Department of Forensic Biology

Quality Assurance Manual

Version 1.0

March 31, 1992

Department of Forensic Biology

Quality Assurance Manual

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Initials: RCO Date: 3/31/92

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I. Historical

Effective this date, this Quality Assurance manual supercedes all previous QA manuals and QC procedures used in the Department of Forenic Biology or Serology Laboratory.

QA Committee Members:

Chairman: Robert C. Shaler, Ph.D. Robert C. Shaler

Co-Chairman: Howard Baum, Ph.D. Howard Baum

FMB QA Coordinator: Robert Wilson

FBH QA Coordinator: Larry Quarino Larry Quarino

Committee Members: Marie Samples Marie Samples

Dora Wolosin

Jocelyn Ferrara Jocelyn Ferrara

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II. Introduction

The Department of Forensic Biology Quality Assurance Manual is designed to provide a program through which all laboratory operations are scrutinized in an effort to provide a reliable laboratory result. The following definitions apply.

A. Quality Control

Those procedures used to maintain acceptable limits of variation for products and services.

B. Quality Assurance

Quality assurance pertains to those procedures used to insure that quality control parameters are appropriate and sufficient measures of variation.

C. An Example

Measuring and recording the pH of a solution is a common quality control to insure that the variation between lots of solutions is maintained within a specified range. But this parameter is a meaningful measure of quality only if the pH meter has been calibrated, the technician making the measurement knows how to operate the pH meter, the water is sufficiently pure, and the technician has added the proper reagents. Quality assurance insures that quality control measures are meaningful measures of variation.

III. Planning and Organization

A. Goals

It is the goal of the Department of Forensic Biology to provide users of its laboratory services access to scientific analyses that compare biological evidentiary specimens with known exemplar biological specimens and to insure the quality, integrity, and accuracy of the department's analyses through the implementation of a detailed quality assurance (QA) program.

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The scientific analyses include but are not limited to the following:

1. Sample identification
2. Species identification
3. Genetic marker analysis
4. Report Preparation
5. Testimony to results

B. QA Objectives

1. Monitor, on a routine basis, the analytical testing procedures for all scientific testing performed in the laboratory by means of Quality Control (QC) standards, proficiency tests, and audits.

2. Verify that all scientific analyses operate within the established performance criteria and that the quality and validity of the analytical data is maintained.

3. Performance criteria are established in the Laboratory Methods Manuals for each of the routine scientific procedures performed in the laboratory.

4. The quality and validity of the data is ensured by the quality control (QC) program for both reagents prepared in the laboratory and obtained commercially and by the quality assurance (QA) program for instrument use and maintenance logs.

5. The qualifications of the laboratory staff are ensured by the position requirements of the Department of Personnel of the City of New York and by the proficiency testing program that is an integral part of the overall QA program of the Department of Forensic Biology.

6. The records for in-house reagent manufacture are maintained as are the QC documentation of their acceptability. Outside vendor QC documents (specification sheets, etc.) are retained.

   Insure that problems are noted and that corrective action is taken and documented. Each problem is recorded in the appropriate log book and the corrective action is noted, dated and signed by the appropriate laboratory supervisor.

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C. Authority and Accountability for the QA Program

The organizational structure (Figure 1.) defines the relationships in the Department of Forensic Biology between individuals and the operational units of the department.

Within the department, a QA/QC committee sets QA/QC policy and is responsible for production and revisions of the QA Manual. Each laboratory division within the Department has a QA Coordinator appointed by either the Department director or assistant director. The QA committee is comprised of the following members.

The chairman of the committee, who has overall responsibility for the QA program is the director of the Department of Forensic Biology.

Assistant Director(s) (co-chairman), forensic scientist(s), assigned QA coordinator(s), and the reagent preparation technician(s) are also members of the committee.

Each Forensic Analyst is responsible to ensure that the QA/QC program guidelines, as they relate to their work and responsibilities, are adhered to.

D. OCME and Department of Forensic Biology Organizational Structure

The OCME is organized (Figure 2.) such that the director of the Department of Forensic Biology reports directly to the head of the agency, the Chief Medical Examiner.

The organization of the Department of Forensic Biology is organized into two operational units, the Forensic Biochemistry and Hematology Laboratory (FBH) and the Forensic Molecular Biology Laboratory (FMB) (Figure 1). Each operational unit has an assistant director who reports directly to the departmental director.

The scientific staff in the FMB laboratory includes 3 forensic scientists and 6 forensic analysts. The FBH laboratory has 2 forensic scientists and 6 forensic analysts.

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Each operational unit is allocated support personnel: one office associate level, and one laboratory helper. The organizational chart of the department defines the responsibility in absences. The laboratory assistant directors are responsible for the operation of their laboratory subunits. The FBH laboratory assistant director is responsible for case coordination between operational units. In case the FBH assistant director is absent, the FMB laboratory assistant director assumes this responsibility. In the absence of the departmental director and both assistant directors, the FBH forensic scientists assume dual responsibility for laboratory operations. In their absence, FMB laboratory forensic scientists assume this responsibility. Under no circumstances will all FBH and FMB forensic scientists be absent from the laboratory.

IV. Documentation

Laboratory personnel record all significant laboratory activities to create a useable audit trail that documents the department's routine scientific testing. The documentation will be kept for the following topic areas:

A. Manuals

1. Scientific Manuals

These documents describe **in detail** the current protocols used for the analytical testing of biological specimens for all the scientific procedures used in the departmental laboratories. They include the following information before they are certified to be used as acceptable procedural manuals:

a. Date the procedure was adopted
b. Date the procedure was authorized
c. Revision dates
d. Signatures of authorized staff + dates

Department Director
Assistant Directors
Forensic Scientists

e. Archives of methods

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2. **Administrative Manual** (Appendix A)

a. Chain of Custody

1. Post-Mortem Evidence
2. Physical Evidence

B. Quality Control/Critical Reagent Documents

The QC documents in the departmental laboratories document that all critical reagents are prepared according to guidelines established within the department and according to accepted procedures. The documents available for each testing procedure include the following.

1. QC Procedures Manuals

Detail the procedures used in determining the quality of reagents prepared in-house or those purchased from outside vendors.

2. Reagent Preparation Manual

Details the procedure to be used in the preparation of critical solutions used in routine testing in the departmental laboratories.

3. Reagent Preparation Records

Lot and Batch Numbers
Date of Preparation
Initials of Preparer
Documentation of QC Pass/Fail and Evaluation
Date of Evaluation
Archive of Evaluation Data

C. Case Files/Case Notes

Case files contain analytical laboratory work sheets and original data (or copies with references to the location of original data), reports reflecting the results and their interpretation, chain-of-custody documentation, analytical summary sheets, police vouchers (if applicable), QC information, and case questionnaire and information sheet.

The case file also contains dates of review by supervisors.

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D. Data Analysis and Reporting

Departmental guidelines require that all case data is interpreted independently by the forensic analyst assigned the case and another laboratory staff scientist. Each independently observed result is dated and initialed.

All original data must be archived by one of several acceptable methods (if possible or if applicable), i.e., densitometry, photography, xerox, and digitization, for future retrieval and analysis.

Where identifications are made, specific matching criteria have been established and are part of the methods manual.

Known standards are recorded and monitored by means of established criteria and are part of the methods manual.

All reports must accurately reflect the data produced and be presented in an unbiased manner.

The format of the report allows the reader to identify the following:

1. Medical Examiner and Forensic Biology Case numbers.
2. Deceased/Victim name (if known).
3. Police Precinct and Complaint Numbers (if applicable).

The review process (in chronological order) for reports is as follows:

1. A draft, hand written report is prepared by the assigned forensic analyst according to the guidelines established for uniform report preparation in either the Forensic Biochemistry and Hematology or Forensic Molecular Biology Laboratories.

2. Review of the draft report by a supervising forensic scientist.

3. Typing of the report by departmental clerical staff.

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4. Second review of the report after typing by the supervising forensic scientist.

At this point additional work may be required which will necessitate a delay in the case being completed. If this occurs, the reporting process will begin again.

5. Final review of the report by the assistant director of the laboratory or by the departmental director.

6. Statistical information to obtain departmental and individual productivity data will be obtained at this time.

7. The original of the report is sent to the OCME records department, a certified True Copy is sent to the submitting agency, and a copy is retained in the case file.

E. Evidence Handling Protocols

1. **Chain-of-Custody** (see also Appendix A)

Chain of custody refers to the documentation that allows evidence tracking from receipt of evidence (either post-mortem autopsy specimens or physical evidence obtained through investigations), through the analytical process, until it leaves the control of the laboratory.

a. Evidence Receipt:

Evidence, whether received as specimens collected during the autopsy or received from user agencies, are signed into the laboratory by an evidence technician, resident in the Forensic Biology Department. An evidence receipt form is filled out. At this point the evidence is controlled by the Evidence Unit.

b. Case Number:

Evidence is assigned a sequential FB--00000 number where FB refers to Forensic Biology, -- refers to the year, i.e., 89, 90, etc, and 00000 identifies a sequential number assigned to one specific investigation.

c. Item Numbers

Each item is assigned a number that is associated with a police control or voucher number.

For example: Multiple items may be received under one police control or voucher number. Each item is assigned a number, i.e., 1,2,3,4, and etc,. Also multiple police or voucher numbers may be used for a single death investigation. All will be assigned the same FB number.

d. Signatures

When the police and other outside agencies bring evidence to the laboratory, the date, police precinct, complaint number, and evidence vouchers are listed on the chain-of-custody form. The signatures of courier and OCME evidence technician are also obtained.

Evidence from autopsies are collected by an assigned evidence technician or laboratory personnel. This evidence is recorded on the chain-of-custody form, dated and the signature of the individual retrieving the evidence is recorded.

e. Storage

Evidence is stored in the departmental cold room (4 degrees celsius) until it is assigned to the forensic analyst who the performs required analyses.

f. Case Assignment

A supervising forensic scientist is responsible for reviewing the case to determine what testing must be performed. The case is then assigned to a staff scientist who obtains the case from the resident evidence technician.

The case is turned over to the staff scientist and the date and signature of the scientist and the evidence technician are recorded on

the chain-of-custody form. At this point the evidence is controlled by the Forensic Biology Department.

During the analysis of the evidence, the scientist describes each item of evidence and initials, dates, and labels each sample of each item that is analyzed.

When the analytical work is completed, the evidence is packaged according to protocols accepted by the NYPD and returned to the Evidence Unit, specifically to the resident evidence technician. The date and signatures are recorded on the chain-of-custody form. The evidence is now controlled by the Evidence Unit.

F. Equipment Calibration and Maintenance Logs

Each piece of essential scientific apparatus has a log usage book. **Essential is defined as being required for use in the procedural or methods manual and has usage and/or QA/QC records available.**

The first step for all preventative maintenance is cleanliness. If there is any kind of spill, inside or outside a piece of equipment, it is to be cleaned up **IMMEDIATELY** (this includes hybridization solution, buffers, salts, and etc.). Some spills may be corrosive to the equipment and cause more damage than necessary. Also, it is much easier to clean some reagents before they dry and have to be chiseled off.

The usage log for each item begins with the date of purchase of the piece of equipment. In addition to daily entries in the log, each calibration of the apparatus is also maintained in the usage log.

For equipment purchased before the institution of the this manual, if the date of purchase is known, that date will be used, if the date of purchase is not known, then the date the manual was placed into service will be used instead. An approximate date of purchase will be entered into the log beside the date.

Any irregularities observed during routine use of any items of equipment are recorded in the comments section of the log and reported to a supervising forensic

scientist, assistant director, or department director. A supervisor, assistant director, or director will investigate the cause of the problem, determine whether the item of equipment is unsuitable for use in casework and take corrective action. The corrective action taken will be recorded in the log. If the item of equipment has been taken out of use, an entry into the log book re-certifying the equipment for casework is made by the supervising forensic scientist, assistant director, or departmental director of forensic biology.

The schedule of equipment maintenance follows:

1. Temperature Maintenance Equipment (refrigerators, freezers, water baths, cooling baths, ovens, and etc).

   Temperatures are recorded daily on a temperature log specified for each piece of equipment. This is done by the technician responsible for the preparation of reagents **OR AS ASSIGNED BY THE QA COORDINATOR OR LABORATORY FORENSIC SCIENTISTS.** The log is dated and initialed by the person performing the temperature recording.

   Each piece of equipment has its own permissible temperature range. Variations which exceed the permissible range will be evaluated relating to its suitability for continued usage on a per situation basis.

2. pH meters

   These are calibrated as they are used and are checked for each measurement by scientific staff performing pH measurements.

   The technician responsible for preparation of reagents keeps a record of the pH measurements used to prepare critical laboratory reagents.

3. Electrophoresis Equipment

   Electrophoresis power supply logs are filled out for each use by the analyst using the equipment and are dated, initialed, purpose, and comments.

Each electrophoresis tank usage is documented on a log sheet as to date, initials, purpose, and comments.

4. Balances

Balances are checked weekly and are calibrated every 6 months by an outside service. The calibration date is recorded in the usage log.

In the event that an outside service is not available or, for other reasons, cannot be contracted for, the calibration will be performed by laboratory personnel. The usage log will reflect this information.

5. Thermocyclers

Each thermocycler has a usage log which is documented as to date, initials, purpose, and comments.

Thermocyclers are checked weekly by a technician or analyst assigned to perform the calibration. The record of the calibration is recorded in the usage log.

6. Microtiter Plate Readers

Each microtiter plate reader has a usage log which is documented as to date, initials, purpose, and comments.

Checking is performed monthly by a technician or analyst assigned to perform the calibration. The record of the calibration is recorded in the usage log.

7. Micropipettes

Micropipettes will be checked using either standard calibration kits or gravimetrically. Each pipet will be assigned a control number and the date of calibration and the initials of the person performing the calibration will be recorded in the log. Each pipette will have a piece of tape on the handle indicating the last time a calibration was conducted. Each pipet will be calibrated three times annually.

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8. Centrifuges

Centrifuges are not normally used for precise centrifugations and do not need to be calibrated.

9. Freezers

Freezers must be defrosted annually. Defrosting of freezers is recorded on the temperature log for that freezer.

10. Hoods

Biological hazard and chemical fume hoods are to be calibrated annually by an outside contractor.

11. Survey Meters

Survey meters for measuring radioactive contamination of work surfaces are calibrated according to the specifications of the radiation license. A record of the calibration is maintained in a maintenance log.

12. Liquid Scintillation Counter

The liquid scintillation counter is checked monthly with a standard radiation source. A use log is maintained and standard reference anomalies are recorded. The instrument is calibrated annually.

G. Proficiency Testing

Proficiency testing is used to periodically demonstrate the quality performance of the scientific service offered by the laboratory and serves as a mechanism for critical self evaluation. This will be accomplished by the analysis and reporting of results from appropriate biological specimens, submitted to the laboratory as open and/or blind case evidence.

All specimens submitted as part of a proficiency test must be analyzed and interpreted according to the analytical protocols being used at the time the proficiency test is taken. All samples of "blind" evidence analyzed will be treated as normal casework.

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The scientific staff will be proficiency tested according to guidelines established by regulating agencies. If not regulated, proficiency tests will be administered twice annually; both proficienies will be open tests. One blind test (if possible) will be administered to the laboratory once annually.

1. Deficiency and Corrective Action

It is the responsibility of the QA Coordinator, or designated individual, to assure that deficiencies are acknowledged and that any corrective or remedial action is documented.

a. Analytical/Interpretative Error

Any error of this type, i.e., mistyping or misinterpreting analytical results whether correct or not, will result in the analyst being suspended from performing that specific test in casework until the cause of the error is identified and corrected.

The supervisor will supervise the scientist in the performance of the specific test until satisfactory performance is obtained. At that time a second proficiency will be administered.

In addition, the QA Coordinator will review the most recent cases signed by the analyst to ascertain whether similar errors have passed through the case review process.

Before the person is permitted to resume casework, an additional set of open proficiency tests must be successfully completed.

b. Systematic Error

Any error found to be the result of equipment, materials, environment, and etc., may require a review of all relevant casework since the unit's last successfully completed proficiency test. Once the cause of the error has been identified and corrected, all analysts will be notified in writing of the appropriate corrective action in order to minimize the

recurrence of the discrepancy.

Any casework performed during the relevant period will be reviewed and selected samples will be repeated in order to verify that the results are correct.

c. Administrative Error

Administrative errors, i.e., clerical, sample switching, improper storage, documentation, etc., once identified as such, will be corrected by instructing the analyst of the problem. Depending on the nature of the error, the analyst may be required to submit to retraining in the relevant area. For example, if the error is in sample storage, the analyst will be retrained concerning the proper storage of biological specimens.

Simple clerical errors will be pointed out to the analyst. Subsequent casework will be closely monitored, more than normal checking, for clerical errors.

In the event of an unresolved disagreement between the designated QA individual and the laboratory, the matter will be resolved by the departmental director.

2. Open Proficiency Testing

Open proficiency test specimens are presented to the laboratory staff as proficiency specimens and are used to demonstrate the capability of the laboratory's analytical methods as well as the interpretive capability of the analyst/scientist. This is the primary means by which the quality performance of the laboratory is judged and is an essential requirement prior to being assigned to perform casework.

a. Personnel

Each member of the scientific staff performing routine analyses on casework are required to take proficiency tests.

b. Specimens

Each open proficiency test may consist of dried specimens of blood and/or other physiological fluids, either singly or as a mixture. Each sample to be analyzed will contain sufficient sample so that a conclusion can be drawn from the results of the analysis.

c. Sample Preparation and Storage

All specimens and proficiency tests should be uniformly prepared using materials and methods that ensure their integrity and identity.

