SO Too BH Department of Forensic

Quality Assurance Man

Version 1.0

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Department of Forensic Biology

Quality Assurance Manual

Version 1.0

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V.

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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:

Date: 3/3	Jocelyn Ferrara	Dora Wolosin	Committee Members: Marie Samples	FBH QA Coordinator: Larry Quarino	FMB QA Coordinator: Robert Wilson	Co-Chairman: Howard Baum, Ph.D.	Chairman: Robert C. Shaler, Ph.D.	
3/3//82	ara localification focusion	ARV	marie Samples	o Lang Quarus	n	James Bang	. Pobut P. Male	



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

B. Quality Assurance

and insure Quality sufficient measures of variation. that assurance quality pertains control parameters 6 those procedures are appropriate

C. An Example

the proper reagents. quality control mose 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 the variation. quality control to insure that Measuring and recording the pH of solutions is maintained within a specified range. But is parameter is a meaningful measure of quality only if pH meter has been measures calibrated, the are the variation between lots meaningful a solution is a common technician making measures

III. Planning and Organization

A. Goals

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

Initials: Date:

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

- Sample identification
- Species identification
- Genetic marker analysis
- Report
- 54.02. Testimony Preparation ony to result results

ä QA Objectives

- • standards, proficiency tests, procedures laboratory on a routine basis, the analytical testing serior all scientific testing performed in ratory by means of Quality Control (QC) and audits.
- **№** yudity and validity of +1 maintained. ce criteria the analvt analyses analytical operate and that data within the S,
- ω scientific Laboratory Methods Manuals Performance procedures performed in the laboratory. criteria are for each of established the routine uT
- 4. commercially and by the program for instrument use prepared quality and validity of the data is ensured by quality control (QC) program for both reagents ij. control (QC) in the la laboratory the quality use and maintenance assurance and obtained logs.
- ហ integral part of the overall Department of Forensic Biology. by the proficiency testing program that integral part of the overall QA program ensured by the position requirements of Department of Personnel of the City of New York qualifications position 0f the laboratory that is ogram of staff and the the are
- 9 acceptability. maintained (specification sheets, etc.) records as are the for in-house Outside ည် reagent documentation of vendor are retained. manufacture 00 of their documents

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

ဂ္ပ Authority and Accountability for the QA Program

department. relationships in the between individuals relationships organizational the structure and Department the operational (Figure 0f Forensic 1.) units defines Biology of the the

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

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

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

QA/QC QA/QC program guidelines, as they and responsibilities, are adhered Each Forensic Analyst 18 responsible to they to. relate ensure to their work that

9 OCME Structure and Department of Forensic Biology Organizational

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

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

analysts. laboratory forensic scientific scientists has staff 2 forensic and in 9 the forensic scientists FMB laboratory analysts. and σ includes forensic The

scientists assume this responsibility. Under no circumstances will all FBH and FMB forensic scientists be director absent operations. scientists this absent, operational units. director is directors are The laboratory responsibility office associate level, and one reconstructions of the department defines responsibility. operational from the the and assume dual subunits. FMB responsible both assistant In their absence, FMB laboratory responsible for the laboratory. laboratory unit In case the FBH assistant director is this In the absence of the departmenta istant directors, the FBH forensial responsibility for laborator D. The for case responsibility. allocated assistant FBH laboratory as coordination laboratory assistant operation director laboratory ry forensic personnel: assistant of artmental forensic helper. between assumes their

IV. Documentation

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

Manuals

Scientific Manuals

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

- the procedure Was adopted
- 0.0 p.a Date the procedure was Revision dates authorized
- Signatures 0f authorized staff + dates

Forensic Assistant Department Scientists Directors Director

0 Archives of methods

N Administrative Manual (Appendix A)

- 9 Chain of Custody
- Post-Mortem Evidence
- 21. Physical Evidence

ğ Quality Control/Critical Reagent Documents

available following. and The according to guidelines established within the department document according to accepted procedures. lable for each testing procedu 200 documents that a11 ij critical the departmental procedure reagents The are include laboratorie documents prepared

1. 200 Procedures Manuals

purchased quality Detail the of from outside vendors. procedures used ļ'n in-house determining 20 those the

2 Reagent Preparation Manual

of the departmental Details critical solutions solutions laboratories. to be used in sused in routine the preparati testing n g

ω Reagent Preparation Records

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

0 Case Files/Case Notes

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

The supervisors. case file also contains dates of review þу

D. Data Analysis and Reporting

Each inde initialed. assigned the case and another laboratory staff scientist. Each independently observed result is dated and initialed. Departmental guidelines re independently require that all case data

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

methods criteria identifications manual. have been established are made, and specific are part matching 0f the

Known standards are established criteria recorded and are part and 0f monitored the methods Уd means manual.

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

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

- ا numbers. Medical Examiner and Forensic Biology Cas
- Deceased/Victim name (if known).
 Police Precinct and Complaint Num
- Police Precinct applicable). and Complaint Numbers (if

The S. S review follows: process (in chronological order) for reports

- 1 guidelines Molecular Biochemistry preparation the assigned forensic analyst guidelines established for hand Biology and written report ij Laboratories. Hematology either according uniform the S P 9 prepared Forensic Forensic to report the
- N Review of forensic of scientist. the draft report þу Ø supervising
- ω Typing staff. of the report уd departmental clerical

Second the sup supervising forensic scie review of the scientist after typing þу

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

- **5**1 departmental director. director Final review of of the the laboratory report Уď the 20 assistant γd
- 9 obtained at Statistical information to obtain departmental and individual productivity data will be individual productivity ned at this time.
- 7. records sent to retained in the case file. The original of the report the department, a the submitting port is sent to certified True agency, and a True the OCME сору Copy 15 S

E. Evidence Handling Protocols

Chain-of-Custody (see also Appendix A)

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

a. Evidence Receipt:

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

b. Case Number:

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

c. Item Numbers

number. associated with item 1s assigned a police Ø control or number that voucher

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

d. Signatures

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

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

e. Storage

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

f. Case Assignment

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

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

Initials: //c

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

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

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

၂ Equipment Calibration and Maintenance Logs

and/or QA/QC use usage book. Each piece of essential scientific in the procedural or methods manual Essential scientific apparatus has a log records available. and has usage

Also, they dry and have to be chiseled off. outside a piece of equipment, it is to be IMMEDIATELY (this includes hybridization buffers, salts, and etc.). Some spills may be cleanliness. the first it equipment and cause more damage than necessarve t is much easier to clean some y and have to step If there is any kind of for all preventative maintenance spill, inside to be cleaned ďn 20

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. The usage log for each item begins with the of

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

0f Any items of equipment the irregularities log and reported are recorded in observed during ç Ø supervising the comments routine use forensic section 0f

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

The schedule of equipment maintenance follows:

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

log the done by the technician responsible for preparation of reagents OR AS ASSIGNED BY THE COORDINATOR OR LABORATORY FORENSIC SCIENTISTS. log specified for each piece of equipment. Temperatures temperature is dated and initialed by the person performing are recording. recorded daily on a temperature BY THE QA This The the 18

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

2. pH meters

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

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

Electrophoresis Equipment

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

comments. log Each electrophoresis sheet as to date, tank usage initials, is documented purpose, no on a and

4. Balances

calibration date every Balances σ are months checked by is 1 recorded an weekly outside in the and service. are usage calibrated log.

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

5. Thermocyclers

comments. documented thermocycler as to date, has Ø initials, usage log purpose, which and n's

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

6. Microtiter Plate Readers

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

analyst assigned to perform Checking record of the ß. performed monthly calibration is the calibration. The recorded in the usage bу þ technician or

7. Micropipettes

conducted. times handle performing the calibration will be reco calibration will be calibration Micropipettes will be checked using either standard annually. indicating ed. Each assigned the calibration will be recorded in the kits or gravimetrically. and or gravimetrically. Each pipet a control number and the date of the the pipet last initials will time be ρ calibrated 0f calibration the on

8. Centrifuges

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

9. Freezers

freezers that free Freezers must be defrosted annually. freezer. D. recorded on the temperature Defrosting of Log for

10. Hoods

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

11. Survey Meters

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

12. Liquid Scintillation Counter

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

G. Proficiency Testing

biological specimens, submitted to the laboratory as open and/or blind case evidence. 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 Proficiency and testing is used reporting of to periodically demonstrate results from appropriate

analytical protocols being proficiency test is taken. evidence analyzed will specimens analyzed will be treated submitted as being interpreted part used A11 of as samples according to മ normal proficiency test casework. of "blind" to the

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

1. Deficiency and Corrective Action

remedial action is documented. designated is the acknowledged responsibility of the QA individual, to assure the constant of assure that deficiencies Coordinator, corrective

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.

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

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

completed. casework, proficiency Before the an person tests additional Js. must permitted bе successfully to of resume

b. Systematic Error

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 notified in writing of the appropria corrective error found to be the result of equipment, action 'n order to minimize appropriate

recurrence of the discrepancy.

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

c. Administrative Error

the 6 proper storage of biological specimens. example, error, problem. etc., once identified corrected by instructing switching, Administrative or, the analyst may be retraining in the remple, if the error is analyst will be retra Improper Depending on errors, be retrained concerning storage, i.e., be relevant 20 the the in required to clerical, sample such, will analyst of nature documentation, area. storage, of submit sample the the the For

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

laboratory, the matter departmental director. between In the the event designated QA 0f an unresolved will be resolved by the individual disagreement and the

Open Proficiency Testing

perform casework. interpretive are the essential performance laboratory's laboratory used proficiency the requirement to of the la capability of demonstrate the c analytical methods staff test laboratory is ent prior to be as proficiency specimens the analyst/scientist. capability ds as well which being judged are specimens presented the assigned and of quality 20 the and the

a. Personnel

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

b. Specimens

Each dried can be drawn from the results of the analysis. physiological fluids, either singly or as a mixture. Each sample to be analyzed will contain sufficient sample so that a conclusion open specimens proficiency 0f test blood may and/or consist other of

c. Sample Preparation and Storage

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

material. A11 cotton test specimens will be prepared cloth, swabs, ೧೭ other on washed suitable

Each specimen identifier th verified by a ensure proper that at assignment. must least should be labeled one 90 other with independently er person to þ unique

A por test laboratory for possible referee anacomparison if circumstances dictate. portion of each specimen used to prepare should эd retained γd the he preparing analysis and

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

Case Retesting

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

The the results results results do of the not of the correlate, reanalysis will be original 'n analysis. other compared words, H with the the

analytical or sample mix-up. determine sample will be performed. analysis will be compare results do not where agree, compared with the 1 the D error third **Was** analysis first two made, the third of the

* Blind Proficiency Testing

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

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

5 Documentation of Open Proficiency Testing Results

Each pr follows: proficiency test will be documented as

- Proficiency Testing Identification Number
- 0 6 Name of analyst
- Dates:

receipt by anal completion date by analyst

- Q. photographs and reports
 All data "";" Copies 0f all sheets, notes,
- 0 numbers, and etc. All data will conformations and include conform lot to numbers, casework ည်

analyst. proficiency testing file will be maintained maintained by forensic results biology of the department for all department all laboratories. proficiency tests separate will on each 0f

H. Personnel Training and Qualification Records

Courses t information scientific universities on-the-job Personnel taken at universities and colleges, worksnops n and staff, training training where seminars experience scientists where falls and theoretical are z: lectures held re invited to obtained and from workshops practical at speak local the

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training meetings. Biology. various topics, requirements Each of these will be discussed in relation to scientific in the literature, Department and professional 0f Forensic

Records o staff is each member of the Ω part 0f training of each member of the scientific staff. of the proficiency file the scientific maintained for maintained

1. Courses at Universities

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

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

know what catalogues will is available and will эd be made obtained so aware can 0f enroll that the staff members courses, should they

