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Chapter 4

Laboratory Operations
Quality Assurance

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Clinical Laboratory Improvement Amendments (CLIA)

- Congress passed in 1988
- Established quality testing standards to ensure consistent patient test results
- Specifies quality standards for:
  - Proficiency testing
  - Patient test management
  - Quality control
  - Personnel qualifications
  - Quality assurance for moderate- and/or high-complexity tests

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Specimen Handling

- *Preanalytical error: Incorrect results caused by events that occurred prior to sample analysis (ex. specimen collection)*

- Specimen should be labeled properly and correlate with identification on requisition form
- Requisition should include:
  - Type of specimen
  - Test ordered
  - Date and time of collection
  - Contact information of ordering physician
- Molecular genetics or parentage testing:
  - Patient consent forms
  - Ethnicity
  - Photo identification
  - Transfusion history or pedigree
- Forensic specimens:
  - Documented chain of custody

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Specimen Handling

- Specimen Rejection:
  - Thawed specimen when it should be kept frozen
  - Collected in wrong anticoagulant, clotted, contaminated or insufficient quantity
  - *Samples improperly handled may be okay for amplification assays, but not for Southern blot where large quantities of high-quality DNA are required*
- Unacceptable specimens: Documentation of disposal or retention of specimen required

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Specimen Quality

- Hemoglobin:
  - Inhibits enzyme activity
  - Inspect specimen for signs of hemolysis
  - Hemoglobin and coagulants are removed in most DNA and RNA isolation procedures
  - Buffers or resins are available to sequester hemoglobin or anticoagulants
- White blood cell lysis:
  - Reduces DNA or RNA yield
  - Can result in false-negative results

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Collection of Blood and Bone Marrow for Molecular Testing

- Heparin:
  - Inhibits enzymes used in molecular testing (eg. reverse transcriptases and DNA polymerases)
  - Heparinized samples have been processed successfully
  - May have more of an affect on amplification of larger PCR products than shorter ones
  - Thus, trisodium EDTA (lavender top) or acid citrate dextrose (yellow top) tubes are preferred
  - If heparinized, wbc's should be separated and washed
- High levels of disodium EDTA (royal blue top tubes) also inhibit enzyme activity and be avoided

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Collection of Blood and Bone Marrow for Molecular Testing

Additive	Color of Tube Top	Nucleic Acid Testing
None	Red	Chemistry, serum, viral antibody studies
Sodium heparin (freeze-dried)	Green	Immunology, virology studies
Sodium heparin	Brown	Cytogenetic and molecular studies
Tripotassium EDTA (7.5%-15% solution)	Lavender	Virology, molecular studies
Acid citrate dextrose solution	Yellow	Molecular biology studies

Buckingham, L and Flaws, ML. Molecular Diagnostics: Fundamentals, Methods, & Clinical Applications. Philadelphia: F.A. Davis Company, 2007.

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Specialized Collection Tubes in Molecular Testing

- Plasma preparation tubes:
  - Designed for stabilization of nucleic acids for molecular testing
  - Contains standard coagulants
  - Contains polymer gel that separates wbc's from rbc's
  - Used for HIV, HCV, genetics and chimerism testing
  - Tube tops have black "tiger" pattern over appropriate anticoagulant color designation

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Specialized Collection Tubes in Molecular Testing

- Tubes for RNA stabilization
  - Especially important for quantitative RT-PCR analysis for level of tumor cells or microorganisms
  - If chaotropic agents (eg. guanidium isothiocyanate) is added immediately, specimen is good for 7 days at room temperature
  - These agents remove proteins and denaturing RNase

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Collection of Tissue

- Solid Tissues: Fresh or Frozen
  - Nucleic acid and gene expression patterns may change based on how tissue is stored
  - Fresh or frozen samples best, especially for Southern and Northern blot or long-range PCR
  - Snap freezing tissue prevents DNA degradation

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Collection of Tissue

- Solid Tissue: Paraffin-embedded or Ethanol Fixed
  - May yield lower quality DNA and RNA, but okay for PRC and RT-PCR
  - Stable at room temperature with minimal degradation
  - Tissues fixed in Zenker's, B5 or Bouin's should not be used due to extensive DNA damage

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Holding and Storage Requirements