All test specimens will be prepared on washed cotton cloth, swabs, or other suitable material.

Each specimen must be labeled with a unique identifier that should be independently verified by at least one other person to ensure proper assignment.

A portion of each specimen used to prepare the test should be retained by the preparing laboratory for possible referee analysis and comparison if circumstances dictate.

One person in the laboratory, as assigned by the assistant director or departmental director should acknowledge receipt of each proficiency test and assign it to the laboratory staff.

3. Case Retesting

Reanalysis of case work will permit an estimate to be made regarding the laboratory error rate. Reanalysis will be performed on casework samples where there is sufficient sample for reanalysis at a later time. Each reanalysis will be conducted by a different analyst, i.e., no analyst will analyze a sample twice.

The results of the reanalysis will be compared with the results of the original analysis. If the results do not correlate, in other words, the

results do not agree, a third analysis of the sample will be performed. The results of the third analysis will be compared with the first two to determine where the error was made, i.e., analytical or sample mix-up.

4. Blind Proficiency Testing

Once a procedure for blind proficiency testing is established, blind proficiency tests will be administered to the laboratory annually and will be presented as a routine case. The samples in the "blind case" will be analyzed as a regular case and reported as such.

Specimens will be of the type commonly encountered in routine casework.

5. Documentation of Open Proficiency Testing Results

Each proficiency test will be documented as follows:

a. Proficiency Testing Identification Number
b. Name of analyst
c. Dates:
   receipt by analyst
   completion date
d. Copies of all data sheets, notes, photographs and reports
e. All data will conform to casework standards and include lot numbers, QC numbers, and etc.

The results of all proficiency tests will be maintained by the department for all department of forensic biology laboratories. A separate proficiency testing file will be maintained on each analyst.

H. Personnel Training and Qualification Records

Personnel training falls into several categories: Courses taken at universities and colleges, workshops designed to educate on specific topics and techniques, on-the-job training where theoretical and practical information and experience is obtained from the scientific staff, seminars and lectures held at local universities where scientists are invited to speak on

various topics, scientific literature, and professional meetings. Each of these will be discussed in relation to training requirements in the Department of Forensic Biology.

Records of the training of each member of the scientific staff is a part of the proficiency file maintained for each member of the scientific staff.

1. Courses at Universities

The Scientific professional staff in the department have met the minimum educational requirements necessary to meet the title descriptions. However, continuing education is important and recognized as a mechanism of maintaining state-of-the-art staff and fostering an academic environment within the service mission of the department.

Because tuition reimbursement through the City of New York is not normally available, the department cannot require staff to attend courses at universities.

Staff will be made aware of the courses, and catalogues will be obtained so that staff members know what is available and can enroll should they desire.

2. Workshops

Workshops are routinely offered in the local area by companies on specific topics, i.e., Cetus on PCR, IBI on sequencing, etc., usually as an aid to their marketing functions. Normally there is a charge for these courses. The staff will be made aware of these workshops, but because reimbursement cannot be guaranteed, attendance will not be mandatory.

Workshops are also offered in conjunction with local universities specializing in forensic science training, i.e., John Jay College of Criminal Justice, University of New Haven, as well as through The Northeastern Association of Forensic Scientists for a reasonable cost. Although the staff cannot be reimbursed for the cost of attending the workshops, very strong recommendations will be made to attend those which

are deemed important to the function of the department.

3. On-The-Job-Training

Most training in the department will be of the on-the-job variety. This training will emphasize theory and practical aspects of the work which is conducted in the department.

a. Theoretical

Theoretical background information required to understand the scientific basis, perform, and interpret the analytical tests performed in the laboratory will be provided to each staff member hired. This training will take place over a number of weeks.

This training will be presented in lecture and/or video tape format. Each member of the scientific staff will have access to literature references and reference books which are maintained by the department. Specific methods used will be referenced to the scientific literature and copies of publications pertaining to in-house methods will be available in a laboratory file.

The OCME has a library in-house which has forensic journals which are available to the scientific staff and original research articles not available in the library will be obtained for staff members by the librarian. Additionally, OCME professional staff has library privileges at the New York University Medical School library which is next door.

Each analyst will participate in moot court. Supervisory scientific staff will conduct the moot court which will be a vehicle for the analyst to demonstrate competency in understanding the theoretical basis of the tests conducted in the department.

b. Practical

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Each analyst will be trained to perform the tests conducted in the departmental laboratories. At the conclusion of training in any particular analytical test, the analyst will be asked to successfully complete open proficiency testing on that analytical procedure.

4. Seminars and Lectures

Seminars and lectures offered at the OCME, at local universities, the Department of Health, and by corporations on selected topics will be announced to staff members.

5. Scientific Literature

All scientific staff are required to read the appropriate scientific literature related to the forensic aspects of the analytical work performed in the department.

The OCME library has forensic journals which are available to staff members. Additionally, the OCME staff librarian will obtain copies of scientific articles for laboratory staff from local scientific libraries.

The supervisory staff will provide copies of articles deemed to enhance the scientific theoretical background necessary for the understanding of current testing procedures or for current research being conducted in the department.

6. Professional Meetings

Each staff scientist is permitted to attend one scientific conference per year, depending on the approval of the Chief Medical Examiner and Mayor's Office. Because of budgetary constraints that exist, reimbursement of expenses cannot be guaranteed.

The annual national convention of forensic scientists (AAFS) and the regional association of forensic scientists (NEAFS) are highly recommended to scientific staff.

Other scientific meetings of interest to the

department, i.e., Society of Human Genetics Meetings, Gene Probe Conference, AAAS conference, Int. Assn. Forensic Scientists, NY Acad. Sci., FEBS and etc., are acceptable substitutions for the forensic conferences.

I. Method Validation Records

Methods used in the departmental laboratories must be validated using accepted validation procedures that demonstrate that the methods used are capable of providing reliable results on the specimen types commonly analyzed in the departmental laboratories.

Procedures used will be approved, if appropriate, for use by any regulating bodies in New York State.

The specific validation protocols for each laboratory procedure must be written and rigorously followed (see I.1.a.2. below). Before validation on any procedures are begun, a conference between senior scientific staff and validation staff members will be held to specify the details of the validation process (see I.1.a.2. below) which details the steps to be completed before the procedure is to be ready for casework.

The analytical test results and the validation protocols used for each test will be kept in a file and/or log book. For data maintained in staff notebooks, the file or log books referred to above will reference the appropriate pages in the research notebooks or will contain xerox copies of the research notebooks.

1. Validation Procedures

a. Existing Procedures

For purposes of categorizing which validation procedures to use, existing procedures are classified as follows:

(1). Those which exist and have been published in peer review journals but not yet validated for forensic testing.

(2). Those which are not published and for which no validation records are known.

(3). Those which have been published and the validation studies have also been published.

For those procedures in category I.1.a.1, the validation for forensic investigations must be performed. The testing to be performed will be carried out according to validation testing procedures as discussed above. Once the validation work has been completed and all records are available, the work will be incorporated as an analytical procedure and will also be submitted for publication in a peer review journal, if applicable.

Those procedures that fall into category I.1.a.2. must also be validated. The process will be the same as discussed above.

The procedures in category I.1.a.3. do not require extensive validation. However, limited validation, including proficiency testing, will be conducted to insure that the test procedure behaves as published.

b. New Procedures

New procedures are those which have been developed as a result of a research project in the Department of Forensic Biology and appear to have potential as analytical tests that might be used in routine testing.

All new procedures must go through an extensive validation process which must include:

(1). Staff review of process including appropriate experiments
(2). Testing on all appropriate sample types
(3). Examination of environmental and aging effects
(4). Variability in results due to experimental protocol drift
(5). Proficiency testing
(6). Collaborative testing
(7). Publish in peer review journal, if applicable

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J. Quality Assurance and Audit Records

Records documenting that the program is implemented and maintained are kept as a normal course of business. The quality assurance coordinator is responsible for maintaining these records. One coordinator is present in each of the departmental laboratories. The departmental director coordinates the departmental quality assurance program.

K. Equipment

1. Inventory

An inventory of all equipment is maintained in an electronic database. The inventory includes a list of essential equipment and includes the following (if available).

a. manufacturer
b. model
c. serial number
d. agency inventory number (if applicable)
e. purchase date (if available)
f. replacement date (if available)

2. Operations Manuals

All equipment operations manuals are kept as a part of a centralized operations manual.

3. Calibration/Maintenance Procedures

Procedures for the calibration and maintenance are part of the operations manual.

4. Calibration/Maintenance Logs

Calibration and Maintenance logs are a part of the usage log books which are kept next to the equipment.

L. Safety Manuals

The Department maintains a separate safety manual. The safety manual is maintained as an agency (OCME) safety manual. This manual is available upon request to departmental staff.

A safety manual has been prepared and is a part of the OCME safety program. A copy of the safety manual is distributed to each staff member.

An OCME safety committee headed by an OCME representative meets monthly. The department of Forensic Biology has one representative on that committee.

The radiation safety manual is a part of the departmental safety manual and is separate from the OCME manual.

The Radiation Safety Committee meets quarterly.

M. Material Safety Data Sheets (MSDS)

MSDS sheets are kept in a separate file for all reagents and chemicals used in the departmental laboratories. The OCME is also required to have a copy of the most current MSDS sheets for those materials used in the OCME building. The sheets are updated monthly.

These are readily available from the departmental safety committee representative.

N. Historical or Archival Records

Records for all laboratory operations are maintained.

Casework records are maintained with the case file under the laboratory case number (FB -).

O. Audits

The Department is audited annually by an independent evaluator who is not responsible for any official function of the Department. Sheets for audits are filled out by the evaluator and submitted to the Department Director for evaluation by the QA Committee.

Figure 1: Forensic Biology
Organizational Structure

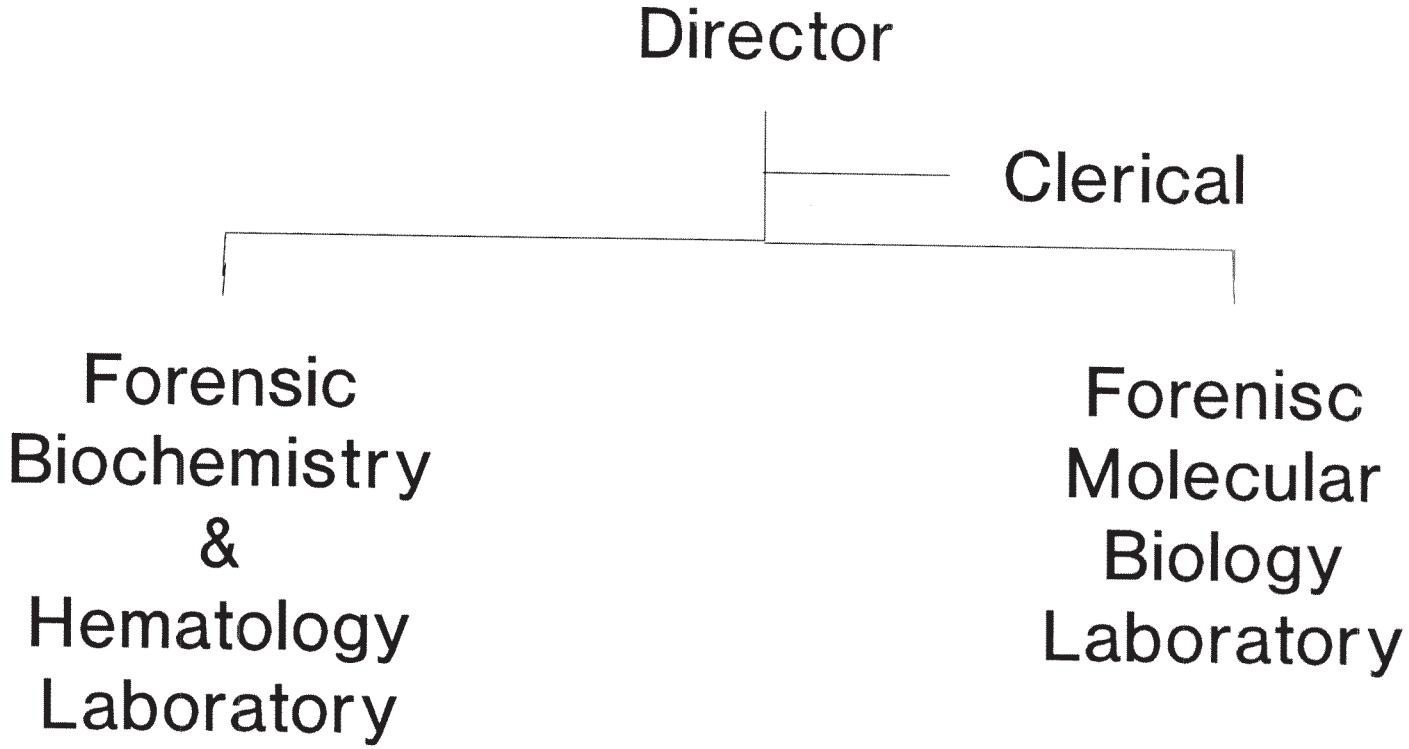
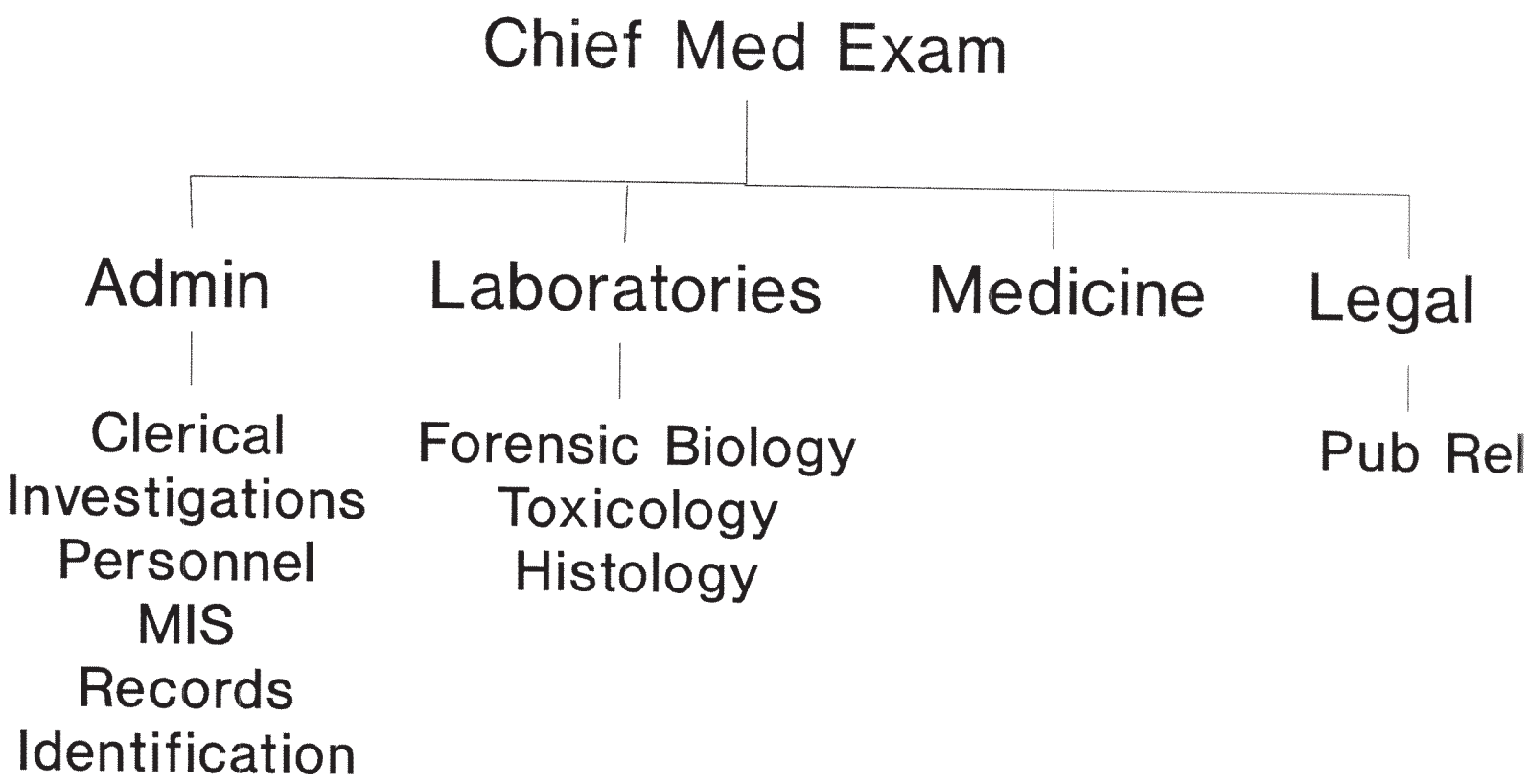


Figure 2: Office Chief Medical Examiner Organizational Structure



Appendix A

Department of Forensic Biology

Administrative Manual

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Chain-of Custody Procedures

A. Post-mortem Evidence

1. Homicide Cases

For cases clearly labeled as homicides, as determined by the Medical Examiner, daily case census, autopsy worksheets or by having NYPD submit investigatory evidence, the following procedures are followed.