2. Workshops

mandatory. charge for these courses. The staff will be made aware of these workshops, but because reimbursement cannot Workshops companies IBI on sequencing, etc., usually as an aid marketing functions. Normally there is ge for these courses. The staff эd are guaranteed, routinely offered in attendance the will local not to on

recommendations attending Scientists Justice, Un through The local universities specializing in forensic science training, i.e., John Jay College of Criminal Justice, University of New Haven, as well as Workshops cannot are for Northeastern Association the be also will മ reasonable reimbursed workshops, be made to offered 'n cost. for attend those conjunction very the Although the cost of Forensic strong which the 0f

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are department deemed important to the function of the

3. On-The-Job-Training

conducted theory the-job training in the and variety. This tra , , the department. department will training of the will be of the work emphasize which on-

a. Theoretical

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

Specific met publications pertaining to in-house m will be available in a laboratory file. literature scientific and/or video This training will be reduced training will be reduced to the format. scientific methods references maintained literature used will and presented Уď Each member of the have əd reference the depar and referenced in department. access copies methods lecture books of tο to

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

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

b. Practical

will proficiency Each procedure. in any particular analytical test, the analyst laboratories. be analyst will asked conducted testing to At successfully complete the conclusion of training Эd ij trained 9 the that to departmental perform analytical open the

4. Seminars and Lectures

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

5. Scientific Literature

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

libraries. articles for laboratory staff from local scientific available to OCME librarian will library has staff members. obtain forensic sic journals w Additionally, copies 0f y, the OCME scientific which OCME

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

6. Professional Meetings

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

forensic scientists (NEAFS) and the to scientific staff The annual national (NEAFS) convention regional are highly association 0f recommended forensic of

Other scientific meetings of interest to the

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forensic conferences. Int. Assn. Forensic Scientists, NY Acad. Sci., Meetings, department, etc., Gene Probe Society Conference, of Human conference, Genetics

Method Validation Records

providing reliable results on the specimen types commonly analyzed in the departmental laboratories. demonstrate validated Methods used using that in the departmental accepted the methods validation procedures used laboratories are capable must that 0f

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

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

appropriate pages in the contain xerox copies of the 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 attack. log For data maintained in staff notebooks, the books referred to research research notebooks. above will notebooks reference will the

1. Validation Procedures

a. Existing Procedures

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

- (1). Those which exist and have been in peer review journals but validated for forensic testing. published not yet
- (2). which Those no validation records which are not published are known. and

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

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

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

require limited testing, The procedure procedures will be conducted to validation, extensive behaves in category I.1 validation. cted to insure as published. including I.1.a.3. proficiency that However,

ρ, New Procedures

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

- include: extensive new procedures validation must process go which through must an
- (1). appropriate expressing on all Staff review experiments including
- (2). (3). appropriate sample types
- effects Examination of environmental and aging
- experimental protoc Proficiency testing Variability protocol results drift due to
- (5) (6) (7) Collaborative testing
- applicable Publish in peer review journal, ĺf

J. Quality Assurance and Audit Records

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

× Equipment

1. Inventory

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

- a manufacturer
- Ď, model
- G serial number
- Q. agency inventory number (if applicable)
- H 0 purchase date (if replacement date
- ? available) (if available)

2 Operations Manuals

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

ω Calibration/Maintenance Procedures

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

4 Calibration/Maintenance Logs

equipment. usage Calibration Log books and Maintenance logs which are kept are next Ø part tο of the the

F. Safety Manuals

departmental staff. manual. safety Department manual This 18 maintains manual maintained z: വ available separate as an agency safety noqu (OCME) manual. request safety The to

Initials: RCS Date: 3/3//92

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

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

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

The Radiation Safety Committee meets quarterly

M. Material Safety Data Sheets (MSDS)

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

committee These are readily available representative. from the departmental safety

N. Historical or Archival Records

Records for all laboratory operations are maintained.

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

O. Audits

function of the Department. Sheets fout by the evaluator and and anti-Director Department for evaluation by 1s audited the Sheets for audits are filled submitted to the Denartmant annually QA Committee. Λq an independent

Figure 1: Forensic Biology Organizational Structure

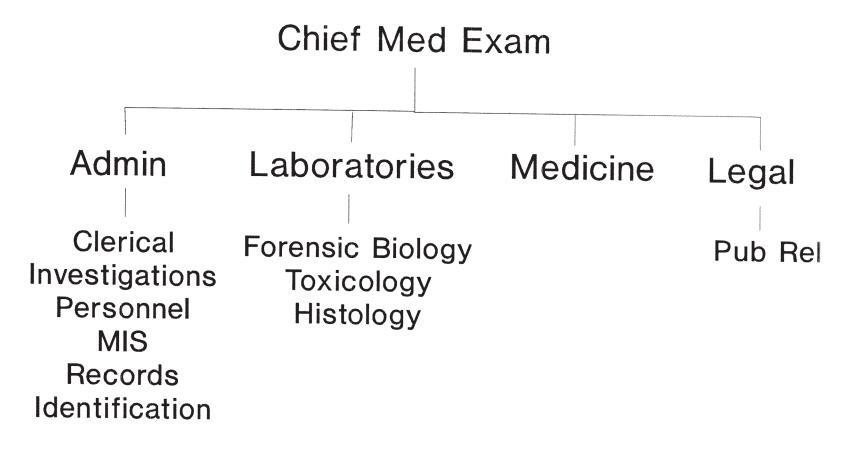
Director

Clerical

Forensic
Biochemistry
&
Hematology
Laboratory

Forenisc Molecular Biology Laboratory

Figure 2: Office Chief Medical Examiner Organizational Structure



Appendix A

Department of Forensic Biology

Administrative Manual

Initials: 20 Date: 3/18/82

Administrative Manual

Chain-of Custody Procedures

A. Post-mortem Evidence

1. Homicide Cases

census, autops investigatory determined followed. autopsy worksheets or γď clearly evidence, the Medical labeled s or by having NYPD submit the following procedures Examiner, homicides, daily case

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

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

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

appear likely to be a homicide or may require NYPD investigation e.g., sexual assaults, are handled as above. n which the cause of death 1s unclear and

The the ME Biology future determination as to whether g processing 18 investigating detectives ascertained through Уď the Department conversations Ω case may who handled of y require Forensic with

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

Initials: 20 Date: 3/18/84

Non-homicides (database samples)

blood, specimens used as database samples receive an MB number. is isolated from these latter samples. 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 transferred to the Forensic Molecular Biology (DNA) specimens These cases do not receive are not mission cases. Forensic If available, from all Medical Exami Most of these do not fall within of the Department of Forensic Biology the subject of an homicide investigati are discarded after two months. and Biology associated department FB case numbers. autopsy workshee worksheets investigation. receives Examiner These These EDTA

ω Cases For Which Specimens Are Not Received

Histology laboratory. freshly preserved specimens are not availal formalin fixed specimens may be obtained from holding time-frame). department's these Sometimes which database specimens instances, appropriate specimens are obtained the Toxicology Department (if within that tment's specimen holding time-frame) or from autopsy specimens physical evidence In unusual instances, and if ecimens are not available, (if within that laboratory's are not received. 1s received cases the

Specimens Received Without Identifying Numbers

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

B. Physical Evidence

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

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

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Appendix B

Department of Forensic Biology

Forensic Biochemistry & Hematology Laboratory

Quality Control Manual

Initials: RO Date: 3/18/84

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																	III.		II.	H.	
14. Takayama Crystal Test for Blood 17 15. When Get Tilly of the triple of triple o	13. Species Determination 15	12. PGM Subtype by IEF	11. P-30 Detection	10. Kastle-Meyer Presumptive Test 13	9. Lewis Typing 12	8. Hemoglobin by IEF	7. Group Specific Component Conventional Typing 10	6. Esterase D/Phosphoglucomutase Conventional Electrophoresis9	5. Esterase D by IEF 8	4. Acid Phosphatase by IEF 7	3. Acid Phosphatase Spot Test 6	2. Alpha-Amylase Diffusion 5	1. Absorption Inhibition 4	C. Procedures 4	B. Guidelines for Quality Control Testing 3	A. Manufactured Materials 1	Methods 1	A. Maintenance of Equipment	Equipment 1	Purpose 1	page

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I. Purpose

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

II. Equipment

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

A. Maintenance of Equipment

<u>ب</u> documenting all maintenance performe of equipment used in the laboratory. An equipment maintenance log must be performed on any kept item

III. Methods

procedure for casework samples. control given Quality control parameters evaluation and validation method must e for that method. If th specifications, they may be tested concurrently studies. are If the defined then be urrently in a quality control tested materials meet quality A11 for materials used in the each method employed method h L 92

A. Manufactured Materials

- conditions. Each time a material is batched, corresponding batch sheet is to be filled out. following information is to be recorded on the sheet: Materials laboratory so as to be able electrode batches. such as buffers, solutions which These are to be stored at appropriate a material is batched, a set is to be filled out. The are to IEF plates, and re prepared by perform multiple tests and IEF the batch The
- Batch number (subsequent batches are listed numerically)
- b. Initials of the preparer
- c. Date of preparation
- d. Amount prepared
- 0 Vendor used to and prepare lot the number batched 0f material. each reagent

B

Initials: AG Date: 11/12/8

- \sim Batch sheets are and stored in an following are to to be kept in respective accessible file cabinet. be batched and given batch files numbers: The
- 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
- **U**-Ethanolamine cathode electrolyte solution
- k. Gc gel buffer
- 1. 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

Initials: Acl Date: 11/12/93

- t. Species agarose gels
- u. Species tank buffer
- v. Takayama crystal test reagent

Initials: 120 Date: 3/18/92

- \sim Batch sheets are to be kept in temperations and stored in an accessible file cabinet. following are to be batched and given batch numbers files The
- 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
- Ų. Ethanolamine cathode electrolyte solution
- k. Gc gel buffer
- 1. Gc tank buffer
- m. Hemoglobin IEF polyacrylamide plates
- n. PGM reaction buffer
- o. PGM reaction mixture
- ò 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
- With the exception of the HHF cathode and anode

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Initials: /20 Date: 3/17/8/

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

8 Guidelines for Quality Control Testing

- <u>|--</u> Quality control control be performed by any member of the lunch has satisfied laboratory proficiency proficiency method. particular method the laboratory who requirements can
- ₽. on worksheets and stored in Each quality control sheet of information: control number along with the following control testing results n respective files. are to be recorded
- a. Initials of analyst who performs test
- b. Date of testing
- 0 Batch numbers for all batched materials
- d. Vendors and lot numbers for all nonbatched reagents
- e. Results of tests

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

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

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

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

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

 \square

 ω

Initials: Ry Date: 3/8/92

5 problem casework samples. However, in not conform to quality control materials can be used in the tested method second control materials. test batch(es) control rejection test does not prod can be performed using If results from the y control used in the te 20 reagent(s) produce acceptable If an initial quality parameters the second must second test the be discarded same group the tested results, then test g does the Ω

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

C. Procedures

F. Absorption Inhibition Quality Control Test

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

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

Procedure

- Titer a lectin anti-A nnti-A antiseum,
 (see procedures manual). anti-B antiserum, and anti
- 2 to Record ord titer results on agglutination scale. quality control worksheet according
- ω secretor, technique scale Using the for titer O secretor, (as each h antisera, perform absorption specified in methods manual) that gives the last +4 on and non-secretor dried absorption inhibition the on A secretor, saliva agglutination stains W
- 4 Record results g quality control sheet

Initials: NO Date: 3 (4)

Quality Control Parameters

Acceptable results are as follows:*

non-secretor-neat -1/10	O secretor-neat -1/10	B secretor-neat -1/10	A secretor-neat -1/10	
3 - 4 3 - 4	3-4	3-4	CI CI-2	A
3 - 4	3-4	CI CI-2	3-4	В
3-4 3-4	CI CI-2	CI-4 CI-4	CI-4 CI-4	0