Test	Sample	Temperature	Time
DNA	Whole blood, buffy coat, bone marrow, fluids	22˚-25˚C 2˚-8˚C -20˚C -70˚C	24 hours 72 hours At least 1 year More than 1 year
	Tissue	22˚-25˚C 2˚-8˚C -20˚C -70˚C	Not recommended Up to 24 hours At least 2 weeks At least 2 years
RNA	Whole blood, buffy coat, bone marrow, fluids	22˚-25˚C 2˚-8˚C -20˚C -70˚C	Not recommended 2-4 hours 2-4 weeks More than 1 year
Microorganisms (DNA or RNA)		22˚-25˚C 2˚-8˚C -20˚C -70˚C	24 hours 72 hours 2-4 weeks More than 1 year

Modified from: Buckingham, L and Flaws, ML. Molecular Diagnostics: Fundamentals, Methods, & Clinical Applications. Philadelphia: F.A. Davis Company, 2007.

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Holding and Storage Requirements

- All nucleic acids should be stored in tightly capped containers
- Working solutions of DNA (purified) should be stored at 4˚C for weeks in TE buffer, Tris, or water
- Long-term storage of DNA can be a -20 to -80˚C in ethanol, with or without salt
- RNA can be stored at -20˚C in water or 0.1% DEPC water or TE buffer, but long-term storage should be in 70% ethanol at -80˚C (never water at -80˚C)
- Freezing and thawing cycles more than 3 times affects stability and recovery of nucleic acids

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Validation of Test Performance

Criteria	Definition	Formula	Example
Sensitivity	Ability of test result to predict a clinical condition	$\frac{TP}{TP+FN} \times 100$	95 of 100 patients with a gene mutation have a disease state, a sensitivity of 95%
Specificity	Disease-associated results only in patients who actually have the disease	$\frac{TN}{TN+FP} \times 100$	1 of 100 normal specimens displayed a gene mutation (1 false positive), specificity of 99%
Precision	Reproducibility of test results		A quantitative method yields 99 results in agreement out of 100 runs, precision of 99%
Accuracy	Production of correct results	$\frac{TN + TP}{TN+TP+FN+FP} \times 100$	99 of 100 specimens with mutations in the HCM gene are detected by sequencing with no mutations detected in normal specimens

TN=true negative; TP=true positive; FN=false negative; FP=false positive

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Assay Measurements

- Linearity
  - Determined by testing sequential known concentrations of analyte and plotting them against the expected value
  - Optimally, concentrations should be evenly spaced and include anticipated minimum and maximum values
  - Expanding concentration range beyond anticipated concentration limits demonstrates the limits of linearity of assay
  - Serial tenfold dilutions are used

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Assay Measurements

- Reportable Range
  - Range of results for which a system has been proven to yield accurate results
  - For qualitative tests range can be positive, negative, or genotypes of patient or organism detected
  - For quantitative tests range includes analytical measurement range (AMR) and clinically reportable range (CCR)

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Assay Measurements

- Reference Range
  - Range of values for a specific analyte found in normal population
  - Allow clinician to
  - Must be established or verified for each reported analyte and specimen source
  - Must be verified if there is a change in methodology, instrumentation or patient population

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Test Validation

- Number of specimens tested varies with procedure and availability of test material
- Archived specimens often used for test validations
- Ensures new test meets performance standards and is appropriate for patient population
- Predeveloped and FDA-approved tests
  - Performance is verified by using purchased reagent sets to test validation specimens
  - If commercial test is modified, validation is required to show equal or superior performance of modified procedure

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Performance Characteristics to be Evaluated According to Type of Test

Type of Test	Required Parameters to Establish	Performance Characteristics
FDA-cleared or FDA-approved tests (unmodified)	Accuracy	≥ 30 specimens compared to "gold standard" or current method
	Precision	Quantitative: 2-3 concentrations plus a negative in duplicate or triplicate, 1 run/day over multiple days with goal of 10-20 data points per concentration Qualitative: 1-2 positive (close to LoD) and 1 negative in duplicate or triplicate, 1 run/day over multiple days with goal of 10-20 data points each
	Reportable range (and linearity, as applicable)	5-7 concentrations within stated AMR in triplicate
	Reference intervals (normal values) for laboratory patient population	10-20 (all samples expected to be negative)
	Analytical sensitivity (lower limit of detection—LoD)***Not required by CLIA	1 concentration at the anticipated LoD, over multiple runs/days for a total of 20 data points
	Analytical specificity (including interfering substances)***Not required by CLIA	

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Performance Characteristics to be Evaluated According to Type of Test

