0

Standard batch size: 500 ml Lot Number: _____

Ingredients

	final concentration	amount
TRIS	1.00 M	60.5 ± 0.1 g
Hydrochloric Acid	------	variable

Procedure

1) Add the TRIS to approximately 400 ml deionized water.

2) Mix well.

3) Adjust the pH to 8.0 with concentrated hydrochloric acid.

4) Bring up to final volume with deionized water.

5) Measure and record the final pH.

6) Prepare a 1/100 dilution (10 mM TRIS-HCl) by mixing 1 ml TRIS-HCl solution and 99 ml deionized water.

7) Autoclave at 250°F for 20 minutes.

8) Store at room temperature.

Data Log	source	lot	amount
TRIS	_____	_____	_____
Hydrochloric Acid	_____	_____	_____

Quality Control

Final pH: _____ (8.0 ± 0.1)

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

UREA (10.8 g)

Standard batch size: ~ 25 tubes x 10.8 g Lot Number: _____

Application
Gel casting for the ABI 377 sequencer (see Protocols for Forensic STR Analysis)

Ingredients	Aliquot	Total Amount
Urea (Electrophoresis Grade)	10.8 ± 0.1 g	270 ± 2.5 g

Procedure

CAUTION: WHEN WORKING WITH POWDERED UREA WEAR GLOVES, EYE PROTECTION, LAB COAT, AND RESPIRATOR FOR SAFETY.

1) Using small weigh boat, weigh 10.8 ± 0.1 g aliquots of urea.

2) Transfer the aliquots to labeled 50 ml conical tubes.

3) Cap all tubes tightly and store in a rack. Label with contents, lot number, date, and your initials.

4) Store at room temperature.

Data Log	source	lot	amount
Urea	_____	_____	_____

Quality Control

QC165 - STR gel electrophoresis (only on new vendor lots/shipments)

Results: ☐ Pass ☐ Fail Initials: _____

Cross reference (date) _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

UREA (18 g)

Standard batch size: ~25 tubes x 18g Lot Number: _____

Application
Gel casting for the ABI 377 sequencer (see Protocols for Forensic STR Analysis)

Ingredients	Aliquot	Total Amount
Urea (Electrophoresis Grade)	18 ± 0.1 g	450 ± 4 g

Procedure

CAUTION: WHEN WORKING WITH POWDERED UREA WEAR GLOVES, EYE PROTECTION, LAB COAT, AND RESPIRATOR FOR SAFETY.

1) Using small weigh boat, weigh 18 ± 0.1 g aliquots of urea.

2) Transfer the aliquots to 50 ml conical tubes.

3) Cap all tubes tightly and store in a rack. Label with contents, lot number, date, and your initials.

4) Store at room temperature.

Data Log	source	lot	amount
Urea	_____	_____	_____

Quality Control

QC165 - STR gel electrophoresis (only on new vendor lots/shipments)

Results: ☐ Pass ☐ Fail Initials: _____

Cross reference (date) _____

Made By: _____ Date: _____

Initials: **Date:** 4/30/2003

YM1 STR/PCR REACTION MIXTURE

Standard batch size: 50 to 200 tubes Lot Number: _____

Ingredients:

	Final Concentration	1 Tube Amount	50 Tubes	100 Tubes	200 Tubes
10X PCR Buffer II	1X	5 µL	250 µL	500 µL	1000 µL
dNTP's (2.5 mM)	200 µM	4 µL	200 µL	400 µL	800 µL
sterile dH_20	--------	7.4µL	370 µL	740 µL	1480µL
BSA (5 mg/ml)	160µg/ml	1.6 µL	80 µL	160 µL	320 µL
DYS19/1 (50 pM/µL)	0.24 µM	0.24 µL	12 µL	24 µL	48 µL
DYS19/2 (50 pM/µL)	0.24 µM	0.24 µL	12 µL	24 µL	48 µL
DYS390/1 (50 pM/µL)	0.24µM	0.24 µL	12 µL	24 µL	48 µL
DYS390/2 (50 pM/µL)	0.24 µM	0.24 µL	12 µL	24 µL	48 µL
DYS389/1 (50 pM/µL)	0.12 µM	0.12 µL	6 µL	12 µL	24 µL
DYS389/2 (50 pM/µL)	0.12 µM	0.12 µL	6 µL	12 µL	24 µL
AmpliTaq Gold (5u/µL)	4 U	0.8 µL	40 µL	80 µL	160 µL
TOTAL		20 µL	1 ml	2 ml	4 ml

Procedure

NOTE: **ALIQUOT ALL TUBES AT ONE TIME AND IN A ROOM FREE FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.**

1) Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

2) Add the ingredients to either a microcentrifuge tube or a 15 ml centrifuge tube using pipetmen dedicated to PCR preparation area only.

3) While wearing clean gloves, remove sufficient amount of tubes from the bag and place them in a clean rack designated for the PCR prep room only.

(Next Page)

Initials: **Date:** 4/30/2003

YM1 STR/PCR REACTION MIXTURE **(Page 2 of 2)**

Procedures (continued)

4) Vortex and spin briefly. Add 20 µL per 0.2ml tube using a dedicated repeat pipettor or tips with hydrophobic filters.

5) Cap all tubes and store in a labeled rack away from all sources of DNA.

6) Store at 2-8˚C.

Data Log	source	lot	amount
10X PCR Buffer II			
dNTP's (2.5 mM)			
sterile dH_20			
BSA (5 mg/ml)			
DYS19/1 (50 pM/µL)			
DYS19/2 (50 pM/µL)			
DYS390/1 (50 pM/µL)			
DYS390/2 (50 pM/µL)			
DYS389/1 (50 pM/µL)			
DYS389/2 (50 pM/µL)			
AmpliTaq Gold (5u/µL)			

Made By: _____ Date: _____

Initials: **Date:** 4/23/2004

POSITIVE PDMS CONTROL

Standard batch size: 50 swabs Lot Number: _____

Application
Lubricant Analysis (see Condom Trace Evidence Analysis Manual)

Ingredients		final concentration	amount
Polydimethylsiloxane	(PDMS)	1.0%	0.5 ml
Methylene Chloride		N/A	49.5 ml

Procedure

CAUTION: **Methylene chloride is mutagenic, teratogenic, and carcinogenic. All work with methylene chloride must be done under a chemical fume hood and proper safety equipment (neoprene gloves, lab coat, etc.) must be worn.**

1. **Stock solution:**
   - A. Transfer between 0.75mL to 1mL of Polydimethylsiloxane into a 1.5mL microcentrifuge tube using a disposable 3 mL transfer pipet. It may be necessary to cut the bottom of the transfer pipet to achieve a larger hole.
   - B. Dispense 49.5 mL of QC'd methylene chloride into a 125 mL Erlenmeyer Flask. Label the flask as "1% PDMS."
   - C. Transfer approximately 0.5 mL of PDMS into the Erlenmeyer Flask using a disposable 3 mL transfer pipet and rinse pipette tip in the solution. Swirl flask and cover.

2) **Preparation of Swabs:**
   - A. Obtain 25 Sterile Cotton-Tipped Applicators. Remove applicators from packaging and place in a suitable holder.
   - B. Dip each swab, one at a time, into the 1% PDMS stock solution and let dry for 30 minutes. Discard any unused portion of the 1% PDMS stock solution into an organic waste container.
   - C. Label 50 micro centrifuge tubes with the DOM, lot number, and initials
   - D. After the swabs have dried, use disinfected scissors and pincers to cut two small pieces off the tip of each swab. Be careful not to cut the wood stick that the swab is attached to during this process. Place each piece into an individual microcentrifuge tube.
   - E. Cap all tubes tightly and store in a rack. Label rack with lot number, initials, and date of manufacture (DOM).

Data Log	source	lot	amount
Polydimethylsiloxane	_____	_____	_____
Methylene Chloride	_____	_____	_____

Quality control
FTIR analysis - Follow the procedures found in the Condom Trace Evidence Analysis Manual to obtain a FT-IR spectra of the positive control. Perform a library search. Search results must indicate PDMS as the first "hit." Print and include the results of this search.

Results: ☐ Pass ☐ Fail Initials: _____

Made by: _____ Date: _____

Initials: **Date:** 4/30/2003

Appendix B

QC procedures used in the OCME Forensic Biology Laboratory are contained in this appendix. These procedures are divided into two parts: 1) General and Analytical Methods, and 2) Calibration and Maintenance. The General and Analytical Methods section refers to QC procedures for the testing of reagents that are used in various analytical methods in the laboratory. Also included in this section are general QC procedures that are used to insure an appropriate laboratory environment for the performance of the various analytical methods. The Calibration and Maintenance section includes QC procedures that are done to monitor and insure the optimum performance of various instruments and apparatus used in the laboratory.

1. QC Procedures: General and Analytical Methods

Initials: **Date:** 4/30/2003

2. QC Procedures: Calibration and Maintenance

Initials: **Date:** 4/30/2003

QC100ACID PHOSPHATASE SPOT TEST REAGENT

Test Materials
Acid Phosphatase Spot Test Reagent

Samples
Whole human semen
Deionized water

Procedure

1. Prepare 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64 dilutions of whole human semen with deionized water or saline.

2. Prepare dried stains of each dilution (including a neat semen stain) on stain cards. Fresh dilutions should be prepared every 3 months.

3. Perform the spot test on each stain and on a negative control (deionized water) stain as specified in the Biochemistry Methods Manual.

Specifications
Positive results should be obtained on each semen dilution stain.
Negative results must be obtained with the negative control stain.

Documentation
Write test results on the reagent sheet and file into the appropriate QC reagent binder.

Initials: **Date:** 4/30/2003

QC105 ALPHA-AMYLASE GEL RADIAL DIFFUSION

Test Materials
Amylase Gel Buffer
Alpha-Amylase Standard (only for new shipments)

Samples
Alpha-Amylase Standards
Deionized Water Negative Control

Procedure

1. Prepare a set of ten-fold serial dilutions of alpha-amylase standards consisting of 20, 2, 0.2, 0.02, and 0.002 units each per 10 µL of deionized water as described in the Forensic Biochemistry Methods Manual.

2. Test 10 µL of each standard and a deionized water negative control as per the Amylase Diffusion Presumptive Test for Saliva method specified in the Forensic Biochemistry Methods Manual.

Specifications
The amount of diffusion for the standards (eg., diameter of the clear circles around standard wells) needs to be linear with respect to the amylase activity expressed logarithmically. Perform a linear regression analysis on the data samples to determine the correlation coefficient (r^2). The r^2 value should be greater than 0.95.

The values of diffusion for the 0.02 and 0.002 unit standards should fall in the ranges of 7-15 and 4-10 mm, respectively. In addition, the amount of diffusion of the 0.02 unit standard must be greater than that of the 0.002 unit standard.

The negative control should be negative.

Documentation
Write the test results on the reagent sheet.

Attach the Amylase Diffusion worksheet and Amylase Diffusion Assay spreadsheet to the reagent sheet and file into the appropriate QC reagent binder.

Initials: **Date:** 4/30/2003

QC110 AMPLIFICATION KITS

Test Materials

Components of the PowerPlex 16, Cofiler and Profiler Plus Kits to include the following:

- Reaction Mix
- Positive Control
- Primer Mix
- Allelic Ladder
- Taq Gold

Samples

Two whole blood or bloodstain samples of known type
One amplification negative
One positive control sample from the PCR typing kit

Procedure

1. Amplify the samples and a positive control from the kit according to the amplification protocol. No extract is added to the amplification negative.

2. Separate the amplification products on a gel or capillary electrophoresis instrument following the appropriate protocol in the Protocols for Forensic STR Analysis Manual.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation

Document on appropriate amplification and electrophoresis worksheets.

Attach the completed worksheets to the Kit Control Log (F160).

File the Kit Control Log and the worksheets together in the appropriate QC reagent binder.

Initials: **Date:** 4/30/2003

QC115 AUTOCLAVING

Glassware/equipment

All glassware must be clean and dry prior to autoclaving (refer to QC175 for standard glassware cleaning procedure).

Cover glassware openings with aluminum foil.

Attach a strip of autoclave time tape to the aluminum foil on each piece.

Bottles should be loosely capped.

Small items may be autoclaved inside a beaker covered with foil.

Solutions

Conical tubes and glass bottles should be loosely capped. Small tubes are autoclaved inside a beaker.

Attach a strip of autoclave time tape to the object being autoclaved.

Do not fill bottles and tubes more than 75% of capacity.

Operation

The drain should be closed. The chamber should be filled with deionized water to the fill line (approximately 4 L). Load the chamber and close the door. Select exhaust, temperature and set the timer. Use fast exhaust for glassware and equipment and slow exhaust for solutions. The autoclave starts automatically and should not be opened until all of the pressure is released. If additional autoclaving is needed, refill water chamber and repeat procedure.

Maintenance

Once all autoclaving has been done, the chamber should be drained of water by opening the drain knob and the door should be left open.

Specification

Lettering on autoclave time tape should turn color (black).

Initials: **Date:** 4/30/2003

QC130 CAPILLARY ELECTROPHORESIS (ABI 310)

Test Materials

Performance Optimized Polymer 4
310 Genetic Analyzer Buffer with EDTA
Formamide (Deionized)
CXR Size Standard
Cofiler Kit Reagents (see QC110)

Samples

Run amplified products from two DNA samples; an allelic ladder, amplified positive control DNA, and a reagent blank (amplification negative control).

Procedure

1. Electrophorese samples according to the capillary electrophoresis protocol.

2. Analyze samples according to the Genescan Analysis and Genotyper protocols as described in the Protocols for Forensic STR Analysis Manual.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation

Document on appropriate capillary electrophoresis run worksheets.

Attach the completed worksheets to a Raw Material Quality Control Test Form (F183).

File reagent sheet and CE run worksheets together in the appropriate QC reagent binder.

Initials: **Date:** 4/30/2003

QC140 CENTRIFUGE CLEANING

Centrifuges are cleaned with a 10% bleach solution on a monthly basis. This insures that the centrifuge surface will be relatively clean of DNA that may have built up through normal laboratory use.

Both the inside chamber, rotor, and outside of the centrifuge should be wiped with the 10% bleach solution. This first wipe is then followed by another wipe, now using 95% ethanol. The ethanol is used to clean the surfaces from bleach and to complete the decontamination/disinfection process.

Cleaning of centrifuges is recorded on a Maintenance Log Sheet (F165) and filed in the Centrifuge Maintenance Log Binder.

Initials: **Date:** 4/30/2003

QC145 CHELEX EXTRACTION

Test Materials
Chelex, 5%

Samples
Two whole blood or bloodstain samples of known type
One negative control sample

Procedure
1. Extract the two known samples and the extraction negative control sample according to the Chelex extraction procedure for whole blood and bloodstains as described in the Protocols for Forensic STR Analysis Manual.

2. Amplify the samples according to the appropriate amplification protocol.

3. Electrophorese the samples according to the appropriate protocol.

Specifications
Each sample must match the assigned type within the current interpretation guidelines.
The extraction negative control sample must show no evidence of contamination.

Documentation
Fill out the appropriate worksheets.

Attach the completed worksheets to the appropriate reagent sheet.

File the reagent sheet and the worksheets in the appropriate QC reagent binder.

Initials: **Date:** 4/30/2003

QC150 CHRISTMAS TREE STAIN FOR SPERMATAZOA

Test Materials
Nuclear Fast Red
Picric Indigo Carmine

Samples
One positive control sperm sample heat fixed to a slide.

Procedure
Apply the Nuclear Fast Red and Picric Indigo Carmine to the cells and view the slide as described in the Forensic Biochemistry Methods Manual.

Specifications
There should be a visible acrosome and nucleus stained red.
The tail should be stained green.

Documentation
The slide should be enclosed in a slide mailer with all pertinent information listed on the front, encased in a plastic Kapak bag and attached to the appropriate reagent sheet.

File the reagent sheet and slide mailer in the appropriate QC reagent binder.