CI = complete inhibition

* II Numbers reflect values on agglutination scale

repeated using properly adjusted and ole range, the partisera titers. procedure must be

N Alpha-Amylase Gel Diffusion Quality Control Tes

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

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

Procedure

- |---Record the amylase gel buffer batch number, control numbers sheet. for agarose, starch, and amylase standards and vendor on and lot quality
- 2 Prepare a set of alpha-amylase standamylase activity/8ul, 2 units/8ul, 0 units/8ul, 0.002 units/8ul, 0.0002 units/8ul in saline, respectively. comparable ten-fold dilution series standards ndards containing 20 un 0.2 units/8ul, 0.02 units/8ul, and 0.00002 Alternatively, any can эd used. units
- ω stain 1/10 d Extract a 5x5mm n in saline.
 dilution in (approximate) saline. From an aliquot of section this of pooled human saliva
 extract, prepare a

Initials: RS Date: 3/17/97

- 4 Test stain extracts, and diffusion procedure 8ul of each standard, the neat and 1/10 diluted tracts, and a saline blank as per amylase gel n procedure specified in methods manual.
- \mathcal{S} the clear diffusion (expressed logarithmically Prepare Ø circles around standard gel (plotted on y axis). standard thmically on x axis) versus the diamete around standard wells in the developed curve of the units of amylase activity diameter Of
- 9 saliva the dia Determine diameter stain amylase activity ain extract from the of the clear of the sta of the neat the standard around curve d both and 1/10 diluted sample after measuring wells

Quality Control Parameters

- logarithmically. The diameter of the clear to be linear with respect circles to the a amylase around standard activity wells need expressed
- 2 The diameter of the clear needs to fall between the linear portion of the the lowest standard cu curve around both and highest sample points wells on the
- ω saliva stallowable calculated amylase stain extract s ble error of 5%. se activity of the r should differ by a neat neat and 1/10 factor of 10 given diluted an

3 Acid Phosphatase Quality Control Test

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

needs previously to be checked evaluated acid phosphatase yearly. spot tes (reagent

Procedure

- reagent Record vendor and lot number of on quality control worksheet acid phosphatas Õ spo $\tilde{\tau}$ tes
- 2 Prepare 1/10, 1/100 1/1000000 dilutions 1/100, of 1/1000, pooled human semen 1/10000, 1/100000, using and saline
- ω Test one dilution methods manual. drop of the as per acid pooled human semen (neat)
 phosphatase spot test spec phosphatase spot eat) and each specified in
- 4. est a 20ul procedures manual dried pooled semen stain according to the

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W

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Initials: 24 Date: 3/14/94

- 4 Test 8ul of each standard, the neat and 1/10 diluistain extracts, and a saline blank as per amylase diffusion procedure specified in methods manual. and 1/10 diluted gel saliva
- **5**1 (expressed logarithmically on x axis) verthe clear circles around standard wells diffusion gel (plotted on y axis). Prepare മ gel (plotted on y axis). standard curve of the units versus the delay of amylase developed activity diameter of
- 9 Determine amylase activity of saliva stain extract from the the diameter of the clear circ the standard circle around the neat around curve after measuri both sample wells. and 1/10 diluted measuring

Quality Control Parameters

- to The logarithmically. be linear diameter of the clear with respect of the circles around to the amylase amylase standard activity wells expressed needs
- 2 The diameter of the clear needs to fall between the linear portion of the star standard circle lowest curve around both and highest sample points g wells the
- ω saliva stain extract sallowable error of 5%. calculated amylase se activity of should differ bу the മ neat factor and 1/10 0f 10 diluted given an

Acid Phosphatase Quality Control Test

needs Commercially to Эď evaluated prepared acid phosphatase spot tes before use in case work. rt reagent

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

Procedure

- -Record vendor and lot number of acid reagent on quality control worksheet. acid phosphatase spot tes
- 2 Prepare 1/10, 1/100, 1/1000000 dilutions of pooled pooled 1/10000, human semen using 1/100000, and saline.
- ω Test one drop of the dilution as per acid methods manual. per pooled human semen (neat)
 phosphatase spot test spe spot eat) and each specified in
- 4 Test manual Ø 20ul dried pooled semen stain as per the procedures

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Initials: PS Date: 11/12/83

2A. Amylase₁ and Amylase₂ Dif Inhibition Quality Control Test Differentiation bу Differential

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

agarose, imilarly, starch, each lot and 0f iodine must commercially be evaluated prepared before wheat use lectin (WL)

Procedure

- Pour amylase diffusion gel as per methods manual (pp.12-13).
- 2. Serially dilute 1/2, 1/4, 1/8, and 1/16 crude extract, crude salivary extract, and commercially bacterial amylase with distilled water, KBE, and WL. pancreatic prepared
- 3. the diffusion Place 10ul of gel. each diluted extract from step \sim מַן punched wells
- 4 temperature Place gel in moisture chamber and incubate overnight Ω 4 room
- 5 ш Lood gel with Lugol's Iodine to develop plat
- φ Determine diffusion area $(A=\pi r^2)$ of each extract

Parameters

ra tio Using data for each from the dilution by using salivary extract, calcul by using the following calculate the lowing formula: inhibition

Area WL Dilution/Area Water Dilution Area KBE Dilution/Area Water Dilution

Values greater than \sim for a11 4 dilutions must be obtained

- than to calculate the n 1 for all 4 Using data from the rom the pancreatic inhibition ratio idilutions must be for each dilution. obtained. use the above Values formula less
- KBE negligible inhibition and Diluted WI samples of bacterial amylase (inhibition ratio to app approximate should show no or 1) with both

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202-324-4363

Initials: AC Date:

Quality Control Parameters

- 1a. Positive and in the results must be seen in e 1/10 and 1/100serially pooled s diluted semen fractions
- 9 Compare over QC sample and in the 1/10 and 1/100 serially diluted fract Sensitivity of the spot test must be greater than that the P-30 detection Crossed-Over electrophoresis method. for last dilution with the sensitivity the current lot number. of the P30 Crossed-
- 2 semen stain. positive result must be obtained on the 20ul dried pooled

Acid Phosphatase bу IEF Quality Control

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

Each lot of methylumbelliferyl phosphate must be tested before use

Procedure

- methumbelliferyl cathode solution quality control Record be batch solution, and ACP reaction control sheet. The vendor recorded. numbers sheet. The vendor phosphate used in for ACP IEF plates, and ACP reaction buffer.
 The vendor and lot the quality fer to be tested on lot number of the anode control solution test
- 2 ACP ACP Bloodstains þу BA, ΙEF respectively) are to IEF method specified containing known ACP to in methods ACP types (ACP A, be extracted and manual tested as and per
- р . tested in containing Sample test ACP A and volume of 10ul ACP B S L Both extracts to be used for the are to extracts
- **b** The triplicate 2.5ul). ed in duplicate.
 extract containing ACP with varying volume BA is size to be (10ul, tested 5ul, in and

Qua Control Parameters

The on ACP following phenotypes: J'S the allowable separation for adjacent bands

	BA	В	A	ACP type
b2 to a a to Hb	b1 to b2	b1 to b2	a to Hb	Bands
> 1 0mm > 1 mm	>8mm	>8mm	> 1 mm	Allowable Separation

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Quality Control Parameters

la. sample Positive results must be seen in the neat serially pooled s diluted semen

- ъ the over Compare Sensitivity ole and in the 1/10 and 1/100 serially dilute sitivity of the spot test must be greater that P-30 detection crossed-over electrophoresis 200 for last dilution with the sensitivity of the current lot number. greater than that for the method P30 Crossed-
- N semen stain. positive result must be obtained on the 20ul dried pooled

Acid Phosphatase Уď IEF Quality Control Test

use solution, and Each מב batch casework. of ACP ACP TEF plates, reaction bu buffer must solution, caused before

Each lot of methylumbelliferyl phosphate must be tested before use

Procedure

- quality control sheet. The methumbelliferyl phosphate must cathode Record batch solution, and ACP reaction control sheet. The vendor recorded. numbers for The vendor ACP IEF plates, used in and lot number of the the quality control to buffer quality anode to be tested solution, control g test
- 2 ACP ACP Bloodstains bу BA, respectively) by IEF method spe containing ely) are to specified : known ACP in methods P types (ACP A, ACP B extracted and tested manual. as and per
- <u>م</u> containing tested in o Sample test duplicate. ACP volume A and of 10ul ACP ₿. ß. Both to be extracts used for are the to extracts be
- Ď. triplicate 2.5ul). The extract containing ACP licate with varying vol volume BA ST. size to be (10ul, tested 5ul, in and

Quality Control Parameters

The ACP phenot phenotypes: z. the allowable separation for adjacent bands

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

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Initials: RS Date: 3/17/83

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

5. Esterase D by IEF

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

Each lot 0f methumbelliferyl acetate must be evaluated before use

Procedure

- quality control sheet. The vendor as methumbelliferyl acetate used in the must also be recorded. cathode solution, quality control s Record batch numbers ers for ESD IEF plates, and est reaction buffer to be tested on heet. The vendor and lot number of the heet. The vendor and lot postrol test the
- 2 Bloodstains ESD 2-1) are ESD 2-1) are to be extracted specified in methods manual. containing known to be extracted ESD and types ((ESD 1, Sp per ESD ESD 5-1, IEF method and
- ω Sample test volume of 10ul is containing ESD 1 and ESD 2-1. tested in duplicate. to be Both used for extracts are the to extracts be
- Ь. The triplicate 2.5ul). extract with varying containing ESD volume 5-1 N N sizes to (10ul, be tested 5ul, in and

Quality Control Parameters

The on ESD following is t SD phenotypes: the allowable separation for adjacent bands

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

 \sim Typable tested. W Weak, results m Weak, but must clearly be observed typable with all results a are sample volumes acceptable.

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 \square

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a to Hk

>1mm

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

5. Esterase D by IEF

Each ESD 1 reaction batch of buffer ESD IEF must plates, must be * evaluated before anode solution, cathode use in casework. solution, and

Each lot of methumbelliferyl acetate must be evaluated before use

Procedure

- must Record batch numbers for ESD cathode solution, and ESD read quality control sheet. The vermethumbelliferyl acetate used also be recorded. for ESD IEF plates, anode solut ESD reaction buffer to be test.
 The vendor and lot number of in the quality control solution, e tested on test the on the
- N ESD 2-1) a specified Bloodstains are in methods manual. containing known to bе extracted and ESD types ((ESD as D 1, per ESD ESD 5-1, method and
- Ω Sample test containing tested test n L duplicate. ESD volume of \vdash and ESD 10ul 11 is 2-1. to be Both used for extracts the are extract: to be
- 9 2.5ul). The extract containing triplicate with varying ESD volume 5-1 is to sizes be (10ul, tested 5ul, in and

Quality Control Parameters

The 9 ESD following phenotypes z. the allowable separation for adjacent bands

	<u>5</u>	1	7-7) 	ESD Type
middle-bottom	top-middle	middle-bottom	top-middle	top-bottom	Bands
>3 mm	>3 mm	>1mm	>1mm	>3 mm	Allowable Separation

2 Typ@able tested. W Weak, results must but must be c observed y typable with all results a with are sample acceptable. volumes

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ESD/PGM by Conventional Electrophoresis

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

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

Procedure

- * ----7 Record batch numbers for ESD/PGM tank purice, buffer, ESD reaction buffer, PGM reaction buffer, and PG reaction mixture on quality control sheet. The vendor a lot number of the methylumbelliferyl acetate, glucose-6-charachate dehydrogenase, meldola blue, and agarose used M gel PGM and
- 2 Two bloodstains having known ESD and PGM 1, and ESD 2-1 and PGM 2-1, respectively) ESD/PGM method specified in methods manual are tested in triplicate using two, four, respectively. manual. four, types and Both Six tested as per (ESD threads, bloodstains and PGM

