Department of Forensic Biology

Forensic Molecular Biology Laboratory

DNA
Quality Control Manual
Version 2.0

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QUALITY CONTROL SYSTEMS AND PROCEDURES

The DNA quality program maintains performance standards with four systems:

1. General Laboratory Quality Control
1. Equipment Quality Control
2. Solution Quality Control
3. PCR Contamination Control

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

A system may incorporate one or more procedures. A procedure is a list of instructions for a specific task within the laboratory. Examples of procedures are QC008 Thermocycler Block Cleaning and QC015 Chelex Extraction. Worksheets used for documentation are located in the appendix. This manual describes how the systems and procedures operate and how they are integrated to make up the QA/QC program for the DNA test.

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

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GENERAL LABORATORY QUALITY CONTROL

QC001 Glassware Cleaning

General Procedure

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

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

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

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

Alternative Cleaning Procedures

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

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

Stubborn films and residues which adhere to the inside of flasks and bottles may often be removed by rinsing with dilute nitric acid. Some glassware may need to soak in dilute nitric acid overnight. Any nitric acid rinse must be followed by multiple rinses with distilled water to remove acid residues.

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QC002 Autoclaving-Barnstead C2250 Autoclaves

GLASSWARE/EQUIPMENT

All glassware must be clean and dry prior to autoclaving (refer to QC001 for standard glassware cleaning procedure).

Cover glassware openings loosely with foil.

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

Flasks should be loosely capped.

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

SOLUTIONS

Flasks should be loosely capped.

Do not fill flasks more than 75% of capacity.

OPERATION

Consult the Barnstead Operator's Manual for details of operation. This manual must be followed closely, particularly with respect to the warnings indicating a risk of personal injury. All scientific personnel who routinely operate the autoclaves should read the Operator's Manual.

MAINTENANCE

Before each day's run, the door gasket should be wiped down with deionized water. After heavy use, the chamber should be cleaned with the cleaner and the pressure relief valve checked according to the Barnstead Operator's Manual.

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QC003 Temperature Control

REFRIGERATORS/ FREEZERS/ VWR OVEN

The Cole-Parmer 1559-72 Multimeter temperature probe (type K) is used to measure refrigerator/freezer/VWR oven temperatures. Refrigerator/freezer temperatures are recorded daily during the work week and the VWR oven temperature is recorded before blot washing and other uses.

Place the calibrated probe into the refrigerator/freezer/VWR oven and close the door. Make sure the door seal closes tightly around the probe wire.

Allow the probe to equilibrate 5 - 10 minutes.

Measure the temperature and log the reading on the monthly TEMPERATURE CONTROL LOG (F015) sheet for that unit.

WATER BATHS/HYBAID OVENS/ HEAT BLOCKS

An Omega thermistor probe (type T) is used to measure the temperature of the Bellco water baths, HYBAID ovens, and heat blocks. The probe is mounted in the water bath , HYBAID oven or heat block.. Temperature measurements are recorded each day the waterbath, oven or heatblock is used and before each hybridization/wash.

To measure the temperature, turn the water bath, HYBAID oven or heat block on (if necessary) and allow it to equilibrate for at least 15 minutes.

When the temperature has stabilized, record the temperature reading on the appropriate TEMPERATURE CONTROL LOG sheet. To measure the thermistor temperature, plug the probe into the correct position on the meter. Record the reading. The thermistor reading can be corrected using the slope and y-intercept values calculated from the probe calibration QC005). The corrected thermistor reading must be 55 ± 1°C for PCR hybridizations.. The corrected thermistor reading must be 65 ± 1°C for RFLP hybridizations. The corrected thermistor reading must be 50 ± 1°C for QuantiBlot.

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QC004 Omega Type T Thermocouple Preparation

Introduction

Temperature probes have two components. The thermocouple, which responds to temperature changes, is mounted inside either an 0.5 ml amplification tube or a 1.5 ml centrifuge tube. Thermocouples are used because of their fast response time and the availability of thin thermocouple wire. Sensors made with thin wire will not significantly affect the temperature reading. The laboratory probes are made from Teflon coated, 36 gage, type T thermocouples purchased from Omega Engineering.

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

Procedure

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

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

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

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

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QC005 Thermocouple Calibration

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

Mercury Thermometer Standardization

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

Place the thermometer in a boiling water bath. The etched line around the bottom of the thermometer must be at or below the level of the liquid. The thermometer must read between 99.8 and 100.2°C.

Record the results of the temperature check on the Thermocouple Calibration Sheet (F010) .

Thermocouple Temperature Response

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

Place the beaker on a stir plate.

Set up a clamp and ring stand behind the beaker.

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

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

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

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

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

Plug the thermocouple into the socket of the electronic thermometer to be used during routine measurements.

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

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

The first measurement is made at room temperature. Record the reading from the thermocouple and the mercury thermometer on the Thermocouple Calibration Sheet (F010). The probe measurements are recorded under the x-axis column, and the readings from the mercury thermometer are recorded under the y-axis column.

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

When the temperature has risen several degrees, turn down the heat.

Check the immersion level of the thermometer. The position of the thermometer may have to be adjusted to compensate for evaporative loss of water.

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

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

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

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

Repeat this process until seven or eight temperature measurements have been recorded from 25°C to 95°C. For best results, the number of comparisons within a set should be a bit greater at the top of the range to compensate for a higher uncertainty of measurement. The

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multiple readings will partially overcome the uncertainty in reading the mercury thermometer and provide some confidence in the performance of the system over a range of temperatures.

Calibration Line

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

$$y = mx + b$$

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

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

Calculations

The following are calculated and recorded on the Thermocouple Calibration Sheet (F010). The variable n is the number of data points collected during the calibration experiment, typically seven or eight.

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

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

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

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

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

$$\text{sum}(x^2) = \Sigma (x_i^2)$$

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

$$S_{xx} = \text{sum}(x^2) - \frac{[\text{sum}(x)]^2}{n}$$

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

$$\text{sum}(xy) = \Sigma x_i y_i$$

S_{xy} is defined as the sum of the x and y products minus the sum of the x values times the sum of the y values divided by n.

$$S_{xy} = \text{sum}(xy) - \frac{\text{sum}(x)\ \text{sum}(y)}{n}$$

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

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

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

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

Finally, the correlation coefficient is calculated using

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

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

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Final Adjustments

Release the thermocouple wire from the mercury thermometer.