Blood: A stain is prepared on clean cotton cloth and dried. Cells and serum are separated and frozen from an aliquot of the original sample. The remainder is discarded after two months. The frozen cells and serum are retained for two years. The dried stain is retained indefinitely.

Hairs, Fingernails, Swabs, etc.: These are placed in the cold room. After two years they are vouchered for entry into the NYPD evidence control system and transferred to the Evidence Unit.

Other specimens (tissues, bone etc.): These are frozen and retained in the main serology lab.

Cases in which the cause of death is unclear and appear likely to be a homicide or may require NYPD investigation e.g., sexual assaults, are handled as above.

The determination as to whether a case may require future processing by the Department of Forensic Biology is ascertained through conversations with the ME or investigating detectives who handled the case.

Those cases which are not clearly homicides but may require future investigation receive FB numbers and are handled as evidentiary specimens.

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2. Non-homicides (database samples)

The Forensic Biology department receives EDTA blood, if available, from all Medical Examiner cases. Most of these do not fall within the mission of the Department of Forensic Biology and are not the subject of an homicide investigation. These cases do not receive FB case numbers. These specimens and associated autopsy worksheets are transferred to the Forensic Molecular Biology (DNA) Laboratory and are tracked in the DNA laboratory through the use of a log book as having being received. Of these, those which are cleared to be used as database samples receive an MB number. DNA is isolated from these latter samples. These specimens are discarded after two months.

3. Cases For Which Specimens Are Not Received

Sometimes physical evidence is received on cases for which autopsy specimens are not received. In these instances, appropriate specimens are obtained from the Toxicology Department (if within that department's specimen holding time-frame) or from DNA database specimens (if within that laboratory's holding time-frame). In unusual instances, and if freshly preserved specimens are not available, formalin fixed specimens may be obtained from the Histology laboratory.

4. Specimens Received Without Identifying Numbers

Sometimes autopsy specimens are received with no identifying case numbers, specimen types or other identifying information. These specimens are discarded.

B. Physical Evidence

Physical evidence is received primarily from the NYPD but other agencies and jurisdictions submit cases as well.

Physical evidence submitted to the laboratory receives an FB case number. This number serves as the control number while the evidence is in the possession of the laboratoy. At the conclusion of the scientific testing the evidence is transferred to the Evidence Unit.

Appendix B

Department of Forensic Biology

Forensic Biochemistry & Hematology Laboratory

Quality Control Manual

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TABLE OF CONTENTS

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I. Purpose

The purpose of this manual is to specify the types of guidelines, criteria, and procedures used to ensure that the quality and safety of the analytical testing in the biochemistry and hematology laboratory in the Department of Forensic Biology is optimal.

II. Equipment

Periodic evaluation of certain laboratory equipment must be performed to ensure quality assurance (see QA Manual section IV-K).

A. Maintenance of Equipment

1. An equipment maintenance log must be kept documenting all maintenance performed on any item of equipment used in the laboratory.

III. Methods

Quality control parameters are defined for each method based on evaluation and validation studies. All materials employed in a given method must be tested concurrently in a quality control procedure for that method. If the tested materials meet quality control specifications, they may then be used in the method on casework samples.

A. Manufactured Materials

1. Materials such as buffers, IEF plates, and IEF electrode solutions which are prepared by the laboratory so as to be able to perform multiple tests are batches. These are to be stored at appropriate conditions. Each time a material is batched, a corresponding *batch sheet* is to be filled out. The following information is to be recorded on the batch sheet:

    a. Batch number (subsequent batches are listed numerically)

    b. Initials of the preparer

    c. Date of preparation

    d. Amount prepared

    e. Vendor and lot number of each reagent used to prepare the batched material.

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2. Batch sheets are to be kept in respective files and stored in an accessible file cabinet. The following are to be batched and given batch numbers:

   a. Acetic acid anode electrolyte solution

   b. ACP IEF polyacrylamide plates

   c. ACP reaction buffer

   d. Amylase gel buffer

   e. Anti-H antiserum

      Crude pancreatic extract

      Crude salivary extract

   f. ESD IEF polyacrylamide plates

   g. ESD reaction buffer

   h. ESD/PGM gel buffer

   i. ESD/PGM tank buffer

   j. Ethanolamine cathode electrolyte solution

   k. Gc gel buffer

   l. Gc tank buffer

   m. Hemoglobin IEF polyacrylamide plates

      Kidney bean extract

   n. PGM reaction buffer

   o. PGM reaction mixture

   p. PGM subtype IEF polyacrylamide plates

   q. P30 agarose gels

   r. P30 tank buffer

   s. Phenolphthalein reagent

      Physiological saline

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t. Species agarose gels

u. Species tank buffer

v. Takayama crystal test reagent

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2. Batch sheets are to be kept in respective files and stored in an accessible file cabinet. The following are to be batched and given batch numbers:

   a. Acetic acid anode electrolyte solution

   b. ACP IEF polyacrylamide plates

   c. ACP reaction buffer

   d. Amylase gel buffer

   e. Anti-H antiserum

   f. ESD IEF polyacrylamide plates

   g. ESD reaction buffer

   h. ESD/PGM gel buffer

   i. ESD/PGM tank buffer

   j. Ethanolamine cathode electrolyte solution

   k. Gc gel buffer

   l. Gc tank buffer

   m. Hemoglobin IEF polyacrylamide plates

   n. PGM reaction buffer

   o. PGM reaction mixture

   p. PGM subtype IEF polyacrylamide plates

   q. P30 agarose gels

   r. P30 tank buffer

   s. Phenolphthalein reagent

   t. Species agarose gels

   u. Species tank buffer

   v. Takayama crystal test reagent

3. With the exception of the IEF cathode and anode

electrolyte solutions (a and j above) (which can be stored at room temperature), all batched materials must be stored refrigerated.

B. Guidelines for Quality Control Testing

1. Quality control testing for a particular method can be performed by any member of the laboratory who has satisfied laboratory proficiency requirements for that particular method.

2. Quality control testing results are to be recorded on worksheets and stored in respective files. Each quality control sheet gets assigned a quality control number along with the following information:

    a. Initials of analyst who performs test

    b. Date of testing

    c. Batch numbers for all batched materials

    d. Vendors and lot numbers for all non-batched reagents

    e. Results of tests

    Quality control files are listed by method and are kept in an accessible file cabinet.

    Each time a method is used on casework samples, its current quality control number is recorded on a case summary sheet and placed in the case file.

3. Quality control results must be reviewed by the laboratory supervisor and the laboratory director. If the laboratory director is absent, then the assistant director will review the results.

    Materials cannot be used in laboratory methods on casework samples until the corresponding quality control results have been reviewed and appropriate signatures are on the QC sheets.

4. All results which require measurement, e.g. band separations on electrophoresis plates, must be photographed with a ruler.

5. *Quality control rejection* - If an initial quality control test does not produce acceptable results, a second test can be performed using the same group of materials. If results from the second test adhere to quality control criteria, then the tested materials can be used in the tested method on casework samples. However, if the second test does not conform to quality control parameters then the problem batch(es) or reagent(s) must be discarded.

   With electrophoretic methods, quality control rejection can be based not only on predetermined parameters (such as allowable band separation distance and sensitivity limits) but also on the overall appearance of the phenotype patterns. Distorted bands or the presence of extra bands are examples of phenotype pattern problems that may be grounds for rejection. If rejection occurs based on these or similar problems, observations must be recorded in the comments section of the quality control worksheet.

C. Procedures

1. Absorption Inhibition Quality Control Test

Quality control evaluation must be performed on all laboratory and commercially prepared antisera before use in the absorption inhibition method.

Quality control evaluation of previously evaluated antisera must be checked monthly.

Procedure

1. Titer anti-A antiseum, anti-B antiserum, and anti-H lectin (see procedures manual).

2. Record titer results on quality control worksheet according to agglutination scale.

3. Using the titer that gives the last +4 on the agglutination scale for each antisera, perform absorption inhibition technique (as specified in methods manual) on A secretor, B secretor, O secretor, and non-secretor dried saliva stains.

4. Record results on quality control sheet.

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Quality Control Parameters

Acceptable results are as follows:*

	A	B	O
A secretor-neat	CI	3-4	CI-4
-1/10	CI-2	3-4	CI-4
B secretor-neat	3-4	CI	CI-4
-1/10	3-4	CI-2	CI-4
O secretor-neat	3-4	3-4	CI
-1/10	3-4	3-4	CI-2
non-secretor-neat	3-4	3-4	3-4
-1/10	3-4	3-4	3-4

CI = complete inhibition

* = Numbers reflect values on agglutination scale.

If results fall outside acceptable range, the procedure must be repeated using properly adjusted antisera titers.

2. Alpha-Amylase Gel Diffusion Quality Control Test

Each batch of amylase gel buffer must be evaluated before use in method.

Each lot of commercially prepared agarose, starch, and amylase standard must be tested before use.

Procedure

1. Record the amylase gel buffer batch number, and vendor and lot numbers for agarose, starch, and amylase standards on quality control sheet.

2. Prepare a set of alpha-amylase standards containing 20 units amylase activity/8ul, 2 units/8ul, 0.2 units/8ul, 0.02 units/8ul, 0.002 units/8ul, 0.0002 units/8ul, and 0.00002 units/8ul in saline, respectively. Alternatively, any comparable ten-fold dilution series can be used.

3. Extract a 5x5mm (approximate) section of a pooled human saliva stain in saline. From an aliquot of this extract, prepare a 1/10 dilution in saline.

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4. Test 8ul of each standard, the neat and 1/10 diluted saliva stain extracts, and a saline blank as per amylase gel diffusion procedure specified in methods manual.

5. Prepare a standard curve of the units of amylase activity (expressed logarithmically on x axis) versus the diameter of the clear circles around standard wells in the developed diffusion gel (plotted on y axis).

6. Determine amylase activity of the neat and 1/10 diluted saliva stain extract from the standard curve after measuring the diameter of the clear circle around both sample wells.

Quality Control Parameters

1. The diameter of the clear circles around standard wells needs to be linear with respect to the amylase activity expressed logarithmically.

2. The diameter of the clear circle around both sample wells needs to fall between the lowest and highest points on the linear portion of the standard curve.

3. The calculated amylase activity of the neat and 1/10 diluted saliva stain extract should differ by a factor of 10 given an allowable error of 5%.

3. Acid Phosphatase Quality Control Test

Commercially prepared acid phosphatase spot test reagent needs to be evaluated before use in case work.

A previously evaluated acid phosphatase spot test reagent needs to be checked yearly.

Procedure

1. Record vendor and lot number of acid phosphatase spot test reagent on quality control worksheet.

2. Prepare 1/10, 1/100, 1/1000, 1/10000, 1/100000, and 1/1000000 dilutions of pooled human semen using saline.

3. Test one drop of the pooled human semen (neat) and each dilution as per acid phosphatase spot test specified in methods manual.

4. Test a 20ul dried, pooled semen stain according to the procedures manual.

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4. Test 8ul of each standard, the neat and 1/10 diluted saliva stain extracts, and a saline blank as per amylase gel diffusion procedure specified in methods manual.

5. Prepare a standard curve of the units of amylase activity (expressed logarithmically on x axis) versus the diameter of the clear circles around standard wells in the developed diffusion gel (plotted on y axis).

6. Determine amylase activity of the neat and 1/10 diluted saliva stain extract from the standard curve after measuring the diameter of the clear circle around both sample wells.

Quality Control Parameters

1. The diameter of the clear circles around standard wells needs to be linear with respect to the amylase activity expressed logarithmically.

2. The diameter of the clear circle around both sample wells needs to fall between the lowest and highest points on the linear portion of the standard curve.

3. The calculated amylase activity of the neat and 1/10 diluted saliva stain extract should differ by a factor of 10 given an allowable error of 5%.

3. Acid Phosphatase Quality Control Test

Commercially prepared acid phosphatase spot test reagent needs to be evaluated before use in case work.

A previously evaluated acid phosphatase spot test reagent needs to be checked yearly.

Procedure

1. Record vendor and lot number of acid phosphatase spot test reagent on quality control worksheet.

2. Prepare 1/10, 1/100, 1/1000, 1/10000, 1/100000, and 1/1000000 dilutions of pooled human semen using saline.

3. Test one drop of the pooled human semen (neat) and each dilution as per acid phosphatase spot test specified in methods manual.

4. Test a 20ul dried pooled semen stain according to as per the procedures manual.

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2A. Amylase$_1$ and Amylase$_2$ Differentiation by Differential Inhibition Quality Control Test

Each batch of kidney bean extract (KBE), crude pancreatic extract, and crude salivary extract must be evaluated before use.

Similarly, each lot of commercially prepared wheat lectin (WL), agarose, starch, and iodine must be evaluated before use.

Procedure

1. Pour amylase diffusion gel as per methods manual (pp.12-13).

2. Serially dilute 1/2, 1/4, 1/8, and 1/16 crude pancreatic extract, crude salivary extract, and commercially prepared bacterial amylase with distilled water, KBE, and WL.

3. Place 10ul of each diluted extract from step 2 in punched wells in the diffusion gel.

4. Place gel in moisture chamber and incubate overnight at room temperature.

5. Flood gel with Lugol's Iodine to develop plate.

6. Determine diffusion area ($A=\pi r^2$) of each extract.

Parameters

1. Using data from the salivary extract, calculate the inhibition ratio for each dilution by using the following formula:

$$\frac{\text{Area WL Dilution/Area Water Dilution}}{\text{Area KBE Dilution/Area Water Dilution}}$$

Values greater than 2 for all 4 dilutions must be obtained.

2. Using data from the pancreatic extract, use the above formula to calculate the inhibition ratio for each dilution. Values less than 1 for all 4 dilutions must be obtained.

3. Diluted samples of bacterial amylase should show no or negligible inhibition (inhibition ratio to approximate 1) with both KBE and WL.

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Quality Control Parameters

1a. Positive results must be seen in the neat pooled semen sample and in the 1/10 and 1/100 serially diluted fractions.
b. Sensitivity of the spot test must be greater than that for the P-30 detection Crossed-Over electrophoresis method. Compare last dilution with the sensitivity of the P30 Crossed-over QC for the current lot number.

2. A positive result must be obtained on the 20ul dried pooled semen stain.

4. Acid Phosphatase by IEF Quality Control Test

Each batch of ACP IEF plates, anode solution, cathode solution, and ACP reaction buffer must be evaluated before use in casework.

Each lot of methylumbelliferyl phosphate must be tested before use.

Procedure

1. Record batch numbers for ACP IEF plates, anode solution, cathode solution, and ACP reaction buffer to be tested on quality control sheet. The vendor and lot number of the methumbelliferyl phosphate used in the quality control test must be recorded.

2. Bloodstains containing known ACP types (ACP A, ACP B, and ACP BA, respectively) are to be extracted and tested as per ACP by IEF method specified in methods manual.

   a. Sample test volume of 10ul is to be used for the extracts containing ACP A and ACP B. Both extracts are to be tested in duplicate.
   b. The extract containing ACP BA is to be tested in triplicate with varying volume size (10ul, 5ul, and 2.5ul).

Quality Control Parameters

1. The following is the allowable separation for adjacent bands on ACP phenotypes:

ACP type	Bands	Allowable Separation
A	a to Hb	>1mm
B	b1 to b2	>8mm
BA	b1 to b2	>8mm
	b2 to a	>10mm
	a to Hb	>1mm

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Quality Control Parameters

1a. Positive results must be seen in the neat pooled semen sample and in the 1/10 and 1/100 serially diluted fractions.

b. Sensitivity of the spot test must be greater than that for the P-30 detection crossed-over electrophoresis method. Compare last dilution with the sensitivity of the P30 Crossed-over QC for the current lot number.

2. A positive result must be obtained on the 20ul dried pooled semen stain.

4. Acid Phosphatase by IEF Quality Control Test

Each batch of ACP IEF plates, anode solution, cathode solution, and ACP reaction buffer must be evaluated before use in casework.

Each lot of methylumbelliferyl phosphate must be tested before use.

Procedure

1. Record batch numbers for ACP IEF plates, anode solution, cathode solution, and ACP reaction buffer to be tested on quality control sheet. The vendor and lot number of the methumbelliferyl phosphate used in the quality control test must be recorded.

2. Bloodstains containing known ACP types (ACP A, ACP B, and ACP BA, respectively) are to be extracted and tested as per ACP by IEF method specified in methods manual.

   a. Sample test volume of 10ul is to be used for the extracts containing ACP A and ACP B. Both extracts are to be tested in duplicate.

   b. The extract containing ACP BA is to be tested in triplicate with varying volume size (10ul, 5ul, and 2.5ul).

Quality Control Parameters

1. The following is the allowable separation for adjacent bands on ACP phenotypes:

ACP type	Bands	Allowable Separation
A	a to Hb	>1mm
B	b1 to b2	>8mm
BA	b1 to b2	>8mm
	b2 to a	>10mm

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2. Typable results must be observed with all sample volumes tested. Weak, but clearly typeable results are acceptable.

5. Esterase D by IEF

Each batch of ESD IEF plates, anode solution, cathode solution, and ESD reaction buffer must be evaluated before use in casework.

Each lot of methumbelliferyl acetate must be evaluated before use.

