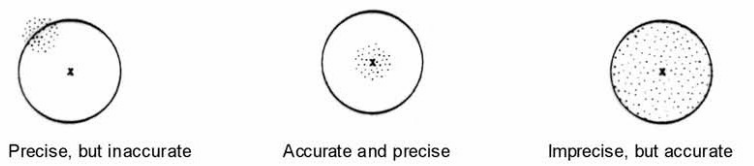
Quality Control Lecture Handout

I. Quality Control (QC)
   A. Why do we need QC and How is it Used?
      1. Monitors the performance of the analytical procedure; used to ensure the reliability of each measurement performed
         a. Reliability of measurement: ability to maintain both precision and accuracy
         b. Accuracy: refers to the closeness of the measured result to the true value
         c. Precision: refers to the **reproducibility** of the result when repeatedly measured



      2. The value of the quality control specimen is **known** and is represented by an 'acceptable range' of values that has been statistically calculated

      3. QC specimens and patient samples are analyzed side by side. Then the value of each control is compared to its specific 'acceptable range'

      4. If the control value falls within the 'acceptable range, then the technologist can assume the procedure performed correctly and can feel certain the patient specimens were also analyzed correctly. The patient values can be reported.

      5. If the control value falls outside the 'acceptable range', then the technologist must assume the possibility an error has occurred that affected the control specimen and must consider the patient specimens also compromised. The patient values cannot be reported.

      6. Note the difference between 'standards' and 'controls':
         a. Standards (also called calibrators, adjustors) are of known concentration, of purified content and are used to determine the concentration of substance in the test samples
         b. Controls have a target value provided by the manufacturer and are used to validate the standard curve and test procedure. The laboratory statistically derives the 'acceptable range' for each control

   B. Types of Quality Control Material
      1. In-house preparation
         a. Pooled patient specimens (serum, urine)
         b. Decreased stability as compared to commercial preparations

      2. Commercial preparation
         a. Lyophilized material (freeze-dried)
            1) Must be reconstituted carefully, following label directions
            2) Mixing too quickly or <u>too vigorously</u> may interfere with the solubilization of the lyophilized material

b. Stabilized low-temperature liquid material (most often used)
    1) Eliminates pipetting error by eliminating reconstitution step
    2) May be frozen for long term stability; then carefully processed prior to use
    3) Control vials that are used daily often stored at refrigerator temperatures for specific amount of days
c. Always follow manufacturer's storage, handling and processing recommendations

3. FDA requires all control material to test negative for the HIV and Hepatitis B virus

C. Criteria for Selection of Control Material
1. 'Mimic' characteristics of the patient sample to be tested (similar matrix)

2. Include at least two concentrations of the analyte to be tested with at least one control focused at the medical decision level
    a. Normal
    b. Abnormal high
    c. Abnormal low

3. Have enough control material of the same lot number to last a length of time (6 months, 1 year)
    a. Each new lot number must be evaluated in parallel with the current lot number in use to establish its 'acceptable range' before the new lot number of control can be put into daily use
    b. Want to minimize the number of times parallel testing performed (cost effective: materials, technologist time, etc)

4. Be available in aliquots that are convenient to use and minimizes waste

5. Have no variability between vials of the same lot number: stable, homogenous

6. Include target values of each analyte to be tested at normal and medical decision levels

D. Target Values
1. These are estimated concentrations of each analyte contained within the control material (pool); supplied by the manufacturer and found in the package insert

2. **Each laboratory must establish the 'acceptable range'** for each analyte through statistical analysis. The 'acceptable range' established by the laboratory must contain the target value found in the package insert
    a. Analyze new QC material in parallel with current QC material in use
    b. When the current QC value is within its 'acceptable range', we can feel confident the analytical procedure performed properly and the value obtained on the new lot number of control is also valid.
    c. The value for the new lot # of control can be placed into the 'new lot # data base' only if the current control values are within their 'acceptable range' (established limits of the current lot # control)

3. Ideally, collect 30 values (N = 30) on 30 consecutive days and calculate the mean, standard deviation and coefficient of variation. The mean and +/- 2 standard deviation represent the 'acceptable range'.