Quality Control Parameters

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

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

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

9

Initials: RU Date: 3/19/92

9 ESD/PGM by Conventional Electrophoresis

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

dehydrogenase, use. Of: meldola blue, and methumbelliferyl agarose must acetate, glucose-6-phosphate be evaluated before

Procedure

- buffer, ESD reaction buffer, PGM reaction buffer, a reaction mixture on quality control sheet. The verlot number of the methylumbelliferyl acetate, glucce phosphate dehydrogenase, meldola blue, and agarose the quality control test must also be recorded. Record batch numbers for ESD/PGM PGM reaction buffer, and control sheet. The vendor tank sheet. The vendor and acetate, glucose-6buffer, ESD/PGM and PGM used and
- 2 Two bloodstains having known ESD and PGM types 1, and ESD 2-1 and PGM 2-1, respectively) are ESD/PGM method specified in methods manual. I are tested in triplicate using two, four, and respectively. two, PGM types Both bloodsta l six threads, tested as (ESD bloodstains and per PGM

Quality Control Parameters

• 9 The ESD following and PGM phenotypes: ıs. the allowable separation for adjacent bands

			PGM 2-1	PGM 1		ESD 2-1	<u>Phenotype</u>
a-d	b-d	' a = C	a-b	a-c	middle-bottom	top-middle	Bands
>14 mm	>7mm	>7mm	>3 mm	>7mm	>4 mm	>4 mm	Allowable Separation

2 Typeable results tested for both results are results acceptable. bloodstains. must be obtained with Weak, but a11 clearly sample le sizes typable

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Initials: 2 Date: ON Personal profits MAGEST erion, enO jnd

Group Specific Electrophoresis Component (GC) bу Conventional

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

before use. Each batch of G tank buffer and gel buffer must ре evaluated

Anti sera Evaluation

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

1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Dilution
300ul 200ul 150ul 120ul 100ul	Amount of anti-Gc
300ul 400ul 450ul 480ul 500ul	Amount of distilled water

- N heating. Dissolve 0 , 8g agarose (Sigma type I) ij 80ml ge1 buffer þу
- ω plate While covering still warm, ing it cast the the gel solution on Ø 15x20cm glass
- .₽ а After comb gel with 1cm cools, , prepare teeth in sample the cer center slits of in the the ge ge Λq inserting
- ហ 2-1.Cut <u>ဒ</u> threads 1cm 'n length from Ø bloodstain having G C type
- 7. 9 each Moisten of threads 5 sample sample with slits. distilled water and place 9 0 H them in
- Cut approximately Electrophorese at set five cellulose maximal values; however, 50mA). 400V for acetate strips 2.5 hours 5cm current (power in should be and Wide can
- ∞ enough to just cover മ sample lane. length
- 9 and To each d 1:6 cellulose acetate strip, diluted antiserum, respe respectively. soak in <u>ب</u> 2, -. W • ب 4 1:5
- 10 end Place from each the strip Wick in down. വ respective sample lane 9 the anodic

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Initials: Rt) Date: 3/A/93

- for 2 hours. Following incuracetate strips and submerge $(2-5\,^{\circ}\text{C})$ overnight. Incubate the gel in a moisture chamber at room temperature Following incubation, remove the cellulose s and submerge the gel in saline and refrigerate
- N The methods manual. next day, develop the gel asper Ga method specified in

Evaluation Parameters

The working dilution to be used subsequent in casework. last dilution which gives of in casework. optimal band n the quality visibility is control method 1.8 the and

Procedure for GC Electrophoresis

- anti-Gc serum and test must also be Record the batch numbers of the the quality control sheet. The anti-Gc serum and agarose used i also be recorded. in 1 vendor and lot number Gc tank the quality control and gel buffers of the
- Ν. Three 2-1, 1 respectively. 2-1, respectively) are tested as methods manual. Each bloodstain using two threads, four threads, bloodstains having known Gc c types per Gc is test and six threads, pes (Gc 1, Gc 2, and Gc Gc method specified tested in triplicate Gc in

Quality Control Parameters

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

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

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

Ø

<u>_</u>

Initials: AU Date: 3/15/84

- 11 for 2 acetate strips and submerge (2-5°C) overnight. Incubate the hours. gel in a moisture chamber at two Following incubation, remove the cellulose following incubation and refriger temperature refrigerate
- 12 The methods manual. next day, develop the gel as per G G method specified H:

Evaluation Parameters

}--å * The last dilution which gives working dilution to be used in subsequent in casework. optimal band n the quality visibility control method and rs. the

Procedure for GC Electrophoresis

- <u>--</u> anti-Gc serum and test must also be the Record the batch numbers of the Gc tank and gel buffers quality control sheet. The vendor and lot number also be recorded. of the
- 2 using two threads, four threads, 2-1, respective methods manual. respectively. Three e bloodstains having known Gc respectively) are tested as I ods manual. Each bloodstain i and per is t types per Gc ypes (Gc 1, Gc 2, and c Gc method specified tested in triplicate six threads, (Gc Gc in

Quality Control Parameters

20 The GC following is phenotypes: the allowable separation for adjacent bands

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

. . Typeable results r bloodstains using Weak, but clearly must four and typeable be obtained with six thread sample results are a11 acceptable. three

Initials: βS Date: 3/17/93

8. Hemoglobin by IEF

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

All lots standard lots must of potassium be assium cyanide evaluated befo before and use the 'n AFSC casework. hemoglobin control

Procedure

- Record batch numbers solution, and cathode of hemoglobin solution on IEF plates, quality con control sheet anode
- 2 Ten microliters (10ul) diluted with 100ul 0.05 0.05% of potassium the AFSC cyanide hemoglobin control r S
- ω Ten microliter (10ul), standard is tested as in methods manual. Th manual. The per 5ul, 10ul hemoglobin IEF method Oul sample is tested in and 2.5ul aliquots of in as as specified triplicate. the diluted

Quality Control Parameters

on The the following AFSC hemo i s the control: allowable separation for adjacent bands

ŭ-C	אַ נ אַ	A-F	Bands
>6mm	> 3 mm	>2mm	Allowable Separation

 \sim Typeble ested. results must be obtained with all sample volumes

9. Lewis Typing Quality Control Test

Quality control evaluation must commercially prepared anti-Le* and casework samples be performed anti-Le^b anti-se antisera before on all use lots with 0f

Procedure

- . Leb control Record vendor antiserum, Le^{a+b} s+: sheet standard, number and Le^{ab+} c+ standard on antiserum, antithe quality
- \sim Test both technique both specified antisera in on methods both standards manual. Ω ซ per Lewis typing

March 17, 1993 Page No:

 \square

12

Initials: /tll Date: 3 (7/92

8. Hemoglobin by IEF

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

A11 standard lots must of potassium рe evaluated cyanide before use and the 'n AFSC casework. hemoglobin control

Procedure

- , , solution, Record batch numbers and cathode of hemoglobin solution on IEF plates, quality con control anode sheet
- \sim Ten microliters (10ul) diluted with 100ul 0.05% of potassium cyanide. the AFSC hemoglobin control Ľ.
- Ψ Ten microliter (10ul), 5ul, and 2.5ul standard is tested as per hemoglobin in methods manual. The 10ul sample manual. 10ul and 2.5ul aliquots of sample LS. IEF tested method 'n as the diluted s specified triplicate.

Quality Control Parameters

g The the following AFSC hemo ıs. the control: allowable separation for adjacent bands

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

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

9. Lewis Typing Quality Control Test

casework Quality control evaluation must commercially prepared anti-Le* and samples. nust be performed and anti-Le^b antise antisera g n all before use with lots of

Procedure

- <u>ب</u> Le^b antiserum, control sheet. Record vendor and lot Lea+b- sta standard, number and Le^{ab+} c+standard on the antiserum, quality anti-
- N Test both technique both specified antisera ij 9 methods both standards manual. as per Lewis typing

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Initials: AS Date: 7/0/83

Quality Control Parameters

but produce agglutination with the Leath standard. Anti-Le* i-Le* must produce not with the Le*-b+ standard. standard. agglutination with Lea-b+ Conversely, sab+ standard, the the but not with Lea+b anti-Le^b standard must

10. Kastle-Meyer Quality Control Test

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

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

Procedure

- Record the batch num quality control shee ethanol and hydrogen must also the batch number be ydrogen peroxide used in the quality control test recorded. sheet. of The the phenolphthalin vendor and lot number of t on the the
- 2. whole Using distilled water, blood ending with distilled മ prepare 1/10 serial 1/1000000 dilution. 1/10 serial dilutions of
- ω One paper drop and allowed of each to dilution dry. J'S placed on ρ Ø tr dt. 0 H fi lter
- 4 Each specified in methods dried drop ı Z then manual. tested SP per phenolphthalin test

Quality Control Parameters

- 1 a Reagent sensitivity must not be less than 1/1000
- Ď. Cross-Over Sensitivity of test midentification method. species must for Compare with the be current greater the lot number. than sensitivity that for species of the
- 2 Positive before th the reactions addition of hydrogen must not be peroxide. observed in any dilution

Initials: (4) Date: 3 (4)

Quality Control Parameters

produce agglutination with the Leabt standard with the Anti-Le* i-Le* must produce not with the Le*-b+ agglutination with the but not with the Lea+b anti-Leb standard must

10. Kastle-Meyer Quality Control Test

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

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

Procedure

- |--3 ethanol and hydrogen peroxide used in the quality control must also be recorded. quality Record the control sheet. batch number of The the vendor phenolphthalin and lot number of the on test the
- 2 whole Using blood ending distilled water, ng with മ prepare 1/10 seria 1/1000000 dilution. serial dilutions of
- ω One paper drop and allowed of each to dilution dry. 18 placed 20 Ø strip of \vdash ilter
- 4. Each specified in methods dried drop L'S then manual. tested as per phenolphthalin test

Quality Control Parameters

- 1a. Reagent sensitivity must not рe less than 1/1000
- à Sensitivity of test muidentification method. Cross-Over species QC 1 species 200 must for Compare with the Эď greater current the lot than sensitivity number. that for species of the
- N before Positive the reactions addition 0f must hydrogen not be peroxide. observed uŢ any dilution

Initials: (20) Date: 10/12/94

10a. Leucomalachite Green Solution Quality Control Test

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

Procedure

- the hydrogen also be recor on the Record the batch number of th quality control sheet. rogen peroxide recorded. used in the the The leucomalachite green reagent The vendor and lot number of he quality control test must
- 2. I Using distilled water, prepare 1/10 ending with a 1/1000000 dilution. 1/10 serial dilutions of whole
- and ω allowed One drop of each dilution is llowed to dry. placed on Ω strip of filter paper
- 4. Each specified in methods dried drop manual. 1'S then tested as per leucomalachi te test

Quality Control Parameters

- 1a. Reagent sensitivity must not be less than 1/1000
- identification method Sensitivity of test must be (check currer current specie species than QC) that for species
- the Positive reactions must not be observed in any dilution before addition of hydrogen peroxide. hydrogen peroxide.