Procedure

1. Record batch numbers for ESD IEF plates, anode solution, cathode solution, and ESD reaction buffer to be tested on the quality control sheet. The vendor and lot number of the methumbelliferyl acetate used in the quality control test must also be recorded.

2. Bloodstains containing known ESD types (ESD 1, ESD 5-1, and ESD 2-1) are to be extracted and tested as per ESD IEF method specified in methods manual.

   a. Sample test volume of 10ul is to be used for the extracts containing ESD 1 and ESD 2-1. Both extracts are to be tested in duplicate.

   b. The extract containing ESD 5-1 is to be tested in triplicate with varying volume sizes (10ul, 5ul, and 2.5ul).

Quality Control Parameters

1. The following is the allowable separation for adjacent bands on ESD phenotypes:

ESD Type	Bands	Allowable Separation
1	top-bottom	>3mm
2-1	top-middle	>1mm
	middle-bottom	>1mm
5-1	top-middle	>3mm
	middle-bottom	>3mm

2. Typable results must be observed with all sample volumes tested. Weak, but clearly typable results are acceptable.

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a to Hb	>1mm

2. Typeable results must be observed with all sample volumes tested. Weak, but clearly typable results are acceptable.

5. Esterase D by IEF

Each batch of ESD IEF plates, anode solution, cathode solution, and ESD reaction buffer must be evaluated before use in casework.

Each lot of methumbelliferyl acetate must be evaluated before use.

Procedure

1. Record batch numbers for ESD IEF plates, anode solution, cathode solution, and ESD reaction buffer to be tested on the quality control sheet. The vendor and lot number of the methumbelliferyl acetate used in the quality control test must also be recorded.

2. Bloodstains containing known ESD types (ESD 1, ESD 5-1, and ESD 2-1) are to be extracted and tested as per ESD IEF method specified in methods manual.

   a. Sample test volume of 10ul is to be used for the extracts containing ESD 1 and ESD 2-1. Both extracts are to be tested in duplicate.

   b. The extract containing ESD 5-1 is to be tested in triplicate with varying volume sizes (10ul, 5ul, and 2.5ul).

Quality Control Parameters

1. The following is the allowable separation for adjacent bands on ESD phenotypes:

ESD Type	Bands	Allowable Separation
1	top-bottom	>3mm
2-1	top-middle	>1mm
	middle-bottom	>1mm
5-1	top-middle	>3mm
	middle-bottom	>3mm

2. Typeable results must be observed with all sample volumes tested. Weak, but clearly typable results are acceptable.

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6. ESD/PGM by Conventional Electrophoresis

Each batch of ESD/PGM tank buffer, ESD/PGM gel buffer, ESD reaction buffer, PGM reaction buffer, and PGM reaction mixture must be evaluated before use in casework.

Each lot of methumbelliferyl acetate, glucose-6-phosphate dehydrogenase, meldola blue, and agarose must be evaluated before use.

Procedure

1. Record batch numbers for ESD/PGM tank buffer, ESD/PGM gel buffer, ESD reaction buffer, PGM reaction buffer, and PGM reaction mixture on quality control sheet. The vendor and lot number of the methylumbelliferyl acetate, glucose-6-phosphate dehydrogenase, meldola blue, and agarose used in the quality control test must also be recorded.

2. Two bloodstains having known ESD and PGM types (ESD 1 and PGM 1, and ESD 2-1 and PGM 2-1, respectively) are tested as per ESD/PGM method specified in methods manual. Both bloodstains are tested in triplicate using two, four, and six threads, respectively.

Quality Control Parameters

1. The following is the allowable separation for adjacent bands on ESD and PGM phenotypes:

Phenotype	Bands	Allowable Separation
ESD 2-1	top-middle	>4mm
	middle-bottom	>4mm
PGM 1	a-c	>7mm
PGM 2-1	a-b	>3mm
	a-c	>7mm
	b-d	>7mm
	a-d	>14mm

2. Typable results must be obtained with all sample sizes tested for both bloodstains. Weak, but clearly typable results are acceptable.

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6. ESD/PGM by Conventional Electrophoresis

Each batch of ESD/PGM tank buffer, ESD/PGM gel buffer, ESD reaction buffer, PGM reaction buffer, and PGM reaction mixture must be evaluated before use in casework.

Each lot of methumbelliferyl acetate, glucose-6-phosphate dehydrogenase, meldola blue, and agarose must be evaluated before use.

Procedure

1. Record batch numbers for ESD/PGM tank buffer, ESD/PGM gel buffer, ESD reaction buffer, PGM reaction buffer, and PGM reaction mixture on quality control sheet. The vendor and lot number of the methylumbelliferyl acetate, glucose-6-phosphate dehydrogenase, meldola blue, and agarose used in the quality control test must also be recorded.

2. Two bloodstains having known ESD and PGM types (ESD 1 and PGM 1, and ESD 2-1 and PGM 2-1, respectively) are tested as per ESD/PGM method specified in methods manual. Both bloodstains are tested in triplicate using two, four, and six threads, respectively.

Quality Control Parameters

1. The following is the allowable separation for adjacent bands on ESD and PGM phenotypes:

Phenotype	Bands	Allowable Separation
ESD 2-1	top-middle	>4mm
	middle-bottom	>4mm
PGM 1	a-c	>7mm
PGM 2-1	a-b	>3mm
	a-c	>7mm
	b-d	>7mm
	a-d	>14mm

2. Typeable results must be obtained with all sample sizes tested for both bloodstains. Weak, but clearly typable results are acceptable.

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7. Group Specific Component (GC) by Conventional Electrophoresis

Quality control evaluation must be done on all lots of commercially prepared anti-Gc antiserum before use in casework.

Each batch of Gc tank buffer and gel buffer must be evaluated before use.

Antisera Evaluation

1. Dilute commercially prepared anti-Gc antiserum 1:2, 1:3, 1:4, 1:5, and 1:6 with distilled water. The table below can be used as a guide in preparing these dilutions.

Dilution	Amount of anti-Gc	Amount of distilled water
1:2	300ul	300ul
1:3	200ul	400ul
1:4	150ul	450ul
1:5	120ul	480ul
1:6	100ul	500ul

2. Dissolve 0.8g agarose (Sigma type I) in 80ml gel buffer by heating.

3. While still warm, cast the gel solution on a 15x20cm glass plate covering it evenly.

4. After gel cools, prepare sample slits in the gel by inserting a comb with 1cm teeth in the center of the gel.

5. Cut 30 threads 1cm in length from a bloodstain having Gc type 2-1.

6. Moisten threads with distilled water and place 6 of them in each of 5 sample slits.

7. Electrophorese at 400V for 2.5 hours (power and current can be set at maximal values; however, current should be approximately 50mA).

8. Cut five cellulose acetate strips 5cm in length and wide enough to just cover a sample lane.

9. To each cellulose acetate strip, soak in 1:2, 1:3, 1:4, 1:5, and 1:6 diluted antiserum, respectively.

10. Place each strip in a respective sample lane on the anodic end from the wick down.

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11. Incubate the gel in a moisture chamber at room temperature for 2 hours. Following incubation, remove the cellulose acetate strips and submerge the gel in saline and refrigerate (2-5°C) overnight.

12. The next day, develop the gel as per Gc method specified in methods manual.

Evaluation Parameters

1. The last dilution which gives optimal band visibility is the working dilution to be used in the quality control method and subsequent in casework.

Procedure for GC Electrophoresis

1. Record the batch numbers of the Gc tank and gel buffers on the quality control sheet. The vendor and lot number of the anti-Gc serum and agarose used in the quality control test must also be recorded.

2. Three bloodstains having known Gc types (Gc 1, Gc 2, and Gc 2-1, respectively) are tested as per Gc method specified in methods manual. Each bloodstain is tested in triplicate using two threads, four threads, and six threads, respectively.

Quality Control Parameters

1. The following is the allowable separation for adjacent bands on Gc phenotypes:

Phenotype	Bands	Allowable Separation
Gc 2-1	top-middle	>3mm
	middle-bottom	>3mm
Gc 1	top-middle	>3mm

2. Typable results must be obtained with all three bloodstains using four and six thread sample sizes. Weak, but clearly typeable results are acceptable.

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11. Incubate the gel in a moisture chamber at room temperature for 2 hours. Following incubation, remove the cellulose acetate strips and submerge the gel in saline and refrigerate (2-5°C) overnight.

12. The next day, develop the gel as per Gc method specified in methods manual.

Evaluation Parameters

1. The last dilution which gives optimal band visibility is the working dilution to be used in the quality control method and subsequent in casework.

Procedure for GC Electrophoresis

1. Record the batch numbers of the Gc tank and gel buffers on the quality control sheet. The vendor and lot number of the anti-Gc serum and agarose used in the quality control test must also be recorded.

2. Three bloodstains having known Gc types (Gc 1, Gc 2, and Gc 2-1, respectively) are tested as per Gc method specified in methods manual. Each bloodstain is tested in triplicate using two threads, four threads, and six threads, respectively.

Quality Control Parameters

1. The following is the allowable separation for adjacent bands on Gc phenotypes:

Phenotype	Bands	Allowable Separation
Gc 2-1	top-middle	>3mm
	middle-bottom	>3mm
Gc 1	top-middle	>3mm

2. Typeable results must be obtained with all three bloodstains using four and six thread sample sizes. Weak, but clearly typeable results are acceptable.

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8. Hemoglobin by IEF

Each batch of hemoglobin IEF plates, anode solution, and cathode solution must be evaluated before use in casework.

All lots of potassium cyanide and the AFSC hemoglobin control standard must be evaluated before use in casework.

Procedure

1. Record batch numbers of hemoglobin IEF plates, anode solution, and cathode solution on quality control sheet.

2. Ten microliters (10ul) of the AFSC hemoglobin control is diluted with 100ul 0.05% potassium cyanide.

3. Ten microliter (10ul), 5ul, and 2.5ul aliquots of the diluted standard is tested as per hemoglobin IEF method as specified in methods manual. The 10ul sample is tested in triplicate.

Quality Control Parameters

1. The following is the allowable separation for adjacent bands on the AFSC hemo control:

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

2. Typeble results must be obtained with all sample volumes tested.

9. Lewis Typing Quality Control Test

Quality control evaluation must be performed on all lots of commercially prepared anti-Le^a and anti-Le^b antisera before use with casework samples.

Procedure

1. Record vendor and lot number of the anti-Le^a antiserum, anti-Le^b antiserum, Le^{a+b-} standard, and Le^{a-b+} standard on the quality control sheet.

2. Test both antisera on both standards as per Lewis typing technique specified in methods manual.

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8. Hemoglobin by IEF

Each batch of hemoglobin IEF plates, anode solution, and cathode solution must be evaluated before use in casework.

All lots of potassium cyanide and the AFSC hemoglobin control standard must be evaluated before use in casework.

Procedure

1. Record batch numbers of hemoglobin IEF plates, anode solution, and cathode solution on quality control sheet.

2. Ten microliters (10ul) of the AFSC hemoglobin control is diluted with 100ul 0.05% potassium cyanide.

3. Ten microliter (10ul), 5ul, and 2.5ul aliquots of the diluted standard is tested as per hemoglobin IEF method as specified in methods manual. The 10ul sample is tested in triplicate.

Quality Control Parameters

1. The following is the allowable separation for adjacent bands on the AFSC hemo control:

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

2. Typeable results must be obtained with all sample volumes tested.

9. Lewis Typing Quality Control Test

Quality control evaluation must be performed on all lots of commercially prepared anti-Le^a and anti-Le^b antisera before use with casework samples.

Procedure

1. Record vendor and lot number of the anti-Le^a antiserum, anti-Le^b antiserum, Le^{a+b-} standard, and Le^{a-b+} standard on the quality control sheet.

2. Test both antisera on both standards as per Lewis typing technique specified in methods manual.

Initials: RCJ Date: 3/17/93

Quality Control Parameters

1. Anti-Le^a must produce agglutination with the Le^{a+b-} standard, but not with the Le^{a-b+} standard. Conversely, the anti-Le^b must produce agglutination with the Le^{a-b+} standard, but not with the Le^{a+b-} standard.

10. Kastle-Meyer Quality Control Test

Each batch of phenolphthalin reagent must be evaluated before using method for testing casework samples.

Each lot of ethanol and hydrogen peroxide must be evaluated with appropriate controls before each use.

Procedure

1. Record the batch number of the phenolphthalin reagent on the quality control sheet. The vendor and lot number of the ethanol and hydrogen peroxide used in the quality control test must also be recorded.

2. Using distilled water, prepare 1/10 serial dilutions of whole blood ending with a 1/1000000 dilution.

3. One drop of each dilution is placed on a strip of filter paper and allowed to dry.

4. Each dried drop is then tested as per phenolphthalin test specified in methods manual.

Quality Control Parameters

1a. Reagent sensitivity must not be less than 1/1000.

b. Sensitivity of test must be greater than that for species identification method. Compare with the sensitivity of the Cross-Over species QC for the current lot number.

2. Positive reactions must not be observed in any dilution before the addition of hydrogen peroxide.

Initials: RG Date: 3/18/92

Quality Control Parameters

1. Anti-Le^a must produce agglutination with the Le^{a+b-} standard, but not with the Le^{a-b+} standard. Conversely, the anti-Le^b must produce agglutination with the Le^{a-b+} standard, but not with the Le^{a+b-} standard.

10. Kastle-Meyer Quality Control Test

Each batch of phenolphthalin reagent must be evaluated before using method for testing casework samples.

Each lot of ethanol and hydrogen peroxide must be evaluated with appropriate controls before each use.

Procedure

1. Record the batch number of the phenolphthalin reagent on the quality control sheet. The vendor and lot number of the ethanol and hydrogen peroxide used in the quality control test must also be recorded.

2. Using distilled water, prepare 1/10 serial dilutions of whole blood ending with a 1/1000000 dilution.

3. One drop of each dilution is placed on a strip of filter paper and allowed to dry.

4. Each dried drop is then tested as per phenolphthalin test specified in methods manual.

Quality Control Parameters

1a. Reagent sensitivity must not be less than 1/1000.

b. Sensitivity of test must be greater than that for species identification method. Compare with the sensitivity of the Cross-over species QC for the current lot number.

2. Positive reactions must not be observed in any dilution before the addition of hydrogen peroxide.

Initials: RCJ Date: 10/12/94

10a. Leucomalachite Green Solution Quality Control Test

Each batch of leucomalachite greem solution must be evaluated before use in casework. Similarly, each lot of hydrogen peroxide must be evaluated before use.

Procedure

1. Record the batch number of the leucomalachite green reagent on the quality control sheet. The vendor and lot number of the hydrogen peroxide used in the quality control test must also be recorded.

2. Using distilled water, prepare 1/10 serial dilutions of whole blood ending with a 1/1000000 dilution.

3. One drop of each dilution is placed on a strip of filter paper and allowed to dry.

4. Each dried drop is then tested as per leucomalachite test specified in methods manual.

Quality Control Parameters

1a. Reagent sensitivity must not be less than 1/1000.

b. Sensitivity of test must be greater than that for species identification method (check current species QC).

c. Positive reactions must not be observed in any dilution before the addition of hydrogen peroxide.

Initials: RCL Date: 4/23/93

11. P-30 Detection Quality Control Test

Each new lot of commercially prepared anti-P30 antiserum must be evaluated before use with casework samples. Similarly, each new batch of P-30 tank buffer and gels must be tested before use.

Once a lot of anti-P30 antiserum has been successfully evaluated, each subsequent batch of P-30 tank buffer and gels can be evaluated by testing against pooled human semen only (see below).

Procedure

1. Prepare 1/10, 1/100, 1/1000, 1/10000, 1/100000, and 1/1000000 serial dilutions of pooled human semen, blood, urine, and saliva with saline.

2. Test neat and diluted body fluid samples against neat anti-P30 antiserum as per P30 method specified in methods manual.

Quality Control Parameters

1. Neat and all diluted samples of the pooled blood, urine, and saliva must give negative results.

2. Neat and 1/10 diluted sample of the pooled semen must give positive results.

11A. P-30 (ELISA) Detection Quality Control Test

New lots of commercially prepared mouse monoclonal anti-human PSA, rabbit polyclonal anti-human PSA, and swine anti-rabbit IgG alkaline phosphatase conjugate must be evaluated before use with casework samples. Similarly, new batches of PBS-casein, PBS-BSA, and alkaline substrate buffer and lots of PBS tablets, P30 antigen, and MOPC 21 must be tested before use.

Once lots of anti-sera have been successfully evaluated, subsequent batches and lots of other reagents can be evaluated by testing against P30 antigen standards (see below).

Procedure - mouse monoclonal anti-human PSA

Use previously evaluated lots of rabbit polyclonal anti-human PSA, swine anti-rabbit IgG APC, and other reagents to perform the evaluation.

1. Prepare 1/5000 - 1/10000 dilutions of mouse monoclonal anti-human PSA with phosphate buffered saline.

Initials: RCJ Date: 3/19/92

11. P-30 Detection Quality Control Test

Each new lot of commercially prepared anti-P30 antiserum must be evaluated before use with casework samples. Similarly, each new batch of P-30 tank buffer and gels must be tested before use.

Once a lot of anti-P30 antiserum has been successfully evaluated, each subsequent batch of P-30 tank buffer and gels can be evaluated by testing against pooled human semen only (see below).