Type of Test	Required Parameters to Establish	Performance Characteristics
Modified FDA-cleared or FDA-approved tests or non-FDA-cleared tests (i.e., LDTs)	Accuracy	≥ 50 specimens compared to "gold standard" or current method
	Precision	Quantitative: 2-3 concentrations plus a negative in duplicate or triplicate, 1 run/day over multiple days with goal of 15-20 data points per concentration Qualitative: 1-2 positive (close to LoD) and 1 negative in duplicate or triplicate, 1 run/day over multiple days with goal of 15-20 data points each
	Reportable range (and linearity, as applicable)	7-11 concentrations below, within, and above expected AMR in triplicate
	Reference intervals (normal values) for laboratory patient population	≥20 (all samples expected to be negative)
	Analytical sensitivity (lower limit of detection--LoD)	20 data points at and on either side of the anticipated LoD, over multiple runs/days
	Analytical specificity (including interfering substances)	Evaluate related sequences *in silico*; test potential interfering endogenous and exogenous substances (drugs, specimen transport media, stabilizers, nucleic acids, microorganisms, etc.(and potential sources of contamination that could cause false positive results
	Any other characteristic required for test performance	

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Quality Assurance: Reagents

- New lot of reagent should be tested on positive and negative specimen and run controls
- When new working stocks are prepared, they are treated as new reagent lots
- Written protocol for all reagents should include:
  - Preparation of reagents and quantities of primers, probes, working stocks, sequences, binding sites of primers, polymorphisms or translocation breakpoints that affect primer binding, and expected size of amplicons
- Lot numbers, working stocks, and sequences of probes and primers need to be documented and matched to test performance
- Primers are supplied in freeze-dried forms so supplier will provide necessary information for rehydration of primers for stock solution
- Probes for real-time PCR are supplied in solution
- Master mixes of primers, probe, buffer, nucleotides, and enzyme may be prepared or purchased

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Reagent Preparation

- Now for the scary part: MATH!!!!!
- Diluting a Stock Solution to a Particular Concentration:

$$\frac{\text{Concentration you want}}{\text{Concentration you have}} \times \text{Final Volume} = \text{Volume of concentrated stock to add to mixture}$$

This is the formula $M_1V_1 = M_2V_2$

Adams, DS. Lab Math: A handbook of measurements, calculations, and other quantitative skill for use at the bench. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 2003.

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Diluting a Stock Solution to a Particular Concentration

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☑ **Example**

You want 25 ml of the following solution:

0.5 M $CaCl_2$
1.0 M $MgSO_4$

You have the following stock solutions:

5.0 M $CaCl_2$
2.5 M $MgSO_4$

Step 1. Figure out how much of the $CaCl_2$ stock to add:

$$\frac{0.5\ \text{M}}{5.0\ \text{M}} \times 25\ \text{ml} = 2.5\ \text{ml of } CaCl_2 \text{ stock}$$

Step 2. Figure out how much of the $MgSO_4$ stock to add:

$$\frac{1.0\ \text{M}}{2.5\ \text{M}} \times 25\ \text{ml} = 10\ \text{ml of } MgSO_4 \text{ stock}$$

Step 3. Q.S. to 25 ml:

$$25\ \text{ml} - (2.5\ \text{ml} + 10\ \text{ml}) = 12.5\ \text{ml of water}$$

Adams, DS. Lab Math: A handbook of measurements, calculations, and other quantitative skill for use at the bench. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 2003.

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Serial Dilutions

- Decide what final volume you need (V_f) of each dilution.
- Decide how many different concentrations (N) you need, and line up that many containers.
- Decide what the dilution factor (X) will be. If you are doing a first experiment to determine the appropriate concentration, a good rule of thumb is to cover a few orders of magnitude, meaning that you will be doing 1:10 dilutions. If you are doing an experiment to narrow down the concentration, do 1:2 dilutions.
- Calculate what the concentration will be in each container when you are done:
  - The first will have a concentration of $\frac{1}{X}$ X initial concentration (C_i), where X is the dilution factor.
  - The second will have a concentration of $\frac{1}{X^2}$ X initial concentration (C_i).
  - The third will have a concentration of $\frac{1}{X^3}$ X initial concentration (C_i).
  - And so on.
- Label the containers with the final concentration they will hold.
- Put V_f solvent into each container.
- Set your pipette to $\frac{V_f}{X-1}$.