October

Initials: Wh Date: 4/13/93

11. P-30 Detection Quality Control Test

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

bу each testing subsequent batch of P-30 tank buffer and gels can be esting against pooled human semen only (see below). 0f anti-P30 antiserum has semen only (see below). been successfully evaluated evaluated

Procedure

- serial dilutions o saliva with saline. Prepare e 1/10, 1/10 dilutions 1/100, ions of 1/1000, pooled human semen, 1/10000, 1/100000, blood, urine, and 1/1000000 and
- \sim antiserum Test neat and diluted body fluid samples against neat anti as per P30 method specified in methods manual. -P30

Quality Control Parameters

- saliva must give negative results. and all diluted samples of the pooled blood, urine, and
- 2 Neat positive and results. 1/10 diluted sample of the pooled semen must give

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

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

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

ı mouse monoclonal anti-human PSA

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

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

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Initials: Rcl Date: 3/19/92

11. P-30 Detection Quality Control Test

evaluated batch of new P-30 lot before use with casework samples. 0f tank commercially buffer and prepared anti-P30 gels must Ьe tested Similarly, antiserum must before use. each new be

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

Procedure

- <u>--</u>serial dilutions o saliva with saline. Prepare 1/10, 1/1 dilutions 1/100, lons of 1/1000, pooled human 1/10000, semen, 1/100000, blood, and 1/1000000 urine, and
- 2 antiserum Test neat and diluted as per P30 method method specified in methods manual. ·P30

Quality Control Parameters

- |---Neat saliva must and all give diluted negative re results. of the pooled blood, urine and
- N Neat positive and results. 1/10 diluted sample of the pooled semen must give

12. PGM Subtype by IEF

reaction buffer, and reaction IEF plates, anode solution, and PGM reaction mixture mus samples. must cathode solution, эd evaluated before PGM

agarose must Each lot 0f glucose-6-phosphate
 be evaluated before evaluated before dehydrogenase, use meldola blue, and

Procedure

- ի---• dehydrogenase, meldola control test must also reaction mixture to be tested on quality solution, vendor Record batch and cathode numbers lot solution, number blue, and ag be recorded. for PGM PGM 0f subtype agarose subtype IEF plates reaction buffer, the glucose-6-phosphate used in the quality control sheet. and quality anode PGM The
- 2 per and Bloodstains PGM 1+1-, re subtype containing respectively) IEF method known specified are to PGM types be in methods extracted and tested (PGM 2+1-, manual PGM 2-1+, ಬ್

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Initials: M Date: 4(1)(8)

- 2 worksheet. Perform a P30 ELISA test as per the methods manual, using the diagram shown on the "monoclonal anti-PSA antisera evaluation"
- W next Determine the result for 2 ng strongest weakest dilution dilution. PSA standard. Choose of anti-sera as the working titer the which gives

Procedure rabbit polyclonal anti-human PSA

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

- human PSA with phosphate buffered Prepare 1/500 1/3000 dilutions of rabbit saline. of polyclonal anti-
- \sim worksheet. Perform a P30 ELISA test as per the methods manual, using the diagram shown on the "polyclonal anti-PSA antisera evaluation"
- ω Determine the wearcar result for 2 ng PSA standard. Choose anti-sera which anti-sera which gives a as the working titer the

Procedure swine anti-rabbit alkaline phosphatase conjugate

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

- Prepare 1/500-1/3000 dilutions of swine anti-rabbit alkaline phosphatase conjugate with phosphate buffered saline.
- 2 diagram shown on the "swine anti conjugate evaluation" worksheet. Perform a P30 ELISA test as per the methods manual, hown on the "swine anti-rabbit alkaline p alkaline phosphatase using
- ω Determine 2 ng PSA strongest standard. dilution. the weakest dilution of APC which gives a result Choose as the working titer the next for

<u> Procedure - P30 ELISA evaluation</u>

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

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Initials: Date: 4/10/95

- from semen, b the protocol. Prepare 1/25 $5\,-\,1/25,000$ serial dilutions of stains prepared blood, urine, and saliva of healthy males as per
- N Perform a P30 ELISA t diagram shown on the P30 ELISA test "P30 ELISA evaluation" workshe the methods worksheet. using the

Quality Control Parameters

- . All negative diluted results. samples of blood, urine, and saliva must give
- 2 of The semen must 2 ng, 6 ng, give positive results. and 10 ng PSA standards and the $1/10^{2}$ dilution

12. PGM Subtype by IEF

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

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

Procedure

- dehydrogenase, meldola control test must also vendor reaction mixture solution, Record batch and cathode numbers lot le solution, PGM reaction be tested on quality control sheet.

 to be tested on quality control sheet.

 The plucose-6-phose of the glucose-6-phose of the glucose-6-phose of the quality control sheet. blue, and ag be recorded. for PGM and agarose subtype subtype IEF plates, reaction buffer, a used glucose-6-phosphate used in the quality and anode PGM The
- \sim per and Bloodstains containing known PGM types PGM PGM subtype 1+1-, respectively) are to be pe IEF method specified be in methods extracted and tested as (PGM 2+1-, manual. PGM 2-1+

Initials: (20) Date: 3/19/83

- 9 extracts are to be extracts Sample test volume of 10ul is to be used for containing PGM 2+1- and 2-are to be tested in duplicate. 2-1+.the Both
- 9 The extract contact triplicate but v 5ul, and 2.5ul). containing PGM 1+1with varying volume is to Sizes be tested in (10ul,

Quality Control Parameters

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

2+1- 2-1+ 1+1-	<u>Bands</u>
> 4mm > 6mm > 2mm	Allowable Separation

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

13. Species Determination Quality Control Test

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

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

Antiserum Evaluation

- Record vendor and lot number of the anti-human antiserum to tested on the pecies tank buffer evaluation worksheet. and gels must also The batch numbers of be recorded. the be
- 2. Detection Limits
- a. Preparation of standards
- human serum with saline. 1/50000, Prepare 1/10, 1/100, 1/1000, 1/10000, 1/20000, 50000, and 1/100000 dilutions of pooled
- (2) 5ul aliquots will contain 1000ug, 1ug, 0.1ug, 0.01ug, 0.001ug, and human Prepare solutions of human albumin, respectively. 0.001ug, albumin , 100ug, 1 0.0001ug in which 10ug,

Initials: RO Date: 3/15/92

٠ ص extracts containing PGM 2+1- and 2-extracts are to be tested in duplicate. Sample test volume of 10ul rs L to and 2-1+ 2-1+. for the Both

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

Quality Control Parameters

نسز g The PGM following subtype S. phenotype: the allowable separation for adjacent bands

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

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

13. Species Determination Quality Control Test

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

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

Antiserum Evaluation

- • species Record vendor and lot tested on the tank buffer evaluation worksheet. and number of the anti-human antiserum to gels must also The batch эd recorded. numbers of the be
- 2. Detection Limits
- a. Preparation of standards
- 1/1000, and 1/100000 dilutions human serum with saling 1/10000, of 00, 1/20000, pooled
- (2) lug, 5ul aliquots will contain 1000ug, 1ug, 0.1ug, 0.01ug, 0.001ug, and human Prepare albumin, solutions of human albumin in respectively. , 100ug, 1 0.0001ug 10ug, which

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

b. Evaluation Test

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

c. Evaluation Parameters

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

3. Specificity Testing

- о О and Commercial animal the anti-human an Ouchterlony ercial animal sera should anti-human antiserum for k techniques both be tested against the crossed-o crossed-over
- (1) Ten species produced. and the animal in should be tested, which the including antiserum Was monkey
- (2). the human serum detection limit and the human and serum detection at dilutions which correspond to normal animal limit. sera should эd tested ten times

b. Evaluation Parameters

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

Quality Control Test for Species Cross-Over Electrophoresis

- --- Record quality batch control sheet. numbers for species gels and tank buffer 92
- \sim Prepare dilutions of pooled human serum with saline 20

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Initials: LA Date: 4/21/93

 ω against previously evaluated con per crossed-over and Ouchterlony methods. neat and commercial anti-human antiserum of the pooled human serum

Quality Control Parameters

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

14. Takayama Quality Control Test

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

Takayama reagent should be checked monthly.

Procedure

- sheet. Record batch number of Takayama reagent on quality control
- Extract a specified in bloodstain methods manual and test as per Takayama crystal test

Quality Control Parameter

Bloodstain extract must give മ positive result with tes \leftarrow

15. Urea Gel Diffusion Quality Control Test

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

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

Procedure

- plates, and the vendor and lot numbers for the bromothymol blue, urease, and phosphoric acid to be the quality control sheet. the batch number or the urea diffusion test tested on and blank agarose,
- \sim Prepare a sourcea/100ml, set of urea standards containing 5g urea/100ml 0.05g urea/100ml, and 0.005g urea/ $^{\prime}$ urea/100ml,

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Initials: /C) Date: 3/19/92

described above.

ů as Test against previously evaluated commercial anti-human antiserum per crossed-over neat and diluted and Ouchterlony methods. samples

Quality Control Parameters

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

14. Takayama Quality Control Test

Takayama method batch of Takayama with casework reagent samples must рe evaluated before using

Takayama reagent should Эď checked monthly.

Procedure

- <u>ا</u> sheet. Record batch number 0f Takayama reagent 9 quality control
- 2 Extract a specified in methods manual. bloodstain and test as per Takayama crystal test

Quality Control Parameter

Bloodstain extract must give Ø positive result with test

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Initials: NO Date: 4/67/69

respectively, in distilled water.

- ω Extract a 1cmx1cm known urine and prepare a 1:2 dilution of stain in 200ul distilled water the extract in distilled water.
- 4. stain extracts, and diffusion procedure Test 9ul of each urea standard, the neat and 1:2 specified in methods manual a distilled water blank as per urea diluted urine gel
- \mathcal{G} (determined by subtracting the mean diffusion radius of standard on blank plate from mean diffusion radius of test plate). Prepare a standard curve of ;urea concentration (expressed
- 9 extracts Plot the of known urine mean diffusion stain on standard the neat and curve. •• \sim diluted

Quality Control Parameters

- concentration expressed logarithmically. standard wells diffusion needs to radius be linear with respect of to area the urea around
- 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 highest points on the standard to fall between the curve. lowest and
- ω urine stain extracts s allowable error of 5%. The calculated urea concentration of the neat and 1:2 diluted ne stain extracts should differ by a factor of 2 given an

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Department of Forensic Biology

Quality Assurance Manual

Revision Sheets

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

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
	Ö	B2		Vew test	
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APPENDIX C

DEPARTMENT OF FORENSIC BIOLOGY

MOLECULAR BIOLOGY LABORATORY

HLA-DQa QUALITY ASSURANCE MANUAL VERSION 1.0

Initials: β C) Date: 3/(48)

CONTAMINATION CONTROL 4.1 Prevention	SOLUTION MANUAL 3.1 Solution Numbers 3.2 Standard Batch Size 3.3 Lot Numbers 3.4 Ingredients 3.5 Procedure 3.6 Data Log 3.7 Quality Control 3.8 Raw Materials Testi 3.8 Raw Materials Testi 3.9 Documentation 3.9 QC001 DQ\alpha Ext 3.9 QC002 DQ\alpha Am 3.0 QC003 DQ\alpha Hyl 3.1 QC004 DQ\alpha Diff	EQUIPMENT QUA 2.1 Thermocycler . 2.2 Water Bath QA001 Ther QA002 Ther QA003 Ther QA004 Ther QA005 Ther QA006 Ther QA006 Ther QA007 Ther QA008 Syste	QUALITY ASS 1.1 Definitions
ON CONTROI on Protocol ting	ANUAL fumbers satch Size ers ntrol ntrol cials Testing tion DQa Extraction DQa Amplification DQa Hybridization DQa Differential E DQa Differential E	LITY ASS mocycler mocycler mocycler mocouple mocouple mocycler mocycler mocycler mocycler mocycler	QUALITY ASSURANCE SYSTEMS AND PROCEDURES
	Size	ASSURANCE cler Block Cleaning cler Diagnostic Tests cler Cycle Time uple Preparation uple Calibration cler Well Temperature cler Well Test	TEMS AND P
		s	ROCEDURE
			S
26 26 26 26	20 20 20 20 21 21 21 22 22 22 22 23 25		* , *