4. Can calculate 'tentative acceptable limits' on N = 10, or N = 20; when N = 30, must establish the 'permanent acceptable limits'. The higher the value of N, the more statistically valid the calculation will be.

Tube Identification	Absorbance
1. Blank 2. #1 standard 3. #2 standard 4. #3 standard 5. Control: Level I (current lot # 12A) 6. Control: Level I (new lot # 65A) ↓ 15. Control: Level II (current lot # 13A) 16. Control: Level II (new lot # 66B)	

Data Base: New Lot Control Values		
Day / Date	Level I (lot # 65A)	Level II (lot # 66B)
1. Monday, May 22 2. Tuesday, May 23 3. Wednesday, May 24 ↓ 20. ↓ 30.	43 42 46	15 13 16

II. Statistics of Quality Control

A. Variability of measurements

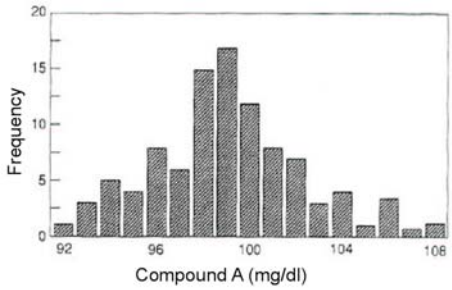
1. In the ideal situation, repeated analysis of a control sample should produce the same value each time; in the real world, there will always be a certain amount of variability in repeated measurements

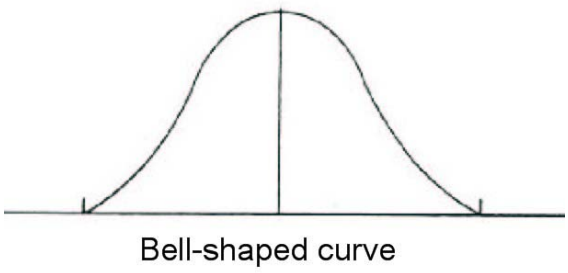
2. Variability caused by
   a. heterogeneity of the sample over time
   b. variation in the technique of the analyst(s)
   c. heterogeneity of reagents over time
   d. instrument variation

3. Data obtained from repeated measurements will have a distribution or spread in the values that reflects how easy it was to repeat the measurement and obtain the same value

4. We want the distribution plot of the repeated control values to display a <u>central tendency and normal distribution pattern</u>

5. Consider the following frequency distribution from 100 values obtained by repeated analysis of a test sample

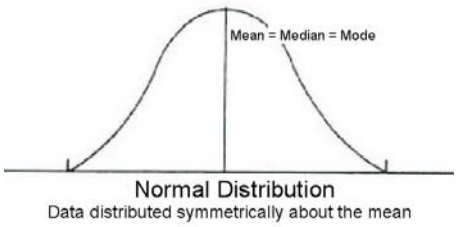




B. Statistics used to Measure Central Tendency

1. Mean = average of all data points

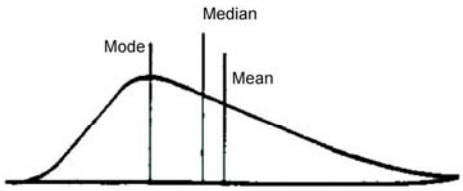
   Mean = $\frac{\text{sum of data points}}{\text{number of results}} = \frac{\sum (x)}{N}$



Normal Distribution
Data distributed symmetrically about the mean

2. Median = the middle data point observed once the data are arranged in descending or ascending order

3. Mode = the value that occurs with the greatest frequency



Non-normal Distribution
Distribution of data is not symmetrical about the mean

4. Normal Gaussian Distribution, symmetric about the mean, Obtained when the Mean = Median = Mode

5. Example: consider the following values: 6, 11, 8, 5, 6, 7, 9, 10, 11, 8, 6

   a. Mean =

   b. Median =

   c. Mode =

   d. Normal or skewed distribution?