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

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

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

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

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EQUIPMENT QUALITY CONTROL AND OPERATING INSTRUCTION

QC006 Balances

ROUTINE WEIGHT MEASUREMENTS

Press the control bar once to turn on the power. Allow the readout to stabilize.

Place the weigh paper or weigh boat on the pan of the balance. Allow the readout to stabilize.

Press the control bar once to tare the balance.

Make the desired measurement.

When finished, pull the control bar up to turn off the power. Clean out the weighing chamber with the small brush or a damp paper towel, being careful not to disturb the pan.

METTLER AE260 ANALYTICAL BALANCE TWO-POINT STANDARDIZATION

A two-point standardization can be performed before routine measurements to ensure consistent performance day to day.

Press the control bar once to turn on the power. Allow the readout to stabilize.

Close all the doors surrounding the weighing chamber.

Press and hold the control bar until the readout says CALIB. The balance is calibrating at zero grams.

When the readout flashes 100, slide the lever on the right side back to release the internal 100 gram standard weight. Allow the balance to calibrate at 100 grams.

When the readout flashes 0, slide the lever forward. Allow the readout to stabilize.

The balance is calibrated for weekly use.

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BALANCE FOUR-POINT STANDARDIZATION

Each week, the balance is standardized using four standard weights.

Weigh the first standard. Record the standard weight and the measured weight on the BALANCE LOG sheet.

Repeat the measurements for the other three standard weights. Record all measurements.

MAINTENANCE

This balance is serviced every year by an outside contractor.

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QC022 pH Meter

TWO-POINT CALIBRATION

Choose standard buffer solutions for a two-point calibration which bracket the expected final pH of the solution to be measured. i.e. use pH 7 and 10 standard buffers for a solution with final pH of 8.

Press STNDBY/MEAS button. Allow the reading to stabilize.

Press TWO POINT CAL button.

Fill the electrode with saturated KCl solution if necessary.

Rinse the electrode with deionized water. Blot dry.

Place the electrode in fresh standard buffer solution.

The display asks for the pH of the first standard solution. Enter the pH value of the standard solution and press ENTER.

The meter will stabilize the mV reading at that pH.

When the readout is stable, press ENTER.

The display asks for the temperature of the reading. Enter the room temperature (a value of 25°C is adequate for our measurements).

Rinse the electrode with deionized water. Blot dry.

Place the electrode in the second standard buffer solution.

The display asks for the pH of the second standard solution. Enter the pH value and press ENTER.

Allow the meter to stabilize the mV reading at that pH.

When the readout is stable, press ENTER.

Enter the temperature.

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Rinse the electrode with deionized water. Blot dry.

The meter is calibrated before routine measurements.

ROUTINE PH MEASUREMENTS

Fill the electrode with saturated KCl solution if necessary. When fresh KCl is added, it is a good idea to mix the solution in the electrode by slowly inverting the electrode several times before continuing.

Calibrate the pH meter.

Rinse the electrode with deionized water. Blot dry.

Place the electrode in solution. Allow the reading to stabilize, and record the measurement.

MAINTENANCE

The pH electrode must be kept filled with saturated KCl solution. This solution is approximately 30% KCl. The electrode is stored in a 2% KCl solution made from the saturated KCl filling solution (NOT deionized water or pH 7.00 standard solution).

When measuring the pH of large volumes, the pH electrode must be held in place. The electrode can be damaged if it is hung over the edge of the container and allowed to stir with the solution.

If the pH reading drifts or requires a long time to stabilize, the electrode bulb may need to be regenerated or the electrode may need to be replaced. Refer to the Beckman insert for further details of electrode maintenance.

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QC007 Micropipette Standardization

Definitions

Errors in analysis may be classified as systematic (determinant) or random (indeterminate). systematic error are caused by a defect in the analytical method or by an improperly functioning instrument or analyst. For example, a dirty balance pan will cause a systematic error. Systematic error is consistent. It represents the effect of instrument calibration, operating characteristics, and conditions that are constant or that change only in a consistent, predictable way during a series of liquid measurements. Systematic error normally biases all measurements at a given setting toward volumes that are either higher or lower than the set value. The only way to deal with this type of error is to rectify the cause.

Random errors are unavoidable because there is some uncertainty in every physical measurement. The careful analyst can only read a 50 ml graduated cylinder accurately to the nearest 0.5 or 1 ml, for example. However a truly random error is just as likely to be positive as negative. This fact makes the average of several replicate measurements more reliable than any individual measurement. Unfortunately random errors do set a definite limit on accuracy even when the measurement is repeated many times.

Statistics

The gravimetric method is used most often to assess the performance of a micropipette. Distilled water is used as the standard. The water measured at a given volume setting is weighed on an analytical balance. The actual volume measured can be determined from the weight by correcting for the density. If the frequency of occurrence is plotted versus measured volume for a large number of samples, a distribution similar to that in Figure 1 is obtained.

Two calculated statistical values are useful in summarizing and interpreting this type of data. The mean (V_{mg}) is calculated in milligrams according to Equation 1.

$$\text{mean (mg)} = \bar{V}_{mg} = \frac{\Sigma V_i}{n} \qquad \text{Equation 1}$$

The V_i are the individual measurements in milligrams, and n is the total number of measurements recorded.

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This mean weight is converted to volume by dividing by the density of water (δ) at the recorded temperature.

$$\text{mean } (\mu l) = \bar{V}_{\mu l} = \frac{\bar{V}_{mg}}{\delta} \qquad \text{Equation 2}$$

The mean volume is the peak of the normal distribution of measured volumes. The standard deviation quantifies the magnitude of scatter due to random error. Volumes close to the mean volume will occur with much higher frequency than volumes farther from the mean volume.

The standard deviation (SD) is calculated according to Equation 3.

$$SD = \left[\frac{\Sigma (V_i - \bar{V}_{mg})^2}{n-1}\right]^{1/2} \qquad \text{Equation 3}$$

Specifications

Each micropipette must pass specifications for both accuracy and precision before it can be released to the laboratory for general use. Accuracy in liquid measurement refers to how close a measurement is to the volume specified by the setting of the pipette. Precision refers to the reproducibility of a volume measurement. Figure 2 illustrates the relationship between the normal distribution of measured volumes and the specifications of the liquid measurement system at a given volume setting.