Adams, DS. Lab Math: A handbook of measurements, calculations, and other quantitative skill for use at the bench. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 2003.

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Serial Dilutions, cont'd.

- Go:
  - Put a new pipette tip on your pipette, or get a new pipette.
  - Pick up $\frac{V_f}{X-1}$ of the most concentrated solution.
  - Dispense it into the first vessel, the one whose label indicates that the concentration will be $\frac{1}{X}$ X initial concentration.
  - Mix.
  - Change pipette tip or pipette.
- Pick up $\frac{V_f}{X-1}$ of the solution you just made. (You will be leaving behind exactly the volume you need of that dilution.)
- Dispense it into the next vessel, the one whose label indicates that the concentration will be $\frac{1}{X^2}$ X initial concentration.
- Mix. Change pipette tip or pipette.
- Pick up $\frac{V_f}{X-1}$ of the solution you just made.
  - Dispense it into the next vessel, the one whose label indicates that the concentration will be $\frac{1}{X^3}$ X initial concentration.
  - Mix.
  - Change pipette tip or pipette.
- And so on.

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Example of Serial Dilution 1:10

- For a 1:10 serial dilution of a 10 M (C_i) solution, you need:
  - 10 ml of each dilution (V_t = 10 ml)
  - 6 different concentrations (N = 6)
  - 1:10 dilutions (X = 10)

Note: 1:10 dilutions are the simplest to calculate, because each dilution is 1/10th the concentration of the last, which means, whatever the concentration was, just move the decimal point one digit to the left to get the concentration of the next most dilute solution.

Container 1: $\frac{10\ M}{10}$ = 1 M

Container 2: $\frac{10\ M}{10^2}$ = 0.1 M (or $\frac{1\ M}{10}$ = 0.1 M)

Container 3: $\frac{10\ M}{10^3}$ = 0.01 M (or $\frac{0.1\ M}{10}$ = 0.01 M)

Container 4: $\frac{10\ M}{10^4}$ = 0.001 M (or $\frac{0.01\ M}{10}$ = 0.001 M) = 10^{-3} M = 1 m

Container 5: $\frac{10\ M}{10^5}$ = 0.0001 M (or $\frac{0.001\ M}{10}$ = 0.0001 M) = 10^{-4} M

Container 6: $\frac{10\ M}{10^6}$ = 0.00001 M (or $\frac{0.0001\ M}{10}$ = 0.00001 M) = 10^{-5} M = 10 µM

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Example of Serial Dilution 1:10, con't.

- Label the containers

  #1: 1 M

  #2: 0.1 M (= 1 X 10^{-1} M)

  #3: 0.01 M (= 1 X 10^{-2} M)

  #4: 0.001 M (= 1 X 10^{-3} M = 1 mM)

  #5: 1 X 10^{-4} M

  #6: 1 X 10^{-5} M (= 10 µM)

- Put 10 ml (V_f) of solvent into each container.
- Set your pipette to $\frac{V_f}{X-1} = \frac{10\ ml}{10-1} = \frac{10\ ml}{9}$ = 1.111 ml.
- Go:
  - Put a new pipette tip on your pipetter.
  - Pick up 1.111 ml of the 10 M solution.
  - Dispense it into the container labeled #1: 1 M.
  - Mix.
  - Change the pipette tip.

Adams, DS. Lab Math: A handbook of measurements, calculations, and other quantitative skill for use at the bench. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 2003.

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Example of Serial Dilution 1:10, con't.

- Pick up 1.111 ml of the #1: 1 M solution.
  - Dispense it into the container labeled #2: 1 X 10^{-1} M.
  - Mix.
  - Change the pipette tip.

- Pick up 1.111 ml of the #2: 1 X 10^{-1} M solution.
  - Dispense it into the container labeled #3: 1 X 10^{-2} M.
  - Mix.
  - Change the pipette tip.

- Pick up 1.111 ml of the #3: 1 X 10^{-2} M solution.
  - Dispense it into the container labeled #4: 1 X 10^{-3} M (= 1 mM).
  - Mix.
  - Change the pipette tip.

- Pick up 1.111 ml of the #4: 1 X 10^{-3} M solution.
  - Dispense it into the container labeled #5: 1 X 10^{-4} M.
  - Mix.
  - Change the pipette tip.