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31	31	7.0
بر 1	31	5.2 Concepts
30		5.1.2 Proficiency
30		, ;
30		5.1 Training Outline
30		TRAINING AND PROFICIENCY
28		QA009 Clean Run
26		4.5 I roubleshooting
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26		7
25		QC003 DQ α Hybridization
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. 22		tation
. 22		3.8 Raw Materials Testing
. 21		3.7 Quality Control
. 21		3.6 Data Log
. 21		3.5 Procedure
. 20		3.4 Ingredients
. 20		3.3 Lot Numbers
. 20		3.2 Standard Batch Size
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. 20		SOLUTION MANUAL
. 18		QA008 System Review
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14	re Profile	QA006 Thermocycler Well Temperature
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· ∞		QA004 Thermocouple Preparation
. 7		QA003 Thermocycler Cycle Time
	s	QA002 Thermocycler Diagnostic Tests
3		_
	2	2.2 Water Bath
. 2		2.1 Thermocycler
. 2		EQUIPMENT QUALITY ASSURANCE
· 		1.1 Definitions
· 	ROCEDURES	QUALITY ASSURANCE SYSTEMS AND PROCEDURES

Initials: Date: 3/17/97

60		:	•					•			:	•			:		:	•	•			CES	REFERENCES	FEI	RE
58	•	•	•	•	:	:	:	•			•			•	•	•	:	•	ffer	But	Digest Buffer		S094		
57						:	:	:	•	:	:	•		:	•	•	:	•		1 M	DTT, 1M		S093		
56				:				:			:	•					:	•	0%	x, 2	Chelex, 20%		S082		
55	•		•	:	:					:	•		•		Phosphate Buffered Saline (PBS	line	d Sa	ffere	But	hate	ıospi	4 P	S034		
54				:			•	•		:	:	•		•		%	e, S	Hydrogen Peroxide, 3%	Per	gen	ydro		S079		
53	:	•		:			:	•		:	:	•	•	•			:	:	ater	W	Sterile Water		S059		
51			:	:	:				:	:	•		•	•	•	•	•		ırker	Ma	Phi-X Marker		S042		
50		•	•		:	:				•			•			•			%	x, 5	Chelex, 5%		S022		
49	:			:	:	:	:	:		:		•	:	•	ıffer	g Bu	adin	Analytical Gel Loading Buffer	l Ge	tical	naly		S018		
48				:		:	:	:	•		:	•			Proteinase-K Enzyme, 10mg/ml	10r	/me,	Enzy	-K	nase	rotei		S014		
47	:		•	:			:		:	:		•	•			•		•	.5M	1, 0	EDTA, 0.5M		S009		
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45	:	:	:				:		:				•		on .	oluti	n Sc	$DQ\alpha$ Hybridization Solution	oridi	Hyb	Qα		S004		
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Initials: PD Date: 4/14/84

REFERENCES	S081 DTT, 1M	S080 Sodium Acetate, 1M	S079 Hydrogen Peroxide, 3%	S059 Sterile Water	S042 Phi-X Marker	S022 Chelex, 5%	S018 Analytical Gel Loading Buffer	S014 Proteinase-K Enzyme, 10mg/ml	S009 EDTA, 0.5M	S005 DQ\alpha Wash Solution			S002 SSPE, 20X	S001 SDS, 20%	Thermocycler Well Calibration Sheet	Thermocouple Calibration Summary	Thermocouple Calibration Sheet	Thermocycler Diagnostics Specification Sheet	DQa Neview WOLKSHeet	DO: Doi: W. data	DOα Hybridization Worksheet	$DQ\alpha$ Amplification Worksheet	DQa Extraction Worksheet	APPENDIX
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QUALITY ASSURANCE SYSTEMS AND PROCEDURES

Definitions

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

- Equipment Quality Assurance
- Solution Manual
- Contamination Control
- Training and Proficiency

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

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 instructions for a specific task within the laboratory. Examples of procedures are QA001 HLA-DQ α test. Thermocycler Block Cleaning and QC001 DQ\alpha Extraction. Worksheets used for A system may incorporate one or more procedures. A procedure is a list of

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

mitials: PC Date: 4-46

EQUIPMENT QUALITY ASSURANCE

2.1 Thermocycler

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

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

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

2.2 Water Bath

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

Initials: 205 Date: 4 Mil

QA001 Thermocycler Block Cleaning

properly. Maximum contact ensures optimum heat transfer from the block to the sample. contaminating agents collect in the sample wells, preventing the reaction tubes from seating The wells of the sample block must be cleaned each month. Dirt, oil, and other

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

sides of each well with the isopropanol solution. cotton swabs. Rotating the swab helps to loosen material dried in the bottom. Add one or two drops of the isopropanol solution to each well and carefully clean using

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

thermocycler to soak at 37°C. At a slightly warmer temperature, hardened deposits are easier to remove. If the deposits of dirt are heavy, it may be difficult to clean the wells. In this case, set the

cotton swabs or kimwipes. 10% bleach solution, followed by a distilled water rinse. If the sample block has been contaminated with biological material, clean the wells using a Dry the sample wells with dry

Initials: AC Date: 4/14/92

QA002 Thermocycler Diagnostic Tests

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

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)

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

To leave a test, press Stop.

Test 1: Display/Keypad Test

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

Test 3: Heater Test

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

Initials: 19 Date: 4/1/92

constant of the sensor/block assembly. installation. indicated. The temperature difference is an indication of proper sensor operation and 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 Compare the results to the specifications. If its value is not correct, a mechanical problem is

Test 4: Chiller Test

Compare the results to the specifications. 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. This test measures the maximum cooling rate. The machine displays the sensor

Test 5: Sensor Test

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

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 shown on the display after 15 seconds. Compare the results to the specifications. The overshoot past 94°C is

Test 8: Undershoot Test

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

Evaluation of Results

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

Trivials: 20 Date: 41192

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

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

Initials: Par Date: 41182

QA003 Thermocycler Cycle Time

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

Procedure

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

Start the program.

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

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

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

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

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

nitials. Date:

QA004 Thermocouple Preparation

Introduction

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

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

Procedure

Poke a small hole through the center of the cap of a sterile reaction tube using a sterile

so the soldered tip of the wire will be inside the tube when the cap is closed Without bending the wire, pass the thermocouple through the hole from the top of the cap,

measurements. The knot prevents the wire from being pulled out of the tube during temperature fits inside the cap of the tube. Tie an overhand knot in the insulated part of the wire. Carefully tighten the knot so that it The knot should not be so tight as to kink or break the wire

the length is too long or too short. within 1 mm or so of the bottom of the tube; it may touch the tube wall slightly. Adjust if Enough of the thermocouple wire should remain below the knot so that the thermocouple is Check the length by closing the tube and pulling the knot against the inside of the cap

Initials: ROS Date: 4/14/92

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 process mercury thermometer is checked by measuring two standard temperatures. Before beginning the calibration procedure, the

Mercury Thermometer Standardization

thermometer must be at or below the level of the liquid. Allow the temperature to equilibrate. Place the thermometer in an ice water slurry. The thermometer must read between -0.2 and 0.2°C. 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. Place the thermometer in a boiling water bath. The etched line around the bottom of the

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.

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

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

thermocouple bead is close to but not touching the bulb. With a twist tie, attach thermocouple near the bulb of the thermometer so that the

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

Initials: PC Date: 4/14/94

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

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

mixing will reduce temperature gradients near the thermometer. necessary, adjust the stirrer during the procedure to keep the water well stirred. Turn on the stir plate. Stir the water to the point where a shallow vortex forms. Thorough

should be made, over a range of 25°C to 94°C. 95°C because the formation of small vapor bubbles can cause fluctuations leading to variable Seven or eight comparisons of the mercury thermometer and the electronic thermometer Temperatures must not be taken above

probe measurements are recorded under the x-axis column, and the readings from the thermocouple and the mercury thermometer on the Thermocouple Calibration Sheet. mercury thermometer are recorded under the y-axis column. The first measurement is made at room temperature. Record the reading from the

the stir plate Raise the temperature of the water approximately 10°C above room temperature by heating

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 be adjusted to compensate for evaporative loss of water.

part of the thermocouple wire with a pencil to release them. gas bubbles have formed on the thermometer or the thermocouple, gently tap the lower

than 0.2°C in a 15 Check the temperature of the thermometer until successive readings show changes of less second period

probe, record the readings of both thermometers. Once the temperature has stabilized, but at least one minute after any adjustment of the

immersion level, remove any gas Heat the water about 10°C more. bubbles, and record the second set of readings Lower the heat until the temperature stabilizes, check the

Repeat this process until seven or eight temperature measurements have been recorded from to 95°C. For best results, the number of comparisons within a set should be a bit

mitials: Date:

and provide some confidence in the performance of the system over a range of temperatures. multiple readings will partially overcome the uncertainty in reading the mercury thermometer greater at the top of the range to compensate for a higher uncertainty of measurement. The

Calibration Line

observed temperature values measured by the thermocouple probe to standard temperatures The calibration line is defined mathematically by the equation points would fall along a straight line. This line is the calibration curve which relates thermocouple values along the x-axis and thermometer values along the y-axis, the If the pairs of readings taken during the calibration procedure were plotted on a

$$y = mx + b$$

where m is the slope and b is the y-intercept.

coefficient of 1. to the best fit line, the higher the correlation coefficient. A perfect fit has a correlation coefficient gives a quantitative estimate of the goodness of fit. The closer the data points are readings into standard temperatures as well as the correlation coefficcient, r. The least squares calculation yields the slope and intercept necessary to convert thermocouple The best fit line for the data can be calculated directly using a least squares method. The correlation

Calculations

typically seven or eight. The variable n is the number of data points collected during the calibration experiment, The following are calculated and recorded on the Thermocouple Calibration Sheet.

together the x-axis values. This is written in standard notation as calculations for the y values will be performed. discussion describes the calculations with respect to the x values only, assuming parallel The following are calculated the same way for the sets of x and y values. Summation (x) is calculated by adding

$$sum(x) = \sum x_i$$

Initials: 20 Date: 41/82

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

$$\overline{x} = \underline{\operatorname{sum}(x)}$$

squared first and then the squares are added together. This is written Summation (x^2) is the sum of the squares of the x values. All of the x values are

$$sum(x^2) = \sum (x_i^2)$$

squared divided by n. S_{xx} is defined as the sum of the squares of the x values minus the sum of the x values

$$S_{xx} = sum(x^2) - [sum(x)]^2$$

adding the products together. Summation (XY) is calculated by multiplying the pairs of x and y values together and

$$sum(xy) = \sum x_i y_i$$

 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.

$$S_{xy} = sum(xy) - \underline{sum(x)} \underline{sum(y)}$$

The slope of the best fit line, m, is defined as

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

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

Finally, the correlation coefficient is calculated using

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

Initials: AC Date: 414/92

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

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.

the tube. two drops of mineral oil. The mineral oil prevents evaporative cooling of the liquid inside For the thermocycler probe, place 120 μ l of deionized water into the tube and overlay with

For the water bath probe, place approximately 1 ml of mineral oil into the tube

thermocouple wires, the pressure in the tube at high temperatures will force liquid up between the sheath and the wire. end of the tube. Do not seal the hole in the cap. If the cap is sealed around the Close the cap of the tube. The thermocouple tip should be just above or lightly touching the

Initials: PG Date: 4/14/12

QA006 Thermocycler Well Temperature Profile

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

Procedure

Allow the thermocycler to warm up at least 15 minutes.

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

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

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

Load amplification file 14.

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

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

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

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

Wait until the sample block reaches 60 degrees.

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

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

mitials: 20 Date: 4/14/92

Wait until the sample block reaches 72 degrees

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

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

$$y = mx + \bar{b}$$

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

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

Specifications

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

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

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

Initials: PO Date: 411/82

QA007 Thermocycler Well Test

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

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

Samples

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

Procedure

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

Hybridize the samples according to the HLA-DQ α hybridization protocol

Specifications

The amplification negative must show no evidence of contamination

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

Initials: PC Date: 4/14/82

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

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

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

Documentation

Write the test up on DQ α Amplification and Hybridization Worksheets.