Mean error is the difference between the mean volume of actual measurements and the volume set on the instrument. Mean error is a measure of the systematic error component of individual liquid measurements. The accuracy specification defines an acceptable range of systematic error.

The precision specification places an upper limit on the standard deviation of measurements performed at a given volume setting. It defines an acceptable range of random error.

Both accuracy and precision may be expressed as percent figures. Percent error is the mean error expressed as a percent of set volume. The percent error is calculated according to Equation 4.

$$\text{percent error} = \frac{(\bar{V}_{\mu l} - V_{set})}{V_{set}} \cdot 100 \qquad \text{Equation 4}$$

where V_{set} is the volume setting on the instrument.

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The coefficient of variation (CV) gives the standard deviation as a proportion of the mean.

$$CV = \frac{SD}{\bar{V}_{mg}} \cdot 100 \qquad \text{Equation 5}$$

Method

Three times a year, ten measurements of distilled water are made at three volume settings for each micropipette. For Eppendorf repeater pipettes, five settings are measured.

Record the model and serial number for the instrument to be calibrated on a Micropipette Calibration Sheet (F009).

Measure and record the temperature of the distilled water.

Record the density of water at the measured temperature. Refer to Table 2 for the density of water at one atmosphere.

Calibrate the analytical balance at zero and 100 grams using the internal standard weight.

Place the weighing vessel on the pan. The weighing vessel should not exceed 25 times the volume of the sample to be measured. Vessels which are as small as possible relative to the set volume reduce the error due to evaporation. Ideally, the vessel should be cylindrical so that the liquid surface area will stay fairly constant as it fills. The caps from screw-top microcentrifuge tubes are useful for the smallest volumes (1-10μl), and caps from larger bottles are used for larger volumes.

Close the glass panels on the balance and allow the reading to stabilize.

Adjust the volume of the pipette to the desired setting. Refer to Table 1 for the volume settings used for each of the different models.

Tare the balance.

Open the top panel of the balance.

Carefully measure a volume of deionized water into the weighing vessel.

Close the top panel and allow the balance to equilibrate.

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Record the weight. The reading must be taken as soon as possible after the measurement has been made so that significant evaporation does not occur. Typically, the reading is taken when the "off-balance" light goes out.

Using a new pipet tip each time, repeat the procedure until ten measurements have been recorded at that volume setting.

Repeat the procedure for the two remaining volume settings.

Calculate the percent accuracy and coefficient of variation for each set of measurements.

Compare the results to the specifications for that pipette model. Refer to Table 1 for the specifications.

If the measurements are within specification, relabel the handle with the new date of calibration and return the pipette to the laboratory. File the completed Pipette Calibration Sheet in the Micropipette Performance Log (F008).

If any of the measurements is outside specification, the pipette must be withheld while the results are reviewed. Often when an instrument fails a performance test, the problem is not due to a loss of proper calibration. Consistent measurements require practice and technical ability. Frequently, a pipette may not pass specifications because of inexperience on the part of the technician performing the test. Instruments may be retested and a decision may be based upon additional results.

To convert the mean sample weights to mean volumes, the sample weights are divided by the density of water under the test conditions. The table of densities assumes that the air temperature is the same as that of the water in the sampling reservoir and that the barometric pressure is one atmosphere.

Because of evaporative cooling, the steady-state temperature of water in the sampling reservoir will be lower than the air temperature by as much as two or three degrees. Also, the density of air will vary slightly with barometric pressure and humidity. These smaller effects are not taken into account since they are significant at a level well below the level of discrimination needed to monitor pipette performance.

Correction for evaporation will also increase the accuracy of the data and can be important for small volume measurements. An evaporation blank is used to estimate the amount of water lost during a single measurement. The amount of evaporative loss is estimated by performing the identical sequence of manipulations for a single sample measurement without sample aspiration. This amount is then added to the mean sample weight. Evaporation blanks are currently not performed as a routine part of pipette standardization since at the smallest volumes, the accuracy of the Mettler AE-260 balance is limiting. Evaporative loss is minimized by using the smallest practical weighing vessels.

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Table 1: Pipette Performance Specifications

model	volume setting (μl)	percent error	CV
P-1000	1000	≤ ±2.0	≤ 1.0
	500	≤ ±2.0	≤ 1.0
	200	≤ ±2.0	≤ 1.0
P-200	200	≤ ±2.0	≤ 1.0
	100	≤ ±2.0	≤ 1.0
	50	≤ ±2.0	≤ 1.0
P-100	100	≤ ±2.0	≤ 1.0
	50	≤ ±2.0	≤ 1.0
	20	≤ ±2.0	≤ 1.0
P-20	20	≤ ±2.0	≤ 1.0
	10	≤ ±2.0	≤ 1.0
	2	≤ ±10	≤ 5.0
E-10	10	≤ ±2.0	≤ 1.0
	5	≤ ±5.0	≤ 2.0
	2	≤ ±10	≤ 5.0
Repeater	10 (500μl tip)	≤ ±2.0	≤ 1.0
	30 (500μl tip)	≤ ±2.0	≤ 1.0
	50 (500μl tip)	≤ ±2.0	≤ 1.0
	50 (2.5ml tip)	≤ ±2.0	≤ 1.0
	250 (12.5ml tip)	≤ ±2.0	≤ 1.0

P - Rainin Pipetman
E - Eppendorf Ultra-micropipette
Repeater - Eppendorf Repeater Pipette

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Table 2: Density of Water

temp (°C)	density δ (mg/μl)	temp (°C)	density δ (mg/μl)
4	1.00000	17	0.99880
5	0.99999	18	0.99862
6	0.99997	19	0.99844
7	0.99993	20	0.99823
8	0.99988	21	0.99802
9	0.99981	22	0.99780
10	0.99973	23	0.99757
11	0.99963	24	0.99733
12	0.99953	25	0.99708
13	0.99941	26	0.99681
14	0.99927	27	0.99654
15	0.99913	28	0.99626
16	0.99897	29	0.99598

References

Adapted from CRC Handbook of Chemistry and Physics, 53rd ed., R.C. Weast, Ed., CRC Press, Inc., 1972-1973, page F-5.