- Pick up 1.111 ml of the #5: 1 X 10^{-4} M solution.
  - Dispense it into the container labeled #6: 1 X 10^{-5} M (= 10 µM).
  - Mix.
  - Throw away the last pipette tip.

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Example of Serial Dilution 1:2

- For 1:2 serial dilution of a 12.6 mg/ml solution, you need:

➢ 150 ml of each dilution
➢ 3 different concentrations
➢ X = 2

- Container 1: $\frac{12.6\ mg/ml}{2}$ = 6.30 mg/ml
- Container 2: $\frac{12.6\ mg/ml}{2^2}$ = 3.15 mg/ml (or $\frac{6.30\ mg/ml}{2}$ = 3.15 mg/ml)
- Container 3: $\frac{12.6\ mg/ml}{2^3}$ = 1.57 mg/ml (or $\frac{3.15\ mg/ml}{2}$ = 1.57 mg/ml)

- Label the containers:
- #1: 6.30 mg/ml
- #2 3.15 mg/ml
- #3 1.57 mg/ml

- Put 150 ml of solvent into each container.

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Example of Serial Dilution 1:2, con't.

- Pick a graduated cylinder that holds $\frac{150\ ml}{2-1}$ = 150 ml.

- Go:
  - Measure out 150 ml of 12.6 mg/ml solution.
  - Dispense it into the container labeled #1: 6.30 mg/ml.
  - Mix.
  - Clean the cylinder or get an unused one.

- Measure out 150 ml of #1: 6.30 mg/ml solution.
  - Dispense it into the container labeled #2: 3.15 mg/ml.
  - Mix.
  - Clean the cylinder or get an unused one.

- Measure out 150 ml of #2: 3.15 mg/ml solution.
  - Dispense it into the container labeled #3: 1.57 mg/ml.
  - Mix.
  - Wash the glassware.

Adams, DS. Lab Math: A handbook of measurements, calculations, and other quantitative skill for use at the bench. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 2003.

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Ok, that wasn't too bad was it?

Take a deep breath and let's summarize.

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How to Do a Serial Dilution

- Set up N containers with capacities to hold $V_f + \frac{V_f}{X-1}$
- Label them $\frac{C_i}{X}$ $\frac{C_i}{X^2}$ $\frac{C_i}{X^3}$,,,,, $\frac{C_i}{X^N}$
- Put V_f solvent into each container.
- Put $\frac{V_f}{X-1}$ of solution C_i into container $\frac{C_i}{X}$. Mix. Change pipette
- Put $\frac{V_f}{X-1}$ of solution $\frac{C_i}{X}$ into container $\frac{C_i}{X^2}$. Mix. Change pipette.
- Put $\frac{V_f}{X-1}$ of solution $\frac{C_i}{X^2}$ into container $\frac{C_i}{X^3}$. Mix. Change pipette.
- Put $\frac{V_f}{X-1}$ of solution $\frac{C_i}{X^{N-1}}$ into container $\frac{C_i}{X^N}$. Mix. Toss pipette.
- N = Number of different concentrations
- V_f = Final volume of each diluted solution
- X = Dilution factor
- C_i = Initial concentration [M]

Adams, DS. Lab Math: A handbook of measurements, calculations, and other quantitative skill for use at the bench. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 2003.

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Quality Assurance: Probes

- Hybridization procedures:
  - Labeled probe solutions are treated as working stock
  - Verify by parallel analysis with old lots
  - FISH probes: validated according to recommended procedures
  - Description of probes includes
    - Type of probe (cDNA, oligonucleotide, etc)
    - Species of origin
    - Probe sequence
    - Restriction enzyme map of that region
    - Known polymorphisms
    - Sites resistant to endonuclease digestion
    - Cross-hybridizing bands

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Purchased Reagents

- Confirm product/shipping label
- Verify proper shipping conditions
- Indicate date received
- Indicate date placed into service
- List expiration date
- Storage conditions
- File MSDS form
- File manufacturer QC form
- Monitor usage/expiration dates

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Labeling "in-house" Reagents

- Content-concentration/pH
- Date prepared
- Initials of technologists
- Date placed into service
- Expiration date
- Storage conditions
- Safety precautions

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Analyte Specific Reagent (ASR)

- Analyte specific reagent:
  - The active ingredient of an in-house ("home brew") test
  - Are raw materials and components used to develop laboratory assays
  - FDA definition of ASR: "*antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents which, through specific binding or chemical reaction with substances in a specimen, are intended to use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens.*"
  - Must be test validated