5
6
6
6
7
8
8
9
10
11
11

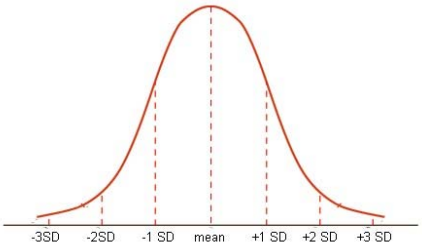
C. Measures of Variation (Refer textbook, page 71, figure 3-17)

1. Desirable to have control data show a slim distribution about the mean, reflecting low variability and low random error

2. Standard Deviation (SD)

   a. Measurement statistic that describes the average distance each data point in a normal distribution is from the mean

   b. $SD = \sqrt{\frac{\sum (x - \text{mean})^2}{N - 1}}$



   c. SD is always expressed in the same units as the measured analyte

   d. One SD unit of measure covers approx 1/6 the total distance of the x-axis on a normal distribution curve (since the laboratory generally uses ± 3SD)

3. Coefficient of Variation (CV)

   a. CV is the standard deviation expressed as a percentage of the mean

   b. $CV = \frac{SD}{\text{mean}} \times 100$

c. Using the CV to evaluate precision allows comparison without influence from the magnitude of the data base

Which of the following two methods is more precise (reproducible) showing the least amount of variability and thus the least amount of random error?

Glucose Method A	Glucose Method B
Mean = 500 mg/dl	Mean = 100 mg/dl
SD = 20 mg/dl	SD = 6 mg/dl
CV = _____	CV = _____

d. The usual limits for acceptability are that the CV on repeat laboratory measurements should be <5%

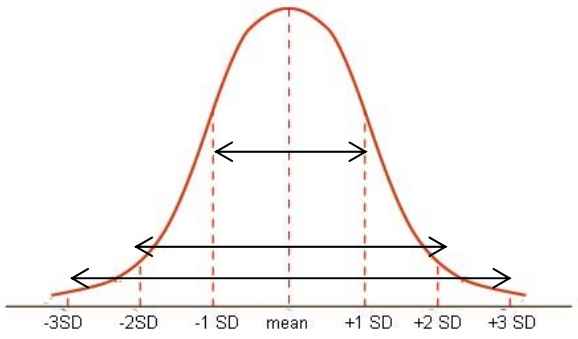
1) This indicates that the distribution of values about the mean is tight rather than broad
2) The CV will be dependent upon the instrument and the type of methodology used: generally,
   a. Modern instrumentation: CV often <3%
   b. Manual methods: CV approximately 8-10% or higher
   c. Automated immunoassay methods: CV often >10% (at this time)

D. Confidence Intervals (also referred to as 'acceptable range', 'established limits')

1. Defined as the limits between which we expect a specified proportion or percentage of a population of values to lie

2. Most of the data in a normal distribution lies close to the mean

3. Confidence limits are the standard deviations expressed as percentages (68.2%, 95.5%, or 99.7%), indicating the percentage of values falling within that area of the curve