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QC020 Freeze Drier/Vacuum Pump

Routine Use

If there is water at the bottom of the coils, drain from the black rubber tubing.

Turn on the Freeze Dry Temperature switch.

Wait until the temperature is at least -40°C. Turn on the vacuum.

Wait until the vacuum gauge is in the green area. Place samples in the centrifuge.

Set drying rate at medium and turn the rotor on.

Open the vacuum.

Allow samples to dry for the appropriate amount of time.

Close the vacuum. Place on vent.

After venting is complete, shut off the rotor and remove the samples.

Open the lid of a centrifuge and turn on the vacuum to that centrifuge. There should be a loud whooshing of air.

Turn off the vacuum power switch.

When the whooshing stops, place the centrifuge on vent.

Turn off the freeze dryer temperature switch.

Maintenance

The vacuum pump oil should be changed once a month with pump oil (NOT Flushing Oil). Every three months, fill with flushing oil, run for 1 hour, drain and replace with regular vacuum pump oil. After adding new oil, open the ballast and let the pump run for 3-4 hours. When changing the oil, drain the oil from the mist filter on top of the vacuum pump. The drain is a small black screw which is removed. The mist filter should be replaced once a year. When replacing the oil, the freeze drier coils should be drained and dried with paper towel.

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QC021 Operation of the Biosafety Hood

Routine Use

NOTE: Do not work with any organic solvents (except ethanol) in the biosafety hood.

Turn the blower on and **WAIT** 15 minutes before using the hood. Leave the blower on while you are working in the hood.

Turn on the fluorescent light, NOT the u.v. light.

Wipe all exposed hood surfaces with 70% ethanol. This must be done by every individual, each time they start to work in the hood.

Line the work surface with absorbent pads. Put the plastic side down and the paper side up. Do not block the vents.

Work on the absorbent pads following all of the safety precautions listed above.

In case of a spill onto the hood surface, decontaminate with 10% bleach for 10 minutes. Absorb the bleach onto a paper towel and rinse the surface with 70% ethanol.

NOTE: All the bleach must be rinsed from the hood surface with the ethanol. Otherwise the hood will corrode.

If the blower stops running, DISCONTINUE all work and safely seal up all samples. **The hood no longer offers any protection.**

When you are done working, discard the absorbent pads and change your top layer of gloves.

Wipe all exposed surfaces with 70% ethanol and then discard your gloves layer by layer in the red biohazard bags.

Turn the u.v. on for 1 hour. Do NOT expose yourself to the u.v.

Shut off the blower and u.v. Do NOT leave on overnight.

Maintenance

The hood is inspected by an outside vendor once a year. The airflow should be recorded monthly.

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2.1 Thermocycler

The performance of the Perkin Elmer thermocyclers is routinely monitored. Once a month, six internal diagnostic tests are run (QC009), the time for the HLA-DQα amplification cycle is measured (QC010), and the thermocycler block is cleaned (QC008). Each week, the temperature profile for one well is measured (QC011) using a thermocouple probe to measure the temperature response of a sample in the thermocycler block as the machine cycles through the amplification program. When the DNA laboratory is closed, the measurements are not taken.

Temperature profiles for every well of the Perkin Elmer thermocyclers have been measured. These profiles are used as specifications of future performance. Each week, a different well is chosen, and temperature profiles for both machines are measured. The new profiles are compared to the specifications to determine if the performance of the well falls within the established temperature range. When a complete set of profiles has been collected, the results are compiled and the system is recalibrated (QC013) for the next cycle.

Initials: RCJ Date: 4/2/94

QC008 Thermocycler Block Cleaning

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

Procedure

NOTE: PROTECTIVE EYEWEAR MUST BE WORN WHEN CLEANING THE SAMPLE BLOCK. LIQUID MAY SPRAY OUT OF THE SAMPLE WELLS AS THEY ARE CLEANED WITH COTTON SWABS.

Prepare a 50% v/v isopropanol/water solution.

Clean excess oil out of the wells using kimwipes or cotton swabs.

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

Remove excess liquid using a kimwipe or a dry cotton swab.

Check that there are no deposits left in the sample wells.

Clean the channels between the rows of the block using the same procedure.

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

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

Initials: RCJ Date: 4/7/94

QC009 Thermocycler Diagnostic Tests

There are six diagnostic tests run once a month. The test results are recorded on a Thermocycler Diagnostics Specification Sheet (F016/F016A).

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

Press **File, Yes.**

The following will appear on the display.

Select Function
CONFIG-DIAGNOSTIC

Press **No.**

This moves the cursor to the "Diagnostic" option.

Press **Enter.**

The following will appear on the display.

Diagnostic Tests
Enter test # (1-8)

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

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

Test 1: Display/Keypad Test

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

Initials: RCJ Date: 4/7/94

Test 3: Heater Test

This test measures the maximum heating rate. At the end of the test, the machine displays the time in seconds required for the first 15 degrees of temperature change, the temperature difference between the upper and lower temperature sensors just before the heaters go off, and the heating rate. The heating time is a measure of the thermal time constant of the sensor/block assembly. If its value is not correct, a mechanical problem is indicated. The temperature difference is an indication of proper sensor operation and installation. Compare the results to the specifications.

Test 4: Chiller Test

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

Test 5: Sensor Test

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

Test 7 (F016); 6 (F016A): Overshoot Test

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

Test 8 (F016); 7(F016A): Undershoot Test

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

Initials: RCJ Date: 4/12/94

Evaluation of Results

If all the results meet specifications, the thermocycler passes diagnostic testing. The Thermocycler Diagnostics Specification Sheet (F016/F016A) is filed in the Thermocycler Calibration Log.

If the results for any of the diagnostic tests fail to meet specifications, the thermocycler must be taken off-line for casework. Recent casework must be reviewed and selected samples may be retyped to confirm the results. Further testing may be necessary to rule out the possibility of human error. The test may not have been run properly or the results may not have been interpreted correctly. If after review the results fall consistently outside specification, the thermocycler must be tested before it can be put back on-line (see QC012 Thermocycler Well Test). If all the wells pass the test, casework may resume. If any of the wells fail the test, the thermocycler must be serviced and retested before going back on-line.