Initials: **Date:** 4/30/2003

QC155 CLEAN RUN

This procedure is used to pinpoint sources of contamination when a typing problem arises.

Samples
two whole blood or bloodstain samples of known type
one extraction negative
one amplification negative
one electrophoresis negative
one positive control sample from the DNA typing kit (if applicable)

Procedure

1. Extract the control samples and the extraction negative according to the Chelex extraction procedure for whole blood and bloodstains as described in the Protocols for Forensic STR Analysis Manual. The extraction negative control is a reagent control containing deionized water in place of sample. This sample should be handled the same way as the other samples, but no substrate is added.

2. Amplify the samples with the appropriate positive control and an amplification negative according to the appropriate amplification protocol. No Chelex extract is added to the amplification negative. This negative is used to evaluate contamination from the reagents and equipment in the amplification area.

3. Electrophorese the samples with an electrophoresis negative control, according to the appropriate protocol. No amplified or chelex extract is added to the electrophoresis or amplification negative controls.

Evaluation
If only the extraction negative shows contamination, the problem has occurred during the extraction step.

If the amplification negative shows contamination while the extraction negative is clean, the problem has occurred during the amplification setup.

If only the positive control appears contaminated, the problem might be a contaminated positive control.

Individual clean runs have to be evaluated on a case by case basis. It may be useful to determine what components have been changed since the last successful typing and to work from there.

Documentation
Document the clean run on a set of appropriate worksheets and place into the QC Troubleshooting/ Investigative Binder.

Initials: **Date:** 4/30/2003

QC160 DIFFERENTIAL EXTRACTION

Test Materials
Chelex, 20%

Samples
One swab with epithelial and sperm cells of known type.
One extraction negative control sample.
One positive DNA control sample from the DNA typing kit (if applicable).

Procedure
1. Extract the known swab and the extraction negative control sample according to the differential extraction procedure in the Protocols for Forensic STR Analysis manual.

2. Amplify the samples and a DNA positive control from the kit according to the appropriate amplification protocol.

3. Electrophorese the samples according to the appropriate protocol.

Specifications
Each sample fraction must match the assigned type within the current interpretation guidelines.
The negative control sample must show no evidence of contamination.

Documentation
Document on a set of appropriate worksheets.

Attach the completed worksheets to the reagent sheet.

File the reagent sheet and worksheets in the appropriate QC reagent binder.

Initials: **Date:** 4/30/2003

QC165 GEL ELECTROPHORESIS (ABI377)

Test Materials:

Ammonium Persulfate (APS)
BSA
dNTPs
Formamide (deionized)
Formamide + Loading Buffer (5:1)
GS500 ROX
Long Ranger
$MgCl_2$
10X PCR buffer
Cofiler/Profiler Plus Kit Reagents (see QC110)
Quad positive control
Quad primers
Sequencing Loading Buffer
Taq Gold DNA Polymerase
TEMED
Urea
Y STR female negative control
Y STR male positive control
Y STR primers

Samples
Two whole blood or stain samples of known type.
One amplification negative.
One positive control sample used for Quad, Cofiler, Profiler Plus or YM1 STR analysis

Procedure
1. Amplify the samples and a positive control according to the amplification protocol. No extract is added to the amplification negative.

2. Electrophorese samples according to the appropriate protocol.

3. Analyze samples according to the STR Gel Analysis and Genotyper protocols as described in the Protocols for Forensic STR Analysis Manual.

Specifications
Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation
Document on appropriate amplification and STR gel worksheets.
Attach the completed worksheets to the appropriate reagent sheet or raw material log sheet (F183).

File the reagent sheet or raw material log sheet and the worksheets in the appropriate QC reagent binder.

Initials: **Date:** 4/30/2003

QC175 GLASSWARE CLEANING

General Procedure

Most pieces of laboratory glassware can be cleaned by washing and brushing with a solution of detergent. Detergent is available from the OCME stockroom.

Rinse each piece at least three times with tap water to remove all detergent residue.

Rinse each piece several times with deionized water. If the surface is clean, the water will wet the surface uniformly. On soiled glass the water stands in droplets. If spotting is observed during the deionized water rinse, the detergent wash should be repeated. If spotting is observed after a second detergent wash, an acid rinse may be necessary (see below).

Allow the glassware to dry at room temperature on a drying rack.

Dishwasher

Load the dishwasher with glassware and put a scoop (approximately 42 g) of non-foaming, laboratory dishwasher detergent in the detergent cup. **Do not use regular laboratory detergent!**

Turn on the dishwasher using the steam scrubbing cycle. When the cycle is finished, remove the clean glassware.

Alternative Cleaning Procedures

When glassware cannot be completely cleaned by scrubbing with a detergent solution, other cleaning methods must be used.

Agarose

Solidified agarose in flasks can be redissolved by adding water to the flask and heating in the microwave. Solidified agarose in graduated cylinders can be removed with a brush. It is best not to use boiling water to redissolve solidified agarose in graduated cylinders, since this may affect the calibration of the cylinder over time.

Acid Rinse

Stubborn films and residues which adhere to the inside of flasks and bottles may often be removed by rinsing with dilute (approx 1-10 M) acetic or nitric acid . Some glassware may need to soak in dilute acid overnight. Any acid rinse must be followed by multiple rinses with deionized water to remove any acid residue.

Initials: **Date:** 4/30/2003

QC190 ISOELECTRIC FOCUSING: HEMOGLOBIN

Test Materials
pH 3-10, 6-8, 7-9 Ampholytes
AFSC Standard

Samples
AFSC Standard
Potassium Cyanide

Procedure
1. Dilute 5uL of the AFSC hemoglobin control with 45 µL 0.05% potassium cyanide.

2. Ten microliter (10uL) aliquot of the diluted standard is tested as per the hemoglobin IEF method as specified in the Forensic Biochemistry Methods Manual.

Specification
All four bands must be visible and sharply defined in at least one standard. The volume giving optimal banding will be used in casework.

Band separation must be as follows:

Bands	Allowable Separation
A to F	>2mm
F to S	>3mm
S to C	>6mm

Documentation
Document on the appropriate worksheet and attach photographic documentation.

File in the appropriate QC reagent binder.

Initials: **Date:** 4/30/2003

QC200 KASTLE -MEYER PRESUMPTIVE TEST FOR BLOOD

Test Materials
Kastle-Meyer Reagent

Samples
Whole Blood
Deionized Water Negative Control

Procedure

1. Prepare serial dilution of whole blood in deionized water beginning with 1/10 and ending with a 1/1,000,000 dilution.

2. Place one drop of each dilution on a stain card (including a heat sample) and deionized water and allow to dry.

3. Test each dried drop with Kastle-Meyer reagent as per the Forensic Biochemistry Methods Manual.

Specifications
Reagent sensitivity must not be less than 1/1000 dilution of whole blood.

The deionized water must give a negative result.

Positive reactions must be observed in any dilution only after the addition of 3% hydrogen peroxide.

Documentation
Write test results on Reagent Sheet.

Initials: **Date:** 4/30/2003

QC205 LEUCOMALACHITE GREEN PRESUMPTIVE TEST FOR BLOOD

Test Materials
Leucomalachite Green Reagent

Samples
Whole Blood
Deionized Water Negative Control

Procedure
1. Prepare serial dilution of whole blood in deionized water beginning with 1/10 and ending with a 1/1,000,000 dilution.

2. Place one drop of each dilution on a stain card (including a neat sample) and deionized water and allow to dry.

3. Test each dried drop with Leucomalachite Green reagent as per the Forensic Biochemistry Methods Manual.

Specifications
Reagent sensitivity must not be less than 1/1000 dilution of whole blood.

The deionized water must give a negative result.

Positive reactions must be observed in any dilution only after the addition of 3% hydrogen peroxide.

Documentation
Write test results on Reagent Sheet.

Initials: **Effective Date:** 8/1/2003

QC210 MATRIX/SPECTRAL FILE

Making a matrix/spectral

Introduction

A matrix file is required by the ABI 3100, ABI 310 and ABI 377 fluorescent fragment detection software in order to subtract overlapping wavelength components from the different color signals (for the ABI 3100 platform, a matrix file is referred to as a "spectral calibration"). Therefore the matrix consists of a table of numbers that quantitatively reflect the amount of each dye detected in each color filter.

The necessity to make a matrix arises anything might change the optical properties of an instrument; this might be a repair or replacement of a component of the optical system or a change in the gel composition. Since there are subtle differences between the different instruments each instrument has to have its own matrix file and gels or runs performed have to be analyzed with the matrix belonging to the instrument that was used.

Due to minor shifts in the quality of the CCD camera, the laser, the glass plates, or the reagents, it can become necessary to make a new matrix, even though no changes were made. The following occurrences are indications that the old matrix does not achieve the correct amount of spectral overlap:

- pull up peaks underneath peaks of a height less than 2000fu
- pull down events in a different color caused by peaks in another color
- elevated baseline of a different color between two peaks in another color

The matrix file is made by running the pure dyes and then performing the Genescan software step "New Matrix" that is described below. Different labeling chemistries of course require different matrices to be used during the analysis.

The table below shows the different labels used for fluorescent system employed by the Department of Forensic Biology for casework and research. The table also displays how the matrix standards are supplied by either Applied Biosystems or Promega, and which virtual filterwheel on the instrument corresponds to which dye.

When making a new matrix **select the appropriate four samples for each system**. Standards for different systems can be run together. The matrix standards have to be run under the regular conditions, but with no matrix applied to the run. Matrix standards can be coloaded with other samples, which can be analyzed separately afterwards.

Initials: **Effective Date:** 8/1/2003

Table 1: Available Matrix Standards

Multiplex systems	Color	Label	ABI kit	Filterwheel required
QUAD, YM1	Blue	6-FAM	Fluorescent Amidite Matrix Standard Kit	A
	Green	JOE	Dye Primer Matrix Standards	
	Yellow	NED	NED Matrix Standard	
	Red	ROX	Dye Primer Matrix Standards	
Cofiler and Profiler Plus	Blue	5-FAM	Dye Primer Matrix Standards	A or F
	Green	JOE	Dye Primer Matrix Standards	
	Yellow	NED	NED Matrix Standard	
	Red	ROX	Dye Primer Matrix Standards	
Powerplex systems	Blue	Fluorescein	Promega Powerplex kit	A
	Green	HEX	Fluorescent Amidite Matrix Standard Kit	
	Yellow	TMR	Promega Powerplex kit	
	Red	ROX	CXR standard from Promega Powerplex kit	
dRhodamine Sequencing Big Dye Sequencing	Dye primer C	dR110	dRhodamine Matirx Standards	E
	Dye primer A	dR6G	dRhodamine Matirx Standards	
	Dye primer G	dTAMRA	dRhodamine Matirx Standards	
	Dye primer T	dROX	dRhodamine Matirx Standards	

Initials: **Effective Date:** 8/1/2003

3100 Spectral Calibration preparation for Cofiler/Profiler Plus systems

NOTE: Matrix standards must be mixed with Hi-Di Formamide.

1. Thoroughly mix the contents of each tube and spin briefly.

2. Combine 1.25ul of standard from each of the 4 tubes supplied (5FAM, JOE, NED, ROX) and 195ul of Hi-Di Formamide in a 1.5ml centrifuge tube.

3. Mix thoroughly and spin briefly. Dipense 10ul of matrix standard/formamide mixture into two columns of a 96 well plate. Denature by placing 96 well plate onto the 9700 thermal cycler at 95 C for 5 minutes. Immediately place on ice. Or use the denature/chill option programmed in the thermal cycler.

Matrix Standard preparation

NOTE: Matrix standards must be mixed with formamide and denatured, but **DO NOT** add the red size standard.

1. For 310 Mix 1µL of each matrix standard with 25 µL of deionized formamide only. Denature at 95°C for 2-3 minutes, then chill on ice and place in the 48-well sample tray. Do two injections each.

2. For 377 Mix 4µL of each matrix standard with 4µL of formamide only. Denature at 95°C for 2-3 minutes, then chill on ice before loading. Load twice, 3µL each..

Don't forget to load both 5-FAM and 6-FAM when making a STR matrix.

Electrophoresis and Making a Matrix file

1. For 3100 Place the 96 well plate onto the 3100 autosampler. Within "Plate View" of the 3100 Data Collection software, click "New". In the "Plate Editor" dialog box: a) name the plate, b) select "spectral calibration", c) select 96 well for plate type, d) click "Finish". Complete the "Plate Editor" spreadsheet: a) assign sample names b) select dye set "F", c) select run module "Spect36_POP4DefaultModule," select the spectral parameter "MtxStd{GeneScanSetF}.par."

Follow the Department of Forensic Biology Protocols for Forensic STR Analysis manual for instructions on how to run samples.

At the end of the run, while the data is being analyzed, the Spectral Calibration Result dialog box opens to indicate which capillaries have passed and which have failed. Failed capillaries are represented by an "X." Passed capillaries are represented by a "." dot. Click "OK."

If a capillary fails, it is automatically assigned the spectral profile of its nearest passing capillary to the left. If there are no passing capillaries to the left, it will be assigned the profile of the nearest passing capillary to the right. These capillaries are marked yellow instead of green in the Array View. It is recommended that each capillary has a passing spectral. Repeat the calibration if necessary.

Review the spectral calibration profile by choosing "Tools," "Display Spectral Calibration," "Dye Set." Select the dye set that corresponds to the correct matrix run (dye set F is for Cofiler/Profiler.) Click "OK."

Use the arrow buttons to review the data for each capillary. For a good quality calibration for dye set F, the condition number should fall between 4 and 7. The Q-value has to be greater than 0.95. Once each capillary has been reviewed, click "OK."

The spectral is automatically saved as the default, and there is no need to print out the profiles.

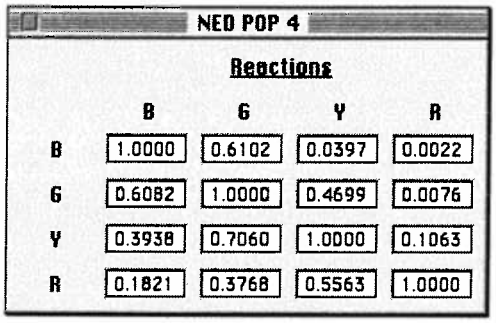
2. For 310 Set up sample sheet, injection list as usual (see STR Manual). The only modification is that in the injection list under Matrix file you have to select "none". Prepare the samples a stated above and start the run.

The duplicates of the standards are only meant as backup. It is not necessary to use both sets. For each standard select the more intense one of the duplicates.

After the run is complete the Genescan analysis software should be open already. Under **File** select **New** and there select **Matrix** .

In the window that appears indicate the sample file that corresponds to each dye color. Refer to **Table 1** for which color has which name and in order to decide which colors to combine for each systems. It may be necessary to browse and open the run folder. Select starting scan numbers of 3300 for each sample. This starting number is intended to exclude the primer peaks.

Under points enter 10,000 and click O.K. The computer makes the matrix and the following window appears:



Under **File** select **Save**. Save the new matrix twice: once in the GS Matrix folder in the Genescan analysis folder (on hard drive), and **IMPORTANT** in the ABI folder in the Macintosh System folder (on hard drive). In order to save a copy in each of these folders, highlight the icon after it has been saved once, under **File** select **Duplicate**. Then drag one of the copies in the other folder. Only if the matrix is saved in the system folder it will be available as an option in the injection list.

As a filename use the instrument name and the creation date:

e.g. CE3 5/03

Proceed with the section **Quality Control Testing of Genescan Matrix Files** (see next section) in order to test the new matrix and print out the documentation.

If runs are analyzed on separate terminals the matrix for the different instruments have to be made available. Copy the file in the GS Matrix folder in Genescan folder on the hard drive.

3. For 377 Genescan

Set up the gel and the electrophoresis conditions as usual (see STR Manual). The only modification is that under Matrix file you have to select "none".