Mean - 1 SD = 34.1%
Mean +1 SD = 34.1%

Mean ± 1 SD = 68.2% of data

Mean - 2 SD = 34.1 + 13.65 = 47.75 %
Mean +2 SD = 34.1 + 13.65 = 47.75 %

Mean ± 2 SD = 95.5 % of data

Mean - 3 SD = 47.75 + 2.1 = 49.85 %
Mean +3 SD = 47.75 + 2.1 = 49.85 %

Mean ± 3 SD = 99.7 % of data

III. Internal Quality Control

A. Control Monitoring System

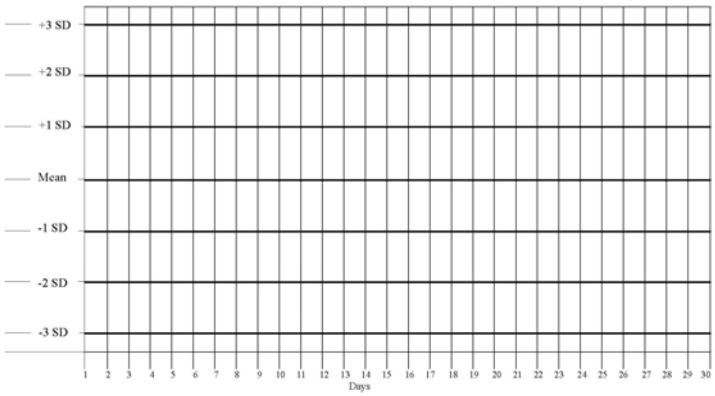
1. Recall: control values are monitored to verify accuracy and precision in measurement
2. Monitoring system
   a. Must give <u>immediate</u> information to the technologist regarding accuracy of patient results

   b. Should provide periodic information to supervisory personnel regarding the <u>overall performance of a method</u>, reflecting precision and long-term accuracy of measurement

   c. Want visual assessment that is fast and reliable

B. Levy-Jennings Plot

1. Most common plot used to visually monitor control values over time

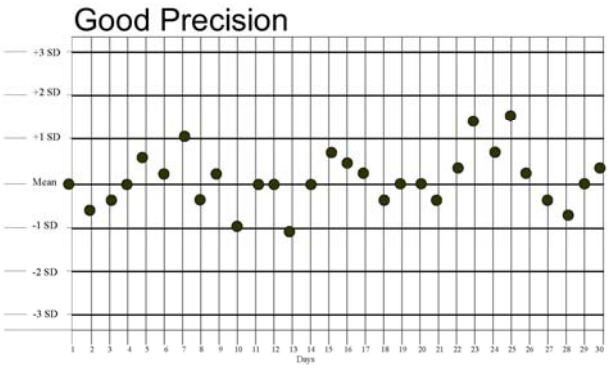


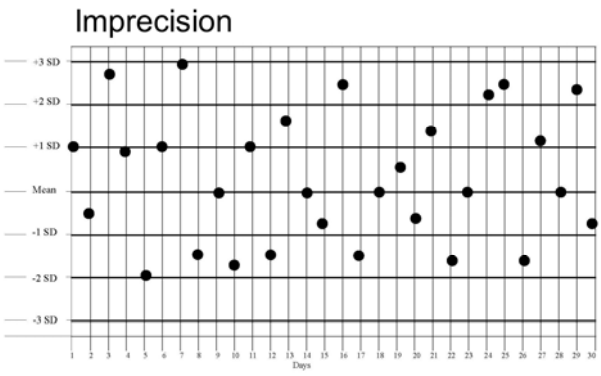
2. Immediate decisions about the 'correctness' of patient results are based on the ability of control values to remain within a pre-established limit: usually the mean <u>+</u> 2SD (95% confidence interval)

3. The control value is plotted on a Levy-Jennings chart. This value, along with subsequent values is monitored over time

C. Visual assessment of the Levy-Jennings chart

1. **Precision and long-term accuracy** are confirmed by control values remaining clustered about the mean with <u>very little variation</u> in an upward or downward direction. **This tells us there is little random error in the procedure**