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

Initials: PC) Date: 4/14/92

QA008 System Review

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

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

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

Procedure

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

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

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

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

$$sum(m) = \sum m_i$$

Initials: PS Date: 4/4/92

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

$$\bar{m} = \underline{sum(m)}$$

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

$$sum(b) = \sum b_i$$

Mean b equals summation (b) divided by n.

$$\overline{b} = \underline{sum(b)}$$

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

Initials: RC Date: 4/19/92

SOLUTION MANUAL

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

3.1 Solution Numbers

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

3.2 Standard Batch Size

in demand persist over time, the solution sheet may be adjusted to a new batch size and carefully record the adjusted amount of each ingredient added to the solution. batch size. In such cases, the analyst must note the total volume clearly on the solution sheet Occasionally, it may be convenient to prepare a batch larger or smaller than the standard The quantities listed in the ingredients section have been calculated for this standard batch. Each sheet indicates the standard batch size which is routinely prepared for each lot. If changes

3.3 Lot Numbers

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

3.4 Ingredients

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

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

Initials: PC Date: 4/14/92

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

3.5 Procedure

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

3.6 Data Log

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

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

3.7 Quality Control

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

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

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

Initials: RO Date: 4/14/82

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

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

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

the same as the positive controls. extract is added to a hybridization negative, but in every other respect, the strip is processed 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 run through the entire test. Only the hybridization procedure is critical. QC003 begins with $DQ\alpha$ Wash Solution. To evaluate the performance of this component, it is not necessary to For example, QC003 DQ α Hybridization is listed in the quality control section for

sufficient for all of the components. hybridization necessary for the wash solution. $DQ\alpha$ Extraction is the appropriate test for the chelex, and the procedure encompasses the Chelex and DQ α Wash Solution, the quality test must begin with the extraction. QC001 More than one solution may be tested at a time. In this case, the quality test must be For example, if a single run is to be performed for 5%

3.8 Raw Materials Testing

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

3.9 Documentation

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

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 quality control documentation are filed in the Solution Inventory. If more than one solution solution sheet. After a solution has passed quality control and been released, the solution sheet and

Initials: RO Date: 4/14/92

QC001 DQa Extraction

Test Materials

S022 Chelex, 5%

Samples

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

Procedure

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

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

Hybridize the samples according to the HLA-DQ α hybridization protocol.

Specifications

guidelines Each sample must match the assigned HLA-DQ α type within the current interpretation

The negative control sample must show no evidence of contamination.

Documentation

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

Attach the completed worksheets to the Solution Log Sheet.

Initials: PG Date: 4/14/92

QC002 DQa Amplification

Test Materials

RM114 DQa Amplitype kit

Samples

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

Procedure

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

Hybridize the samples according to the HLA-DQ α hybridization protocol.

Specifications

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

The amplification negative must show no evidence of contamination.

Documentation

Write the test up on DQ\alpha Amplification and Hybridization Worksheets.

Attach the completed worksheets to the Solution Log Sheet.

Initials: LO Date: 4/14/92

QC003 DQa 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-DQlpha type one hybridization negative

Procedure

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

Specifications

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

The hybridization negative must show no evidence of contamination.

Documentation

Write the test up on a DQ α Hybridization Worksheet.

Attach the completed worksheet to the Solution Log Sheet.



QC004 DQa Differential Extraction

Test Materials

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

Samples

S094 Digest Buffer

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

Procedure

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

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

Hybridize the samples according to the HLA-DQ α hybridization protocol.

Specifications

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

The negative control sample must show no evidence of contamination.

Documentation

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

Attach the completed worksheets to the Solution Log Sheet.



Initials: AC Date: 4/14/92

CONTAMINATION CONTROL

4.1 Prevention

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

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

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

4.2 Contamination Protocol

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

4.3 Troubleshooting

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

Initials: Date: 4/14/84

Generally, fresh reagents will eliminate this problem. negative extract is greater than the threshold of detectability for the hybridization. extractions. The weak signal appears when the concentration of DNA in the amplified This contamination represents a build up of DNA in the reagents over the course of many

along with the sample. must be aliquoted. with a 10% bleach solution. All of the kit reagents must be changed and new reaction tubes To solve this problem, the pre-amplification room must be cleaned out and the bench washed 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. indicate a more serious contamination problem at the amplification step. If tubes or reagents Strips which appear to have the same mixture of DNA types across all the samples

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



QA009 Clean Run

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

Samples

one positive control sample from the HLA-DQ α typing kit one hybridization negative one amplification negative one extraction negative two whole blood or bloodstain samples of known HLA-DQa type

Procedure

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

and equipment in the amplification area. amplification negative. This negative is used to evaluate contamination from the reagents according to the HLA-DQ α amplification protocol. No chelex extract is added to the Amplify the samples with the positive control from the kit and an amplification negative

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

Evaluation

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

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

Initials: (R) Date: 4/14/92

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

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

Documentation

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

Initials: Pd Date: 4/14/97

TRAINING AND PROFICIENCY

5.1 Training Outline

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

5.1.1 Demonstration

equipment necessary to perform the test. 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 aspects of the amplification and hybridization procedures. By the end of the extraction used to introduce each of the different extraction procedures and to reinforce the important procedures. The test requires a day to run from beginning to end. The first few days are kinds of samples. New analysts are trained to perform a variety of extraction procedures for different All samples are typed using the same amplification and hybridization

5.1.2 Practice

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

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

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

Initials: 20 Date: 4/19/2

5.1.3 Proficiency

in the analyst's folder. may choose to perform each type of extraction separately. The final proficiency test is filed Although it is possible to extract some samples together using different protocols, the analyst 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.

5.2 Concepts

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

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

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

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

5.3 Supplemental Training

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

Initials: (1) Date: 4/1/12

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: RC) Date: 4/14/82

APPENDIX

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

Initials: LO Date: 3/10/93

DQα Extraction Worksheet

Particular recommendation of the Commendation	Da+0	T'i me	Performed by
conc ng/µl	on	case number - sample description	
		Digest Buffer	Chelex, 20%
	PBS	nl DTT, 1M	Pro-K, 10mg/ml
Trickiummussa, com cyclopytologische propositionische des propositionisc		Sterile Water	Chelex, 5%

Initials: RCT Date: 4/14/82

DQα Extraction Worksheet

	1 0 0 0 1	DICCHARGE MACCE
Proteinase-K	DTT	
tube label	case number - sample description	ption conc

April 14, 1992	Performed by
C34	Time Date

C34

Date: 6/11/92 Initials: AG

DQα Amplification Worksheet

Positive Control	MgCl ₂	Kit
Thermocycler	Mineral Oil	Reaction Tubes

	T	T	,		
					tube
					sample
					μl extract
					sample well

Performed by
ру
Time
Date

C-A 35

June 11, 1992

Initials: AC) Date: 4/14/92

DQα Amplification Worksheet

Positive Control	MgCl ₂	Kit
Thermocycler	Mineral Oil	Reaction Tubes

	T	T	T	T	T	T	T	7
								tube
								sample
								μl extract
								sample well

Performed by
by
Time
Date

Date: C/1/82 Initials: PCS

Strips

DOα Hybridization Worksheet

strip	tube	sample	HLA-DQα type	comments
Hyb. Solution	lon		Enzyme Conjugate	jugate
Wash Solution	on			
Chromogen _			Reconstitution Date	tion Date
H ₂ O ₂			Citrate Buffer	ffer
Developing time	time	minutes		
Performed by			Time	Date

Initials: RG Date: 4/14/82

Strips

DQa Hybridization Worksheet

Developing time minutes	H ₂ O ₂	Chromogen	Wash Solution	Hyb. Solution					strip tube sample HL	
	Citrate Buffer	Reconstitution		Enzyme Conjugate					HLA-DQα type	
	ffer	tion Date		jugate					comments	

DQα Review Worksheet

Reviewed by					
ed by					strip
					HLA-DQα type
					comments
Date					

Initials: ACI Date: 4/14/82

DOa Review Worksheet

	T	_	,			
						strip
						HLA-DQα type comments
						comments

Reviewed by

Date

Initials: 20 Date: 4/11/82

Diagnostics Spec
Spe
cification Shee

Thermocycler	Date	
	Performed By	
QA002 Diagnostic Tests		
Test 1: Display/Keypad Test		
All panels of display illuminate Comments	properly yes	no
All keys correspond to the correct Comments	command yes	no
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: 6/4/62 Initials: RU

Thermocouple Calibration Sheet

probe _____

date_____

thermometer

performed by

position

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)

sum (x) = _____

× I ||

 $sum (x^2) = \underline{\hspace{1cm}}$

spec > 0.9999

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The State of the Land
Secretary and Section
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Initials: Date: 4/14/92

Thermocouple Calibration Sheet

probe

date thermometer

meter 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 probe reading (°C) thermometer reading (°C)

sum(x) =

sum(y) =

$$\operatorname{Sum}(x^2) = \underline{\hspace{1cm}}$$

$$\operatorname{S}_{xx} = \underline{\hspace{1cm}}$$

 $sum (y^2) =$

 $S_{yy} =$

р

Initials: (1) Date: 4/14/97

Thermocouple Calibration Summary

meter	probe
	date

	calibration
	date
Мартирання в под	
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position

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calibration

date

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11	11

Reference Table

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

Initials: μ Date: μ

Thermocycler Well Calibration Sheet

ecification Set 2

Date	Thermocycler
Performed By	Well Number

Performed By

RUN 1

60	45	30	15	0		time (s)
					94	(s)
					std	
91.9-94.4	90.9-94.5	89.2-94.4	86.1-93.2	81.3-90.5	spec	
	1				60	
	[[std	tempera
 		56.4-62.9	55.4-66.1	53.9-72.8	spec	temperature (°C)
					72	
	-				std	
		68.6-72.0	66.9-70.8	63.5-67.8	spec	

RUN 2

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

Initials: PC Date: 4/4/82

andard batch size: 1	S001 SDS, 20%	
Г		
	Lot	
	number:	
	demonstration and management properties and military and management and management of the properties o	

Ingredients concentration final amount

RM007 sodium dodecyl sulfate 20 200 G

Q

Procedure

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

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

Add Add the fraction of SDS until it the SDS, allowing the is all in solution solids to dissolve before adding more.

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

Filter sterilize the warm solution.

Dispense into sterile 500 mL bottles

Store at room temperature.

made by:	RM007 sodium dodecyl sulfate	Data Log
	sulfate	
		source
date:		lot
		amount

Initials: LO Date: 1/11/82

S002 SSPE, 20X

andard batch size: 4 L

lot number:

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 ± 1	0 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 the EDTA.	y 6.0 with 10N	sodium hydroxide	to help dissolve
Add the sodium phosphate first	and then the	sodium chloride.	
Adjust the pH to 7.4 with 10N	10N sodium hydroxide	de (about 40 ml).	
Adjust the final volume to 4	liters with deionized water.	onized 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:	sp	specification 7.4	+ 0.2
made by:	da	date:	

April 14, 1992

C43

Q	
000	jasoni kang pad pad
Citra	mals: 80
(T (D)	Date
üffer	(9)
	1

S003 DQα Citrate Buffer andard batch size: 4 L Ingredients	final concentration	lot number:
andard batch size: 4 L		
Ingredients	final concentration	amount
RM001 trisodium citrate	manus manus manus manus manus	$73.6 \pm 0.1 \text{ g}$
RM002 citric acid	same units major state space	24 ± 1 g (guideline

Procedure

Adjust the pH to 5.0 by addition of Dissolve the sodium citrate in approximately 3 liters distilled water. 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	5.0 ± 0.2
QC003 DQα hybridization			
made by:		date:	

Initials: PS Date: 4/14/82

S004 DQa Hybridization Solution

lot number:

andard batch size: 4 L

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

Procedure

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

Add the SDS.

Warm the solution until all solids are dissolved.

Mix well.