Load 3µL each twice. Avoid spillover. If possible leave an empty lane between the standards.

The duplicates of the standards are only meant as backup. It is not necessary to use both sets. For each standard select the more intense one of the duplicates.

After the gel run, open **Genescan analysis**, open the gel file, select a gel range starting at about 1500, fill out the sample sheet and extract the lanes as usual. At this point you will see the Analysis Control Project window.

Under **File** select **New** and there select **Matrix** .

In the window that appears indicate the sample file that corresponds to each dye color. Refer to **Table 1** for which color has which name and in order to decide which colors to combine for each systems. **ATTENTION**: use 6-FAM once with all three other colors, then repeat using 5-FAM and all three other colors. It may be necessary to browse and open the run folder. Select starting scan numbers that correspond with the above selected analysis range for each sample. This starting number is intended to exclude the primer peaks.

Under value enter 10,000 points and click O.K. The computer makes the matrix and a window as shown above appears.

Under **File** select **Save**. Save the new matrix twice: once in the GS Matrix folder in the Genescan analysis folder, and **IMPORTANT** in the ABI folder in the Macintosh System folder. In order to save a copy in each of these folders, highlight the icon after it has been saved once, under **File** select **Duplicate**. Then drag one of the copies in the other folder. Only if the matrix is saved in the system folder it will be available as an option in the injection list.

As a filename use the instrument name, the FAM used and the creation date:
e.g. Jeffreys 6-FAM 5/03

Repeat the making of the new matrix for the second blue color.

Proceed with the section **Quality Control Testing of Genescan Matrix Files** (see next section) in order to test the new matrix and print out the documentation.

If runs are analyzed on separate terminals the matrix for the different instruments have to be made available. Copy the file in the GS Matrix folder in Genescan folder on the hard drive

3. For 377 dRhodamine and Big Dye sequencing

Set up the gel and the electrophoresis conditions as usual. The only modification is that under Matrix file you have to select "none".

Load 3µL each twice. Avoid spillover. If possible leave an empty lane between the standards.

After the gel run, under **Sequence Analysis** open the gel file, select the gel range to exclude the primer peaks, fill out the sample sheet and extract the lanes as usual.

Open the **Data utility** application and from the **Utilities** menu select **Make Matrix** .

For a sequencing matrix each matrix standard has to be selected in different boxes three times. Follow the instructions below. As the starting scan number, select a the number that corresponds with the above selected analysis range for each sample. This starting number is intended to exclude the primer peaks.

A. Make the Dye Primer Matrix
Select each box and click on the sample file corresponding to the standards below:

C ...	dR110
A...	dR6G
G...	dTAMRA
T...	dROX

Click **New File**. Name the file dRhod and save it in the **ABI folder** within the **System** folder
Click the Dye Primer Matrix radial button. Click O.K.

B. Make the Taq Terminator Matrix:
From the **Utilities** menu select **Make Matrix** .
Select each box and click on the sample file corresponding to the standards below:

C ...	dROX
A...	dR6G
G...	dR110
T...	dTAMRA

Click **Update File**. Choose dRhod and save it in the **ABI folder** within the **System** folder
Click the Taq Terminator Matrix radial button. Click O.K.

C. Make the T7 Terminator Matrix:
From the **Utilities** menu select **Make Matrix** .
Select each box and click on the sample file corresponding to the standards below:

C ... dR6G
A... dTAMRA
G... dROX
T... dR110

Click **Update File**. Choose dRhod and save it in the **ABI folder** within the **System** folder
Click the T7 Terminator Matrix radial button. Click O.K.

To check the matrix file, select **Copy Matrix** from the **Utilities** menu. Under source select **Instrument File** and choose **dRhod** from the **ABI folder** within the **System** folder. The matrix will be displayed on the screen, all three boxes should be filled, the corresponding numbers for each of the three boxes will be the same. Click **Cancel**.

NOTE: Not all three matrices are necessary for sequencing analysis, but they are necessary for terminator reactions sequencing data collection. The run will not start if only a terminator matrix is present. The error message that will appear if the primer matrix is missing will read "Taq is not found".

If sequencing runs are analyzed on separate terminals then make sure that you use the correct matrix for the different instruments. If necessary, copy the file into the Sequencing Analysis folder onto the hard drive

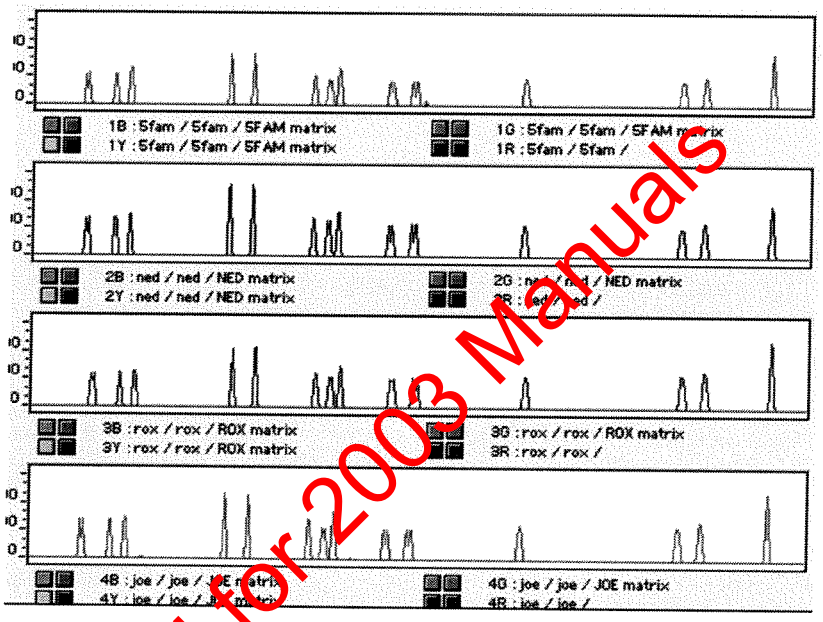
Quality control testing of Genescan STR matrix files

In order to test, if the new matrix is working correctly, it should be applied to the matrix standard sample files.

Open the project with the extracted matrix standards. Under **Samples** choose **Install new matrix**. Install the matrix you just made.

Click on the top blue, green, yellow, and red boxes to select the all colors for the analysis for all lanes. Click on the **Analyze** button in the upper left corner. All selected samples will be analyzed. There will be an error message in the analysis log window because the samples do not have a size standard. Ignore this message.

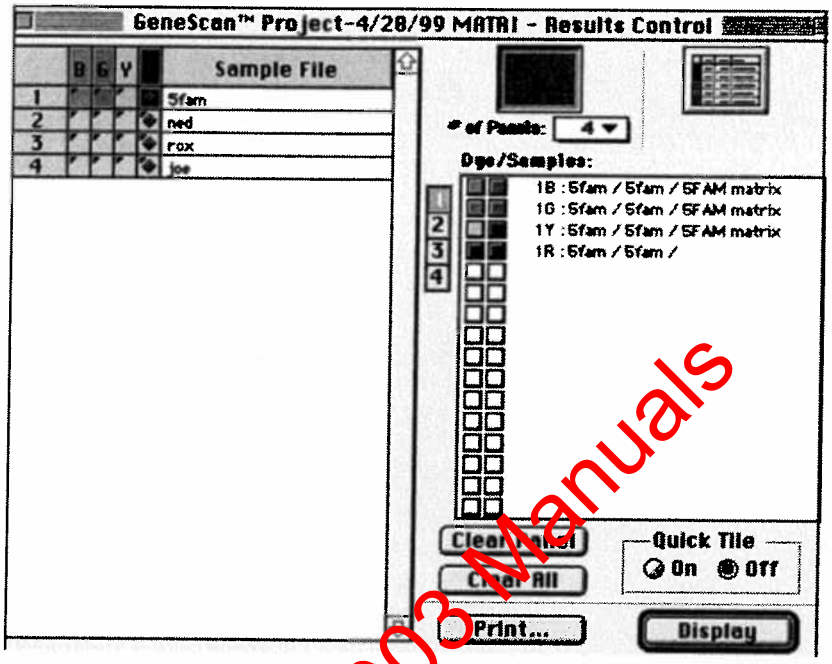
Open the results control window.



In the upper right hand corner, deselect the **Display Table** option by clicking on the icon, so that it is not indented anymore. Also switch **Quick Tile** to **Off**.

Display all colors in sample one in field one, sample two in filed two, and so on...

If the matrix is correct, no pull-up peaks should be visible, all colors should only consist of one color. See example on the next page.



Print out the following documentation for the **Matrix Log Book**:

For STRs: the Matrix number box (double click on the icon in the Matrix Folder in Genescan analysis folder to open the file and select print) , the electropherogram of the analyzed matrix standards (see above).

For Sequencing: the three Matrix number boxes

File these sheets together with the run control or gel sheets in the Matrix Log book.

Initials: **Date:** 4/30/2003

QC220 OUCHTERLONY RADIAL DIFFUSION: SPECIES DETERMINATION

Test Materials
Serum
α-Serum

Samples
One serum sample positive control.
One corresponding α-serum sample.
One negative control (deionized water or saline).

Procedure
Prepare the tank buffer and agarose gel as described in the Quality Manual.
Punch holes in the solidified gel, load samples and develop gel as described in the Forensic Biochemistry Methods Manual.

Specifications
The positive control must give a positive result.
The negative control must give a negative result.

Documentation
Document on an Ouchterlony Test Worksheet and attach it to the appropriate reagent sheet.

Note: Either QC220 or QC255 may be used to QC serum and α-serum.

Initials: **Effective Date:** 12/29/2003

QC225 P30 ELISA

Test Materials

P30 Antigen
Polyclonal Anti-human P30
IgG1, Kappa Chain (MOPC 21)
Alkaline Substrate Buffer
Casein Stock Solution
Monoclonal Anti-human P30
Alkaline Phosphatase Conjugate
p-Nitrophenol Phosphate Tablets
Phosphate Buffered Saline Tablets

Procedure - Monoclonal Anti-human P30 QC

Prepare 1/5,000 - 1/10,000 dilutions of monoclonal anti-human P30 with phosphate buffered saline. Set up a microtiter plate as diagramed below and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
B	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
C	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
D	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
E	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
F	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
G	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
H	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	

PBS = phosphate buffered saline
W = wash buffer (PBS-casein)
2ng, 6ng, 10ng - quantity of P30 antigen

3 C-D, 3 G-H & 4 C-D: 1/5,000 monoclonal anti-human P30
4 G-H, 5 C-D & 5 G-H: 1/6,000 monoclonal anti-human P30
6 C-D, 6 G-H & 7 C-D: 1/7,000 monoclonal anti-human P30
7 G-H, 8 C-D & 8 G-H: 1/8,000 monoclonal anti-human P30
9 C-D, 9 G-H & 10 C-D: 1/9,000 monoclonal anti-human P30
10 G-H, 11 C-D & 11 G-H: 1/10,000 monoclonal anti-human P30

Note: 2-12, A-B and E-F are coated with 1/8000 MOPC as described in the Biochemistry Methods Manual.

Initials: **Effective Date:** 12/29/2003

Specifications
Determine the weakest dilution of antisera which gives a result for the 2ng P30 standard. Choose as the working titer the next strongest dilution. Once the proper working titer has been established, also perform specificity procedure (see below).

Documentation
Document test on a P30 ELISA worksheet.
Fill out a P30 Antisera and Reagents QC sheet (including working titer).
Attach P30 ELISA worksheet to QC sheet and file into the appropriate QC binder.

Procedure - Polyclonal Anti-human P30 QC
Prepare 1/500 - 1/3000 dilutions of polyclonal anti-human P30 with phosphate buffered saline.
Set up a microtiter plate as diagramed below and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
B	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
C	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
D	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
E	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
F	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
G	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
H	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	

PBS = phosphate buffered saline
W = wash buffer (PBS-casein)
2ng, 6ng, 10ng - quantity of P30 antigen

3 C-D, 3 G-H & 4 C-D: 1/500 polyclonal anti-human P30
4 G-H, 5 C-D & 5 G-H: 1/1,000 polyclonal anti-human P30
6 C-D, 6 G-H & 7 C-D: 1/1,500 polyclonal anti-human P30
7 G-H, 8 C-D & 8 G-H: 1/2,000 polyclonal anti-human P30
9 C-D, 9 G-H & 10 C-D: 1/2,500 polyclonal anti-human P30
10 G-H, 11 C-D & 11 G-H: 1/3,000 polyclonal anti-human P30

Note: 2-12, A-B and E-F are coated with 1/8000 MOPC as described in the Biochemistry Methods Manual.

Initials: **Effective Date:** 12/29/2003

Specifications

Determine the weakest dilution of antisera which gives a result for the 2ng P30 standard. Choose as the working titer the next strongest dilution. Once the proper working titer has been established, also perform specificity procedure (see below).

Documentation

Document test on a P30 ELISA worksheet.
Fill out a P30 Antisera and Reagents QC sheet (including working titer) .
Attach P30 ELISA worksheet to QC sheet and file into the appropriate QC binder.

Procedure - Alkaline Phosphatase Conjugate QC

Prepare 1/500 - 1/3,000 dilutions of alkaline phosphatase conjugate with phosphate buffered saline.

Set up a microtiter plate as diagramed below and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
B	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
C	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
D	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
E	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
F	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
G	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
H	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	

PBS = phosphate buffered saline W = wash buffer (PBS-casein)
2ng, 6ng, 10ng - quantity of P30 antigen

3 C-D, 3 G-H & 4 C-D:	1/500 alkaline phosphatase conjugate
4 G-H, 5 C-D & 5 G-H:	1/1,000 alkaline phosphatase conjugate
6 C-D, 6 G-H & 7 C-D:	1/1,500 alkaline phosphatase conjugate
7 G-H, 8 C-D & 8 G-H:	1/2,000 alkaline phosphatase conjugate
9 C-D, 9 G-H & 10 C-D:	1/2,500 alkaline phosphatase conjugate
10 G-H, 11 C-D & 11 G-H:	1/3,000 alkaline phosphatase conjugate

Note: 2-12, A-B and E-F are coated with 1/8000 MOPC as described in the Biochemistry Methods Manual.

Initials: **Effective Date:** 12/29/2003

Specifications
Determine the weakest dilution of alkaline phosphatase conjugate which gives a result for the 2ng P30 standard. Choose as the working titer the next strongest dilution.

Once the proper working titer has been established, also perform specificity procedure (see below).

Documentation
Document test on a P30 ELISA worksheet.
Fill out a P30 Antisera and Reagents QC sheet (including working titer).
Attach P30 ELISA worksheet to QC sheet and file into the appropriate QC binder.

Specificity Procedure - All Other Reagents
Prepare a 1/25 dilution of stains prepared from semen, blood, urine, and saliva from healthy males.

Prepare concentrations of standard P30 antigen as follows:

0.5ng/mL: 2.5 uL P30 (1 ug/mL) + 5mL PBS-casein
1ng/mL: 5 uL P30 (1 ug/mL) + 5mL PBS-casein
2ng/mL: 10 uL P30 (1 ug/mL) + 5mL PBS-casein
6ng/mL: 30 uL P30 (1 ug/mL) + 5mL PBS-casein
10ng/mL: 50 uL P30 (1 ug/mL) + 5mL PBS-casein
14ng/mL: 70 uL P30 (1 ug/mL) + 5mL PBS-casein
18ng/mL: 90 uL P30 (1 ug/mL) + 5mL PBS-casein

Set up a microtiter plate as diagrammed below and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	W	2ng	1ng	6ng	14ng	sem	u				
B	PBS	W	2ng	1ng	6ng	14ng	sem	u				
C	PBS	W	2ng	1ng	6ng	14ng	sem	u				
D	PBS	W	2ng	1ng	6ng	14ng	sem	u				
E	PBS	W	0.5ng	2ng	10ng	18ng	b	sal				
F	PBS	W	0.5ng	2ng	10ng	18ng	b	sal				
G	PBS	W	0.5ng	2ng	10ng	18ng	b	sal				
H	PBS	W	0.5ng	2ng	10ng	18ng	b	sal				

Initials: **Effective Date:** 12/29/2003

PBS = phosphate buffered saline
W = wash buffer (PBS-casein)
0.5ng, 1ng, 2ng, etc. - quantity of standard P30 antigen
sem = 1/25 semen
b = 1/25 blood
u = 1/25 urine
sal = 1/25 saliva

Specifications

All samples of blood, urine, and saliva must give negative results.