Initials: [handwritten initials] Date: 4/7/94

QC010 Thermocycler Cycle Time

The amplification cycle on a thermocycler is timed once a month to confirm the reproducibility of the thermocycler.

Procedure

To measure the cycling time, load amplification program file 14- 94°C for 1 min; 60°C for 30 sec; 72°C for 30 sec; 32 cycles.

Start the program.

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

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

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

Record the time for a single cycle (F016/F016A).

Repeat this procedure for an additional cycle.

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

Compare the results to the specifications.

Evaluation of Results

If the results meet specifications, the thermocycler passes the cycle time test. The Thermocycler Diagnostics Specification Sheet (F016/F016A) is filed in the Thermocycler Calibration Log.

If the results fail to meet specifications, the thermocycler must be taken off-line for casework. Recent casework must be reviewed and selected samples may be retyped to confirm the results. Further testing may be necessary to rule out the possibility of human error. The test may not have been run properly or the results may not have been interpreted correctly. If after review the results fall consistently outside specification, the thermocycler must be tested before it can be put back on-line. QC012 Thermocycler Well Test must be performed for every third well of the block. If all the wells pass the test, casework may resume. If any of the wells fail the test, the thermocycler must be serviced and retested before going back on-line.

Initials: *ZCJ* Date: *4/7/94*

QC011 Thermocycler Well Temperature Profile

The thermocouple probe is used to approximate the temperature response of a sample during an actual amplification cycle. Each well is measured during two cycles, listed as run 1 and run 2 on the Thermocycler Well Calibration Sheet (F017).

Procedure

Allow the thermocycler to warm up at least 15 minutes.

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

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

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

Load amplification file 14- 94°C for 1 min; 60°C for 30 sec; 72°C for 30 sec; 32 cycles

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

Wait until the sample block reaches 94°C for the second time.

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

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

Wait until the sample block reaches 60°C.

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

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

Initials: RCS Date: 4/12/94

Wait until the sample block reaches 72°C.

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

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

Record temperatures for a second cycle.

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

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

where x is the observed probe temperature, $\bar{m}$ is the average slope, and $\bar{b}$ is the average intercept from the current Thermocouple Calibration Summary for the thermocycler probe. The y values are the standard temperatures. The corresponding standard temperatures are recorded in the 'std' columns of the Thermocycler Well Calibration Sheet (F017).

Compare the results to the specifications on the Thermocycler Well Calibration Sheet (F017).

Specifications

If all of the temperature measurements fall within the specified ranges, the well passes the temperature check. The Thermocycler Well Calibration Sheet (F017) is filed in the Thermocycler Calibration Log.

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

If after review the well exhibits a consistent shift in performance outside the specified temperature ranges, the well must be tested empirically according to QC012 Thermocycler Well Test.

Initials: RCS Date: 4/12/94

QC012 Thermocycler Well Test

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

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

Samples

One sample of known type which is susceptible to allelic drop-out or differential amplification
One amplification negative

Procedure

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

Hybridize or electrophorese the samples according to the appropriate protocol.

Specifications

The amplification negative must show no evidence of contamination.

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

Initials: RCJ Date: 4/7/94

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

If the samples meet the specifications, the well passes the typing test and may be used for casework. See QC013 System Review for adjustment of the specifications. If the samples do not meet specifications, the well is taken off-line for casework until the next service call. After service, the performance of the well may be reevaluated.

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

Documentation

Write the test up on the appropriate worksheets.

Attach the completed worksheets to the Thermocycler Well Calibration Sheet (F017) and file in the Thermocycler Calibration Log.

Initials: ρCJ Date: 4/7/94

QC013 System Review

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

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

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

Procedure

If any wells have successfully passed thermocycler well tests, adjust the specifications to include the new acceptable temperature extremes. Record the new specifications on the Thermocycler Well Calibration Sheet (F017).

Recalibrate the thermocouple probes for the thermocycler and the water bath, and calculate new calibration curves according to QC005.

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

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

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

Initials: RCJ Date: 4/7/94

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

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

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

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

Mean b equals summation (b) divided by n.

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

Record the results on the Thermocouple Calibration Summary (F011). The average values are used to standardize temperature probe readings for the next eight month cycle.

Initials: RU Date: 4/7/94

SOLUTION QUALITY CONTROL

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

3.1 Solution Numbers

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

3.2 Standard Batch Size

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

3.3 Lot Numbers

Each batch of a solution is assigned a lot number beginning with 1. Information about each lot of the solutions is recorded in the Solution Inventory (F003/F003A). The inventory indicates the date each solution lot was prepared, who prepared it, and where it is stored. The solution sheets for each lot are filed in the Solution Inventory along with any supporting quality control documentation.

3.4 Ingredients

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

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

Initials: LCI Date: 4/7/94

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

3.5 Procedure

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

3.6 Data Log

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

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

3.7 Quality Control

The quality control section lists the tests to be performed, if any, before the solution is released for use in the laboratory. These test procedures have been assigned QC numbers and names (format QC014 Chelex Extraction).

Quality tests are started at different stages of protocols, depending upon the reagent under study. For example, for HLA-DQα, there are three QC procedures beginning with the extraction, amplification, and hybridization steps. The QC procedure assigned to a solution represents the appropriate level of testing required to pass the reagent. (i.e. QC016 DQα Hybridization is listed in the quality control section for DQα Wash Solution. To evaluate the performance of this component, it is not necessary to run through the entire test. Only the hybridization procedure is critical. QC016 begins with samples which have previously been amplified and repeats the hybridization using the new wash solution. A hybridization negative is the appropriate negative control. No amplified extract is added to a hybridization negative, but in every other respect, the strip is processed the same as the positive controls.)

Initials: RCS Date: 4/7/94

More than one solution may be tested at a time. In this case, the quality test must be sufficient for all of the components. For example, if a single run is to be performed for 5% Chelex and DQα Wash Solution, the quality test must begin with the extraction. QC014 Chelex Extraction is the appropriate test for the chelex, and the procedure encompasses the hybridization necessary for the wash solution.