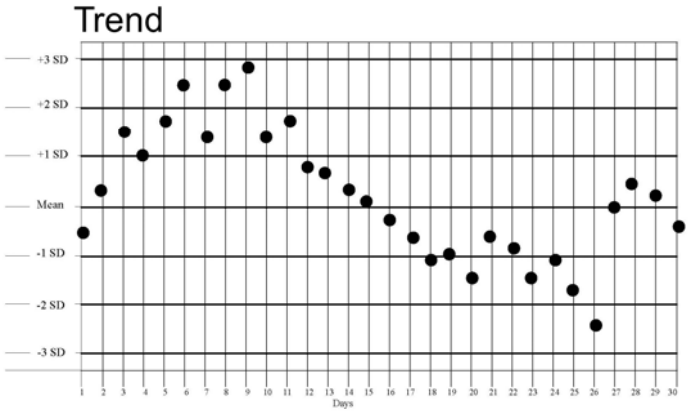
2. **Imprecision** in measurement is indicated by a large amount of scatter (or variability) about the mean and usually an uneven distribution above and below the mean. **This tells us there is a lot of random error in the procedure**

   Most often caused by technique errors:
   a. Variability in pipetting
   b. Inattention to detail by the technologist
   c. Bubbles in reagents or reagent lines
   d. Inadequate mixing of reagents
   e. Unstable temperatures, incubations, or electrical supply
   f. Individual operator variation in pipetting, timing, etc
   g. Defect unit test device
   h. Air bubble in sample cup
   i. Air bubble in syringe

3. Out of control: (trend, shift, outlier, second of 2 consecutive values between 2-3SD high or 2-3 SD low)

   Long-term in-accuracy is indicated by either a **trend or shift.** A shift or trend indicates the 'run' is out of control and patient results should not be reported until the 'problem' is resolved

   a. A **trend** is a gradual change in the mean that proceeds in one direction

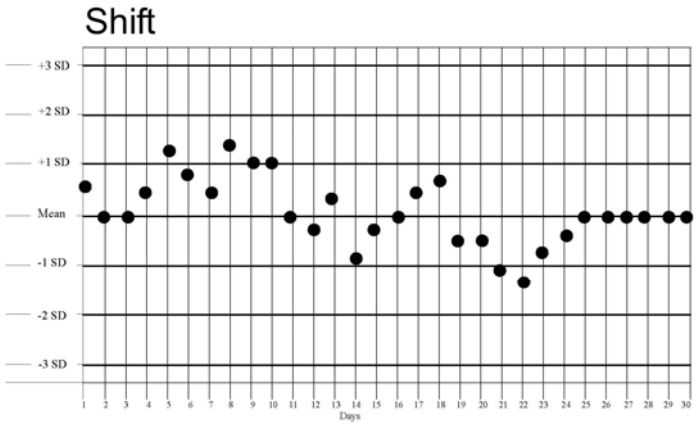


   1) Indicated by **six or more consecutive plots** being distributed in one general direction upward or downward. **This tells us there is a systematic error present** which needs to be investigated before proceeding and/or reporting patient results.

   2) **Values may cross the mean from one side to the other**

   3) A trend may be caused by a gradual:
      a) Deterioration in reagents or standard/calibrators (or expired)
      b) Deterioration in instrument performance
      c) Gradual change in pH or operating temperature
      d) Gradual change in operating temperature
      e) Deterioration of light source

b. A **shift** is an <u>abrupt change</u> in the mean that <u>becomes continuous</u>; tends to lack a general increase or decrease pattern as seen with a trend.



1) Indicated by **six or more consecutive** values distributed on **either side** of the mean or distributed **<u>on</u>** <u>the mean.</u> **This tells us there is a systematic error present** which needs to be investigated before proceeding and/or reporting patient results

2) A <u>shift upward</u> or a <u>shift downward</u> is **ended when a value is plotted on the mean**