Semen results must yield positive results.

P30 standard results must reflect standard quantities with values indicative of its concentration.

Documentation

Fill out and attach P30 ELISA worksheet to an appropriate reagent sheet and file into the appropriate QC binder.

Initials: **Date:** 4/30/2003

QC240 PCR AMPLIFICATION

Test Materials

BSA
Cofiler Kit Reagents (see QC110)
dNTPs set
$MgCl_2$
10X PCR Buffer
Profiler Plus Kit Reagents (see QC110)
Quad and Y STR Primers
Quad STR Positive Control
Taq Gold
Y STR Male Positive and Female Negative Controls

Samples

Two whole blood or stain samples of known type.
One amplification negative.
One positive control sample from amplification materials.

Procedure

1) Amplify the samples and a positive control using reaction mixture according to the amplification protocol. No extract is added to the amplification negative.

2) Electrophorese samples according to the gel electrophoresis protocol.

3) Analyse samples according to the STR Analysis and Genotyper Instructions protocols.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation

Document on an appropriate amplification and STR gel worksheets.

Attach the completed worksheets to the appropriate reagent sheet or raw material log sheet (F183).

File the reagent sheet or raw material log sheet and the worksheets in the appropriate QC reagent binder.

Initials: **Date:** 4/30/2003

QC250 QUANTIBLOT HYBRIDIZATION

Test Materials

BSA, 5 mg/ml
Chromagen
dNTPs Set
Digest Buffer
DTT, 1 M
$MgCl_2$ (25 µL)
PCR Buffer (25 µL)
Phosphate Buffered Saline (PBS)
Primers Used for Quad & Y STR Analysis
Proteinase-K Enzyme, 20 mg/ml
QuantiBlot DNA Standards
QuantiBlot Hybridization Solution
QuantiBlot Kits
  Calibrators 1 & 2
  DNA Probe
  Enzyme Conjugate
QuantiBlot Wash Solution
Sterile Water
Taq DNA Polymerase (20 µL)
TE^{-4}, 1X

Samples

Solution to be tested for the presence of DNA at the volume indicated above or in the QC section of the reagent sheet.

Procedure

Hybridize the samples according to the Quantiblot protocol.

Specifications

Each QuantiBlot Calibrator must have an intensity bounded by the appropriate QuantiBlot DNA standard.
All of the QuantiBlot standards must be visible.
The tested solution must show no evidence of contamination. There must be no hybridization to the slot containing the tested solution.
The negative control must show no evidence of contamination.

Documentation

Document on a QuantiBlot Hybridization Worksheet.
Attach the completed worksheet to the appropriate reagent sheet or raw material log sheet.
File the reagent sheet or raw material log sheet and the worksheets in the appropriate QC reagent binder.

Note: Chromagen and components of the QuantiBlot Kits (with the exception of the QuantiBlot DNA Standards which are tested for each new lot) should be tested for each new vendor lot/ shipment.

Initials: **Date:** 4/30/2003

QC255 SPECIES CROSSOVER ELECTROPHORESIS

Test Materials:
Serum
α-Serum

Samples
One positive control serum sample.
One corresponding α-serum sample.
One negative control (distilled water or saline).

Procedure
Prepare tank buffer and agarose gel as described in the Quality Manual; Appendix A.
Punch holes in solidified gel, load samples and develop gel as described in the Forensic Biochemistry Methods Manual.

Specifications
The positive control must give a positive result.
The negative control must give a negative result.

Documentation
Document on Crossover Electrophoresis Worksheet and attach the completed sheet to the appropriate reagent sheet.

Note: Either QC220 or QC255 may be used to QC serum and α-serum.

Initials: **Date:** 4/30/2003

QC265 TAKAYAMA HEMOGLOBIN TEST

Test Materials
Takayama Reagent

Samples
One positive control consisting of a whole blood or bloodstain sample.
One negative control consisting of saline or deionized water.

Procedures
Perform the Takayama test on the positive and negative controls as described in the Forensic Biochemistry Methods Manual.

Specifications
The positive control must give a positive result.
The negative control must give a negative result.

Documentation
The test should be documented on a Takayama reagent sheet.

Initials: **Date:** 4/30/2003

QC305 UREA GEL DIFFUSION

Test Materials
Urease standard

Samples
Urea standards
Dried urine stain

Procedure

1. Prepare urea standards containing 5g/100ml, 0.5g urea/100ml, 0.05g urea/100ml, and 0.005g urea/100ml respectively, in deionized water.

2. Extract a 1cmx1cm urine stain in 200ml deionized water and prepared a 1/10 dilution of the extract in deionized water.

3. Test each urea standard, the neat and 1/10 urine stain extract dilution, and a deionized water blank as per the urine gel diffusion procedure specified in the Forensic Biochemistry Methods Manual.

4. Prepare a standard curve of urea concentration (expressed logarithmically on x axis) versus the adjusted diffusion radius (determined by subtracting the mean diffusion radius of each standard on the blank plate from the mean diffusion radius on the test plate).

5. Plot the adjusted diffusion radius of the neat and 1/10 diluted extracts of the known urine stain on the standard curve.

Specifications
The adjusted diffusion radius of the standard needs to be linear with respect to the urea concentration expressed logarithmically.

The adjusted diffusion radius of the neat and 1/10 diluted urine stain extracts needs to fall between the highest and lowest points on the standard curve.

The calculated urea concentration of the neat and 1/10 diluted urine stain extracts needs to differ by an approximate factor of 10.

Documentation
Write test results on the appropriate reagent sheet.
Attach appropriate worksheets to the reagent sheet.

Initials: **Date:** 4/30/2003

QC120 BALANCES: VERIFICATION AND MAINTENANCE

Routine Weight Measurements

1. Press the control bar once to turn on the power. Allow the readout to stabilize to 0.000.
2. Place the weigh paper or weigh boat on the pan of the balance. Allow the readout to stabilize.
3. Press the control bar once to tare the balance.
4. Make the desired measurement.
5. When finished, pull the control bar up to turn off the power. Clean out the weighing chamber with the small brush or a damp paper towel, being careful not to disturb the pan.

Mettler AE260 Analytical Balance Two-point Calibration

A two-point standardization should be performed each calendar month using the protocol described below:

1. Press the control bar once to turn on the power.
2. Close all the doors surrounding the weighing chamber and allow the readout to stabilize to 0.000.
3. Press and hold the control bar until the readout says CALIB.
4. When the readout flashes 100, slide the lever on the right side back to release the internal 100 gram standard weight. Allow the balance to calibrate at 100 grams.
5. When the readout flashes 0, slide the lever forward. Allow the readout to stabilize.

The balance is calibrated and ready for use.

Balance Four-point Weight Verification

Each calendar month, the balance is verified using four standard weights.

Do not handle the weights directly. Use Kimwipes or forceps to handle weights.

1. Weigh the first standard. Record the standard weight and the measured weight on the Balance Verification and Maintenance Log (F100).
2. Repeat the measurements for the other three standard weights. Record all measurements.
3. File Balance Verification and Maintenance Logs into the Scale Log Binder.

Calibration and Maintenance

Balances must be calibrated yearly by an outside contractor.

Initials: **Date:** 4/30/2003

QC120 BALANCES:VERIFICATION AND MAINTENANCE (CONT.)

Specification

Specification for weight verification should be +/- 0.1%.

Standard (g)	Range of tolerance (g)
4000	3996.0 - 4004.0
1000	999.0 - 1001.0
500	499.5 - 500.5
100	99.9 - 100.1
50	49.95 - 50.05
20	19.98 - 20.02
2	1.998 - 2.002

If a value falls out of range, repeat. If still out of rance for the AE260 Analytical Balance, then perform calibration using the internal 100 g weight. Repeat verification. If still out of range, phone for instrument calibration by an outside vendor.

Initials: **Date:** 4/30/2003

QC125 BIOLOGICAL SAFETY CABINET/FUME HOOD: OPERATION AND MAINTENANCE

Routine Use

Turn the blower on and **WAIT** 15 minutes before using the hood. Leave the blower on while you are working in the hood.

Turn on the fluorescent light (NOT the UV light of the Biological Safety Cabinet).

Wipe all exposed hood surfaces with 70% ethanol. This must be done by every individual, each time they start to work in the hood.

Line the work surface with absorbent pads. Put the plastic side down and the paper side up. Do not block the vents.

Work on the absorbent pads following all of the safety precautions listed above.

In case of a spill onto the hood surface, decontaminate with 10% bleach for 10 minutes. Absorb the bleach onto a paper towel and rinse the surface with 70% ethanol.

NOTE: All the bleach must be rinsed from the hood surface with the ethanol. Otherwise the hood will corrode.

If the blower stops running, DISCONTINUE all work and safely seal up all samples. **The hood no longer offers any protection.**

When you are done working, discard the absorbent pads and change your top layer of gloves.

Wipe all exposed surfaces with 70% ethanol and then discard your gloves layer by layer in the red biohazard bags.

If using a Biological Safety Cabinet that is equipped with a UV light, turn the UV light on for 1 hour. Do not expose yourself to the UV.

Shut off the blower and UV (if applicable). Do NOT leave on overnight.

NOTE: **Do not work with any organic solvents (except ethanol) in the biosafety hood.** Use the Fume Hood for this purpose.

Initials: **Date:** 4/30/2003

QC126 BIOLOGICAL SAFETY CABINET/FUME HOOD: OPERATION AND MAINTENANCE (MISONIX FE-2620 WORKSTATION)

Routine Use
Turn the blower on and adjust air speed (if necessary).

Line the work surface with absorbent pads. Put the plastic side down and the paper side up.

Work on the absorbent pads following all of the safety precautions of the laboratory.

In case of a spill onto the surface, decontaminate with 10% bleach for 10 minutes. Absorb the bleach onto a paper towel and rinse the surface with 70% ethanol.

If the blower stops running, DISCONTINUE all work and safely seal up all samples. **The hood no longer offers any protection.**

When you are done working, discard the absorbent pads and change your gloves.

Wipe all exposed surfaces with 70% ethanol and then discard your gloves in the red biohazard bags.

Shut off the blower. Do NOT leave on overnight.

NOTE: **Organic solvents can be used in the workstations as long as they contain an "A/C" level carbon filter.**

Maintenance
The Misonix FE-2620 Work Station contains two filters - a Pre-filter and a Carbon Filter. It is recommended by Misonix that the carbon filters be changed once a year and the pre-filters as often as necessary. Consult the Misonix FE-2620 Operating Manual for instructions on how this can be done. The workstations will be inspected once a year by an outside company.

Initials: **Date:** 4/30/2003

QC135 CAPILLARY ELECTROPHORESIS (ABI 310): MAINTENANCE

When problems are experienced with the ABI 310 Capillary Electrophoresis unit, there are two diagnostic tests that may be done according to the protocols presented below. The purpose of these tests is to check the operation of the laser and CCD camera.

The test results are recorded on a 310 Capillary Electrophoresis Diagnostic Log sheet. These tests can be run while there is a capillary in the instrument. Make sure that the capillary is not damaged during the testing. Especially since the second test requires the removal of the capillary from the laser window.

The first test cannot be run with the 310 Collection Software open!

LASER TEST

1) Quit 310 Collection Software if necessary.

2) To access the diagnostic test files, open the **310 diagnostics** folder located on the hard drive. And click on the 310 diagnostics icon. At this point you will receive a warning, that the 310 diagnostics software cannot run if the Prism collection software is already running. You can check this by going to the upper left hand corner, and clicking on the finder icon. If it is not running, click **Continue,** otherwise click **Quit** and start with step1).

   At this point you may receive the message "Establishing serial communication link with 310 instrument. This may take several seconds. Do not click Abort!!! Afterwards you might get the message "Instrument is not responding. Wait 10 seconds and then click o.k." Do wait and click **o.k.**.

   From the first menu of options choose **Test Components.** From the second menu of test components choose **Laser Power**.

3) Click on **start**. The values for the laserpower mW and the laserpower Amps will appear on the screen, ignore the first two readings and record the 3rd, the 4th, and the 5th reading on the Capillary Electrophoresis Diagnostic Log. Also record the pass or fail status.

4) After the 5th set of values appeared, wait till the indicator on the left side shows 100% done, then click on **Done**. The message that will appear says results not logged. To the question "log now" click **no**.

5) On the 310 components menu press **Return.** On the main diagnostics menu press **Quit.**

If the laser fails readings 3-5 take the instrument out of service and call the PE/ABD technical service representative.

Initials: **Date:** 4/30/2003

QC135 CAPILLARY ELECTROPHORESIS (ABI 310): MAINTENANCE (CONT.)

CCD CAMERA SENSITIVITY TEST

For this test the regular capillary is replaced with a sensitivity standard capillary and a mock run is performed. The capillary does not have to be taken out, it is sufficient to temporarily remove it from the CCD camera lens window.

1) Open the 310 Collection Software.

2) Under **file** select **new** then select **sequence sample sheet for 48 tubes**. In the first row (A1) put one sample name e.g. CCD test. If there is no module and no matrix selected, import any of the existing possibilities. The sections have to be filled, but the files will not be applied and are just fake. Close the sample sheet and save it as e.g. CCD test.

3) Under **file** select **new** then select **sequence injection run**. Import the sample sheet that was created under 2. Select **Test CCD sensitivity** as run module. Deselect Autoanalyze if necessary.

4) Open the 310 instrument door, open the heat plate cover door, and the laser window door. Be careful not to damage the regularly installed capillary during the next steps. Move the capillary out of the laser window notch and bend it out of the way so that the laser window door and the heat plate cover can be closed without damaging the capillary.

5) Take the sensitivity standard capillary provided by ABD/PE (part # 401928) and place its window in front of the camera lens. The yellow tag should be on top. Carefully close the laser window door, the heat plate cover and the instrument door.

6) Click on **Run**. Under **Window** open **Status** to observe the progress. The program will collect data for 5 min. Then a second data collection set for 3 min will start. An alert message "EP current is zero" will pop up, click **o.k.**. Data collection will continue.

7) When the alert prompt " Remove capillary" appears, open the instrument door, open the heat plate cover and the laser window door and remove the sensitivity standard. Do not put the old capillary back yet!! Close all doors, click **o.k.**, the run will resume automatically. Data will be collected for 3 minutes. Click **o.k.** to the alert prompt that the EP current is zero.

8) After the data collection is completed, close the run, save the injection list, and quit the data collection program.

9) On the hard drive open the **310 diagnostics** folder and click on the **310 diagnostics icon**. From the main menu select **Analysis**. From the Analysis menu select **Signal to Noise Auto**.

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QC135 CAPILLARY ELECTROPHORESIS (ABI 310): MAINTENANCE (CONT.)

10) Click on **Start**. Import the mock run from before, which should be in the current run folder. Highlight the sample file and click ok. The data will be analyzed automatically. Record the relevant values on the 310 Capillary Electrophoresis Diagnostic Log; the relevant values are 586 S/N ratio, 625 S/N ratio, 586 signal w/cap, and 586 signal net. These are the only ones listed on this form.

11) Click on **done**. On the 310 components menu press **Return.**
On the main diagnostics menu press **Quit.**

12) Open the instrument door, the heat plate door, and the laser window door and place the regular capillary in front of the camera lens. Close all doors.

If any of the values fail call technical service.