3.8 Raw Materials Testing

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

RM#	Raw Material	QC Procedure
RM114	HLA-DQα Kits	QC015, QC016
RM378	QuantiBlot Kits	QC018

3.9 Documentation

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

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

Initials: RCJ Date: 4/7/94

QC014 Chelex Extraction

Test Materials

S022 Chelex, 5%

Samples

Two whole blood or stain samples of known type
One negative control sample
One positive control sample from the typing kit (if applicable)

Procedure

Extract the two known samples and the negative control sample according to the Chelex extraction procedure for whole blood and bloodstains in the forensic DNA manual.

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

Hybridize or electrophorese the samples according to the appropriate protocol.

Specifications

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

The negative control sample must show no evidence of contamination.

Documentation

Write the test up on a set of the appropriate worksheets.

Attach the completed worksheets to the Solution Log Sheet.

Initials: RO Date: 4/7/94

QC015 DQα Amplification

Test Materials

RM114 DQα Amplitype kit
S104 Chromogen Solution
S105 HLA-DQα PCR Reaction Mixture

Samples

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

Procedure

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

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

Specifications

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

The amplification negative must show no evidence of contamination.

Documentation

Write the test up on DQα Amplification and Hybridization Worksheets.

Attach the completed worksheets to the Solution Log Sheet.

Initials: RCS Date: 4/7/94

QC016 DQα Hybridization

Test Materials

S003 DQα Citrate Buffer
S004 DQα Hybridization Solution
S005 DQα Wash Solution
S079 Hydrogen Peroxide, 3%
S104 Chromogen Solution

Samples

Three amplified samples of known HLA-DQα type
One hybridization negative

Procedure

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

Specifications

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

The hybridization negative must show no evidence of contamination.

Documentation

Write the test up on a DQα Hybridization Worksheet.

Attach the completed worksheet to the Solution Log Sheet.

Initials: LS Date: 4/7/94

QC017 Differential Extraction

Test Materials

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

Samples

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

Procedure

Extract the known swab and the negative control sample according to the differential extraction procedure in the forensic DNA manual.

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

Hybridize or electrophorese the samples according to the appropriate protocol.

Specifications

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

The negative control sample must show no evidence of contamination.

Documentation

Write the test up on a set of appropriate worksheets.

Attach the completed worksheets to the Solution Log Sheet

Initials: RCJ Date: 8/19/94

QC018 QuantiBlot Hybridization

Test Materials

S003 DQα Citrate Buffer
S004 DQα Hybridization Solution
S079 Hydrogen Peroxide, 3%
S097 Pre-Wetting Solution
S098 Spotting Solution
S099 QuantiBlot Wash Solution
S100 QuantiBlot DNA Standards
S101 SDS 0.1%
S104 Chromogen Solution

Samples

QuantiBlot Calibrators 1 and 2

Procedure

Hybridize the samples according to the Quantiblot protocol.

Specifications

Each Calibrator must have an intensity bounded by the appropriate QuantiBlot DNA standard.
All of the QuantiBlot standards must be visible.
The QuantiBlot Standard intensities must show a correlation with DNA concentrations.
The negative control must show no evidence of contamination.

Documentation

Write the test up on a QuantiBlot Hybridization Worksheet.
Attach the completed worksheet to the Solution Log Sheet.

Initials: RCJ Date: 5/23/94

QC018 QuantiBlot Hybridization

Test Materials

S003 DQα Citrate Buffer
S004 DQα Hybridization Solution
S079 Hydrogen Peroxide, 3%
S097 Pre-Wetting Solution
S098 Spotting Solution
S099 QuantiBlot Wash Solution
S100 QuantiBlot DNA Standards
S101 SDS 0.1%
S104 Chromogen Solution
S106 Quantitation Check Standards

Samples

S106 Quantitation Check Standards- use 5 μl each of Q1, Q2 and Q3.
Q1 has 2ng/5μl, Q2 has 1ng/5μl, and Q3 has 0.2 ng/5μl
QuantiBlot Calibrators 1 and 2

Procedure

Hybridize the samples according to the Quantiblot protocol.

Specifications

Each Quantitation Standard and Calibrator must have an intensity bounded by the appropriate QuantiBlot DNA standard.
All of the QuantiBlot standards must be visible.
The QuantiBlot Standard intensities must show a correlation with DNA concentrations.
The negative control must show no evidence of contamination.

Documentation

Write the test up on a QuantiBlot Hybridization Worksheet.
Attach the completed worksheet to the Solution Log Sheet.

Initials: RCI Date: 4/12/94

QC018 QuantiBlot Hybridization

Test Materials

S003 DQα Citrate Buffer
S004 DQα Hybridization Solution
S079 Hydrogen Peroxide, 3%
S097 Pre-Wetting Solution
S098 Spotting Solution
S099 QuantiBlot Wash Solution
S100 QuantiBlot DNA Standards
S101 SDS 0.1%
S104 Chromogen Solution
S105 HLA-DQα PCR Reaction Mixture
S106 Quantitation Check Standards

Samples

S106 Quantitation Standards- use 5 μl each of Q1, Q2 and Q3.
Q1 has 2ng/5μl, Q2 has 1ng/5μl, and Q3 has 0.2 ng/5μl
QuantiBlot Calibrators 1 and 2

Procedure

Hybridize the samples according to the Quantiblot protocol.

Specifications

Each Quantitation Standard and Calibrator must have an intensity bounded by the appropriate QuantiBlot DNA standard.
All of the QuantiBlot standards must be visible.
The QuantiBlot Standard intensities must show a correlation with DNA concentrations.
The negative control must show no evidence of contamination.

Documentation

Write the test up on a QuantiBlot Hybridization Worksheet.
Attach the completed worksheet to the Solution Log Sheet.

Initials: RCJ Date: 5/23/94

QC023 QuantiBlot Quality Control of Solutions

Test Materials

S003 DQα Citrate Buffer
S004 DQα Hybridization Solution
S079 Hydrogen Peroxide, 3%
S097 Pre-Wetting Solution
S098 Spotting Solution
S099 QuantiBlot Wash Solution
S100 QuantiBlot DNA Standards
S101 SDS 0.1%
S104 Chromogen Solution

Samples

Solution to be tested for the presense of DNA at the volume indicated in the QC section of the solution sheet.
QuantiBlot Calibrators 1 and 2

Procedure

Hybridize the samples according to the Quantiblot protocol.