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Controls

- Controls:
  - Samples of known type or amount
  - Treated like and run with patient specimens
  - Used as a standard to verify acceptable test performance

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Negative/(No-Template)/Reagent Contamination/Blank Controls

- Controls for possibility of intersample contamination
- Assesses background noise generated by reagents in assay
- Generally consists of water or buffer in place of nucleic acid template
- Ideally, should comprise a specimen that lacks targeted sequence
- Should be taken through entire assay process

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Positive Controls

- Need at least one for all assays
- May consist of matrix, negative sample spiked with target, or previous positive sample
- Use concentration close to the lower limit of detection (LoD)
- For genetic tests, heterozygous variant controls are used since normal and variant alleles are represented
- If target sequence is unavailable as a control, bidirectional sequencing can be used
- Quantitative assays need 2 positive controls (low and high concentrations)
- Take control through entire process, including lysis and extraction

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Inhibition (Internal) Controls

- Run in the same reaction mix as test specimen
  - Housekeeping genes: are constitutive genes (always turned ON) required for maintenance of basal cellular function; found in all human cells
  - Supplies a base for normalization of results
- Used to monitor specimens for substances that could interfere with amplification giving a false-negative result
- Failed internal controls are documented and call for repeat of assay
- Can be discontinued if it is determined that inhibition rates are medically acceptable
- Concentration should be at minimal level

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Controls

- Qualitative tests:
  - Positive, negative and sometimes sensitivity controls
    - Sensitivity control: defines the lower limit of detection for more meaningful interpretation of negative results
      - Sometimes called amplification control when used with PCR techniques
      - Amplification control specifically is a target that should always amplify
        - » Used to distinguish true negative amplification results from false negatives resulting from amplification failure

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Controls

- Quantitative tests:
  - High positive, low positive, and negative controls included with each run
  - High and low levels should be similar to critical points in assay (eg. lowest detectable level of analyte)

- Calibrators/Calibration
  - Used with quantitative tests to evaluate and adjust a test analyte being detected relative to actual concentration of analyte present (calibration verification)
  - Assures that assay has remained stable throughout the reportable range, thus patient results are set accurately
  - Constructed with known concentrations of analyte

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Quantitative Tests and LoQ

- Quantitative molecular assays:
  - Have limited range over which the measured value of an analyte is accurate and precise without dilution or concentration or other pretreatment of primary specimen
  - Range is referred to as linear range or Analytical Measurement Range (AMR)
  - Extremes of range are referred to as upper and lower limits of quantification (LoQ)
  - LoQ is not the same as lower LoD

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Controls

- Real-time PCR:
  - Requires measurement of a standard curve or dilution series of analyte levels
  - On instruments, standard curve must be run simultaneously with the specimen

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Reporting Results

- Report must clearly convey:
  - Method or manufactured kit used
  - Locus, mutation or organism tested
  - Analytical interpretation of raw data
  - Clinical interpretation of the analytical result
  - Interpretation includes:
    - Penetrance of mutations (probability of having a mutation but not getting the associated disease)
  - Likelihood of false-positive or false-negative results
  - Mutation detection rate and residual risk of undetected mutations
  - Sensitivity of the test
  - Parentage reports:
    - Combined paternity index
    - Probability of paternity as a percentage
    - Population used for comparison

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Reporting Results

- Laboratory director, pathologist, or other clinical expert:
  - Reviews analytical interpretation
  - Determines clinical interpretation
  - Verifies the final results with actual or electronic signature on test report
- Disclaimer statements are not required for tests using commercially prepared reagents, but are needed for ASRs

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Procedures

- Procedure description should include:
  - Primer and probe sequences
  - Purification conditions
  - Labeling
  - Copy of standard form used to set up reaction mixes
  - Clear description of formulas
  - Reporting units for quantitative results
  - Acceptable ranges such as band patterns
  - Product sizes
  - Melting temperatures
  - Reasons for rejecting results
  - Methods used to score FISH or array results relative to internal control loci
- Discontinued procedures:
  - Note dates of initial use and retirement of written procedure
  - Keep for at least 2 years

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Procedure Manual

- Principle of test
- Pertinent clinical significance
- Specimen requirements
- Criteria for acceptability/rejection
- Reagents needed
- Steps to perform
- QC measures
- Verification ranges
- Interpretation of results
- References and pertinent notes

According to CAP guidelines, the format and style of procedure manual is at discretion of Laboratory Director