Initials: **Date:** 4/30/2003

QC162 DNA SEQUENCER (ABI 377): MAINTENANCE

There are no diagnostic tests to be performed for the ABI 377 DNA Sequencer. Check, and if necessary clean all instruments, and sign the maintenance log. However, the water reservoirs should be checked and refilled on a monthly basis. This information should be documented on a Maintenance Log sheet (F165) and filed in the ABI 377 Maintenance Log Binder.

Refilling the Water Reservoir - this is done once a month and if the water level drops below one third. The ideal level for the water reservoir is between one third and two thirds full.

1. The water reservoir is located in a compartment on the right side of the instrument.

2. Make sure the pump is not running.

3. Open the compartment door. Unscrew the plastic bottle and remove it by pulling downward. Place a papertowel under the tubes connecting the reservoir to the pump.

4. Discard the old fluid, and rinse out the bottle. Fill the reservoir up to the mark (corresponds to 600 ml) with dH_2O, and add several drops of algicide.

5. Replace the reservoir, being sure to insert the two tubes before you screw it into place.

Initials: **Date:** 4/30/2003

QC167 GEL ELECTROPHORESIS (ABI 377): PLATE PREPARATION

Each new set of plates has to be treated with NaOH. This process does not have to be repeated.

A set of plates consists of one backplate and a notched front plate. The insides that will be in contact with the gel have to be treated. To mark which sides have to be the insides, the outside of the plates get etched in the following way:

Notched plate - an "L" for left on the left upper side, an "R" for right on the right upper side.
Plain plate - a mirror image "L" on the right side, and a mirror image "R" on the left side.
This way the "L"s and "R"s should be readable when the plates are placed correctly.

Place the plates on a sheet of bench paper with the side of the plates that is not etched facing upwards. **CAUTION:** Wear protective goggles, gloves and a lab coat before handling sodium hydroxide!!! Pour 10ml of 10N NaOH on the plate and distribute it evenly using a bundle of large Kimwipes. Rub the plate for approximately one minute in every direction. Rinse the plate off with plenty of tap water followed by a final rinse with deionized water. Repeat for the second plate.

Wash plates by hand throughout the entire procedure. Do not use the dishwasher.

The plates can be used immediately after treatment.

Initials: **Date:** 4/30/2003

QC215 MICROPIPETTE CALIBRATION AND MAINTENANCE

Calibration & Maintenance

Micropipettes are sent to an outside vendor twice a year for calibration.

Each station is equipped with a set amount of pipetman. During the time of calibration, complete sets of pipetman are replaced with a substitute set consisting of pre-calibrated pipetman that are reserved for this particular function. The pipetman from several stations can be removed and sent for calibration at one time.

Any micropipette transfer to or from service for any reason (i.e. repair, calibration, return from calibration) must be documented on the respective Micropipette Maintenance Log (F170). These sheets are located in the Micropipette Calibration QC Log binder. This binder is organized by workstation (e.g. pipetman at the chelex station, pipetman at the amplification station, etc.).

Micropipettes are prepared by wiping the outer shaft with 10% bleach and then followed with a final wipe using 95% ethanol.

Package micropipettes in bubble wrap packaging material before shipping out.

The substitute set is rotated to the next station once the pipetmen that were sent out for calibration are returned back to their respective station.

Gravimetric Check of Pipetman Accuracy

The table on the following page shows the performance specifications for the various pipetman that are being used in the laboratory. These specifications show levels of tolerance at various points on a given pipetman's range. If measured values differ significantly from the specifications, the pipetman in question will be removed from laboratory use and included in the next shipment of pipetman for calibration.

Initials: **Date:** 4/30/2003

QC215 MICROPIPETTE CALIBRATION AND MAINTENANCE

Table: Pipette Performance Specifications

Type	Volume Setting (µL)	Percent Error	Allowable Range (µL)
P-1000	1000	≤ ±2.0	980-1020
	500	≤ ±2.0	490-510
	200	≤ ±2.0	196-204
P-200	200	≤ ±2.0	196-204
	100	≤ ±2.0	98-102
	50	≤ ±2.0	49-51
P-100	100	≤ ±2.0	98-102
	50	≤ ±2.0	49-51
	20	≤ ±2.0	19.6-20.4
P-20	20	≤ ±2.0	19.6-20.4
	10	≤ ±2.0	9.8-10.2
	2	≤ ±10	1.8-2.2
E-10	10	≤ ±2.0	9.8-10.2
	5	≤ ±5.0	4.75-5.25
	2	≤ ±10	1.8-2.2
Repeater	10 (500µL tip)	≤ ±2.0	9.8-10.2
	30 (500µL tip)	≤ ±2.0	29.4-30.6
	50 (500µL tip)	≤ ±2.0	49-51
	50 (2.5ml tip)	≤ ±2.0	49-51
	250 (12.5ml tip)	≤ ±2.0	245-255

P - Rainin Pipetman
E - Microcentrifuge ULtra-micropipette
Repeater - Microcentrifuge Repeater Pipette

Initials: **Date:** 4/30/2003

QC230 P30 PLATE READER DIAGNOSTIC TESTS

Microwell (microtiter) plate reader(s) should be tested monthly for **linearity**, **repeatability** of readings, and **calibration**.

Linearity is determined by the relationship of the calibrator absorbance (well No. 2) to the p-nitrophenol (PNP) concentrations in the remaining wells.

Repeatability is determined by comparing the absorbance of a given well in the strip when the strip is read twice in succession.

Calibration is determined by measuring the absorbance of the calibration well (well No. 2) and comparing it to the acceptable absorbance range assigned to the Microwell reader. The acceptable range is determined by the Microwell reader manufacturer.

NOTE: PNP IS TOXIC. IT IS HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. IT IS IRRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. IT IS ALSO A POSSIBLE MUTAGEN. USE APPROPRIATE PRECAUTIONS WHEN HANDLING. WASH HANDS THOROUGHLY AFTER USE.

Test Materials/Supplies
AccuChrome™ 405 Microwells Kit
Deionized Water
Parafilm™
Linearity/Repeatability and Calibration Record Sheets (found in Microwell kit)

Procedure

1) Remove one Microwell strip from the kit. Gently tap the bottom of the strip on the counter to settle PNP in the wells (this is to prevent loss of powder on opening). DO NOT remove the tab on the Microwell strip.

2) Gently remove plastic and paper covering the strip. Keep the strip right side up.

3) Reconstitute each well with 200 µl of deionized water. Pipet carefully to avoid splashing, bubbles, or overfill. Use a calibrated micropipet. **DO NOT** touch the bottom of the microwell with the pipet tip. **DO NOT MIX.**

4) Place the wells strip into row A of the microtiter plate designed for the wells strip (supplied with kit). Notice that the wells strip has a tab on one side of the strip. The correct placement of the wells strip into the microtiter plate is so that the tab is positioned next to column 12 of the microtiter plate.

Initials: **Date:** 4/30/2003

QC230 P30 PLATE READER DIAGNOSTIC TESTS (CONT.)

5.Gently cover all wells of the strip with Parafilm ™ to prevent evaporation. Let stand on benchtop for two hours at room temperature (18-26°C). **DO NOT** disturb during incubation. Warm up the microtiter plate reader the required amount of time before the end of the two hour incubation time. After two hours, remove the Parafilm™, being careful not to splash any of the samples.

6.Place the microtiter plate with the test wells into the plate reader. Read the test samples according to the standard plate reader protocol used for casework samples (measurement filter = 405 nm; reference filter = 655 nm) and print the results.

7.Repeat the reading of the wells a second time and then print the second set of results as well.

Calculations

1. **Linearity Data Record (measures accuracy)**

   a Calculate the average concentrations for replicate wells. Then calculate the average concentration of wells 3,4; of wells 5,6; of wells 7,8; and wells 9,10,11.

   Example:

   Average Concentration of well 3 = 25.4
   Average Concentration of well 4 = 25.6
   Average concentration of wells 3 & 4: (25.4 + 25.6) / 2 = 25.5

   b. Using the Linearity Graph Paper provided with the kit, plot the calculated average concentration on the vertical axis and the assigned concentration (see below) on the horizontal axis for each set of replicate wells.

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QC230 P30 PLATE READER DIAGNOSTIC TESTS (CONT.)

Well No.	**PNP Concentration (Units)**
Well1:	0 (blank)
Well2:	50 (calibrator)
Well3:	25
Well4:	25
Well5:	50
Well6:	50
Well7:	100
Well8:	100
Well9:	200
Well10:	200
Well11:	200
Well12:	0 (blank)

3. All values must fall within the shaded area on the Linearity Graph Paper. This means the instrument has acceptable linearity (+/- 10%) variation.

Specifications

Loss of linearity is an indicator of stray light due to filter deterioration.
If the values fall outside the shaded area on the Linearity Graph Paper, the test must be repeated. If the repeat test values are still outside the shaded area on the Linearity Graph Paper, the instrument must be serviced and not allowed to be used for casework until it has passed the test.

2. **Repeatability Data Record (measures precision)**

a. Calculate the difference between the absorbance readings for each of the strips.

Example:

Reading	Well No.	Absorption	Difference
1st	3	.243	
			0.000
2nd	3	.243	
1st	4	.244	
			0.001
2nd	4	.245	

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QC230 P30 PLATE READER DIAGNOSTIC TESTS (CONT.)

b. Record the difference for each well in the appropriate space on the second page of the report (the Repeatability Record Sheet on the back of the Linearity Record Sheet).

Specifications

To ensure repeatability of readings, the difference in absorbance of each well between the two readings must be within the acceptable range as indicated on the Linearity Graph Paper (Repeatability section). If the difference is not within the acceptable range, there is a loss of repeatability of the readings.

If the repeatability is not within the accepted range, the test must be repeated. If the repeat test results are still out of the accepted range, the instrument must be serviced and not be used for casework.

3. **Calibration Data Record**

a. AccuChrome™ Microwell strips calibration assignments are lot specific. Use calibration ranges assigned on the Calibration Sheet included in each kit.

b. Recorded absorbance of the calibrator (well No. 2) of the first strip in the column labeled Strip 1 if you are using the first strip in a new kit. If previous strips have already been used, record the average absorbance of well number two for this run in the appropriate strip # column on the Calibration Record Sheet.

c. When the first strip in a kit is used set upper and lower limits for absorbance by drawing a line 0.040 absorbance units above and below the observed absorbance for the calibrator (well No.2). Absobances of all remaining strips should fall within the drawn absorbance limits.

Specifications

If the absorbance of the calibrator (well No.2) falls within the range on the Calibration Record Sheet contained in the kit (as established by Sigma Diagnostics) there is no significant change in the calibration performance of the instrument. The acceptable range incorporated the expected variation due to the strips, the dye, and run-to-run variation.

If the calibrator does not fall within the range on the Calibration Record Sheet, the test must be repeated. If the repeat test value falls outside the range on the Calibration Record Sheet, the instrument must be serviced and is not to be used for casework.

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QC230 P30 PLATE READER DIAGNOSTIC TESTS (CONT.)

Documentation

File the Linearity/Repeatability Record Sheet that was filled out for this QC run with the Calibration Sheet that accompanied the kit for this lot of microwells. All sheets should be filed together in the P30 Plate Reader Maintenance Binder.

Initials: **Date:** 4/30/2003

QC235 P30 ELISA DISINFECTION

Disinfection of the P30 plate washer should be done weekly to insure good working order of this instrument. Documentation for the performance of this procedure is recorded on the Plate Washer Maintenance Log Sheet (F180) and filed in the Plate Washer Maintenance Log Binder.

Procedure

1) Prepare a 10% solution of bleach (100 ml of bleach, 900 ml of dH_2O).

2) Under the SELECT function press the up arrow to reach the DISINFECTION program. Press YES.

3) The machine will prompt the connection of the disinfectant (the 10% bleach solution). Place the designated wash hose into the bottle of prepared bleach mixture (DO NOT pour the bleach mixture into the designated wash container that came with the machine or it will have to be thoroughly rinsed when disinfection is complete). Press YES.

4) The machine will indicate that the pump is priming. Disinfection will then occur for 30 minutes.

5) The machine will prompt the connection of the rinse. Place the wash hose into either the washer's designated rinse bottle filled with dH_2O or a plain bottle filled with dH_2O. Press YES.

6) The machine will indicate that the pump is priming. Prime the plate washer multiple times to ensure that the machine and the wash hose are free of the 10% bleach solution.

7) The SELECT function will return at the RUN program. You may now turn the plate washer off.

Initials: **Date:** 4/30/2003

QC245 pH METER

A two-point calibration is done weekly using the pH meter and standard pH solutions. This information is documented on a pH Meter Calibration Log sheet and filed in the pH Log & Water System Binder.

Two-point Calibration

Choose standard buffer solutions for a two-point calibration which bracket the expected final pH of the solution to be measured. (i.e. use pH 7 and 10 standard buffers for a solution with final pH of 8.) Press STNDBY/MEAS button before the electrode is removed from any solution. Do not allow electrode to dry out.

Fill the electrode with saturated KCl solution if necessary.

Press STNDBY/MEAS button.

Press TWO POINT CAL button. The display asks for the pH of the first standard solution. Enter the pH value of the standard solution and press ENTER.

Press STNDBY/MEAS button.

Rinse the electrode with deionized water. Blot dry outside of electrode.

Place the electrode in fresh standard buffer solution and press STNDBY/MEAS button

The meter will stabilize the mV reading at that pH.

When the readout is stable and 3 asteriks are visible, press ENTER.

The display asks for the temperature of the reading. Enter the room temperature (a value of 24.0°C is adequate for these measurements).

The display asks for the pH of the second standard solution. Enter the pH value and press ENTER.

Press STNDBY/MEAS button.

Rinse the electrode with deionized water. Blot dry outside of electrode.

Place the electrode in the second standard buffer solution and press STNDBY/MEAS button.

The meter will stabilize the mV reading at that pH.

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QC245 pH METER (CONT.)

When the readout is stable and 3 asteriks are visible, press ENTER.

Enter the temperature.

Once the measurement has stabilized and 3 asterisks appear, rinse the electrode with deionized water. Blot dry outside of electrode.

The meter is calibrated before routine measurements.

Routine pH Measurements

Fill the electrode with saturated KCl solution if necessary. When fresh KCl is added, it is a good idea to mix the solution in the electrode by slowly inverting the electrode several times before continuing.

Calibrate the pH meter.

Rinse the electrode with deionized water. Blot dry outside of electrode.

Place the electrode in the solution. When the measurement has stabilized and 3 asteriks appear, record the measurement.

Calibration & Maintenance

The pH electrode must be kept filled with saturated KCl solution. This solution is approximately 30% KCl. The electrode is stored in a 2% KCl solution made from the saturated KCl filling solution (NOT deionized water or pH 7.00 standard solution). Do not leave electrode in deionized water for long periods of time.

When measuring the pH of large volumes, the pH electrode must be held in place. The electrode can be damaged if it is hung over the edge of the container and allowed to stir with the solution.

If the pH reading drifts or requires a long time to stabilize, the electrode bulb may need to be rejuvenated in 1 M HCl or the electrode may need to be replaced. Refer to the Beckman insert for further details of electrode maintenance.

Specification

During a two point calibration the pH meter calculates the slope for the given two standards. If the slope does not pass meter specifications an error message - EFFICIENCY OUT OF TOLERANCE - flashes on the display.

Initials: **Date:** 4/30/2003

QC270 TEMPERATURE CONTROL

Refrigerators & -20°C Freezers

A digital thermometer is used to measure refrigerators and -20°C freezers. The refrigerator and -20°C freezer temperatures are recorded daily during the work week.

Each refrigerator/freezer has its own dedicated temperature probe.

Measure the temperature and document in the respective Refrigerator and Freezer (-20°C) Temperature Control Log sheet for that unit.

-80°C Freezers

An Omega thermocouple thermometer and an Omega thermocouple probe (type T-Brown) is used to measure -80°C freezers. The -80°C freezers are monitored daily during the work week.

Measure the temperature and record reading in the monthly Freezer (-80°C) Temperature Control Log (F120) sheet for that unit.