Procedure

1. Prepare 1/10, 1/100, 1/1000, 1/10000, 1/100000, and 1/1000000 serial dilutions of pooled human semen, blood, urine, and saliva with saline.

2. Test neat and diluted body fluid samples against neat anti-P30 antiserum as per P30 method specified in methods manual.

Quality Control Parameters

1. Neat and all diluted samples of the pooled blood, urine, and saliva must give negative results.

2. Neat and 1/10 diluted sample of the pooled semen must give positive results.

12. PGM Subtype by IEF

Each batch of PGM IEF plates, anode solution, cathode solution, PGM reaction buffer, and PGM reaction mixture must be evaluated before using method with casework samples.

Each lot of glucose-6-phosphate dehydrogenase, meldola blue, and agarose must be evaluated before use.

Procedure

1. Record batch numbers for PGM subtype IEF plates, anode solution, cathode solution, PGM reaction buffer, and PGM reaction mixture to be tested on quality control sheet. The vendor and lot number of the glucose-6-phosphate dehydrogenase, meldola blue, and agarose used in the quality control test must also be recorded.

2. Bloodstains containing known PGM types (PGM 2+1-, PGM 2-1+, and PGM 1+1-, respectively) are to be extracted and tested as per PGM subtype IEF method specified in methods manual.

Initials: [illegible] Date: 4/23/93

2. Perform a P30 ELISA test as per the methods manual, using the diagram shown on the "monoclonal anti-PSA antisera evaluation" worksheet.

3. Determine the weakest dilution of anti-sera which gives a result for 2 ng PSA standard. Choose as the working titer the next strongest dilution.

Procedure - rabbit polyclonal anti-human PSA

Use previously evaluated lots of mouse monoclonal anti-human PSA, swine anti-rabbit IgG APC, and other reagents to perform the evaluation.

1. Prepare 1/500 - 1/3000 dilutions of rabbit polyclonal anti-human PSA with phosphate buffered saline.

2. Perform a P30 ELISA test as per the methods manual, using the diagram shown on the "polyclonal anti-PSA antisera evaluation" worksheet.

3. Determine the weakest dilution of anti-sera which gives a result for 2 ng PSA standard. Choose as the working titer the next strongest dilution.

Procedure - swine anti-rabbit alkaline phosphatase conjugate

Use previously evaluated lots of mouse monoclonal anti-human PSA, rabbit polyclonal anti-human PSA, and other reagents to perform the evaluation.

1. Prepare 1/500 - 1/3000 dilutions of swine anti-rabbit alkaline phosphatase conjugate with phosphate buffered saline.

2. Perform a P30 ELISA test as per the methods manual, using the diagram shown on the "swine anti-rabbit alkaline phosphatase conjugate evaluation" worksheet.

3. Determine the weakest dilution of APC which gives a result for 2 ng PSA standard. Choose as the working titer the next strongest dilution.

Procedure - P30 ELISA evaluation

Use the previously determined dilutions of mouse monoclonal anti-human PSA, rabbit polyclonal anti-human PSA, and swine anti-rabbit alkaline phosphatase conjugate to do the evaluation of the entire P30 ELISA test.

Initials: RJ Date: 4/22/93

1. Prepare 1/25 - 1/25,000 serial dilutions of stains prepared from semen, blood, urine, and saliva of healthy males as per the protocol.

2. Perform a P30 ELISA test as per the methods manual, using the diagram shown on the "P30 ELISA evaluation" worksheet.

Quality Control Parameters

1. All diluted samples of blood, urine, and saliva must give negative results.

2. The 2 ng, 6 ng, and 10 ng PSA standards and the $1/10^2$ dilution of semen must give positive results.

12. PGM Subtype by IEF

Each batch of PGM IEF plates, anode solution, cathode solution, PGM reaction buffer, and PGM reaction mixture must be evaluated before using method with casework samples.

Each lot of glucose-6-phosphate dehydrogenase, meldola blue, and agarose must be evaluated before use.

Procedure

1. Record batch numbers for PGM subtype IEF plates, anode solution, cathode solution, PGM reaction buffer, and PGM reaction mixture to be tested on quality control sheet. The vendor and lot number of the glucose-6-phosphate dehydrogenase, meldola blue, and agarose used in the quality control test must also be recorded.

2. Bloodstains containing known PGM types (PGM 2+1-, PGM 2-1+, and PGM 1+1-, respectively) are to be extracted and tested as per PGM subtype IEF method specified in methods manual.

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a. Sample test volume of 10ul is to be used for the extracts containing PGM 2+1- and 2-1+. Both extracts are to be tested in duplicate.

b. The extract containing PGM 1+1- is to be tested in triplicate but with varying volume sizes (10ul, 5ul, and 2.5ul).

Quality Control Parameters

1. The following is the allowable separation for adjacent bands on PGM subtype phenotype:

Bands	Allowable Separation
2+1-	>4mm
2-1+	>6mm
1+1-	>2mm

2. Typeable results must be observed with all sample volumes tested. Weak, but clearly typeable results are acceptable.

13. Species Determination Quality Control Test

Quality control evaluation must be done on all lots of commercially prepared anti-human antiserum before use with casework samples.

Each new batch of species tank buffer and gels must be evaluated be successfully evaluated against anti-human antiserum.

Antiserum Evaluation

1. Record vendor and lot number of the anti-human antiserum to be tested on the evaluation worksheet. The batch numbers of the species tank buffer and gels must also be recorded.

2. Detection Limits

a. Preparation of standards

(1). Prepare 1/10, 1/100, 1/1000, 1/10000, 1/20000, 1/50000, and 1/100000 dilutions of pooled human serum with saline.

(2). Prepare solutions of human albumin in which 5ul aliquots will contain 1000ug, 100ug, 10ug, 1ug, 0.1ug, 0.01ug, 0.001ug, and 0.0001ug of human albumin, respectively.

a. Sample test volume of 10ul is to be used for the extracts containing PGM 2+1- and 2-1+. Both extracts are to be tested in duplicate.

b. The extract containing PGM 1+1- is to be tested in triplicate but with varying volume sizes (10ul, 5ul, and 2.5ul).

Quality Control Parameters

1. The following is the allowable separation for adjacent bands on PGM subtype phenotype:

Bands	Allowable Separation
2+2-	>4mm
2-1+	>6mm
1+1-	>2mm

2. Typeable results must be observed with all sample volumes tested. Weak, but clearly typeable results are acceptable.

13. Species Determination Quality Control Test

Quality control evaluation must be done on all lots of commercially prepared anti-human antiserum before use with casework samples.

Each new batch of species tank buffer and gels must be evaluated be successfully evaluated against anti-human antiserum.

Antiserum Evaluation

1. Record vendor and lot number of the anti-human antiserum to be tested on the evaluation worksheet. The batch numbers of the species tank buffer and gels must also be recorded.

2. Detection Limits

a. Preparation of standards

(1). Prepare 1/10, 1/100, 1/1000, 1/10000, 1/20000, 1/50000, and 1/100000 dilutions of pooled human serum with saline.

(2). Prepare solutions of human albumin in which 5ul aliquots will contain 1000ug, 100ug, 10ug, 1ug, 0.1ug, 0.01ug, 0.001ug, and 0.0001ug of human albumin, respectively.

(3). Prepare 1/10, 1/100, 1/1000, and 1/10000 dilutions of pooled human semen and saliva with saline.

b. Evaluation Test

(1). Test all prepared standards (including neat samples of pooled human serum, semen, and saliva) by both crossed-over electrophoresis and Ouchterlony diffusion methods for species determination as specified in methods manual.

c. Evaluation Parameters

(1). Positive results must be observed in a dilution of pooled human serum that exceeds 1/100, a 5ul aliquot of human albumin (200ug/ul), a dilution of pooled human semen and pooled human saliva that exceeds 1/10 with both the crossed-over and Ouchterlony techniques.

3. Specificity Testing

a. Commercial animal sera should be tested against the anti-human antiserum for both the crossed-over and Ouchterlony techniques.

(1). Ten species should be tested, including monkey and the animal in which the antiserum was produced.

(2). The normal animal sera should be tested neat and at dilutions which correspond to ten times the human serum detection limit and the human serum detection limit.

b. Evaluation Parameters

(1). Cross reactivity is not acceptable with any animal sera, except monkey, at all tested concentrations.

<u>Quality Control Test for Species Cross-Over Electrophoresis</u>

1. Record batch numbers for species gels and tank buffer on quality control sheet.

2. Prepare dilutions of pooled human serum with saline as

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3. Test neat and diluted samples of the pooled human serum against previously evaluated commercial anti-human antiserum as per crossed-over and Ouchterlony methods.

Quality Control Parameters

1. Sensitivity must be comparable with results obtained with antiserum evaluation against pooled human serum. The sensitivity of a given lot of anti-human antiserum should not significantly vary with different lots of tank buffer and/or agarose gels (a one dilution step variation can be tolerated provided that it meets the parameters specified for the antiserum evaluation).

14. Takayama Quality Control Test

Each batch of Takayama reagent must be evaluated before using Takayama method with casework samples.

Takayama reagent should be checked monthly.

Procedure

1. Record batch number of Takayama reagent on quality control sheet.

2. Extract a bloodstain and test as per Takayama crystal test specified in methods manual.

Quality Control Parameter

1. Bloodstain extract must give a positive result with test.

15. Urea Gel Diffusion Quality Control Test

Each batch of urea diffusion test and blank plates must be evaluated before use in method.

Each lot of commercially prepared bromothymol blue, urease, agarose, urea, and phosphoric acid must be tested before use.

Procedure

1. Record the batch number or the urea diffusion test and blank plates, and the vendor and lot numbers for the agarose, bromothymol blue, urease, and phosphoric acid to be tested on the quality control sheet.

2. Prepare a set of urea standards containing 5g urea/100ml, 0.5g urea/100ml, 0.05g urea/100ml, and 0.005g urea/100ml,

described above.

3. Test neat and diluted samples of the pooled human serum against previously evaluated commercial anti-human antiserum as per crossed-over and Ouchterlony methods.

Quality Control Parameters

1. Sensitivity must be comparable with results obtained with antiserum evaluation against pooled human serum. The sensitivity of a given lot of anti-human antiserum should not significantly vary with different lots of tank buffer and/or agarose gels (a one dilution step variation can be tolerated provided that it meets the parameters specified for the antiserum evaluation).

14. Takayama Quality Control Test

Each batch of Takayama reagent must be evaluated before using Takayama method with casework samples.

Takayama reagent should be checked monthly.

Procedure

1. Record batch number of Takayama reagent on quality control sheet.

2. Extract a bloodstain and test as per Takayama crystal test specified in methods manual.

Quality Control Parameter

1. Bloodstain extract must give a positive result with test.

respectively, in distilled water.

3. Extract a 1cmx1cm known urine stain in 200ul distilled water and prepare a 1:2 dilution of the extract in distilled water.

4. Test 9ul of each urea standard, the neat and 1:2 diluted urine stain extracts, and a distilled water blank as per urea gel diffusion procedure specified in methods manual.

5. Prepare a standard curve of ;urea concentration (expressed logarithmically on x axis) versus the mean diffusion radius (determined by subtracting the mean diffusion radius of standard on blank plate from mean diffusion radius of stand on test plate).

6 Plot the mean diffusion radius of the neat and 1:2 diluted extracts of known urine stain on standard curve.

Quality Control Parameters

1. The mean diffusion radius of the blue circular area around standard wells needs to be linear with respect to the urea concentration expressed logarithmically.

2. The mean diffusion radius of the blue circular area around the wells containing the neat and 1:2 diluted urine stain extracts, respectively, needs to fall between the lowest and highest points on the standard curve.

3. The calculated urea concentration of the neat and 1:2 diluted urine stain extracts should differ by a factor of 2 given an allowable error of 5%.

Department of Forensic Biology

Quality Assurance Manual

Revision Sheets

The following table must be filled out when changes to the Quality Assurance Manual are made. The following definitions apply:

Date:	The date the revision went into effect.
Revision #:	The Revision number of the manual affected.
Change:	This column is checked if the revision reflects a change in procedures.
Addition:	This column is checked if the revision reflects an addition to the manual.
Initials:	Initials of the laboratory director.

Date	Rev. #	Page #	Change	Addition	Initials
11/15/93	1.0	B2		New tests	WJB
11/15/93	1.0	1365		Auxiliary Diff formulation	WJB

APPENDIX C

DEPARTMENT OF FORENSIC BIOLOGY

MOLECULAR BIOLOGY LABORATORY

HLA-DQα
QUALITY ASSURANCE MANUAL
VERSION 1.0

Initials: RC Date: 3/17/93

Initials: RC Date: 3/14/92

Initials: RS Date: 3/17/93

Initials: RD Date: 4/14/92

Initials: RCJ Date: 4/14/92

QUALITY ASSURANCE SYSTEMS AND PROCEDURES

1.1 Definitions

The HLA-DQα quality assurance program maintains performance standards with four systems:

1. Equipment Quality Assurance
2. Solution Manual
3. Contamination Control
4. Training and Proficiency

Each system describes a flow of information. The system defines an area of interest, outlines what information is routinely collected, how it is collected, and how it is evaluated.

A system may incorporate one or more procedures. A procedure is a list of instructions for a specific task within the laboratory. Examples of procedures are QA001 Thermocycler Block Cleaning and QC001 DQα Extraction. Worksheets used for documentation are located in the appendix. This manual describes how the systems and procedures operate and how they are integrated to make up the QA/QC program for the HLA-DQα test.

The quality assurance program has both a bookkeeping function and a revision function. The information collected during routine testing is recorded and compiled to support typing results from the laboratory. The information is also used to evaluate the success of the quality program. Changes may be necessary to improve the efficiency of data collection or to ensure that the system is comprehensive. Consequently, the quality assurance program modifies itself based upon the information it collects during routine testing.

Initials: RCJ Date: 4/14/92

EQUIPMENT QUALITY ASSURANCE

2.1 Thermocycler

The performance of the Perkin Elmer thermocyclers is routinely monitored. Once a month, six internal diagnostic tests are run, the time for the HLA-DQα amplification cycle is measured, and the thermocycler block is cleaned. Each week, the temperature profile for one well is measured using a thermocouple probe.

To construct the probe, a thermocouple is mounted in an amplification tube which fits the contours of the thermocycler well. The probe is calibrated against a standard mercury thermometer, and a calibration curve is calculated. Finally, the probe is used to measure the temperature response of a sample in the thermocycler block as the machine cycles through the amplification program.

Temperature profiles for every well of both Perkin Elmer thermocyclers have been measured. These profiles are used as specifications of future performance. Each week, a different well is chosen, and temperature profiles for both machines are measured. The new profiles are compared to the specifications to determine if the performance of the well falls within the established temperature range. When a complete set of profiles has been collected, the results are compiled and the system is recalibrated for the next cycle.

2.2 Water Bath

The water bath temperature is measured and recorded prior to each hybridization run. The same kind of thermocouple probe used to measure well temperatures in the thermocyclers is used to measure the temperature of the water bath. The probe is constructed in the same way as a thermocycler probe, except in this case a 1.5 ml centrifuge tube is used to house the tip of the thermocouple (QA004). The thermocouple is calibrated against a standard mercury thermometer (QA005).

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QA001 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.

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 distilled water rinse. Dry the sample wells with dry cotton swabs or kimwipes.

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QA002 Thermocycler Diagnostic Tests

There are six diagnostic tests run once a month. The test results are recorded on a Thermocycler Diagnostics Specification Sheet.

To access the diagnostic test files, use the following commands.

Press **File**, **Yes**.

The following will appear on the display.

Select Function
CONFIG-DIAGNOSTIC

Press **No**.

This moves the cursor to the "Diagnostic" option.

Press **Enter**.

The following will appear on the display.

Diagnostic Tests
Enter test # (1-8)

Type the number of the test you want and press **Enter**. Tests 2 and 6 are run only under special circumstances.

To leave a test, press **Stop**.

Test 1: Display/Keypad Test

The machine first illuminates each block on the display board. The operator must watch to see that all the dots light up across the screen. Next, the operator checks each of the keys on the control board. As each key is pressed, the machine should display the corresponding command or number.

Test 3: Heater Test

This test measures the maximum heating rate. At the end of the test, the machine displays the time in seconds required for the first 15 degrees of temperature change, the

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temperature difference between the upper and lower temperature sensors just before the heaters go off, and the heating rate. The heating time is a measure of the thermal time constant of the sensor/block assembly. If its value is not correct, a mechanical problem is indicated. The temperature difference is an indication of proper sensor operation and installation. Compare the results to the specifications.

Test 4: Chiller Test

This test measures the maximum cooling rate. The machine displays the sensor difference and cooling time similar to the heating test. Allow the machine to idle for at least 30 minutes before this test is run so that the coolant has time to reach operating temperature. Compare the results to the specifications.

Test 5: Sensor Test

To check the sensor difference, allow the sample block to soak at a set temperature for at least 10 minutes. For example, run file 1 with a setpoint of 35°C. Record the soak temperature on the Thermocycler Diagnostics Specification Sheet. At the end of the incubation period, quickly abort the file and enter this diagnostic test. The machine will display the current temperature readings of the two sensors and their difference. Compare the results to the specifications.

Test 7: Overshoot Test

This test measures the temperature overshoot on a setpoint step from 37 to 94°C. The block is set to 37°C for 1 minute then ramps up to 94°C. The overshoot past 94°C is shown on the display after 15 seconds. Compare the results to the specifications.