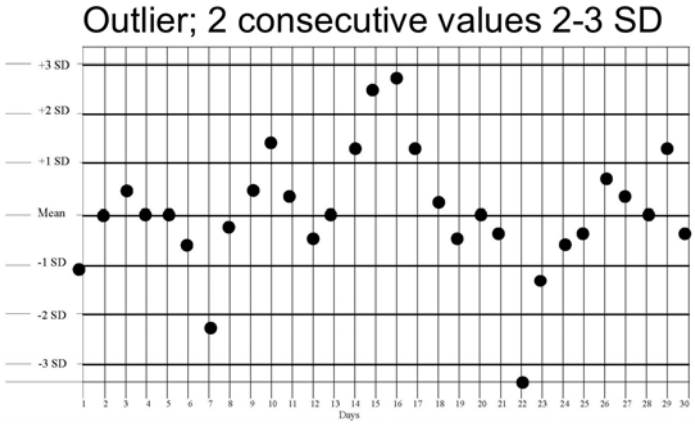
3) A shift may be caused by:
   a) Introduction of something new to the assay: new lot number of reagent, new lot number of standards/calibrators, new antibody strength
   b) Incorrect assignment of calibrator values
   c) Improperly prepared reagents or standards
   d) Inadequate storage of reagents or standards
   e) Sudden malfunction in an instrument that results in an immediate and somewhat permanent change in performance: voltage fluctuation, incubation temperature
   f) Incorrect instrument or pipette calibration that results in a change in sample or reagent volumes (pipettor misadjustments or misalignment)
   g) Change in procedure by an operator

c. An **outlier** is a value that falls outside 3SD from the mean
   1) N**ever report patient results** when control value is <u>outside 3SD</u> from the mean; the run is **<u>always</u>** rejected
   2) Caused by gross technical error: usually random (see causes of imprecision)

d. **The second of two consecutive** data points between 2-3 SD high or 2-3 SD low

1) We anticipate 1 out of 20 values to fall between 2-3 SD due to random error; so the first value that falls between 2-3 SD is okay and is used as a 'warning'. This data point does not require action and patient results may be reported.

2) If 2 consecutive values fall between 2-3 SD, this tells us there is more than just random error present causing variability. We now have a systematic error which needs to be investigated. The second value is the out of control point and requires action; patient results are not reported until the problem is resolved.



IV. QC Decisions

A. Frequency of analysis

1. **Batch assays**, manual or automated, require the use of controls **with each run**

   Random Access assays require controls be analyzed once per shift or once every 24 hours (depends on lab policy and manufacturer guidelines)

2. **Sophisticated instrumentation** with good calibration stabilities may require a specific monitoring frequency dependent upon the instrument and method
   a. Once every 60 minutes
   b. Once every 4 hours
   c. Once every 8 hours (once per shift)
   d. Once every 24 hours

3. Controls should be run
   a. When assay is calibrated to verify calibration is accurate
   b. When new batch lots of commercial reagents are received
   c. After instrument maintenance has been performed
   d. When troubleshooting patient results
   e. When troubleshooting possible instrument malfunctions

B. Statistical behavior of control values to determine if action is required:

1. Control results should fall within a range of ± 2SD of the mean, that is, within the 95.5% confidence limit (acceptable range)
   a. If the control falls within the acceptable range, then we are 95.5% confident the patient results are without error. We can accept the calibration (standard) curve as valid; we can report patient test results

   b. We expect 1 in every 20 (4.5%) of the values to be out of this range because of a chance occurrence (called random error) even though the control may be duplicating values from the target range. When this happens, we use this as a warning to watch future QC values; we do not act on this value and patient results are reported

2. The control values should be distributed evenly on either side of the mean. These values should be 'close' to the mean and show little variability. Random error occurs without any real pattern and is indicated by variability around the mean.

3. Six or more consecutive values on one side of the mean indicate a **shift.**
   A gradual increase or decrease for six or more consecutive values indicate a **trend**
   a. Systematic error is continuous and affects all results equally; patient results are not reported.
   b. This type of error requires investigation to identify the cause.
   c. After the problem is resolved, control and patient samples are re-analyzed

4. Two consecutive values cannot fall between ± 2-3SD range. When two consecutive values fall in the 2-3 SD range, the frequency of this occurrence is more than is statistically anticipated (1 out of 20) and a systematic error is present. Patient results are not reported. We must resolve the problem and then repeat analysis of the control and patient samples.