Air Humidity & Temperature

A digital hygrometer/thermometer is used to measure the north, south, and southeast rooms of the laboratory. The room temperature and percent humidity is recorded daily during the work week.

Place the probe on any surface and allow it to equilibrate for 5 - 10 minutes. Measure the temperature and percent humidity and log in the Temperature Control Log sheet for that room.

Water Baths & Heat Blocks

An Omega thermocouple thermometer and an Omega thermocouple probe (type T-blue) are used to measure the temperature of the water baths and heat blocks. Each probe is calibrated before use (see QC280). Temperature measurements are recorded each day the water bath is used. Temperatures are recorded daily during the work week for the heat block.

Initials: **Date:** 4/30/2003

QC270 TEMPERATURE CONTROL (CONT.)

To measure the temperature, turn the water bath or heat block on (if necessary) and allow it to equilibrate for at least 15 minutes. The probe is mounted in the water bath or positioned in the heat block.

When the temperature has stabilized, record the temperature reading on the appropriate Temperature Control Log sheet or Water Bath Temperature Control Log (F230). To measure the thermocouple temperature, plug the probe into the correct position in the meter (silver-colored constantan wire on the left, copper wire on the right). Record the reading. The thermocouple reading can be corrected using the slope and y-intercept values calculated from the probe calibration (see QC280).

Unit	Acceptable Thermocouple Reading
QuantiBlot Water Bath	50 ± 1°C
56°C Heat Block	56 ± 3°C
65°C Heat Block	65 ± 3°C
95°C Heat Block	95 ± 3°C
100°C Heat Block	100 ± 5°C

Calibration

Digital thermometers with the exception of Omega Model HH21 (see below) and hygrometer/thermometers are sent out for calibration against a NIST traceable standard to an outside vendor once a year. Documentation of calibration is recorded on an appropriate log sheet (F165) and filed in the Temperature Equipment Maintenance Log Binder.

Type T-Blue thermocouples which are used to monitor waterbath and heat block temperatures, are calibrated with designated Omega (Model HH21) digital thermometers against an NIST traceable mercury thermometer (see QC280) annually. After calibration, Type T-Blue thermocouples are always used with the Omega meter that they were used with for calibration.

Type T-Brown thermocouples are used to measure temperatures of the -80°C low temperature freezers. Since an exact low temperature of these freezers is not critical (eg. for storage of forensic DNA extracts), Type T-Brown thermocouples are not calibrated. However, the performance of the Type T-Brown thermocouple is verified yearly as described in QC285.

If a suspicion arises of the performance of any of the digital thermometers, hygrometer/ thermometers, or probes during use, that particular temperature measuring device will be taken offline and recalibrated or reverified to insure that it meets proper specification.

Initials: **Date:** 4/30/2003

QC280 THERMOCOUPLE CALIBRATION (TYPE T-BLUE)

The Type T-Blue thermocouple is calibrated as a set with a designated Omega digital thermometer once a year against a NIST traceable thermometer, graduated to 0.1°C over the range -1.0 to 101.0°C. Before beginning the calibration procedure, the thermometer is checked by measuring two standard temperatures. This procedure may also be used to calibrate a standard thermometer against a NIST traceable thermometer. If this is the case, clamp the thermometer to be calibrated as described below for the NIST traceable thermometer and submerge it in the water near the NIST traceable thermometer. Take readings from the thermometer being calibrated in place of taking readings from the digital meter/probe unit.

Thermocouple Temperature Response

Add 2-3 liters of distilled water to a 4 liter glass beaker and place the beaker on a stir plate.

Set up a clamp and ring stand behind the beaker. Clamp the thermometer onto the ring stand and position it so that it can be submerged in the water.

With a twist tie, attach thermocouple near the bulb of the thermometer so that the thermocouple bead is close to but not touching the bulb.

Lower the thermometer, with attached thermocouple and wire, into the water. Tighten the clamp to hold the thermometer at the correct depth. The thermometer should be immersed at a minimum level of 7.5 cm from the bulb for accurate readings.

Plug the thermocouple into the socket of the thermocouple thermometer to be used during routine measurements.

Turn on the stir plate. Stir the water to the point where a shallow vortex forms. If necessary, adjust the stirrer during the procedure to keep the water well stirred. Thorough mixing will reduce temperature gradients near the thermometer.

Eight comparisons of the thermometer and the thermocouple thermometer should be made, over a range of 25°C to 94°C. Temperatures must not be taken above 95°C because the formation of small vapor bubbles can cause fluctuations leading to variable temperatures.

The first measurement is made at room temperature. Record the reading from the thermometer and the thermocouple thermometer on the Thermocouple Calibration Log (F200). The probe measurements are recorded under the x-axis column, and the readings from the thermometer are recorded under the y-axis column.

Raise the temperature of the water approximately 10°C above room temperature by heating the stir plate.

Initials: **Date:** 4/30/2003

QC280 THERMOCOUPLE CALIBRATION (TYPE T-BLUE) (CONT.)

When the temperature has risen several degrees, turn down the heat.

Check the immersion level of the thermometer. The position of the thermometer may have to be adjusted to compensate for evaporation of water.

If gas bubbles have formed on the thermometer or the thermocouple, gently tap the lower part of the thermocouple wire with a pencil to release them.

Check the temperature of the thermometer until successive readings show changes of less than 0.2°C in a 15 second period.

Once the temperature has stabilized, but at least one minute after any adjustment of the probe, record the readings of both thermometers.

Heat the water about 10°C more. Lower the heat until the temperature stabilizes, check the immersion level, remove any gas bubbles, and record the second set of readings.

Repeat this process until eight temperature measurements have been recorded from 25°C to 95°C. For best results, the number of comparisons within a set should be a bit greater at the top of the range to compensate for a higher uncertainty of measurement. The multiple readings will partially overcome the uncertainty in reading the thermometer and provide confidence in the performance of the system over a range of temperatures.

Calibration Line

If the pairs of readings taken during the calibration procedure were plotted on a graph, thermocouple values along the x-axis and thermometer values along the y-axis, the points wouLd fall along a straight line. This line is the calibration curve which relates observed temperature values measured by the thermocouple probe to standard temperatures. The calibration line is defined mathematically by the equation

$$y = mx + b$$

where m is the slope and b is the y-intercept.

The best fit line for the data can be calculated directly using the least squares method. The least squares calculation yields the slope and intercept necessary to convert thermocouple readings into standard temperatures as well as the correlation coefficient, r. The correlation coefficient gives a quantitative estimate of the goodness of fit. The closer the data points are to the best fit line, the higher the correlation coefficient. A perfect fit has a correlation coefficient of 1.

Initials: **Date:** 4/30/2003

QC280 THERMOCOUPLE CALIBRATION (TYPE T-BLUE) (CONT.)

Calculations

The following are calculated and recorded on the Thermocouple Calibration Sheet (F010). The variable n is the number of data points collected during the calibration experiment, typically seven or eight.

The following are calculated the same way for the sets of x and y values. The discussion describes the calculations with respect to the x values only, assuming parallel calculations for the y values will be performed. Summation (x) is calculated by adding together the x-axis values. This is written in standard notation as

$$\text{sum}(x) = \Sigma\, x_i$$

Mean x equals summation (x) divided by n. This is written

$$\bar{x} = \frac{\text{sum}(x)}{n}$$

Summation (x^2) is the sum of the squares of the x values. All of the x values are squared first and then the squares are added together. This is written

$$\text{sum}(x^2) = \Sigma\, (x_i^2)$$

S_{xx} is defined as the sum of the squares of the x values minus the sum of the x values squared divided by n.

$$S_{xx} = \text{sum}(x^2) - \frac{[\text{sum}(x)]^2}{n}$$

Summation (XY) is calculated by multiplying the pairs of x and y values together and adding the products together.

$$\text{sum}(xy) = \Sigma\, x_i y_i$$

S_{xy} is defined as the sum of the x and y products minus the sum of the x values times the sum of the y values divided by n.

$$S_{xy} = \text{sum}(xy) - \frac{\text{sum}(x)\ \text{sum}(y)}{n}$$

The slope of the best fit line, m, is defined as

$$m = \frac{S_{xy}}{S_{xx}}$$

QC280 THERMOCOUPLE CALIBRATION (TYPE T-BLUE) (CONT.)

The intercept is calculated using the mean x and y values.

$$b = \bar{y} - m\bar{x}$$

Finally, the correlation coefficient is calculated using

$$r = \frac{S_{xy}}{(S_{xx}\, S_{yy})^{1/2}}$$

The slope is written with three significant figures. The intercept is rounded to the tenth's place. The correlation coefficient has a specification of >0.999. If the calibration passes specification, the probe is ready for use.

Procedure for Type T-Blue Thermocouple Preparation
Poke a small hole through the center of the cap of a sterile reaction tube using a sterile needle.

Without bending the wire, pass the thermocouple through the hole from the top of the cap, so the soldered tip of the wire will be inside the tube when the cap is closed.

Tie an overhand knot in the insulated part of the wire. Carefully tighten the knot so that it fits inside the cap of the tube. The knot should not be so tight as to kink or break the wire. The knot prevents the wire from being pulled out of the tube during temperature measurements.

Check the length by closing the tube and pulling the knot against the inside of the cap. Enough of the thermocouple wire should remain below the knot so that the thermocouple is within 1 mm or so of the bottom of the tube; it may touch the tube wall slightly. Adjust if the length is too long or too short.

For the thermocycler probe, place 120 µL of deionized water into the tube and overlay with two drops of mineral oil. The mineral oil prevents evaporative cooling of the liquid inside the tube.

For the water bath probe, place approximately 1 ml of mineral oil into the tube.

Close the cap of the tube. The thermocouple tip should be just above or lightly touching the end of the tube. Do not seal the hole in the cap. If the cap is sealed around the thermocouple wires, the pressure in the tube at high temperatures will force liquid up between the sheath and the wire.

Initials: **Date:** 4/30/2003

QC285 THERMOCOUPLE VERIFICATION (TYPE T-BROWN)

Temperature probe operation is verified once a year.

Before beginning the verification procedure, the NIST traceable thermometer is checked by measuring two standard temperatures.

Mercury Thermometer Standardization

Place the NIST traceable thermometer in an ice water slurry. The etched line around the bottom of the thermometer must be at or below the level of the liquid. Allow the temperature to equilibrate. The thermometer must read between -0.2 and 0.2°C.

Place the thermometer in a boiling water bath. The etched line around the bottom of the thermometer must be at or below the level of the liquid. The thermometer must read between 99.8 and 100.2°C.

Record the results of the temperature check on the Thermocouple (Type T-Brown) Verification Log (F205).

Verification

Place the temperature probe in an ice water slurry along with a NIST traceable thermometer that has been previously standardized. Allow the temperature to equilibrate. The probe must read between -1 and 1°C.

If the probe is going to be used in the 0 to 100°C range, place the temperature probe in a boiling water bath. Allow the temperature to equilibrate. The probe must read between 99 and 101°C.

If the probe is going to be used in the -80 to 0°C range, place the temperature probe in a dry ice ethanol slurry. Allow the temperature to equilibrate. The probe must read between -78 and -74°C.

Record the results of the temperature check on the Thermocouple (Tye T-Brown) Verification Log (205). If the type T-brown probe fails verification, it is removed from service. The probe must meet the above specifications to be certified for use.

Initials: **Date:** 4/30/2003

QC290 THERMOCYCLER BLOCK CLEANING

The wells of the sample block must be cleaned each month. Dirt, oil, and other contaminating agents collect in the sample wells, preventing the reaction tubes from seating properly. Maximum contact ensures optimum heat transfer from the block to the sample.

Documentation of Thermocycler Block Cleaning is kept in the Thermocycler Calibration and Maintenance Log Binder.

Procedure

NOTE: PROTECTIVE EYEWEAR MUST BE WORN WHEN CLEANING THE SAMPLE BLOCK. LIQUID MAY SPRAY OUT OF THE SAMPLE WELLS AS THEY ARE CLEANED WITH COTTON SWABS.

Prepare a 50% v/v isopropanol/water solution.

Clean excess oil out of the wells using kimwipes or cotton swabs.

Add one or two drops of the isopropanol solution to each well and carefully clean using cotton swabs. Rotating the swab helps to loosen material dried in the bottom. Wash the sides of each well with the isopropanol solution.

Remove excess liquid using a kimwipe or a dry cotton swab.

Check that there are no deposits left in the sample wells.

Clean the channels between the rows of the block using the same procedure.

If the deposits of dirt are heavy, it may be difficult to clean the wells. In this case, set the thermocycler to soak at 37°C. At a slightly warmer temperature, hardened deposits are easier to remove.

If the sample block has been contaminated with biological material, clean the wells using a 10% bleach solution, followed by a deionized water rinse. Dry the sample wells with dry cotton swabs or kimwipes.

Initials: **Date:** 4/30/2003

QC300 THERMOCYCLER DIAGNOSTIC TESTS (PE 9600)

There are two diagnostic (heater and chiller) tests that are run for the GeneAmp PCR System 9600 each month. The 9600 Thermocycler must pass all of these tests to be used for online forensic casework.

In addition, temperature verification and uniformity tests are done yearly according to the manufacture's instructions (Perkin Elmer, 1994). These tests are performed using a digital thermometer and probe as part of a Temperature Verification System that was purchased from the manufacturer. The thermocycler must pass the specifications set by the manufacturer to be used online in forensic STR analysis.

Accessing diagnostic test files

Get to the Main menu. Press the **STOP** key once or twice until the Main menu appears. The following will appear on the display:

Select Option 9600
RUN-CREATE-EDIT-UTIL

Press the **OPTION** key three times to move the cursor to UTIL, then press **ENTER.** The Utilities menu appears:

Select function
DIR-CONFIG-DIAG-DEL

Press the **OPTION** key two times to move the cursor to DIAG, then press **ENTER.** The following display appers:

Enter Diag Test #1
REVIEW HISTORY FILE

Before running the heater or chiller test, make sure you place an empty MicroAmp Tray on the sample block, then slide the heated cover forward and turn the cover knob clockwise until the white mark on the knob lines up with white mark on the cover.

Running the Heater Test

Select Diagnostic Test #2 by pressing **2**, and then pressing **ENTER**. The following display appears:

Enter diag Test #2
HEATER TEST

QC300 THERMOCYCLER DIAGNOSTIC TESTS - PE 9600 (CONT.)

Press **ENTER** to start the test. The following display appears:

Heater Test Blk=xx.x
going to 35C...

When the temperature stabilizes, full power is applied to all heaters. The display then reads "ramping...", then "timing..." and the block temperature is monitored.

When the block reaches the setpoint, the following screen appears:

Heater Test Passed.

This display will show "Passed" if the test was successful. If the test was not successful, the display will show "Failed." If this should occur, contact a PerkinElmer Biosystems Service Engineer.

Press **STOP** to return to the first Diagnostic display.

Running the Chiller Test

Select Diagnostic Test #2 by pressing **3**, and then pressing **ENTER**. The following display appears:

Enter diag Test #3
CHILLER TEST

Press **ENTER** to start the test. The following display appears:

Chiller Test Blk=xx.x
going to 50C...

The system first waits for the coolant temperature to get to 10 degrees C. The value "xx.x" on the screen pictured above represents the current temperature (in degrees C) of the sample block.

When the temperature stabilizes, the system drives the sample block cold, the temperature is monitored for a specific amount of time, and the cooling rate is calculated. The following display appears:

Chiller Test Passed

Initials: **Date:** 4/30/2003

QC300 THERMOCYCLER DIAGNOSTIC TESTS - PE 9600 (CONT.)

This display will show "Passed" if the test was successful. If the test was not successful, the display will show "Failed". If this should occur, contact a Perkin Elmer Biosystems Service Engineer.

Press **STOP** to return to the first Diagnostic display.