Dispense into 1 L bottles.

Store at room temperature.

QC003 DQα hybridization	Quality Control	S001 SDS, 20%	S002 SSPE, 20X	Lata Log source lot
				amount

Initials: PC Date: 4/14/82

@H.:

andard batch	5005 DQa
batch	₩ash
size:	DQα Wash Solution
4 L	hand
	Tot
	D

Ingredients S001 SDS, S002 SSPE, 20X 20% concentration 2.5 X 0.10 % final 500 amount 20 1+ 1+ 10 ml m1

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.

S001 SDS, 20%	S002 SSPE, 20X	Eata Log source lot an
		amount

made

by:

date:

April 14, 1992

Initials: RS Date: 4/14/92

S009
EDITA,
O.53

lot number:

andard batch size: 500 ml

made by: da	Quality Control final pH: specification:	RM003 EDTARM004 sodium hydroxide, 10N	Data Log source	Store at room temperature.	Autoclave at 250°F for 20 minutes.	spense into 125 ml bottles.	Check and record the final pH.	Bring up to volume with distilled water.	When the EDTA is dissolved, adjust the pH to 8.0.	Mix well.	Adjust the pH to 8.0 with sodium hydroxide solution	Add the EDTA to approximately 250 ml distilled wat	Procedure	RM004 sodium hydroxide, 10N	RM003 EDTA 0.50 M	Ingredients final concentration
date:	ication: 8.0 ± 0.1		ce lot						to		le solution.	cilled water.		The state and the state and	93 ± 1 g	amount
			amount													

Initials: (LC) Date: 3/14/93

S014
Proteinase-K
Enzyme,
10mg/ml
0
number:

landard batch size: 10 ml

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

Procedure

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

Mix by slowly inverting until completely reconstituted.

Dispense 500 ul aliquots into 1.5 ml eppendorf tubes.

made by:	${\tt QA004~DQ}{lpha}$ differential extraction	Quality Control	RM119 proteinase-K, lyophilized	Data Log
				source
date:				lot
				amount

Initials: Los Date: 4/14/82

andard hatch size: 10 ml	S014 Proteinase-K Enzyme, 10mg/m	
	/ml lot	
	number:	

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

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.

made by:	RM119 proteinase-K, lyophilized	Data Log
		source
date:		lot
		amount

Initials: PC) Date: 4/14/92

S018	
Analytical	
6	
Loading	
Buffer	
lot	
number:	
minorio anima Konsalanti (mayelli Kolostili ya koheli kohena akakata kalata kakoka kanana mata	

andard batch size: 100 ml

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

Procedure

Combine the TAE, EDTA, and ficoll.

Add the bromophenol blue and xylene cyanol. Mix well. The solution may need to be heated gently to dissolve the ficoll.

x well.

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

Dispense 1.5 ml aliquots into 1.5 ml eppendorf tubes.

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: D Date: 1/14/9

S022 Chelex, 5%	0	number.	
standard batch size: 500 ml			
ingredients	final concentration	amount	
RM027 chelex 100	5.	25 ± 2 g	
S059 sterile water		450 ± 50 n	ml (guideline)
Procedure			
Filter sterilize approximately	600 ml distilled	water.	
Pour the water into a 500 ml k	bottle.		
Save the bottom container from	from the disposable fi	filter unit.	
Autoclave the water at 250°F 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 markings on the disposable filter	o a volume of container.	500 ml using the	graduation
x on a magnetic stir plate.			
While the stock solution is mitubes.	mixing, aliquot 10 ml	each into 15	ml centrifuge
Store at 2-8°C.			
Data Log	source	lot	amount
RM027 chelex 100			
S059 sterile water			
Quality Control			
QC001 DQα extraction			
made by:		date:	

S042 Phi-X Marker

lot number:

page of N

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

Calculations

Record the phi-X-174 H Hae III received from t in the $ng/\mu l$ and the manufacturer. and the initial volume in µl of the

Calculate the final volume according to equation 1.

(final volume) = (initial DNA concentration)(initial DNA volume) (50 ng/ μ l) equation

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

Calculate the amount of buffer to be added according to equation N

(buffer volume) 11 0.2(final volume) equation

N

Calculate the amount of sterile water to be added according to equation W

(water volume) = 0.8(final volume) -(initial DNA volume)

equation 3

Record the buffer and water volumes above

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

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

N

lot number:



ocedure

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet eppendorf tubes. tips, dispense 500 μl aliquots into sterile <u>.</u> 5 ml

Pata Log RM156 phi-X-174 Hae III fragments S018 analytical gel loading	source	lot
S018 analytical gel loading buffer		
S059 sterile water		
made by:		date:

Initials: LC Date: 4/14/92

SO59	
Sterile Water	minais,

standard batch size: 500 ml

lot number:

rocedure

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: (6) Date: 7/17/82

8059	
STERILE	W
WATER	

standard batch size: 500 ml

lot number:

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: pc Date: 4 14/82

RM284 hydrogen peroxide, 3%	rngredients	standard batch size: 8	S079 Hydrogen Peroxide, 3%
, ω %	CO	80 x 0.5 ml	96 (7)
ω %	final concentration	gonesol .	
0.5 ml	amount		lot number:
(guideline)			

Procedure

Aliquot approximately 0.5 ml of hydrogen peroxide into tubes. <u>-1</u> .5 ml microcentrifuge

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

Store at $4^{\circ}C$ in the dark.

made by:	CC003 DQα hybridization	Quality Control	RM284 hydrogen peroxide, 3%	Data Log
				source
date:				lot
				amount

Initials: β Date: 3/17/9

	M.,		* 4
	+ano		S080
	lard		CO
	batch		odium
			Acel
	Size:		Acetate,
			3
17	and post		
elephone and an alpha particles and the	Nove		
	1		
Option and the second			
	1	1	
			5
			number:

RM059 sodium acetate,		Ingredients	andard patch size:
1.0 м	concentration	final	

amount

	M059
anhydrous	sodium acetate,

1.0 M

8.2 1+ 0.4 9

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.

* toclave at 250°F for 30 minutes.

Store at room temperature.

made by:	RM093	RM059	Data Log
эу:	RM093 acetic acid, glacial	RM059 sodium acetate, anhydrous	og
			source
date:			lot
			amount

202	
3	Initials:
	To To
) (m	Date:
oda pi Ne	

S080 Sodium Acetate, 3

standard batch Size: 100 0 number:

ingredients	final concentration	amount
RM059 sodium acetate, anhydrous	1.0 M	$8.2 \pm 0.4 g$
RM093 acetic acid, glacial	many data and had ware	water thank state state open open

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.

rytoclave at 250°F for 30 minutes.

Core at room temperature.

nade by:	RM093 acetic acid, glacial	RM059 sodium acetate,	Data Log
			source
date:			lot
			amount

Initials: (20) Date: 3/17/97

standard batch	SO81 DTT,
STZe:	
U1 E	
Phop	
	Pot
	number:
	Финериализминей принценту принциприн

Ingredients

Standard batch size: 5 ml

conce

amount

final concentration

RM101 dithiothreitol

1. 0 M

10. mM

 $0.77 \pm 0.04 \le$

50 ++

H

Procedure

S059

sterile water

S080

sodium acetate,

<u>__</u>

Add the DTT to a centrifuge tube. to approximately 4 ml sterile, distilled water in α 5 ml

Mix well.

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

Filter sterilize.

Dispense 500 µl aliquots into sterile 1.5 ml eppendorf tubes.

made by:	S059 sterile water	S080 sodium acetate, 1M	RM101 dithiothreitol	Data Log
				source
date:				lot
				amount

Initials://c Date: Illfr

SO81 DIT, 1x

lot number:

. 0 Z (J1 B

rngredients	standard batch
	<u>ا</u>

concentratio	final
9	

amount

RM101	
dithiothreitol	

1.0 3

> 0.77 1+ 0.04 9

S080 sodium acetate,

10. mM

3

50 1+ H

S059 sterile water

Procedure

Add the DTT to a centrifuge tube. approximately 4 ml sterile, distilled water in ω <u>_</u>5 ml

Mix well.

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

Filter sterilize.

Dispense 500 µl aliquots into sterile <u>.</u> S m_{\perp} eppendorf tubes.

ore at -20°C.

made by:	S059 ste	\$080 soc	RM101 dit	Data Log
	S059 sterile water	S080 sodium acetate, 1M	RM101 dithiothreitol	
	, ,	ce, 1M	51	
				source
d				СФ
date:				lot
				amount

Initials: βC Date: 3/7493

S034 Phosphate Buffered Saline	(PBS)	lot number:	
standard batch size: 4 L			
ingredients	final concentration	amount	
RM005 sodium chloride	137 mM	$32.0 \pm 0.1 \text{ g}$	
RM053 potassium chloride	3.0 mM	$0.90 \pm 0.01 \text{ g}$	
RM065 sodium phosphate, dibasic	6.0 mM	$3.41 \pm 0.03 \text{ g}$	
RM056 potassium phosphate, monobasic	1.5 mM	0.82 ± 0.02 g	
Procedure			
Add all the components to approx	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.			
spense into 50 ml centrifuge tuk	tubes.		
Store at room temperature.			
Data Log	source	lot amount	
RM005 sodium chloride			
RM053 potassium chloride			American control of the control of t
RM065 sodium phosphate, dibasic			
RM056 potassium phosphate, monobasic			
Quality Control			
final pH:		spec: 7.5 ± 0.1	
QA004 DQ α differential extraction	D		
made by:		date:	

Initials: (C) Date: 3/17/9 3

muna/Co
Date.

lot number:

standard batch size: 500 ml

Ingredients	final concentration	amount	(1
RM027 chelex 100	20. %	100 ±	2 g
S059 sterile water	water course stress	450 ± 50) ml (guideline)
Procedure			
Filter sterilize approximately	600 ml distilled	water.	
Pour the water into a 500 r	ml bottle.		
Save the bottom container 1	from the disposable fi	filter unit.	
Autoclave the water at 250°F	F for 30 minutes.		
Add the chelex to the botto	bottom container of the fi	filter unit.	
Allow the water to cool after	er autoclaving.		
Add sterile water to the ch	chelex to a volume of 5 le filter container.	500 ml using th	he graduation
Mix on a magnetic stir plate	e.		
While the stock solution is tubes.	mixing, aliquot 10 ml	l each into 15	5 ml centrifuge
Store at 2-8°C.			
Data Log	source	lot	amount
RM027 chelex 100			
S059 sterile water			
Quality Control			
QC004 DQα differential extr	extraction		
made bv:	0.	date:	

S093	
DITT,	
h	4

number:

standard batch size: 20 ml	la.	
size: 20 ml	ndar	C
ze: 20 ml	batch	3
	SIZe:	
-		
0		
0		
0		
0		
		0

S059 sterile water	RM101 dithiothreitol	Ingredients
desiry seam once more more	1.0 M	final concentration
manual vegeta entroly control analosis	$3.1 \pm 0.2 \text{ g}$	amount

Procedure

Add the DTT to approximately centrifuge tube. 15 ml sterile, distilled water 'n ρ 50 ml

Mix well.

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

Dispense $250~\mu l$ aliquots into sterile 0.5 ml eppendorf tubes.

ta Log RM101 dithiothreitol S059 sterile water	source	lot	amount
Quality Control			
QA004 $\text{DQ}\alpha$ differential extraction			
made by:		date:	

Initials; (J) Date: 3117(5)

S094 Digest Buffer

lot number:

candard 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	5 ml
RM096 hydrochloric acid			
Procedure			
Add the EDTA, TRIS, sodium chwater.	chloride, and SDS t	to approximately	4 L distilled
Adjust the pH to 7.5.			
Bring up to the final volume v	with distilled wa	water.	
x well.			
Measure and record the final p	pH.		
Aliquot into 50 ml centrifuge tubes.	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
004 differential extraction			



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