5. No value should exceed ± 3SD (the run is always rejected and patient results are not reported)
   a. Since the mean ± 3SD takes in 99.7% of the data, we can be fairly certain (incorrect <0.3% of the time) that any control value outside of this range is not duplicating the target values and indicates that an error in measurement has occurred.

   b. Random error is considered a one time event and is indicated by a control value that is significantly different from the expected value (different than the established limit, or acceptable range).

   c. The run is rejected and no patient results are reported. After the problem has been fixed, the QC and patient specimens are run again

C. Technologist Response to out-of-control values: (shift, trend, >3SD, 2-2SD)
   1. The technologist should troubleshoot the cause of the QC failure
      a. Look at historical QC data by looking at the Levy-Jennings chart for the past 30 days. Evaluate the QC charts and classify the error as random or systematic.
      b. Determine the likely cause of the error: refer to p.9 for causes of RAE; p.9-10 for SAE

      Other troubleshooting actions (not exhaustive):
      c. Check for calculation error; check for transcription error
      d. Check for obvious mechanical malfunctions of instrument
      e. Check to see if sample clots have caused an aspiration error causing invalid results
      f. Check reagents: expired? Bacterial growth?
      g. Check calibration solutions: expired? Bacterial growth?

   2. Notify supervisor of problem; may need to notify physician of possible delay

D. Recording/Plotting Daily QC Results
   1. All control (and patient) results are recorded on the worksheet

   2. All control values are then plotted on the appropriate QC chart (Levy-Jennings plot). If QC results are within acceptable range, the patient results can be reported
      a. Controls should be plotted at the time of assay and in the order of measurement
      b. Separate charts are required for each analyte AND each level of control measured

   3. All techs are responsible on a daily basis to identify and follow-up on QC problems as they occur (shifts, trends, outliers, 2-2SD)

   4. If a control value that falls outside of the $\pm$ 2SD range is accepted, notation on the Levy-Jennings chart must clearly identify the reason for accepting the questionable value

   5. Documentation of new reagents, new lot numbers of standards, new lot number of reagent, instrument repair, introduction of new calibration curve, etc, is required and helps identify causes of problems (troubleshooting)

   6. Supervisor of the department reviews all QC charts monthly

   7. All unacceptable control and patient results should appear on the worksheet. Always indicate clearly that the run was unacceptable and results cannot be reported.

   8. No worksheets are discarded

E. Worksheet documentation of results (refer to example worksheet on following page)

1. All worksheets must be dated and signed by technologist (initials)

2. Enter all information using ink pen (black or blue ball point pen preferred, do not use 'Sharpies' or felt tip markers)

3. Worksheets must be clearly & neatly written so others may understand and read it

4. Provide complete patient specimen information:
   a. Last name, first name, initial
   b. Accession or specimen identification number
   c. Date and time specimen drawn
   d. Type of specimen: serum, plasma, urine, CSF
   e. Volume of urine collection, if applicable (ex: 1850 ml/12 hr)

5. Indicate if a dilution has been made:
   a. Record dilution or dilution factor
   b. Record original 'measured value' (ABS or raw number)
   c. Record complete calculation used to calculate results based on the dilution
   d. Circle final result to be reported

6. Clearly indicate if the run is unacceptable and cannot be reported.
   All unacceptable control and patient results must appear on the worksheet

7. If error is found and corrected, cross out original information using a single 'strike through line' such that the original incorrect value is still legible, and write corrected result next to it, followed by technologist's initials

8. If results (STAT or CRITICAL) need to be communicated to attending physician or nurse in charge:
   a. Report results (and units: mg/dl, ug/dl) promptly
   b. Have results repeated back to you
   c. Note time results were called to attending on the worksheet
   d. Note name (and credentials) of person receiving results

   Note: a critical result indicates a life threatening condition exists such that the patient requires immediate medical attention