Test 8: Undershoot Test

This test measures the temperature undershoot on a setpoint step from 94 to 55°C. The block is set to 94°C for 1 minute and then ramps down to 55°C. The undershoot past 55°C is shown on the display after 15 seconds. Compare the results to the specifications.

Evaluation of Results

If all the results meet specifications, the thermocycler passes diagnostic testing. The

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Thermocycler Diagnostics Specification Sheet is filed in the Thermocycler Calibration Log.

If the results for any of the diagnostic tests fail to meet specifications, the thermocycler must be taken off-line for casework. Recent casework must be reviewed and selected samples may be retyped to confirm the results. Further testing may be necessary to rule out the possibility of human error. The test may not have been run properly or the results may not have been interpreted correctly. If after review the results fall consistently outside specification, the thermocycler must be tested before it can be put back on-line. QA007 Thermocycler Well Test must be performed for every third well of the block. If all the wells pass the test, casework may resume. If any of the wells fail the test, the thermocycler must be serviced and retested before going back on-line.

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QA003 Thermocycler Cycle Time

The amplification cycle for HLA-DQα is timed once a month to confirm the reproducibility of the incubation program.

Procedure

To measure the cycling time, load file 14, the HLA-DQα amplification program.

Start the program.

Allow the machine to complete three amplification cycles before making any measurements.

Begin timing the fourth cycle when the machine first ramps to 94°C from the third incubation at 72°C.

Stop the timer at the end of the 72°C incubation.

Record the time for a single cycle.

Repeat this procedure for an additional cycle.

Calculate the difference in seconds between the first and second cycle measurements.

Compare the results to the specifications.

Evaluation of Results

If the results meet specifications, the thermocycler passes the cycle time test. The Thermocycler Diagnostics Specification Sheet is filed in the Thermocycler Calibration Log.

If the results fail to meet specifications, the thermocycler must be taken off-line for casework. Recent casework must be reviewed and selected samples may be retyped to confirm the results. Further testing may be necessary to rule out the possibility of human error. The test may not have been run properly or the results may not have been interpreted correctly. If after review the results fall consistently outside specification, the thermocycler must be tested before it can be put back on-line. QA007 Thermocycler Well Test must be performed for every third well of the block. If all the wells pass the test, casework may resume. If any of the wells fail the test, the thermocycler must be serviced and retested before going back on-line.

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QA004 Thermocouple Preparation

Introduction

The thermocycler temperature probe has two components. The thermocouple, which responds to temperature changes, is mounted inside either an amplification tube or a 1.5 ml centrifuge tube. Thermocouples are used because of their fast response time and the availability of thin thermocouple wire. Sensors made with thin wire will not significantly affect the temperature reading. The laboratory probes are made from Teflon coated, 36 gage, type T thermocouples purchased from Omega Engineering.

The thermocouple is plugged into an electronic thermometer which displays the temperature read-out. An Omega HH72T electronic thermometer is used for its speed, sensitivity, and accuracy. The combination of the thermocouple and electronic thermometer must be calibrated together as the response with different thermocouples will vary.

Procedure

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.

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QA005 Thermocouple Calibration

The temperature probe is calibrated against an ASTM mercury thermometer, graduated to 1°C over the range 25-100°C. Before beginning the calibration procedure, the mercury thermometer is checked by measuring two standard temperatures.

Mercury Thermometer Standardization

Place the 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 Calibration Sheet.

Thermocouple Temperature Response

Add 3 liters of distilled water to a 4 liter glass beaker.

Place the beaker on a stir plate.

Set up a clamp and ring stand behind the beaker.

Clamp the mercury thermometer onto the ring stand and position it so that the thermometer can be submerged in the water.

Open the cap of the tube with the thermocouple and slide the tube up the wire far enough to be out of the way. The performance of the thermocouple should be checked directly, without interference from the amplification tube.

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 mercury thermometer, with attached thermocouple and wire, into the water. Tighten the clamp to hold the thermometer at the correct depth. The thermometer has an etched line 17 cm from the bulb which is the minimum level the thermometer must be

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immersed for accurate readings. Failure to immerse at the correct depth will result in incorrect results.

Plug the thermocouple into the socket of the electronic thermometer to be used during routine measurements. Generally, socket 1 on electronic thermometer 1 is used.

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.

Seven or eight comparisons of the mercury thermometer and the electronic 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 thermocouple and the mercury thermometer on the Thermocouple Calibration Sheet. The probe measurements are recorded under the x-axis column, and the readings from the mercury 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.

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 evaporative loss 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 seven or 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 mercury thermometer and provide some 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 a 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 coeffiecient, 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.

Calculations

The following are calculated and recorded on the Thermocouple Calibration Sheet. 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

$$\text{sum}(x) = \Sigma x_i$$

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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

$$m = \frac{S_{xy}}{S_{xx}}$$

The intercept is calculated using the mean x and y values.

$$b = \bar{y} - m\bar{x}$$

Finally, the correlation coefficient is calculated using

$$r = \frac{S_{xy}}{(S_{xx}\ S_{yy})^{1/2}}$$

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The slope is written with three significant figures. The intercept is rounded to the tenths place. The correlation coefficient has a specification of >0.9999. If the calibration passes specification, the probe is ready for use.

Final Adjustments

Release the thermocouple wire from the mercury thermometer.

Slide the cap of the tub down the thermocouple wire until it is near the knot.

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.

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QA006 Thermocycler Well Temperature Profile

The thermocouple probe is used to approximate the temperature response of a sample during an actual amplification cycle. Each well is measured during two cycles, listed as run 1 and run 2 on the Thermocycler Well Calibration Sheet.

Procedure

Allow the thermocycler to warm up at least 15 minutes.

Make sure that the tip of the thermocouple is immersed in water and that mineral oil has not collected in the bottom of the tube. There must be sufficient oil above the water to prevent evaporative cooling.

Place one drop of mineral oil in the well to be measured.

Place the tube in the well. Press down on the top of the tube without disturbing the thermocouple wire so that the tube is seated firmly in the well.

Load amplification file 14.

Begin the program. Once the block has reached 94°C, push the tube as far into the well as possible. Allow the machine to complete the first cycle.

Wait until the sample block reaches 94 degrees for the second time.

When the instrument begins the 60 second countdown, record the probe temperature on the Thermocycler Well Calibration Sheet in the column headed 94, the set temperature for this step of the amplification cycle. This is the measurement at time zero.

Record the probe temperature every 15 seconds until the 94 degree incubation is complete. The readings must be timed carefully in order for the temperature measurements to be valid.

Wait until the sample block reaches 60 degrees.

When the instrument begins the 30 second countdown, record the probe temperature on the Thermocycler Well Calibration Sheet in the column headed 60. This is the measurement at time zero.

Record the probe temperature every 15 seconds until the 60 degree incubation is complete.

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Wait until the sample block reaches 72 degrees.

When the instrument begins the 30 second countdown, record the probe temperature on the Thermocycler Well Calibration Sheet in the column headed 72. This is the measurement at time zero.

Record the probe temperature every 15 seconds until the 72 degree incubation is complete.

Record temperatures for a second cycle.

Calculate the standard mercury temperatures for each of the observed probe temperatures using

$$y = \bar{m}x + \bar{b}$$

where x is the observed probe temperature, $\bar{m}$ is the average slope, and $\bar{b}$ is the average intercept from the current Thermocouple Calibration Summary for the thermocycler probe. The y values are the standard temperatures. The corresponding standard temperatures are recorded in the 'std' columns of the Thermocycler Well Calibration Sheet.

Compare the results to the specifications on the Thermocycler Well Calibration Sheet.

Specifications

If all of the temperature measurements fall within the specified ranges, the well passes the temperature check. The Thermocycler Well Calibration Sheet is filed in the Thermocycler Calibration Log.

If any of the temperature measurements falls outside the specified ranges, the well must be taken off-line for casework. Recent casework will be reviewed and selected samples may be retyped to verify the results. The measurements may be repeated to rule out the possiblity of human error or equipment failure. The test may not have been run properly or the results may not have been interpreted correctly. The thermistor probe may be checked to ensure that it is performing properly. Finally, diagnostic tests may be performed to ensure that the thermocycler is performing as expected.

If after review the well exhibits a consistent shift in performance outside the specified temperature ranges, the well must be tested empirically according to QA007 Thermocycler Well Test.

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QA007 Thermocycler Well Test

This test is run to determine if a temperature deviation outside the specified ranges adversely affects typing results. The specifications reflect extremes of performance which have actually been measured using the thermocouple probe. Since the specifications were originally established using only one set of temperature profiles, it is expected that temperature profiles will occasionally be measured which fall outside these ranges. These deviations may or may not affect HLA-DQα typing results.

A sample of known HLA-DQα type which is susceptable to allelic drop-out is amplified in the well outside specifications and in a control well. Acceptable sample types for this test are 1.1,4, 1.2,4, and 1.3,4. The typing result from the well outside specification demonstrates whether differential amplification or allelic drop-out is likely to appear given the measured temperature profile. Comparison of the amplified samples indicates the relative amplification efficiency of the test well relative to the control well.

Samples

one sample of known HLA-DQα type which is susceptable to allelic drop-out
one amplification negative

Procedure

Prepare duplicates of the known sample for amplification and one amplification negative. Amplify one known sample in the well which is outside specification. Amplify the other known sample and the amplification negative in wells which have recently passed temperature specifications.

Hybridize the samples according to the HLA-DQα hybridization protocol.

Specifications

The amplification negative must show no evidence of contamination.

Each known sample must match the assigned HLA-DQα type within the current interpretation guidelines. The intensity of the dots across each strip must be approximately the same. In particular, the 1 dot must not appear weak, indicating differential amplification of the sample.

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The intensity of dots between the duplicate sample strips must also be approximately the same. This indicates that the amplification efficiency of the well outside specification is acceptable.

If the samples meet the specifications, the well passes the typing test and may be used for casework. See QA008 System Review for adjustment of the specifications. If the samples do not meet specifications, the well is taken off-line for casework until the next service call. After service, the performance of the well may be reevaluated.

This procedure is sufficient to pass a well which falls outside temperature specifications. Occassionally, an extracted control sample may not be available, and a control sample will have to be extracted before beginning the amplification test. In that case, an extraction negative is run in place of the amplification negative. In order to pass the well, the extraction negative must show no evidence of contamination.

Documentation

Write the test up on DQα Amplification and Hybridization Worksheets.

Attach the completed worksheets to the Thermocycler Well Calibration Sheet and file in the Thermocycler Calibration Log.

Initials: RC Date: 4/14/92

QA008 System Review

Every eight months, the temperature performance of the thermocyclers and the water bath is evaluated. This procedure describes how the results are compiled and how the system is reset to begin the next cycle.

There are thirty two wells in the thermocycler block which are used for HLA-DQα amplifications. After eight months, a new set of temperature profiles has been collected. The results are compiled and the specifications for the temperature profiles are reviewed. The results from any thermocycler well tests performed during the eight month period may be used to adjust the specifications for the next cycle.

The thermocouple probes for the thermocycler and the water bath are recalibrated. As calibrations are performed for each of the probes, the slope and intercept values are averaged. The cumulative values are used to calculate standard mercury temperatures. The repeated calibrations compensate for any changes in the the performance of the thermocouples over time. By averaging the calibration curves, the uncertainty associated with the calibration procedure is minimized.

Procedure

If any wells have successfully passed thermocycler well tests, adjust the specifications to include the new acceptable temperature extremes. Record the new specifications on the Thermocycler Well Calibration Sheet.

Recalibrate the thermocouple probes for the thermocycler and the water bath, and calculate new calibration curves according to QA005.

Calculate mean slope and intercept values for both probes. The ten most recent calibrations are used for the calculations. If ten calibrations have not been performed for a particular probe, all the available calibrations are included. For each of the calibrations, record the date of the calibration, the slope and the intercept values on the Thermocouple Calibration Summary. The variable n is the total number of calibrations.

Summation (m) is calculated by adding together the slope values. This is written in standard notation as

$$\text{sum(m)} = \Sigma\, m_i$$

Initials: RCI Date: 4/14/92

Mean m equals summation (m) divided by n. This is written

$$\bar{m} = \frac{\text{sum}(m)}{n}$$

Similarly, summation (b) is calculated by adding together the intercept values.

$$\text{sum}(b) = \Sigma\, b_i$$

Mean b equals summation (b) divided by n.

$$\bar{b} = \frac{\text{sum}(b)}{n}$$

Record the results on the Thermocouple Calibration Summary. The average values are used to standardize temperature probe readings for the next eight month cycle.

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SOLUTION MANUAL

The laboratory prepares several solutions to supplement the material provided in the HLA-DQα kits. Every solution has a corresponding Solution Sheet. The sheet indicates the standard batch size, the ingredients of the solution, the procedure to follow when preparing the solution, a section where data is recorded, and a section which lists the quality control procedures to be performed before the solution is released for use in the laboratory. Blank solution sheets are listed in the appendix.

3.1 Solution Numbers

Each solution has been assigned a unique solution number along with the name (format S003 DQα Citrate Buffer). The solution numbers identify a solution with a specific recipe. They can be used as a double check for analysts performing procedures with which they are relatively unfamiliar. They are also a useful labeling shorthand for intermediate vessels during solution preparation.

3.2 Standard Batch Size

Each sheet indicates the standard batch size which is 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 analyst must note the total volume clearly on the solution sheet and carefully record the adjusted amount of each ingredient added to the solution. If changes in demand persist over time, the solution sheet may be adjusted to a new batch size.

3.3 Lot Numbers

Each batch of a solution is assigned a lot number beginning with 1. Information about each lot of the solutions is recorded in the Solution Inventory. The inventory indicates the date each solution lot was prepared, who prepared it, and where it is stored. The solution sheets for each lot are filed in the Solution Inventory along with any supporting quality control documentation.

3.4 Ingredients

The ingredients required for the solution are listed at the top of the page. They indicate the final concentration of the ingredient and the amount of that ingredient required for the standard batch size. An ingredient may be either a raw material, something purchased from an outside vendor, or another solution prepared in the laboratory.

Each amount is listed with an uncertainty of measurement. The uncertainties are calculated to define an acceptable range of variation which will not significantly change the

final concentration. In a few cases, narrower ranges have been adopted based upon recommendations for optimum performance.

3.5 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 noted.

3.6 Data Log

The data log is where information is recorded about the ingredients of the solution. Every raw material ingredient is labeled with a QA sticker when it is received in the laboratory. The label lists an assigned RM number for the material (format RM000), the vendor, the vendor's lot number, and the date. On the solution sheet, the vendor is recorded as the source of the material, the vendor's lot number is recorded, and the amount of the ingredient measured and added to the solution is recorded. The amount measured must fall within the specified range listed in the ingredients section unless the range is marked guideline. A guideline is a suggested range used to make preparation easier and faster. Other ranges are specifications of tolerance.

Solutions prepared in the laboratory may also be listed as ingredients. In those cases, the source is listed as DNA and the laboratory lot number is recorded. Volume measurements which are made in the appropriate size graduated cylinders and which appear to the eye to be exact fall well within the ranges of tolerance listed in the ingredients section. The solution volumes must be recorded in the data log to keep track of the ingredients as they are added.

3.7 Quality Control

The quality control section lists the tests to be performed, if any, before the solution is released for use in the laboratory. These test procedures have been assigned QC numbers and names (format QC001 DQα Extraction).

At this point, there may be some uncertainty regarding the use of the terms quality control and quality assurance. It is common to say that a quality control test has been performed on a solution to determine if a new lot behaves the same way as earlier lots. The term quality control is used correctly in the sense that the test measures variability of performance from lot to lot, allowing different lots to be compared.

However, the final product of the laboratory is not solutions, but test results. Strictly speaking, the quality controls for HLA-DQα are the controls run during the test which indicate its success and permit a comparison to results obtained from other runs. With

respect to the test results, anything else, from thermocycler calibration to solution preparation, falls within the bounds of quality assurance.

In this manual, the procedures measuring variability of solutions from lot to lot have been assigned QC numbers following the common meaning of quality control as a control of variance. With respect to the actual test results, these procedures are not quality controls, but one aspect of quality assurance which guarantees that the test controls (positive, substrate, negative) are meaningful indicators of the quality of the individual test run.

Quality tests are started at different stages of the protocol, depending upon the reagent under study. There are three QC procedures beginning with the extraction, amplification, and hybridization steps. The QC procedure assigned to a solution represents the appropriate level of testing required to pass the reagent.

For example, QC003 DQα Hybridization is listed in the quality control section for DQα Wash Solution. To evaluate the performance of this component, it is not necessary to run through the entire test. Only the hybridization procedure is critical. QC003 begins with samples which have previously been amplified and repeats the hybridization using the new wash solution. A hybridization negative is the appropriate negative control. No amplified extract is added to a hybridization negative, but in every other respect, the strip is processed the same as the positive controls.

More than one solution may be tested at a time. 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 DQα Wash Solution, the quality test must begin with the extraction. QC001 DQα Extraction is the appropriate test for the chelex, and the procedure encompasses the hybridization necessary for the wash solution.

3.8 Raw Materials Testing

In addition to solution quality control, each lot of kits is tested when it is received in the laboratory to ensure that its performance has not been affected during shipping. These quality control records are filed in the Inventory Control Log with raw materials information.