Documentation

The test results are documented on a Thermocycler (PE 9600) Diagnostic Log (F215) and filed in the Thermocycler Calibration and Maintenance Log Binder.

Maintenance

Temperature verification and uniformity tests are done yearly according to the manufacturer's instructions. These tests are performed using a digital thermometer and probe as part of a Temperature Verification System that was purchased from the manufacturer. The thermocycler must pass the specifications set by the manufacturer to be used online in forensic STR analysis as described below.

Equipment Required:

1. A one pound weight
2) Temperature verification System should include the following:
3) Digital Thermometer with 9V battery installed
4) RTD probe
5) Light mineral oil
6) Cotton swabs

The RTD probe assembly consists of two cones. The black cone houses the probe that measures the temperature of the sample well. The other one is a dummy one. This probe is calibrated yearly against NIST standards by Perkin Elmer Biosystems.

Temperature Verification Test for PE9600

Preparation

If the sample block heated cover is in the forward position, turn the knob completely counterclockwise, then slide the cover back.

Initials: **Date:** 4/30/2003

QC300 THERMOCYCLER DIAGNOSTIC TESTS - PE 9600 (CONT.)

Coat wells D1 and E1 with mineral oil using a cotton swab.

Place the probe tray on the sample block so that the probe tray notch faces the front of the instrument.

Place the probe assembly into wells D1 and E1 so that the dummy probe sits in D1. Carefully thread the probe wire through the notch in the probe tray. Connect the probe to the digital thermometer.

Slide the heated cover forward, then turn the cover knob clockwise until the white mark on the knob aligned with the white mark on the cover.

Procedure

NOTE: To ensure maximum accuracy, the temperature of the heated cover and the sample block are the same in this test. This prevents the heated cover from affecting the accuracy of the RTD probe.

Turn the digital thermometer by moving the ON-OFF/RANGE switch to the 200 position.

Turn on the GeneAmp PCR System 9600. The main menu appears

Press the OPTION key three times to move the curse to UTIL, then press ENTER. The utilities menu appears.

Press the OPTION key twice to move the cursor to DIAG, then press ENTER.

Run the Verify Calibration Diagnostic Test by pressing 5 then ENTER.

The temperature of the sample block and heated cover will go to 40°C, **Going to 40 °C, Cvr = xxC Blk = xx.xC** will appear. This display shows the current temperature of the block cover (Cvr = xxC) and sample block (Blk = xx.xC).

When the temperature of the block cover is within ten degrees the sample block temperature, the following display appears:

Wait 3 Minutes, Time = MM:SS Blk = 95.0 C

This display shows the current sample block temperature (Blk =40°C) and a clock, which counts up from zero in minutes and seconds (Time = MM:SS)

Initials: **Date:** 4/30/2003

QC300 THERMOCYCLER DIAGNOSTIC TESTS - PE 9600 (CONT.)

RECORD TEMPERATURE, TIME =MM:SS Blk = 95.0 C display will appears, when the clock reaches three minutes.

Measure the temperature of the well E1 using the digital thermometer. Record this temperature as T(40) on the log sheet.

Press ENTER.

The temperature of the sample block and heated cover will go to 95°C
Going to 95°C... Cvr =xxC Blk = xx.xC will appear.

When the temperature of the block cover (Cvr = xxC) is within ten degrees of the sample block (Blk = xx.xC) temperature the following display will appear:

WAIT 3 MINUTES, TIME = MM:SS BLK = 95.0C

When the clock reaches three minutes, the following display will appear:

Record Temperature, Time = MM:SS Blk + 95. 0C

Measure the temperature of the well E1 using the digital thermometer. Record this temperature as T(95) on the log sheet

Repeat the procedure for the second time. Record the temperature on the log sheet.

Remove the probe assembly from the sample block and move the digital thermometer ON-OFF/RANGE switch to the off position.

Clean the oil from D1 and E1 using cotton swabs.

Calculating Test Results:

Make sure that the serial number on the calibration label matches the serial number on the instrument you are testing.

Use the following formula to calculate the average block temperature at 95°C.

Block Average at 95 oC = T(95) - High Offset

Initials: **Date:** 4/30/2003

QC300 THERMOCYCLER DIAGNOSTIC TESTS - PE 9600 (CONT.)

The offset is the number of degrees Celsius that the temperature of well E1 differed from the average temperature of the block when the instrument was calibrated at the factory. The offset value is printed on the calibration label on the instrument.

Block Average at 40 oC = T(40) - Low Offset

If the block average is differ more than +/- 0.75°C from the programmed target temperature, the instrument must be recalibrated. Call PE Applied Biosystems Service Representative.

Documentation
Record data on F213 Thermocycler (PE9600) Calibration Log.

File all the paperwork in the Thermal Cycler Calibration Log Binder.

Temperature Uniformity for PE9600

Preparation:

If the sample block heated cover is in the forward position, turn the knob completely counterclockwise, then slide the cover back.

Coat all the wells in rows A, C, E and H with mineral oil using a cotton swab.

Place the probe tray on the sample block so that the probe tray notch faces the front of the instrument.

Place the probe assembly into the wells A1 and A2 so that the dummy probe sits in well A2. Carefully thread the probe wires through the notch in the probe tray. Connect the probe to the digital thermometer.

Slide the heated cover forward, then turn the cover knob clockwise until the white mark on the knob aligned with the white mark on the cover.

Procedure:

Turn the digital thermometer on by moving the ON-OFF/RANGE switch to thr 200 position.

Initials: **Date:** 4/30/2003

QC300 THERMOCYCLER DIAGNOSTIC TESTS - PE 9600 (CONT.)

Turn on the GeneAmp PCR System 9600. The main menu appears. Press the OPTION key once to move the cursor to the CREATE position. Press enter and a new menu appears. Again press OPTION once to move the cursor to the CYCL file. Press enter to accept and create a two-temperature CYCL program with the following parameters:

Setpoint #1 Temperature =95
Hold Time = 2:00
Ramp Time = 0:00 minutes

Setpoint #2 Temperature= 40
Hold Time = 2:00
Ramp Time = 0:00

Cycles = 99

On the third cycle, measure the temperature of well A1 90 seconds into Setpoint #1 (95 degrees setpoint temp) using the digital thermometer. The time remaining clock on the run-time display will read "0:30". Record the temperature.

Still on the third cycle, measure the temperature of well A1 90 seconds into Setpoint #2 (40 degrees setpoint temp) using the digital thermometer. The time remaining clock on the run-time display will read "0:30". Record the temperature.

After you measure the second temperature of well A1, turn the cover knob completely counterclockwise, then slide the heated cover back.

Move the probe assembly to wells A4 and A5, placing the dummy probe in A5.

Slide the heated cover forward, then turn the cover knob clockwise until the white mark on the knob and the white mark on the cover are aligned.

Repeat the measurements on the wells A4, A8, A12, C1, C4, C8, C12, E 1, E4, E8, E12, H1, H4, H8, and H12. Make sure you place the measuring cone of the probe assembly into these wells and the dummy probe into the adjacent wells.

After you have completed all measurements, remove the probe assembly from the sample block and turn off the digital thermometer.

Clean the oil from the sample block using cotton swabs.

Initials: **Date:** 4/30/2003

QC300 THERMOCYCLER DIAGNOSTIC TESTS - PE 9600 (CONT.)

Test Results:

For the Setpoint #1 measurements (95 degrees hold), subtract the lowest measured temperature from the highest measured temperature.

For the Setpoint #2 measurements (40 degrees hold), subtract the lowest measured temperature from the highest measured temperature.

* If either result is more than 1 degree Celsius, your GeneAmp PCR Sytem 9600 must be serviced by a Perkin-Elmer Service Representative.

Documentation

Record data on F213 Thermocycler (PE9600) Calibration Log

File all the paperwork in the Thermal Cycler Calibration Log Binder

Initials: **Date:** 4/30/2003

QC302 THERMAL CYCLER DIAGNOSTIC TESTS (PE 9700)

There are three monthly diagnostic tests that are run on the Gene Amp PCR System 9700 to check temperature calibration and verify the integrity of the cooling and heating system. The test are as follows:

1. Temperature Verification Test
2. Rate Test
3. Cycle Test

In addition, a temperature non-uniformity (TNU) test is done yearly to test the temperature uniformity of the sample block in the Gene Amp PCR System 9700.

The temperature verification and TNU tests are performed using a digital thermometer with probe and a 9700 probe tray. The rate and cycle tests require a 96-well plate with full plate cover. The thermal cycler must pass specifications set by the manufacturer to be used on line in forensic STR analysis.

1. Temperature Verification

This test requires the 96-well 0.2 ml Temperature Verification System. Two types of verification systems, cat. #N8010435 and #4317939 can be used for performing this test. The major difference between the two verification systems is whether the probe contains one or two cones.

The temperature verification system cat. #N8010435 consists of two cones, one of which measures the temperature of the sample well. The first cone that the wire is attached to does not measure the temperature of the sample well; this cone is the dummy probe. The other cone measures the well temperature. Temperature verification system cat. #4317939 consists of one cone that measures the well temperature.

Procedure

1. Place a probe tray on the 9700 sample block so that the notch faces the front of the instrument. Thread the probe wire through the notch in the probe tray. Make sure the probe is connected to the digital thermometer.
2. Coat well A6 ligthly with mineral oil. Also coat well B6 with mineral oil if using the two cone temperature verification system.
3. Place the temperature measuring probe of the temperature measuring system into well A6. If using a two cone temperature verification system, also place the dummy probe into well B6.
4. Turn on the digital thermometer by moving the ON-OFF/RANGE switch to the 200 position.

Initials: **Date:** 4/30/2003

QC 302 THERMAL CYCLER DIAGNOSTIC TESTS - PE 9700 (CONT.)

5. Access the temperature verification screen by following this path:

Util (F4) → Diag (F1) → TempVer (F3)

The 9700 thermal cycler has 5 function keys (F1 to F5) that you will be pressing to access various instrument functions. The above schematic shows what function key you will be pressing (in parentheses) to access the indicated funtion.

6. Press Run. The System 9700 screen will look as follows:

Calibration Verification
block temp= xx.x˚C Cover temp=xxx˚C
Setpoint is 85˚C
Cover must be w/i 10˚ C of Setpoint

7. When the block temperature reaches 85˚ C the instrument will begin a countdown. When this value reaches zero enter the actual block temperature (read from the external digital meter of the temperature verification system) on the 9700 instrument using the numeric keypad.

8. Repeat the temperature entry for the 45˚C setpoint as prompted by the instrument.

9. When the System 9700 completes calibration verification one of two screens appear:

Calibration Verification
Calibration is Good

OR

Calibration Verification
Instrument may Require Service
Contact PE/Applied Biosystems
Technical Support

10. Complete this test by removing probe and cleaning the oil from the sample block.

Specification

Instrument must indicate that calibration is good. Contact Applied Biosystems if the other screen is displayed. Instrument must be taken off line if the test has failed.

Initials: **Date:** 4/30/2003

QC 302 THERMAL CYCLER DIAGNOSTIC TESTS - PE 9700 (CONT.)

Documentation

Document the test results on F217 Thermal Cycler (AB 9700) Diagnostic Log and file in the Thermal Cycler Maintenance Log Binder.

2. Rate Test

Before beginning the rate and cycle tests, place an empty 96 well plate with full plate cover on the sample block (this test does not require the 96-well 0.2 ml Temperature Verification System). Slide the heat cover forward and pull down the lever. Access the rate test function by following the path shown below.

Util (F4) → Diag (F1) → System (F2) → Rate (F1)

After accessing the rate test function, the instrument will prompt you to install an empty microplate with a microamp full plate cover. Press the CONTINUE (F1) function key.

The instrument then runs a series of tests stabilizing the sample block at 35° C, 94° C, and 4° C. At the conclusion of the test, the test results appear on the screen and whether the test passes or fails.

Specification

The instrument must indicate on the screen that it passes this test according to the following specifications: heating >3.0° C/second; cooling >3.0° C/second. If the instrument does not pass this test, contact Applied Biosystems. Instrument must be taken off line if the test has failed.

Documentation

Document the test results on F217 Thermal Cycler (AB 9700) Diagnostic Log and file in the Thermal Cycler Maintenance Log Binder.

Initials: **Date:** 4/30/2003

QC 302 THERMAL CYCLER DIAGNOSTIC TESTS - PE 9700 (CONT.)

3. Cycle Test

Access the cycle test function by following the schematic shown below:

Util (F4) → Diag (F1) → System (F2) → Cycle (F2)

After accessing the rate test function, the instrument will prompt you to install an empty microplate with a microamp full plate cover. Press the CONTINUE (F1) function key.

Note: Pressing pause will generate false test results. Test must be allowed to run in its entirety. At the conclusion of the test, the screen displays the test results and whether or not it passes or fails.

At the conclusion of this test, the screen displays the test results and whether or not the instrument passes or fails.

Specification

The instrument must indicate on the screen that it passes this test according to the following specifications: Average Cycle Time < 160 seconds; Cycle Time Standard < 5 seconds. If the instrument does not pass this test, contact Applied Biosystems. Instrument must be taken off line if the test has failed.

Documentation

Document the test results on F217 Thermal Cycler (AB 9700) Diagnostic Log and file in the Thermal Cycler Maintenance Log Binder.

Initials: **Date:** 4/30/2003

QC 302 THERMAL CYCLER DIAGNOSTIC TESTS - PE 9700 (CONT.)

4. Temperature Non-uniformity (TNU) Test

This test requires the 96-well 0.2 ml Temperature Verification Systems (see the Temperature Verification section above for a discussion of temperature verification systems).

Procedure

1. Place a probe tray on the 9700 sample block so that the notch faces the front of the instrument. Thread the probe wire through the notch in the probe tray. Make sure the probe is connected to the digital thermometer.
2. Coat well A1 lightly with mineral oil. Also coat well A2 if using the two-cone temperature verification system.
3. Place the temperature measuring probe of the temperature measuring system into well A1. If using a two cone temperature verification system, also place the dummy probe into well A2.
4. Turn on the digital thermometer by moving the ON-OFF/RANGE switch to the 200 position.
5. Slide heat cover forward and bring lever down to lock in place.
6. Access the TNU screen by the following path:

Util (F4) → Diag (F1) → TempVer (F3) → TNU (F2)

7. When prompted to put probe in well A1, press RUN.
8. When sample block reaches 94° C, the TNU performance screen will show that the block is stabilizing for 30 seconds and will ask for block temperature.
9. Record block temperature from the digital thermometer and using the instrument numeric keypad enter this value. Also, record this value on F218 Thermal Cycler (AB 9700) Diagnostic Log.
10. The sample temperature then approaches the next temperature point, 37° C by shutting off the heat cover.
11. The message "stabilizing block at set point... 00:30" will appear on the screen.
12. When the block has stabilized at 37 °C (eg., timer has counted down to 0:00), record the block temperature from the digital thermometer and enter this value using the instrument's numeric keypad. Record this value on F218 Thermal Cycler (AB 9700) Diagnostic Log. Press ENTER.

Initials: **Date:** 4/30/2003

QC 302 THERMAL CYCLER DIAGNOSTIC TESTS - PE 9700 (CONT.)

Note: Prompts appear for you to move the probe assembly to the respective sample well to be tested.

TNU performance	
sample temp= xxx˚C	Cover temp= xxx˚C
place probe in well xx,	dummy in xx

13. Slide heat cover back. Remove probe(s) from wells and move to the next prompted well(s)
14. Slide heat cover forward and pull lever down.

Repeat these steps for the wells prompted by the instrument. They are as follows: A1/A2, A12/A11, C4/C3, C9/C10, F4/F3, F9/F10, H1/H2, and H12/H11. The first well of each pair indicates the well the measuring probe is placed in. The second well number indicates the well the dummy probe is placed into when using a two cone probe.

The instrument will prompt you to move the probe(s) through this sequence of wells twice, once for the higher temperature (94˚ C) and the second time for the lower temperature (37˚ C).