DATE/TECH: UOtten 6/10/2006 GENERAL CHEMISTRY WORKSHEET

Tube # /PATIENT NAME / Ident #	DATE/TIME of collection VOLUME of collection	Glucose- run 2							
		ABS	mg/dl						
1. Blank		0.000	------						
2. 100 mg/dl STD		0.125	100						
3. 200 mg/dl STD		0.248	200						
4. 300 mg/dl STD		0.380	300						
5. 400 mg/dl STD		0.505	400						
6. 500 mg/dl STD		0.625	500						
7. Control level 1		0.083	66						
8. Otten, Ricki #0962 **1:3**	6/9 @1825	0.322	257.6 **x 3** = 772.8 = **773 mg/dl**						
9. Control level 2		0.396	317						
10. Urine control # 557		0.855	684						
11. Otten, Ricki #1009 (urine)	6/7 –> 6/8 0830 –> 2100 685 ml/12.5 hr	1.233	~~986~~ exceeds linearity, repeat on dilution						
12. Otten, Ricki #1009 (urine) **1:5**	6/7 –> 6/8 0830 –> 2100 685 ml/12.5 hr	0.263	210.4 **x 5** = 1052.0 = **1052 mg/dl** ; 1052 mg/dl X 685 ml/12 hr X 1 dl/100 ml = 7206.2 = **7206 mg/12.5 hr**						

V. External Quality Control:
   A. Proficiency Testing
      1. Provides independent, unbiased validation of the quality of patient results

      2. Biologic samples are submitted to the laboratory for analysis by an outside agency
         Example: College of American Pathologists (CAP)

         a. Laboratory analyzes the proficiency samples 'blind' just as though they were actual patient samples, and returns the results to the agency
         b. Agency compares results of the laboratory to the known value of the sample and informs the laboratory of its accuracy (or inaccuracy)
         c. The laboratory's accuracy is also compared with other labs using the same methodology

VI. Predictive Value of Test Results
   A. Why is it imperative that test results be verified to be accurate by the use of QC?
      1. Clinicians use the data submitted by the laboratory to
         a. Aid in diagnosis of disease
         b. Determine a patient's prognosis
         c. Determine efficacy of treatment
         d. Determine relative risk of contracting disease

B. Possible test outcomes
   1. Ideal situation:
      a. All persons with disease will test positive for the disease
      b. All persons without disease will test negative for the disease
   2. Reality:
      a. All methods have an inherent amount of error present that will affect test results
      b. No method is able to detect all persons with disease accurately
         No method is able to detect all persons without disease accurately
      c. Possible outcome of testing:
         1) True positive (TP): person with disease tests positive
         2) False positive (FP): person without disease tests positive
         3) True negative (TN): person without disease tests negative
         4) False negative (FN): person with disease tests negative

	Patients with Disease	Patients Without Disease
Positive Test Result	+ (TP)	+ (FP)
Negative Test Result	- (FN)	- (TN)

   3. Test Sensitivity (also referred to as Diagnostic Sensitivity)
      a. Definition: percentage of patients who have a disease that test positive

      b. Calculation: $\frac{TP}{TP + FN} \times 100$

   4. Test Specificity (also referred to as Diagnostic Specificity)
      a. Definition: percentage of patients who do not have a disease that test negative

      b. Calculation: $\frac{TN}{TN + FP} \times 100$

   5. No test is 100% Sensitive and 100% Specific

C. Predictive Value
   1. Predictive value of a positive test result
      a. Definition: the probability that a patient with a positive test result has the disease (proportion of positive test results that are truly positive)

      b. Calculation: $\frac{TP}{TP + FP} \times 100$

   2. Predictive value of a negative test result
      a. Definition: the probability that a patient with a negative test result does not have the disease (proportion of negative test results that are truly negative)

      b. Calculation: $\frac{TN}{TN + FN} \times 100$