3.9 Documentation

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

After a solution has passed quality control and been released, the solution sheet and quality control documentation are filed in the Solution Inventory. If more than one solution has been quality controlled in a single test run, the original quality control documents will be filed with one solution sheet and a copy of the original will be filed with each additional solution sheet.

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QC001 DQα Extraction

Test Materials

S022 Chelex, 5%

Samples

two whole blood or stain samples of known HLA-DQα type
one negative control sample
one positive control sample from the HLA-DQα DNA typing kit

Procedure

Extract the two known samples and the negative control sample according to the chelex extraction procedure for whole blood and bloodstains in the HLA-DQα protocol.

Amplify the samples and a positive control from the kit according to the HLA-DQα amplification protocol.

Hybridize the samples according to the HLA-DQα hybridization protocol.

Specifications

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

The negative control sample must show no evidence of contamination.

Documentation

Write the test up on a set of DQα Extraction, Amplification, and Hybridization Worksheets.

Attach the completed worksheets to the Solution Log Sheet.

Initials: RCS Date: 4/14/92

QC002 DQα Amplification

Test Materials

RM114 DQα Amplitype kit

Samples

two whole blood or stain samples of known HLA-DQα type
one amplification negative
one positive control sample from the HLA-DQα DNA typing kit

Procedure

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

Hybridize the samples according to the HLA-DQα hybridization protocol.

Specifications

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

The amplification negative must show no evidence of contamination.

Documentation

Write the test up on DQα Amplification and Hybridization Worksheets.

Attach the completed worksheets to the Solution Log Sheet.

Initials: KCS Date: 4/14/92

QC003 DQα Hybridization

Test Materials

S003 DQα Citrate Buffer
S004 DQα Hybridization Solution
S005 DQα Wash Solution
S079 Hydrogen Peroxide, 3%

Samples

three amplified samples of known HLA-DQα type
one hybridization negative

Procedure

Hybridize the samples according to the HLA-DQα hybridization protocol. No amplified extract is added to the hybridization negative. In all other respects, this strip is processed the same way as the positive control samples.

Specifications

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

The hybridization negative must show no evidence of contamination.

Documentation

Write the test up on a DQα Hybridization Worksheet.

Attach the completed worksheet to the Solution Log Sheet.

Initials: RC Date: 3/10/93

QC004 DQα Differential Extraction

Test Materials

S014 Proteinase-K Enzyme, 10mg/ml
S034 Phosphate Buffered Saline (PBS)
S082 Chelex, 20%
S093 DTT, 1M
S094 Digest Buffer

Samples

one swab with epithelial and sperm cells of known HLA-DQα type
one negative control sample
one positive control sample from the HLA-DQα DNA typing kit

Procedure

Extract the known swab and the negative control sample according to the differential extraction procedure in the HLA-DQα protocol.

Amplify the samples and a positive control from the kit according to the HLA-DQα amplification protocol.

Hybridize the samples according to the HLA-DQα hybridization protocol.

Specifications

Each sample fraction must match the assigned HLA-DQα type within the current interpretation guidelines.

The negative control sample must show no evidence of contamination.

Documentation

Write the test up on a set of DQα Extraction, Amplification, and Hybridization Worksheets.

Attach the completed worksheets to the Solution Log Sheet.

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CONTAMINATION CONTROL

4.1 Prevention

Several measures have been taken to prevent contamination problems. The laboratory is divided into three physically isolated areas for extraction, pre-amplification and post-amplification. Each of these areas has its own dedicated equipment used only for PCR. Samples, once they are accepted into the laboratory, move through these three areas in one direction only. Samples are first processed in the extraction area. They are then moved into the pre-amplification area which is a low DNA concentration area. Here fresh kit reagents are stored and samples are prepared for amplification. Finally, the samples are amplified and hybridized in the third area, which is a high DNA concentration area. This laboratory set-up helps eliminate cross contamination from high concentration DNA areas back into low concentration DNA areas.

To avoid cross contamination between specimens, exemplar samples with high concentrations of higher quality DNA are processed separately from evidence samples which are expected to have lower concentrations of partially degraded DNA.

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

4.2 Contamination Protocol

Contamination problems reflect a system failure. The source may be equipement, materials, or the environment. When contamination is found during a test run, a supervising scientist must be notified. No additional casework will be performed until the source of the contamination has been identified and eliminated. Recent casework will be reviewed, and selected samples may be repeated later to verify the results. Once the error has been corrected, all analysts will be notified in writing that casework may resume. The analysts will also be informed of any corrective action adopted to prevent the recurrence of the problem.

4.3 Troubleshooting

Often the source of a contamination problem can be identified on the basis of experience. A light signal without a visible 'c' dot in the negative control may indicate slight contamination of the chelex or the sterile water used during the extraction procedure.

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This contamination represents 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 amplified negative extract is greater than the threshold of detectability for the hybridization. Generally, fresh reagents will eliminate this problem.

Strips which appear to have the same mixture of DNA types across all the samples indicate a more serious contamination problem at the 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.

In some cases, the source of contamination may be more elusive. Problems which persist may be addressed by performing a clean run. During a clean run, control samples are processed along with a series of negative controls. Negative controls are run at the extraction, amplification, and hybridization 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.

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QA009 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 HLA-DQα type
one extraction negative
one amplification negative
one hybridization negative
one positive control sample from the HLA-DQα typing kit

Procedure

Extract the control samples and the extraction negative according to the chelex extraction procedure for whole blood and bloodstains from the HLA-DQα protocol. The extraction negative control is a reagent control, containing distilled water in place of sample. This sample should be handled the same way as the other samples, but no substrate should be added.

Amplify the samples with the positive control from the kit and an amplification negative according to the HLA-DQα 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.

Hybridize the samples with a hybridization negative according to the HLA-DQα hybridization protocol. No amplified extract is added to the strip for the hybridization negative.

Evaluation

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

If the amplification negative shows contamination, the problem has occurred during the amplification set-up. The extraction negative may or may not appear contaminated as well.

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If only the positive controls appear contaminated, the problem might be the stringency of the hybridization.

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

Write the clean run up on a set of DQα Extraction, Amplification, and Hybridization Worksheets.

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TRAINING AND PROFICIENCY

5.1 Training Outline

The goal of training and proficiency testing is to establish consistency of performance between individual analysts and to maintain the highest possible level of performance over time. The HLA-DQα training program is monitored by the QA coordinator in forensic molecular biology. Training may be performed by the QA coordinator or by a proficient analyst with the appropriate level of experience.

5.1.1 Demonstration

New analysts are trained to perform a variety of extraction procedures for different kinds of samples. All samples are typed using the same amplification and hybridization procedures. The test requires a day to run from beginning to end. The first few days are used to introduce each of the different extraction procedures and to reinforce the important aspects of the amplification and hybridization procedures. By the end of the extraction demonstrations, the analyst will have some hands-on experience, will be able to complete the worksheets used to document sample analyses, and will be familiar with the operation of the equipment necessary to perform the test.

5.1.2 Practice

The analyst will then practice each of the extraction procedures with supervision. At first, the analyst will require direct supervision and assistance throughout the entire test. But after one or two days of practice, the analyst will be comfortable with the amplification and hybridization set-up while still having only limited experience with each of the extraction procedures. At this point, direct supervision may be limited to the extraction procedure and the analyst will complete the test alone. Eventually, the analyst will be able to complete the entire test with limited supervision.

During this phase, the analyst will perform practice runs for each of the extraction methods. Three to six samples are sufficient for each of the practice tests. The results from the analyst's tests will be evaluated in terms of sensitivity, consistency, and contamination. Problems will be addressed at this point and tests repeated if necessary.

Each analyst must complete one practice test for each of the extraction methods without serious contamination. Low-level contamination (contamination which is less intense than the 'c' dot) may not affect the typing results. Such contamination may often be eliminated by simply changing a reagent. However, if the analyst consistently has low-level contamination, the analyst will be observed during a practice run to determine the reason for the problem. These practice tests are filed in the analyst's proficiency folder.

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5.1.3 Proficiency

At the end of the practice stage, the analyst will take a proficiency test. The test consists of two samples for each of the extraction procedures which the analyst has practiced. These samples are run with a single negative control and a positive control from the kit. Although it is possible to extract some samples together using different protocols, the analyst may choose to perform each type of extraction separately. The final proficiency test is filed in the analyst's folder.

5.2 Concepts

The most important concept to stress at the earliest stage of training is the physical separation of the extraction, pre-amplification, and post-amplification areas. It is important to emphasize that dedicated equipment should never be moved from one area to another, except for the transfer racks which are used to move samples. Physical separation includes a habit as simple as changing gloves when entering or leaving a PCR area in order to prevent contamination.

It is important to stress that a contamination problem may require two or three complete testing runs to pinpoint and correct. Consequently, it is better to avoid possible problems by taking plenty of time to do the procedure and by using the best technique. It is also important to remember that carelessness on the part of one analyst may adversely affect others' test results. If there is any doubt about whether a glove, a reagent, or a tip is contaminated, it must be replaced.

Other important issues are raised as the analyst observes different extractions. During the extraction of whole bloods, it is important to emphasize the techniques used to prevent sample to sample cross contamination. During the extraction of stains, cross contamination techniques should be reinforced. In addition, the typing results from stains should be evaluated in terms of sensitivity and consistency which are harder to maintain for these types of samples.

By the end of the training period, the analyst will have aquired a basic theoretical understanding of each step of the test. It is important to know what is happening to the DNA during the extraction procedure, what happens in the reaction tubes during each step of the amplification cycle, the purpose of the various washing steps during hybridization and the basic mechanism for color development.

5.3 Supplemental Training

The analysts who have completed their initial round of proficiency testing will be introduced to new extraction procedures as they are ammended to the HLA-DQα protocol. As before, they will observe a demonstration run, and they will be given time to practice the

Initials: RS Date: 4/14/92

extraction on their own. In this case, practice samples may be processed at the same time as other samples. Once the analysts are comfortable with the new extraction procedure, they will type unknown samples as an internal proficiency. At least two samples must be successfully typed for each new extraction procedure before the analyst is considered proficient.

Initials: RCJ Date: 4/14/92

APPENDIX

This appendix contains blank copies of the worksheets used to document the quality assurance program for HLA-DQα. These documents may be ammended individually as the needs of the program change.

Initials: RCJ Date: 3/10/93

DQα Extraction Worksheet

Chelex, 5% _____ Sterile Water _____

Pro-K, 10mg/ml _____ DTT, 1M _____ PBS _____

Chelex, 20% _____ Digest Buffer _____

	case number - sample description	conc ng/µl

Performed by _____ Time _____ Date _____

Initials: RCS Date: 4/14/92

DQα Extraction Worksheet

Chelex _____ Sterile Water _____

Proteinase-K _____ DTT _____

tube label	case number - sample description	conc ng/µl

Performed by _____ Time _____ Date _____

Date: 6/11/92 Initials: RCS

DQα Amplification Worksheet

Kit _____ Reaction Tubes _____

$MgCl_2$ _____ Mineral Oil _____

Positive Control _____ Thermocycler _____

tube	sample	μl extract	sample well

Performed by _____ Time _____ Date _____

Initials: RCS Date: 4/14/92

DQα Amplification Worksheet

Kit _____ Reaction Tubes _____

$MgCl_2$ _____ Mineral Oil _____

Positive Control _____ Thermocycler _____

tube	sample	µl extract	sample well

Performed by _____ Time _____ Date _____

Date: 6/11/92 Initials: RCS

DQα Hybridization Worksheet

Strips _____

strip	tube	sample	HLA-DQα type	comments

Hyb. Solution _____ Enzyme Conjugate _____

Wash Solution _____

Chromogen _____ Reconstitution Date _____

H_2O_2 _____ Citrate Buffer _____

Developing time ___ minutes

Performed by _____ Time _____ Date _____

Initials: RC Date: 4/14/92

DQα Hybridization Worksheet

Strips _____

strip	tube	sample	HLA-DQα type	comments

Hyb. Solution _____ Enzyme Conjugate _____

Wash Solution _____

Chromogen _____ Reconstitution Date _____

H_2O_2 _____ Citrate Buffer _____

Developing time ___ minutes

Performed by _____ Time _____ Date _____

Date: 6/4/92 Initials: RS

DQα Review Worksheet

strip	HLA-DQα type	comments

Reviewed by _____ Date _____

Initials: RCJ Date: 4/14/92

DQα Review Worksheet

strip	HLA-DQα type	comments

Reviewed by _____ Date _____

Initials: QC Date: 4/14/92

Thermocycler Diagnostics Specification Sheet

Thermocycler _____ Date _____

Performed By _____

QA002 Diagnostic Tests

Test 1: Display/Keypad Test

All panels of display illuminate properly yes_____ no_____
Comments

All keys correspond to the correct command yes_____ no_____
Comments

Test 3: Heater Test

Rate (°C/s)	_____	specification	>0.90°C/s
Diff (°C)	_____	specification	0.0–12.0°C
Time (s)	_____	specification	≤23s

Test 4: Chiller Test

Rate (°C/s)	_____	specification	0.85–1.90°C/s

Test 5: Sensor Test

Temp (°C)	_____		
Diff (°C)	_____	specification	<±0.5°C

Test 7: Overshoot Test

Over (°C)	_____	specification	<2°C

Test 8: Undershoot Test

Under (°C)	_____	specification	<3°C

QA003 Cycle Time

Run 1 (s)	_____	specification	3:25–3:55
Run 2 (s)	_____	specification	3:25–3:55
Diff (s)	_____	specification	<5s

Date: 6/4/92 Initials: RCS

Thermocouple Calibration Sheet

probe _____ date _____

meter _____ thermometer _____

position _____ performed by _____

Mercury Thermometer Standardization

Ice Water Bath

Measured Temperature _____ specification -0.2-0.2°C

Boiling Water Bath

Measured Temperature _____ specification 99.8-100.2°C

Thermistor Calibration

x probe reading (°C)	y thermometer reading (°C)

n = _____

sum (x) = _____ sum (y) = _____

$\bar{x}$ = _____ $\bar{y}$ = _____

sum (x^2) = _____ sum (y^2) = _____

S_{xx} = _____ S_{yy} = _____

sum (xy) = _____ S_{xy} = _____

r = _____ spec > 0.9999

Date: 6/4/92 Initials: RG

m = _____

b = _____

Initials: RCJ Date: 4/14/92

Thermocouple Calibration Sheet

probe _____ date _____

meter _____ thermometer _____

position _____ performed by _____

Mercury Thermometer Standardization

Ice Water Bath

Measured Temperature _____ specification −0.2–0.2°C

Boiling Water Bath

Measured Temperature _____ specification 98.2–100.2°C

Thermistor Calibration

x probe reading (°C)	y thermometer reading (°C)

n = _____

sum (x) = _____ sum (y) = _____

$\bar{x}$ = _____ $\bar{y}$ = _____

sum (x^2) = _____ sum (y^2) = _____

S_{xx} = _____ S_{yy} = _____

sum (xy) = _____ S_{xy} = _____

r = _____ spec > 0.9999

m = _____

b = _____

Initials: RCV Date: 4/14/92

Thermocouple Calibration Summary

probe _____ date _____

meter _____

position _____

1. calibration date _____ m = _____ b = _____
2. calibration date _____ m = _____ b = _____
3. calibration date _____ m = _____ b = _____
4. calibration date _____ m = _____ b = _____
5. calibration date _____ m = _____ b = _____
6. calibration date _____ m = _____ b = _____
7. calibration date _____ m = _____ b = _____
8. calibration date _____ m = _____ b = _____
9. calibration date _____ m = _____ b = _____
10. calibration date _____ m = _____ b = _____

$\overline{m}$ = _____

$\overline{b}$ = _____

Reference Table

probe reading (°C)	standard temperature (°C)

Initials: RCI Date: 4/14/92

Thermocycler Well Calibration Sheet

Specification Set 2

Thermocycler _____ Well Number _____

Date _____ Performed By _____

RUN 1

time (s)	temperature (°C)								
	94	std	spec	60	std	spec	72	std	spec
0			81.3–90.5			53.9–72.8			63.5–67.8
15			86.1–93.2			55.4–66.1			66.9–70.8
30			89.2–94.4			56.4–62.9			68.6–72.0
45			90.9–94.5	---	---	---	---	---	---
60			91.9–94.4	---	---	---	---	---	---

RUN 2

time (s)	temperature (°C)								
	94	std	spec	60	std	spec	72	std	spec
0			81.3–90.5			53.9–72.8			63.5–67.8
15			86.1–93.2			55.4–66.1			66.9–70.8
30			89.2–94.4			56.4–62.9			68.6–72.0
45			90.9–94.5	---	---	---	---	---	---
60			91.9–94.4	---	---	---	---	---	---

Initials: RCl Date: 4/14/92

S001 SDS, 20% lot number: _____

Standard batch size: 1 L

Ingredients	final concentration	amount
RM007 sodium dodecyl sulfate	20 %	200 ± 5 g

Procedure

CAUTION: AN AEROSOL MASK OR FUME HOOD MUST BE USED WHEN MAKING THIS SOLUTION. WEAR GOGGLES FOR EYE PROTECTION.

Warm approximately 750 mL distilled water on a stirring hot plate.

Add a fraction of the SDS, allowing the solids to dissolve before adding more.

Add the SDS until it is all in solution.

When the solution is clear, bring up to volume with distilled water.

Filter sterilize the warm solution.

Dispense into sterile 500 mL bottles.