Specifications

Each QuantiBlot Calibrator must have an intensity bounded by the appropriate QuantiBlot DNA standard.
All of the QuantiBlot standards must be visible.
The tested solution must show no evidence of contamination. There must be no hybridization to the slot containing the tested solution.
The negative control must show no evidence of contamination.

Documentation

Write the test up on a QuantiBlot Hybridization Worksheet.
Attach the completed worksheet to the Solution Log Sheet.

Initials: ACJ Date: 4/7/94

PCR CONTAMINATION CONTROL

4.1 Prevention

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

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

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

4.2 Contamination Protocol

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

4.3 Troubleshooting

Often the source of a contamination problem can be identified on the basis of experience. For example, in the HLA-DQα test, a light signal without a visible 'c' dot in the negative control may indicate slight contamination of the Chelex or the sterile water used during the

Initials: RCJ Date: 4/7/99

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

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

In some cases, the source of contamination may be more elusive. Problems which persist may be addressed by performing a clean run (QC019). During a clean run, control samples are processed along with a series of negative controls. Negative controls are run at the extraction, amplification, and hybridization steps. The results from these samples will indicate the area in which contamination appears. By focusing attention on one area at a time, the source or sources of contamination can be systematically eliminated.

Initials: RCJ Date: 4/7/94

QC019 Clean Run

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

Samples

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

Procedure

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

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

Hybridize or electrophorese the samples with a hybridization or electrophoresis negative according to the appropriate protocol. No amplified extract is added for the hybridization or electrophoresis negative.

Evaluation

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

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

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

Initials: RCI Date: 4/7/94

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

Documentation

Write the clean run up on a set of appropriate worksheets.

APPENDIX A- FORENSIC DNA FORMS, SHEETS AND LOGS

This appendix contains blank copies of the worksheets used to document the quality control program for DNA. These documents may be amended individually as the needs of the program change.

Initials: RCI Date: 4/7/94

MAINTENANCE LOG (F001- 4/4/94) Unit: ______________

Room: ______________

date		initials

Initials: RCI Date: 4/7/94 A-3

INVENTORY CONTROL SHEET (F002- 4/4/94) item: ______________________

date received					
vendor					
lot number					
quantity					
location					

review date						stock on hand

Initials: RCJ Date: 4/7/94

INVENTORY CONTROL SHEET (F002A- 4/4/94) item: ____________________

date received					
vendor					
lot number					
quantity					
location					

date received					
vendor					
lot number					
quantity					
location					

date received					
vendor					
lot number					
quantity					
location					

date received					
vendor					
lot number					
quantity					
location					

Initials: RCS Date: 4/7/94 A-5

SOLUTION CONTROL SHEET **(F003- 4/4/94)** solution: ____________________

date of manufacture (DOM)					
lot number					
quantity					
location					

review date						stock on hand

Initials: RCI Date: 4/7/94

SOLUTION CONTROL SHEET (F003A- 4/4/94) solution: ______________

date of manufacture (DOM)					
lot number					
quantity					
location					

date of manufacture (DOM)					
lot number					
quantity					
location					

date of manufacture (DOM)					
lot number					
quantity					
location					

date of manufacture (DOM)					
lot number					
quantity					
location					

date of manufacture (DOM)					
lot number					
quantity					
location					

Initials: RCJ Date: 4/7/94

DNA LABORATORY SCHEDULE (F004- 4/4/94)

Week of ______________________ **to** ______________________

DAY	EXTRCT 1	EXTRCT 2	P. E. 1	P. E. 2	HYB. 1	HYB. 2
MON	8:00	8:00	8:30	8:30	8:00	8:00
	10:00	10:00	11:00	11:00	10:00	10:00
	1:00	1:00	1:30	1:30	1:00	1:00
	3:00	3:00	4:00	4:00	3:00	3:00
TUE	8:00	8:00	8:30	8:30	8:00	8:00
	10:00 RFLP	10:00 RFLP	11:00	11:00	10:00	10:00
	1:00	1:00	1:30	1:30	1:00	1:00
	3:00	3:00	4:00	4:00	3:00	3:00
WED	8:00	8:00	8:30	8:30	8:00	8:00
	10:00	10:00	11:00	11:00	10:00	10:00
	1:00	1:00	1:30	1:30	1:00	1:00
	3:00	3:00	4:00	4:00	3:00	3:00
THU	8:00	8:00	8:30	8:30	8:00	8:00
	10:00	10:00	11:00	11:00	10:00	10:00
	1:00	1:00	1:30	1:30	1:00	1:00
	3:00	3:00	4:00	4:00	3:00	3:00
FRI	8:00	8:00	8:30	8:30	8:00	8:00
	10:00	10:00	11:00	11:00	10:00	10:00
	1:00	1:00	1:30	1:30	1:00	1:00
	3:00	3:00			3:00	3:00

BALANCE LOG (F005- 4/4/94)

Unit: ____________

date	std 1 (g)	meas 1 (g)	std 2 (g)	meas 2 (g)	std 3 (g)	meas 3 (g)	std 4 (g)	meas 4 (g)	init	comments

Initials: RCJ Date: 4/7/96

PH METER LOG (F006- 4/4/94) Unit: ______________

date	standard 1	pH	standard 2	pH	init	comments

Initials: LCJ Date: 4/7/94 A-10

POWER SUPPLY LOG (F007- 4/4/94) Power Supply: ______________

date	purpose	gel box	init	comments

Initials: RCS Date: 4/17/94

MICROPIPETTE PERFORMANCE LOG (F008- 4/4/94)

Serial Number ________________ Model ________________________

Location ______________________

date	initial	comments

Initials: RCI Date: 4/7/94

MICROPIPETTE CALIBRATION SHEET (F009- 4/4/94) Model: ______ Serial #: ______

Temperature: ______ Density: ______ Date: ______ Analyst: ______

test volume		sample		sample	mean (mg)	mean (μl)	% error	spec	p/f	SD	CV	spec	p/f
	1		6										
	2		7										
	3		8										
	4		9										
	5		10										
	1		6										
	2		7										
	3		8										
	4		9										
	5		10										
	1		6										
	2		7										
	3		8										
	4		9										
	5		10										