Specification

When the System 9700 completes the TNU test, the screen will display all of the TNU values at 94˚C and 37˚C. If all of the values are correct press ACCEPT. If not, repeat the test. The instrument will then display the final TNU values on the screen and will indicate pass or fail according to this specification: ≤ 0.5. This value reflects whether the range of temperature values at a given temperature does not exceed +/- 1 ˚C. If the instrument fails this specification call Applied Biosystems. Instrument must be taken off line if the test has failed.

Documentation

Document the test results on F218 Thermal Cycler (AB 9700) Diagnostic Log and filed in the Thermal Cycler Maintenance Log Binder.

Initials: **Date:** 4/30/2003

QC310 WATER QUALITY MAINTENANCE

Changing Water Filters

Water filters should be changed once every two weeks. This is documented on a Maintenance Log (F165) and filed in the pH Log & Water Systems Binder. Use the procedure that follows to change filters:

1. Turn off the main water valve. Open deionized water valve and depress pressure release button (red button on dispenser) to relieve pressure in the housing.
2. Unscrew filter housing from cap, discard used cartridge and insert new cartridge (1 and 5 um).
3. Screw the housing onto the cap and hand tighten.
4. Open the main water valve slowly. Let the water run for 1-2 min. through the dispenser.
5. Turn off the deionized water dispenser.

Checking Water Quality

Water quality is checked weekly to include readings of total chlorine, free chlorine, total hardness, total alkalinity, pH and resistivity of the water using an Aquacheck strip and Myron L conductivity meter. Information is recorded on a Maintenance Log (F165) along with water filter information (if necessary) and filed together in the pH Log & Water Systems Binder.

Procedure

1. Take one strip from the bottle.
2. Turn on the deionized water
3. Pass the strip under water system
4. Remove (do not shake)
5. Compare total hardness, total alkalinity and pH to the color chart shown on the bottle.
6. Record the readings on the log.
7. Again hold the strip under water system for 10 seconds.
8. Compare chlorine pads to the color chart.
9. Record readings on the log.

Specification

Readings should show a neutral pH (approx pH 7), and very low (total chlorine < 1 ppm; free chlorine <1 ppm; total hardness < 50 ppm; total alkalinity <80 ppm) or no traces of ions. The detection of ions indicates a reduced efficiency of ion removal by the deionizing tanks. A red light on top of the tanks indicates that tank replacement is necessary.

Initials: **Date:** 4/30/2003

QC310 WATER QUALITY MAINTENANCE (CONT.)

Checking Water Resistivity

1. Check batteries of the meter by pressing the button at the lower right corner of the meter. If the light is not visible change batteries.
2. Select range by turning the range knob at the lower left corner (x .1).
3. Rinse the cell cup three times with deionized water.
4. Then fill with deionized water to at least 1/4" above upper electrode.
5. Push button to read directly in microohms or megaohms.

Specification

Record the readings on the same Maintenance Log as for checking the Water Quality. File the Maintenance Log into the pH Log & Water System Binder.
The resistivity reading should be greater than 10 megaohms (on the red lettered scale). When readings fall to 1 megaohm, call vendor for ion exchange tank replacement.

Initials: **Date:** 4/30/2003

QC320 INSTALLATION VALIDATION FOR ADDITIONAL ABI 377 OR ABI 310 INSTRUMENTS

This procedure only refers to new instruments of the same model number and from the same manufacturer as the current data collection platform. For a change of model or manufacturer a more extensive validation is required.

The laboratory has been utilizing ABI 377 and ABI 310 for a couple of years and reproducibility and precision data were established for each platform during the different multiplex validations. The main objective for testing new additional instruments prior to casework is to compare the performance and sensitivity to the current equipment.

For each multiplex system, run a batch of previously amplified and analyzed samples. Include negative controls and allelic ladders where applicable.
Compare the new results to the old runs in regard to:

- allele calls
- peak intensities
- absence of artefacts

The new instrument must yield the same allele calls and similar peak intensities without unspecific signals.

Initials: **Date:** 4/30/2003

QC325 INSTALLATION VALIDATION FOR ADDITIONAL 9600 AND 9700 THERMOCYCLERS

This procedure only refers to new instruments of the same model number and from the same manufacturer as thermal cyclers that were previously put in service. For a change of model or manufacturer, a more extensive validation is required.

Amplification conditions for all casework multiplexes were previously established and validated on the 480, 9600, and 9700 cyclers. A new instrument has to pass the diagnostics test and yield satisfactory amounts of specific PCR product.

Perform diagnostics test as outlined in QC295, QC300 and QC302.

Amplify a positive control sample in every other well of the thermocycler block. Each multiplex system should be used in a representative number of wells. Each batch of samples should include an amplification negative control.

The following guidelines apply:

- all samples must yield the correct type
- no sample should display additional alleles
- all samples should be of similar peak intensity

Initials: **Date:** 4/30/2003

QC330 PERFORMANCE TEST AFTER MAJOR REPAIRS FOR ABI 377 OR ABI 310 INSTRUMENTS

This procedure only applies for repairs affecting the optical system and/or computer parts essential for data collection. Neither a performance test nor a new matrix are required for minor repairs such as the flow pump switch for the 377 or the syringe for the 310.

Run a new matrix following QC 210. On the same run include the amplification product of at least one known sample, one negative control, if not previously run, and if applicable an allelic ladder.

Compare the new results to the old runs in regard to:

- allele calls
- peak intensity
- absence of artefacts

The new instrument must yield the same allele calls and similar peak intensities without unspecific signals. Even if the instrument type is used for more than one kind of casework multiplex it is not necessary to test each multiplex. A performance test in one of the systems is sufficient.

Initials: **Date:** 4/30/2003

QC335 PERFORMANCE TEST AFTER MAJOR REPAIRS FOR 9600 AND 9700 THERMAL CYCLERS

This procedure applies to instruments that have been shipped out for service and have to be tested before reinstating them for use in casework.

Perform diagnostics test as outlined in QC295, QC300 and QC302.

If the cycler passes the diagnostics test, amplify a positive control sample in every other well of the thermocycler block. One well should contain the amplification negative control.

The following guidelines apply:

- all samples must yield the correct type
- no sample should display additional alleles
- all samples should be of similar peak intensity

Even if the instrument type is used for more than one kind of casework multiplex it is not necessary to test each multiplex. A performance test in one of the systems is sufficient.

Initials: **Date:** 4/30/2003

QC340 PERFORMANCE TEST FOR MISCELLANEOUS EQUIPMENT FOLLOWING REPAIR

Instruments such as heat blocks, water baths, freezers, balances, pH meters, refrigerators, freezers, ice machines, incubators, microplate washers, microplate readers, and water stations do not require specific performance tests other than the QC tests that are done routinely or as needed (eg., verifying that the water bath temperature is in range) to demonstrate that the instruments are performing to specification. Where applicable, diagnostic tests (eg., linearity and reapeatability tests for the microplate reader) will also be run to demonstrate that the instrument is performing to specification.

Initials: **Effective Date:** 4/19/2004

QC350 CAPILLARY ELECTROPHORESIS (ABI 3100)

Test Materials

Performance Optimized Polymer 4
310/3100 Genetic Analyzer Buffer with EDTA
Cofiler, Profiler Plus, PowerPlex 16 Kit Reagents (see QC110)
CXR or ILS 600 Size Standard
HI-DI Formamide

Samples

Run amplified products from two DNA samples; an allelic ladder, amplified positive control DNA, and a reagent blank (amplification negative control).

Procedure

1. Electrophorese samples according to the capillary electrophoresis protocol.

2. Analyze samples according to the Genescan Analysis and Genotyper protocols as described in the Protocols for Forensic STR Analysis Manual.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation

Document on appropriate capillary electrophoresis run worksheets.

Attach the completed worksheets to a Raw Material Quality Control Test Form (F183).

File reagent sheet and CE run worksheets together in the appropriate QC reagent binder.

Initials: **Effective Date:** 4/26/2004

QC360 CAPILLARY ELECTROPHORESIS (ABI 3100): MAINTENANCE

Basic cleaning of the instruments should be done once a week by simply wiping down the inside with Kimwipes lightly dampened with deionized water. According to the ABI manual, organic solvents should not be used to clean the instrument. Be sure to wipe down the entire inside of the instrument including inside the oven, under the autosampler, the syringe holder and drip trays as well as the doors. Check for leaks around the syringes and clean any dried polymer.

The polymer blocks should be cleaned every time the capillary is changed by casework analysts. They can be cleaned more often as needed if there are leaks.

Once a month, the air filters should be checked to see if they need to be cleaned or changed, the buffer and water reservoirs should be soaked in warm water or changed, and the septa for these reservoirs should be changed.

Be sure that everything is dry when done.

When problems are experienced with the ABI 3100 Capillary Electrophoresis unit, there are limited diagnostic tests that may be done according to the protocols presented below. The purpose of these tests are to check the operation of the laser and the power supply. The diagnostics software is primarily for use by the ABI service engineers. Do not perform tests other than the ones listed below.

LASER TEST OR POWER SUPPLY TEST

1) Make sure the doors of the instrument are closed.

2) To access the diagnostic test files, select the **PE Biosystems** folder from the start menu. And click on the "3100 service" option. From this menu, select "3100diagnostics.exe," then select "Diagnostics Menu." Here you have several options to choose from. Only choose the laser power or EP power options by clicking on the appropriate box. Click on the start button to run the test. Once the test is finished, a pass/fail grade will be given to each tested item. If any test fails, take the instrument offline and place a service call.

3) A message will appear to log the results. Click "no." Click **Return** to exist out of screen until you reach the main diagnostics menu then press **Exit.**

If it is necessary to shut down the instrument, close the instrument doors and press the ON/OFF button on the front of the instrument. Next, shutdown the computer.

To restart the instrument, first restart the computer (let it completely restart before proceeding) then press the ON/OFF button on the front of the instrument. The firmware and calibration files will reload.

Initials: **Date:** 4/30/2003

Appendix C-1

This appendix shows a list of log usage and maintenance forms that are used in the OCME Forensic Biology Laboratory to provide records of equipment use, calibration, and maintenance. All of these forms can be accessed on the Forensic Biology computer network.

Usage and Maintenance Log List

F100 Balance Verification and Maintenance Log
F105 Capillary Electrophoresis Diagnostic Log
F110 Capillary Electrophoresis (ABI 310) Usage Log
F115 Freezer (-20°C) Temperature Control Log
F120 Freezer (-80°C) Temperature Control Log
F125 Gel Electrophoresis (ABI 377) Parameters Log
F130 Gel Electrophoresis (ABI 377) Usage Log
F135 Heat Block (56°C) Temperature Control Log
F140 Heat Block (65°C) Temperature Control Log
F145 Heat Block (95°C) Temperature Control Log
F150 Heat Block (100°C) Temperature Control Log
F157 Incubator Control Log (37°C)
F160 Kit Control Log
F165 Maintenance Log
F170 Micropipette Maintenance Log
F172 P30 ELISA Raw Material Quality Control Test Form
F175 pH Meter Calibration Log
F180 Plate Washer Maintenance Log
F183 Raw Material Quality Control Test Form
F185 Reagent Inventory Log
F187 Reagents/Machine Verification Quality Control Log
F190 Refrigerator Temperature Control Log
F195 Temperature/Humidity Control Log
F200 Thermocouple (Type T-Blue) Calibration Log
F205 Thermocouple (Type T-Brown) Verification Log
F213 Thermocycler (PE 9600) Calibration Log
F215 Thermocycler (PE 9600) Diagnostic Log
F220 Thermocycler File Log
F225 Thermocycler Usage Log
F230 Water Bath Temperature Control Log

Initials: **Date:** 6/1/2003

Appendix C-2

This appendix shows a list of reagents used in the OCME Forensic Biology Laboratory. They are further classified as "Critical" or "Non-Critical" reagents. As per the FBI Quality Assurance Guidelines, a "Critical reagent" requires testing on established samples before use in order to prevent unnecessary loss of sample.

REAGENT	CRITICAL
Acid Phosphatase Test Reagent	Y
Alkaline Substrate Buffer	Y
Ampholytes (for IEF Hb plates)	Y
Amylase Gel Buffer	Y
Anode Solution (IEF Focusing)	N
Bromothymol Blue	N
Casein Stock Solution	Y
Cathode Solution	N
Coomassie Blue Stain	N
Destain Solution	N
Erythrocyte Acid Phosphatase (ACP) Reaction Buffer	Y
Esterase D (ESD) Reaction Buffer	Y
Iodine Solution, 0.01 N	N
Isoelectric Focusing Acid Phosphatase (ACP) Plates	Y
Isoelectric Focusing Esterase D (ESD) Plates	Y
Isoelectric Focusing Hemoglobin (Hb) Plates	N
Isoelectric Focusing Phosphoglutamase (PGM) Plates	Y
Kastle-Meyer (KM) Reagent	Y
Leucomalachite Green (LMG) Reagent	Y
Nuclear Fast Red	Y
PBS Solution for P30 ELISA (PBS tablets)	Y*

Initials: **Date:** 6/1/2003

PBS-BSA Solution	N
Phosphoglutamase (PGM) Reaction Buffer	N
Phosphoglutamase (PGM) Reaction Mixture	N
Picric Indigo Carmine (PIC)	Y
Potassium Cyanide Solution (KCN), 0.05%	N
Saline (0.85% NaCl)	N
Sodium Acetate, 0.1 M (pH 5.5)	N
Species Agarose Gel	N
Sjpecies Tank Buffer	N
Takayama Reagent	Y
Urea Diffusion Test and Blank Plates	N
Ammonium Persulfate (APS)	Y*
BSA Solution, 5 mg/mL	Y
Cell Lysis Buffer (CLB)	Y
Chelex, 5%	Y
Chelex, 20%	Y
Chloroform-Isoamyl Alcohol	N
Chromogen Solution	Y*
Cofiler PCR Reaction Mixture	Y*
Deoxynucleotide Triphosphates, 2.5 mM (dNTPs)	Y
Digest Buffer	Y
Dithiothreitol (DTT), 1M	Y
EDTA, 0.5 M	N
Formamide, Deionized	Y*
Formamide and Loading Buffer	Y
Hydrogen Peroxide, 3%	N
Negative female control DNA for Y STR analysis	Y

Initials: **Date:** 6/1/2003

PBS for Chelex Extraction	Y
Positive Control - Quad	Y
Positive Male Control DNA for Y STR Analysis	Y
Primer, DYS19/1	Y
Primer, DYS19/2	Y
Primer, DYS389/1	Y
Primer, DYS389/2	Y
Primer, DYS390/1	Y
Primer, DYS390/2	Y
Primer, F13A1/1	Y
Primer, F13A1/2	Y
Primer, FES/FPS/1	Y
Primer, FES/FPS/2	Y
Primer, TH01/1	Y
Primer, TH01/2	Y
Primer, VWA/1	Y
Primer, VWA/2	Y
Profiler Plus PCR Reaction Mixture	Y*
QUAD STR/PCR Reaction Mixture	N
Quantiblot Citrate Buffer	N
Quantiblot DNA Standards	Y
Quantiblot Hybridization Solution	Y
Quantiblot Pre-Wetting Solution	N
Quantiblot Spotting Solution	N
Quantiblot Wash Solution	Y
Sarkosyl, 20%	N
Sequencing Loading Buffer	Y

Initials: **Date:** 6/1/2003

Sodium Acetate, 0.2 M	Y
SDS, 0.1%	N
SDS, 10%	Y
SDS, 20%	N
SSPE, 20X	N
Stain Extraction Buffer	Y
Sterile Deionized Water	Y
TEMED	Y*
Tris-EDTA, 1X	Y
Tris-HCl, 1M (pH 8.0)	N
TNE, 1X	N
TNE, 10X	N
Urea, 10.8 g	Y*
Urea, 18 g	Y*
Urease	Y*
Y1 STR/PCR Reaction Mixture	N

* tested for each new vendor lot/shipment