Store at room temperature.

Data Log	source	lot	amount
RM007 sodium dodecyl sulfate	_____	_____	_____

made by: _____ date: _____

Initials: RCS Date: 4/14/92

S002 SSPE, 20X lot number: _____

Standard batch size: 4 L

Ingredients	final concentration	amount
RM003 EDTA	20. mM	29.8 ± 0.7 g
RM004 sodium hydroxide, 10N	-----	40 ± 5 ml (guideline)
RM005 sodium chloride	3.6 M	840 ± 10 g
RM006 sodium phosphate, monobasic	200 mM	110 ± 3 g

Procedure

Dissolve the EDTA in approximately 3 liters distilled water.

Adjust the pH to approximately 6.0 with 10N sodium hydroxide to help dissolve the EDTA.

Add the sodium phosphate first and then the sodium chloride.

Adjust the pH to 7.4 with 10N sodium hydroxide (about 40 ml).

Adjust the final volume to 4 liters with deionized water.

Measure and record the final pH.

Dispense into 1 L bottles.

Store at room temperature.

Data Log	source	lot	amount
RM003 EDTA	_____	_____	_____
RM004 sodium hydroxide, 10N	_____	_____	_____
RM005 sodium chloride	_____	_____	_____
RM006 sodium phosphate, monobasic	_____	_____	_____

Quality Control

final pH: _____ specification 7.4 ± 0.2

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

S003 DQα Citrate Buffer lot number: _____

Standard batch size: 4 L

Ingredients	final concentration	amount
RM001 trisodium citrate	-----	73.6 ± 0.1 g
RM002 citric acid	-----	24 ± 1 g (guideline)

Procedure

Dissolve the sodium citrate in approximately 3 liters distilled water.

Adjust the pH to 5.0 by addition of citric acid (approximately 24 g).

Adjust the final volume to 4 liters with distilled water.

Mix well.

Measure and record the final pH.

Dispense into a 4 L bottle.

Store at room temperature.

Data Log	source	lot	amount
RM001 trisodium citrate	_____	_____	_____
RM002 citric acid	_____	_____	_____

Quality Control

final pH: _____ specification 5.0 ± 0.2

QC003 DQα hybridization

made by: _____ date: _____

Initials: RCS Date: 4/14/92

S004 DQα Hybridization Solution lot number: _____

Standard batch size: 4 L

Ingredients	final concentration	amount
S002 SSPE, 20X	5.0 X	1000 ± 10 ml
S001 SDS, 20%	0.50 %	100 ± 1 ml

Procedure

Combine the SSPE and 2.9 L distilled water in a 4 L flask.

Add the SDS.

Warm the solution until all solids are dissolved.

Mix well.

Dispense into 1 L bottles.

Store at room temperature.

Data Log	source	lot	amount
S002 SSPE, 20X	_____	_____	_____
S001 SDS, 20%	_____	_____	_____

Quality Control

QC003 DQα hybridization

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

S005 DQα Wash Solution lot number: _____

Standard batch size: 4 L

Ingredients	final concentration	amount
S002 SSPE, 20X	2.5 X	500 ± 10 ml
S001 SDS, 20%	0.10 %	20 ± 1 ml

Procedure

Measure 20 ml 20% SDS in a 50 ml graduated cylinder.

Raise the volume of the SDS solution to 50 ml by adding 30 ml distilled water.

Pour the SDS into a 4 L bottle.

Add 500 ml SSPE and 3450 ml distilled water.

Cap and mix well by inverting.

Store at room temperature.

Data Log	source	lot	amount
S002 SSPE, 20X	_____	_____	_____
S001 SDS, 20%	_____	_____	_____

Quality Control

QC003 DQα hybridization

made by: _____ date: _____

Initials: RCS Date: 4/14/92

S009 EDTA, 0.5M lot number: _____

Standard batch size: 500 ml

Ingredients	final concentration	amount
RM003 EDTA	0.50 M	93 ± 1 g
RM004 sodium hydroxide, 10N	------	------

Procedure

Add the EDTA to approximately 250 ml distilled water.

Adjust the pH to 8.0 with sodium hydroxide solution.

Mix well.

When the EDTA is dissolved, adjust the pH to 8.0.

Bring up to volume with distilled water.

Check and record the final pH.

Dispense into 125 ml bottles.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
RM003 EDTA	_____	_____	_____
RM004 sodium hydroxide, 10N	_____	_____	_____

Quality Control

final pH: _____ specification: 8.0 ± 0.1

made by: _____ date: _____

Initials: RC Date: 3/17/93

S014 Proteinase-K Enzyme, 10mg/ml lot number: _____

Standard batch size: 10 ml

Ingredients	final concentration	amount
RM119 proteinase-K, lyophilized	10 mg/ml	100 ± 1 mg

Procedure

Add 10 ml sterile, distilled water to one bottle (100 mg) lyophilized proteinase-K enzyme.

Mix by slowly inverting until completely reconstituted.

Dispense 500 ul aliquots into 1.5 ml eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM119 proteinase-K, lyophilized	_____	_____	_____

Quality Control

QA004 DQα differential extraction

made by: _____ date: _____

Initials: RCS Date: 9/14/92

S014 Proteinase-K Enzyme, 10mg/ml lot number: _____

Standard batch size: 10 ml

Ingredients	final concentration	amount
RM119 proteinase-K, lyophilized	10 mg/ml	100 ± 1 mg

Procedure

Add 10 ml sterile, distilled water to one bottle (100 mg) lyophilized proteinase-K enzyme.

Mix by slowly inverting until completely reconstituted.

Dispense 500 ul aliquots into 1.5 ml eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM119 proteinase-K, lyophilized	_____	_____	_____

made by: _____ date: _____

Initials: RC Date: 4/14/92

S018 Analytical Gel Loading Buffer lot number: _____

Standard batch size: 100 ml

Ingredients	final concentration	amount
RM020 bromophenol blue	0.25%	0.25 ± 0.01 g
RM217 xylene cyanol	0.25%	0.25 ± 0.01 g
RM040 ficoll 400	12.5%	12.5 ± 0.1 g
S009 EDTA, 0.5M	50. mM	10.0 ± 0.1 ml
RM083 TAE, 10X	5.0 X	50.0 ± 0.5 ml

Procedure

Combine the TAE, EDTA, and ficoll.

Mix well. The solution may need to be heated gently to dissolve the ficoll.

Add the bromophenol blue and xylene cyanol.

Mix well.

When all the solids are dissolved, bring up to volume using distilled water.

Filter sterilize.

Dispense 1.5 ml aliquots into 1.5 ml eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM020 bromophenol blue	_____	_____	_____
RM217 xylene cyanol	_____	_____	_____
RM040 ficoll 400	_____	_____	_____
S009 EDTA, 0.5M	_____	_____	_____
RM083 TAE, 10X	_____	_____	_____

made by: _____ date: _____

Initials: DCJ Date: 4/14/92

S022 Chelex, 5% lot number: _____

standard batch size: 500 ml

Ingredients	final concentration	amount
RM027 chelex 100	5. %	25 ± 2 g
S059 sterile water	---	450 ± 50 ml (guideline)

Procedure

Filter sterilize approximately 600 ml distilled water.

Pour the water into a 500 ml bottle.

Save the bottom container from the disposable filter unit.

Autoclave the water at 250°F for 30 minutes.

Add the chelex to the bottom container of the filter unit.

Allow the water to cool after autoclaving.

Add sterile water to the chelex to a volume of 500 ml using the graduation markings on the disposable filter container.

Mix on a magnetic stir plate.

While the stock solution is mixing, aliquot 10 ml each into 15 ml centrifuge tubes.

Store at 2-8°C.

Data Log	source	lot	amount
RM027 chelex 100	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC001 DQα extraction

made by: _____ date: _____

Initials: RCS Date: 4/14/92

S042 Phi-X Marker lot number: _____

Ingredients	initial concentration (ng/µl)	initial volume (µl)	final concentration	final volume (µl)
RM156 phi-X-174, Hae III fragments			50 ng/µl	
S018 analytical gel loading buffer	5 X		1 X	----
S059 sterile water	-----		----	----

Calculations

Record the initial concentration in ng/µl and the initial volume in µl of the phi-X-174 Hae III received from the manufacturer.

Calculate the final volume according to equation 1.

$$\text{(final volume)} = \frac{\text{(initial DNA concentration)(initial DNA volume)}}{\text{(50 ng/µl)}} \quad \text{equation 1}$$

Record the final volume above. The final volume is the total batch size.

Calculate the amount of buffer to be added according to equation 2.

$$\text{(buffer volume)} = 0.2\text{(final volume)} \quad \text{equation 2}$$

Calculate the amount of sterile water to be added according to equation 3.

$$\text{(water volume)} = 0.8\text{(final volume)} - \text{(initial DNA volume)} \quad \text{equation 3}$$

Record the buffer and water volumes above.

To check the calculations, add together the initial volumes of DNA, loading buffer, and sterile water.

The sum of the initial volumes must be equal to the calculated final volume.

Initials: RCJ Date: 4/14/92

S042 Phi-X Marker lot number: _____

Procedure

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 500 µl aliquots into sterile 1.5 ml eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM156 phi-X-174 Hae III fragments	_____	_____	_____
S018 analytical gel loading buffer	_____	_____	_____
S059 sterile water	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

S059 Sterile Water lot number: _____

standard batch size: 500 ml

Procedure

Filter sterilize 500 ml of distilled water.

Pour into sterile, 125 ml bottles.

Autoclave at 250°F for 30 minutes.

Store at room temperature.

made by: _____ date: _____

Initials: RCS Date: 7/17/92

S059 STERILE WATER lot number: _____

standard batch size: 500 ml

PROCEDURE

Filter sterilize 500 ml of distilled water.

Aliquot 10 ml each into 15 ml centrifuge tubes.

Autoclave at 250°F for 30 minutes.

Store at room temperature.

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

S079 Hydrogen Peroxide, 3% lot number: _____

standard batch size: 80 X 0.5 ml

Ingredients	final concentration	amount
RM284 hydrogen peroxide, 3%	3 %	0.5 ml (guideline)

Procedure

Aliquot approximately 0.5 ml of hydrogen peroxide into 1.5 ml microcentrifuge tubes.

Label each tube with "H_2O_2" and the lot number.

Store at 4°C in the dark.

Data Log	source	lot	amount
RM284 hydrogen peroxide, 3%	_____	_____	_____

Quality Control

QC003 DQα hybridization

made by: _____ date: _____

Initials: RG Date: 3/17/93

S080 Sodium Acetate, 1M lot number: _____

standard batch size: 100 mL DELETE

Ingredients	final concentration	amount
RM059 sodium acetate, anhydrous	1.0 M	8.2 ± 0.4 g
RM093 acetic acid, glacial	-----	-----

Procedure

Add the sodium acetate to approximately 75 ml distilled water.

Mix well.

Adjust the pH to 5.2 with glacial acetic acid.

Bring up to volume with distilled water.

Measure and record the final pH.

Dispense into a 100 ml bottle.

Autoclave at 250°F for 30 minutes.

Store at room temperature.

Data Log	source	lot	amount
RM059 sodium acetate, anhydrous	_____	_____	_____
RM093 acetic acid, glacial	_____	_____	_____

made by: _____ date: _____

Initials: RD Date: 4/14/92

S080 Sodium Acetate, 1M lot number: _____

standard batch size: 100 mL

Ingredients	final concentration	amount
RM059 sodium acetate, anhydrous	1.0 M	8.2 ± 0.4 g
RM093 acetic acid, glacial	-----	-----

Procedure

Add the sodium acetate to approximately 75 ml distilled water.

Mix well.

Adjust the pH to 5.2 with glacial acetic acid.

Bring up to volume with distilled water.

Measure and record the final pH.

Dispense into a 100 ml bottle.

Autoclave at 250°F for 30 minutes.

Store at room temperature.

Data Log	source	lot	amount
RM059 sodium acetate, anhydrous	_____	_____	_____
RM093 acetic acid, glacial	_____	_____	_____

made by: _____ date: _____

Initials: RC Date: 3/17/93

S081 DTT, 1M lot number: _____

standard batch size: 5 ml DELETE

Ingredients	final concentration	amount
RM101 dithiothreitol	1.0 M	0.77 ± 0.04 g
S080 sodium acetate, 1M	10. mM	50 ± 3 µl
S059 sterile water	-----	-----

Procedure

Add the DTT to approximately 4 ml sterile, distilled water in a 15 ml centrifuge tube.

Mix well.

When the DTT is dissolved, add the sodium acetate solution, and bring up to volume with sterile, distilled water.

Filter sterilize.

Dispense 500 µl aliquots into sterile 1.5 ml eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM101 dithiothreitol	_____	_____	_____
S080 sodium acetate, 1M	_____	_____	_____
S059 sterile water	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

S081 DTT, 1M lot number: _____

standard batch size: 5 ml

Ingredients	final concentration	amount
RM101 dithiothreitol	1.0 M	0.77 ± 0.04 g
S080 sodium acetate, 1M	10. mM	50 ± 3 µl
S059 sterile water	-----	-----

Procedure

Add the DTT to approximately 4 ml sterile, distilled water in a 15 ml centrifuge tube.

Mix well.

When the DTT is dissolved, add the sodium acetate solution, and bring up to volume with sterile, distilled water.

Filter sterilize.

Dispense 500 µl aliquots into sterile 1.5 ml eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
RM101 dithiothreitol	_____	_____	_____
S080 sodium acetate, 1M	_____	_____	_____
S059 sterile water	_____	_____	_____

made by: _____ date: _____

Initials: RC Date: 3/17/93

S034 Phosphate Buffered Saline (PBS) lot number: _____

standard batch size: 4 L

Ingredients	final concentration	amount
RM005 sodium chloride	137 mM	32.0 ± 0.1 g
RM053 potassium chloride	3.0 mM	0.90 ± 0.01 g
RM065 sodium phosphate, dibasic	6.0 mM	3.41 ± 0.03 g
RM056 potassium phosphate, monobasic	1.5 mM	0.82 ± 0.02 g

Procedure

Add all the components to approximately 3 L distilled water.

Mix well.

Adjust the pH to 7.5.

Bring up to the final volume with distilled water.

Measure and record the final pH.

Dispense into 50 ml centrifuge tubes.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
RM005 sodium chloride	_____	_____	_____
RM053 potassium chloride	_____	_____	_____
RM065 sodium phosphate, dibasic	_____	_____	_____
RM056 potassium phosphate, monobasic	_____	_____	_____

Quality Control

final pH: _____ spec: 7.5 ± 0.1

QA004 DQα differential extraction

made by: _____ date: _____

Initials: RC Date: 3/17/93

S082 Chelex, 20% lot number: _____

standard batch size: 500 ml

Ingredients	final concentration	amount
RM027 chelex 100	20. %	100 ± 2 g
S059 sterile water	---	450 ± 50 ml (guideline)

Procedure

Filter sterilize approximately 600 ml distilled water.

Pour the water into a 500 ml bottle.

Save the bottom container from the disposable filter unit.

Autoclave the water at 250°F for 30 minutes.

Add the chelex to the bottom container of the filter unit.

Allow the water to cool after autoclaving.

Add sterile water to the chelex to a volume of 500 ml using the graduation markings on the disposable filter container.

Mix on a magnetic stir plate.

While the stock solution is mixing, aliquot 10 ml each into 15 ml centrifuge tubes.

Store at 2-8°C.

Data Log	source	lot	amount
RM027 chelex 100	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC004 DQα differential extraction

made by: _____ date: _____

Initials: RG Date: 3/17/93

S093 DTT, 1M lot number: _____

standard batch size: 20 ml

Ingredients	final concentration	amount
RM101 dithiothreitol	1.0 M	3.1 ± 0.2 g
S059 sterile water	-----	-----

Procedure

Add the DTT to approximately 15 ml sterile, distilled water in a 50 ml centrifuge tube.

Mix well.

When the DTT is dissolved, bring up to volume with sterile, distilled water.

Filter sterilize.

Dispense 250 µl aliquots into sterile 0.5 ml eppendorf tubes.

Store at -20°C.

ta Log	source	lot	amount
RM101 dithiothreitol	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QA004 DQα differential extraction

made by: _____ date: _____

Initials: CY Date: 3/17/93

S094 Digest Buffer lot number: _____

Standard batch size: 6 L

Ingredients	final concentration	amount
S009 EDTA, 0.5M	10. mM	120 ± 6 ml
S036 TRIS-HCl, 1M-pH 7.4	10. mM	60 ± 3 ml
S012 sodium chloride, 5M	50. mM	60 ± 1 ml
S001 SDS, 20%	2.0 %	600 ± 15 ml
RM096 hydrochloric acid	---	---

Procedure

Add the EDTA, TRIS, sodium chloride, and SDS to approximately 4 L distilled water.

Adjust the pH to 7.5.

Bring up to the final volume with distilled water.

Mix well.

Measure and record the final pH.

Aliquot into 50 ml centrifuge tubes.

Store at room temperature.

Data Log	source	lot	amount
S009 EDTA, 0.5M	_____	_____	_____
S036 TRIS-HCl, 1M-pH 7.4	_____	_____	_____
S012 sodium chloride 5M	_____	_____	_____
S001 SDS, 20%	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

Quality Control

final pH: _____ specification: 7.5 ± 0.1

S004 differential extraction

Initials: KO Date: 4/14/92

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