Initials: RCJ Date: 4/7/94

THERMOCOUPLE CALIBRATION SHEET (F010- 4/4/94)

probe ________ date ________________ position ____________

meter ________ thermometer ______ performed by ________

Mercury Thermometer Standardization

Ice Water Bath Measured Temp. __________ spec. -0.2-0.2°C

Boiling Water Bath Measured Temp. _______ spec. 99.8-100.2°C

Thermistor Calibration

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

n = _________

sum (x) = _________ sum (y) = _________

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

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

S_{xx} = _________ S_{yy} = _________

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

r = _________ spec > 0.9999

m = _________ b = _________

Initials: RCS Date: 4/7/94

THERMOCOUPLE CALIBRATION SUMMARY (F011- 4/4/94)

probe ______ date ______________

meter ______ position ____________

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

$\bar{m}$ = ______________ $\bar{b}$ = ______________

Reference Table

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

Initials: RCS Date: 4/7/94

TEMPERATURE CONTROL LOG (F012- 4/4/94)

Unit: ________ Room: ________ Month: ________

date	temperature reading	temperature setting	checked by

Initials: RC)   Date: 4/7/84

HEAT BLOCK CONTROL LOG **(F013- 4/4/94)**   Unit: ________

Probe: ________   Room: ________   Month: ________

date	thermocouple reading	standard temperature	comments	init

Initials: [illegible] Date: 4/7/94

THERMOCYCLER FILE LOG (F014- 4/4/94) INSTRUMENT ___________

FILE NO.	FILE TYPE	USER ID	FILE DESCRIPTION

Initials: RCS Date: 4/7/94

THERMOCYCLER LOG **(F015- 4/4/94)** Thermocycler: ______________

date	purpose	init	comments

Initials: RU Date: 6/17/94

THERMOCYCLER TC1 DIAGNOSTICS SPECIFICATION SHEET (F016- 4/4/94)

Thermocycler __________ Date ____________ Performed By ___________

QA002 Diagnostic Tests

Test 1: Display/Keypad Test

All panels of display illuminate properly yes_____ no_____
Comments

All keys correspond to the correct command yes_____ no_____
Comments

Test 3: Heater Test

Rate (°C/s)	_______	Diff (°C)	_______	Time (s)	_______
specifications	> 0.90°C/s		0.0-12.0°C		≤ 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)	__________	Run 2 (s)	__________	Diff (s)	__________
specifications	3:25-3:55		3:25-3:55		< 5s

THERMOCYCLER 480 DIAGNOSTICS SPECIFICATION SHEET (F016A- 4/4/94)

Thermocycler ________ Date __________ Performed By __________

QA002 Diagnostic Tests

Test 1: Display/Keypad Test

All panels of display illuminate properly yes____ no____
Comments

All keys correspond to the correct command yes____ no____
Comments

Test 3: Heater Test

Rate (°C/s)	______	Diff (°C)	______	Time (s)	______
specifications	> 0.90°C/s		0.0-12.0°C		≤ 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 6: Overshoot Test Over (°C) ________ specification < 0.5°C

Test 7: Undershoot Test Under (°C) ________ specification < 2°C

QA003 Cycle Time

Run 1 (s)	________	Run 2 (s)	________	Diff (s)	________
specifications	3:25-3:55		3:25-3:55		< 5s

WELL CALIBRATION SHEET (F017- 4/4/94)

Thermocycler _____ Well # _____ Date ______________ Performed By ______________

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

time (s)	RUN 2 temperature (°C)								
	94	std	spec	60	std	spec	72	std	spec
0			81.3-90.5			53.9-72.8			63.5-67.8
15			86.1-93.2			55.4-66.1			66.9-70.8
30			89.2-94.4			56.4-62.9			68.6-72.0
45			90.9-94.5	---	---	---	---	---	---
60			91.9-94.4	---	---	---	---	---	---

Initials: LC Date: 4/7/94

POSITIVE CONTROL LOG SHEET (F018- 4/4/94)

TUBE	DATE	CASE #	ANALYST	"C" DOT VISIBLE?

Initials: Date:

DNA PCR PROFICIENCY TEST REVIEW (F019- 4/4/94)

Test: RC **Analyst:** 4/7/94

			Comments
Data Sheets			
Were Extraction, Amplification, and Hybridization/ Electrophoresis sheets filled out correctly?	yes	no	
Were all reagent lot numbers recorded?	yes	no	
Procedure			
Were reagent negatives processed?	yes	no	
Were positive controls processed?	yes	no	
Were substrate controls processed?	yes	no	
Were exemplars extracted separately from evidence?	yes	no	
Were duplicate analyses performed?	yes	no	
Results			
Were the appropriate photos attached to each Hybridization/Electrophoresis sheet?	yes	no	
Were the reagent negatives clean?	yes	no	
Did the positive control type correctly?	yes	no	
Were the alleles written down by the analyst consistent with the photos?	yes	no	
Report			
Is the report written in the standard format?	yes	no	
Does the table of results accurately describe each specimen?	yes	no	
Was the evidence matched correctly to the exemplars?	yes	no	
Is the table of results complete?	yes	no	
Is the summary consistent with the table of results?	yes	no	

Supervisor/Reviewer Additional Comments

Initials: RCS Date: 4/7/94

DNA PCR PROFICIENCY TEST REVIEW (F019- 4/4/94)

Assistant Director's Comments

Director's Comments

Analysts's Comments

Evaluation pass fail

I have read the proficiency summary, and I understand the comments and corrective actions outlined above.

Analyst Signature:____________________ Date: __________

Supervisor/Reviews Signature: ____________________ Date: __________

Assistant Director's Signature: ____________________ Date: __________

Director's Signature: ____________________ Date: __________

Initials: RCJ   Date: 4/7/94

WATER BATH CONTROL LOG (F020- 4/4/94)   Unit: ________

Room: ________   Probe: ________   Month: ________

date	water bath setting	thermistor reading	corrected temperature	checked by

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