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Instrumentation and Quality Control

- QC of instruments:
  - Manufacturer's manual—always accessible
  - Step-by-step instructions for operations
  - Documentation of scheduled calibration
  - Documentation of scheduled performance check
  - Documentation of scheduled maintenance
  - Description of malfunction and corrective action
  - Documentation filed and accessible
  - Written procedure for quality control, operation, and calibration

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Calibrations

- Calibration
  - Fitting an instrument or test system output with actual concentration of a reference analyte by testing and making appropriate adjustments
- Calibration verification
  - Materials of known concentration throughout the reportable range are tested as patient samples to ensure the test system is accurate
  - Perform at least every 6 months or when changes have been made to components, software, or lots of reagents
  - Three levels of measurement: low, medium and high points

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QC: Spectrophotometer

- Measures DNA/RNA concentrations
- Usually requires a warm-up period
- Cuvettes properly maintained
- Blank of solution always run in reference position
- Stray light check before operation
- Scheduled wavelength calibration with known standards and appropriate solutions
- Scheduled preventative maintenance with recalibration

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QC: Centrifuge

- Always balance when in use
- Scheduled routine cleaning
- Centrifuge bucket covers should always be used to minimize formation of aerosols
- Check for excess stains before use
- Scheduled speed check

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QC: Balance

- Always tarred before weighing
- Accuracy appropriate for use
- Clean
- Place in area with low traffic and level countertop
- Scheduled calibration with standard weights
- Located where vibration/drafts do not interfere with readings

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QC: Pipetman

- Daily cleaning before use
- Know upper and lower limits of pipette
- Use appropriate tips
- Differentiate between stop and release buttons
- Scheduled cleaning with 0.25N HCL/0.5N NaOH
- Scheduled calibration after cleaning
- Designated for specific uses, e.g., radioactive, genomic, RNA, PCR, plasmid

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QC: pH Meter

- Check accuracy weekly using known standards
- Use reagents same temperature as solution to be pH'd
- Make sure electrode is completely submerged
- Make sure electrode is saturated with potassium chloride solution
- Calibrate using unexpired buffer solution closest to pH routinely used

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QC: Incubators and Waterbath

- Daily temperature readings
- Scheduled temperature checks at the temperature appropriate for use
- Scheduled routine cleaning

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QC: Geiger Counter

- Measures radioactivity
- Battery check with each use
- Protect monitor surfaces
- Scheduled calibration

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QC: Electrophoretic Equipment

- Routine cleaning as needed
- Scheduled maintenance when required
- Scheduled check of voltage output
- Do not connect or disconnect while power is on
- Always turn off power before opening gel apparatus

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QC: Gel Photographic Equipment

- Photographic equipment cleaned
- Fixed mountings secure and level
- Desired settings posted
- Film stored properly
- Danger of UV document

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QC: Thermocycler

- Directory of files maintained
- Designated electrical line
- Scheduled random well temperature check
- Scheduled calibration of timer

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Decontamination and Sterilization

- Decontaminate with heat, liquid disinfectants, vapors, gasses, and radiation
- Most effective method of sterilization is steam under pressure at 121-132˚C in autoclave
- Alcohols are good surface disinfectants, but do not kill bacterial spores
- Bleach:
  - is active against all microorganisms including spores
  - must be diluted to 10%
  - must be used within 24 hour period
  - make fresh daily
- Other liquid decontaminants:
  - Glutaraldehyde, formaldehyde and phenolic compounds (highly toxic)

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Nucleic Acid Contamination

- Label all tubes throughout isolation
- Use sterile, disposable “glassware”
- Use plugged pipette tips
- Positive displacement pipetman
- Biohazard disposable pads
- Never return an aliquot to the stock tube

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Quality Control and Proficiency Testing (PT)

- Proficiency testing (PT)
  - Refers to analysis of external specimens from a reference source supplied to independent laboratories
  - Used to assess competency of laboratory personnel performing molecular tests and performance of assay itself
  - College of American Pathologists (CAP) supplies some proficiency specimens
  - If no specimens are commercially available
    - Laboratories can exchange blinded split specimens
    - Blinded samples within the lab can be used

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Quality Control and Proficiency Testing (PT)

- PT performed at least twice a year
- Proficiency samples tested within routine patient runs
- Specific procedures should be defined and documented in lab
- Incorrect responses are documented with corrective action taken

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