Slide 1

Advances in instrument and reagent technology, increasingly complex procedures, and legislative changes, focus new attention on the growing need for quality control and quality assurance procedures in the clinical laboratory. Helena Laboratories is pleased to present this slide series to assist the clinical laboratory in implementing, monitoring and interpreting quality assurance.
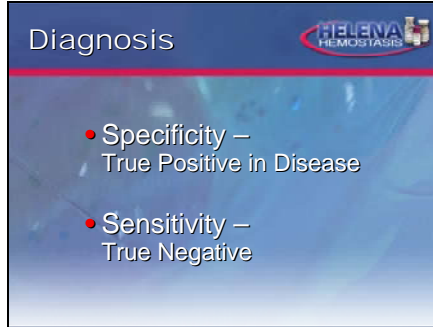
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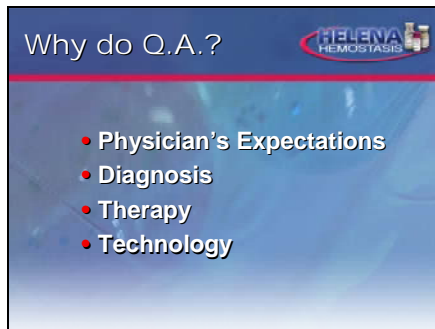
Why do we need quality assurance?
When a laboratory test is ordered, the physician expects the result to be accurate and precise. Laboratory tests are used many different ways – to aid in or establish a diagnosis, to monitor therapy and adjust dosage levels. Improper reporting of results can place extreme stress on the patient. An effective quality assurance program can help the laboratory provide the best possible test result by identifying potential problems and preventing errors.

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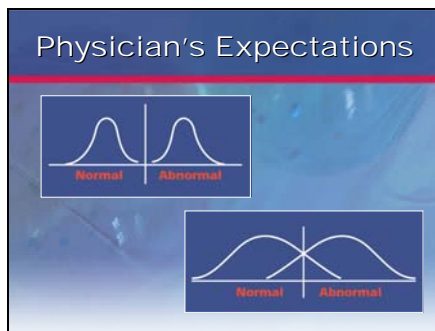
Physicians would like it if all test results fell into one of two categories – normal or abnormal. In truth, the distinction isn't that clear. Normal and abnormal reference ranges show a cross-over zone. This gray area is what makes quality control so imperative. The laboratory must be confident of the precision and accuracy of assay procedures in the borderline area.

Slide 4

Diagnosis

- Specificity –
  True Positive in Disease

- Sensitivity –
  True Negative

Interpretation of laboratory results is dependent on two interactive parameters – specificity and sensitivity. Specificity is the ability of a test to identify true positives in the presence of disease. Sensitivity is the ability to determine true negatives in the absence of disease. An ideal test would yield 100% specificity and 100% sensitivity and thus give the physician an absolute diagnosis. Most instrument/reagent systems have some specificity and sensitivity limitations. The laboratory must include quality control measures to assure test results provide the best specificity and sensitivity possible.

Slide 5

Therapy

- **Adjustment of Medication**

Laboratory tests can effect the type and scope of therapy. The course of therapy is often adjusted based on test results. In hemostasis, over or under adjustment of medications like coumadin, aspirin, or heparin, can trigger thrombotic or hemorrhagic episodes.

Slide 6

Technology

- Complex Instruments
- Complex Reagents
- Micro Techniques

Technology has changed significantly since 1935 when Dr. Quick first described a manual tilt tube procedure for prothrombin times. Hemostasis laboratories now have instruments with sophisticated optical systems and computers. Commercial reagents offer a wide array of sensitivities and specificities. Advances in specific protein biochemistry have added synthetic (or chromogenic) procedures to the mix. And platelet function testing is also a major part of today's hemostasis laboratory. Cost-containment demands have brought changes as well, including a shift to systems that use micro techniques. As the scope of hemostasis testing increases so does the need for quality control.

Slide 7

What is Q.A.?

- Samples
- Control Material
- Assay Conditions
  a. Reagents
  b. Instruments
- Daily Review
- Retrospective Peer Review

Quality assurance begins the moment a laboratory test is ordered. Specimen collections, storage and sample preparation are as important as using appropriate control materials, monitoring assay conditions (reagents and instruments), and daily review of quality control. The complexities of specimen collection and preparation merit its own discussion and are not addressed in this presentation. Selection of proper control materials to monitor instrument and reagent function is a relatively easy task for the laboratory. Where we often fall short is the utilization of daily quality control data to identify, and even circumvent problems. This series focuses on daily Q.C. review, retrospective review and peer review as they relate to evaluation of instrument and reagent performance.

Slide 8

Controls

- Similar to patient samples
- Respond like samples
- Stability like samples

Controls used in the clinical laboratory should be very similar in base protein structure to patient samples. For hemostasis testing, this usually means pooled human plasmas for normal controls and treated plasmas, e.g. heparin, or plasmas with known factor deficiencies for abnormal controls. Controls shouldn't contain excessive amounts of stabilizers or preservatives, since they need to be sensitive to the same conditions as patient samples. For example, over or under incubation of the sample, or over or under delivery of reagent, should have the same effect on patient and control. Likewise, the stability of controls should approximate that of patient samples.

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Daily Review

- Decision Rules
- Random Error
- Systematic Error

Daily review of quality control data is an absolute must. Daily evaluation of control performance should be made using a system of pre-determined decision rules. Decision rules are essential for immediate recognition of out-of-control situations and immediate corrective action. Decision rules help identify problems as systematic variance or random error, making it substantially easier to troubleshoot. Systematic variance can be described in general terms as a fluctuation in data consistently in one direction. Random error is more difficult to define, showing data fluctuations in either direction, rather than only in one. We'll go into more detail on random and systematic errors later.

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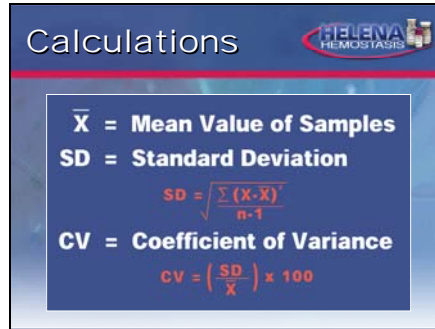
Fundamental calculations for quality control analysis include mean, standard deviation (S.D.), and coefficient of variance (C.V.).

The mean is an arithmetic average defined simply as the sum of all values divided by the number of values.

Standard deviation can be thought of as the variance or scatter of values about the mean. The formula for calculating S.D. is the square root of the sum of all the differences squared divided by the number of values minus one.

The coefficient of variance makes it possible to compare different data sets by expressing the standard deviation of each set as a percentage of the mean. The C.V. provides a measure of relative variability regardless of the units or the magnitude of the units in which the results are expressed. For example, if the C.V. for a 12 second prothrombin time control is 2%, then the C.V. for a 30 second prothrombin time control of equal quality should also be approximately 2%.

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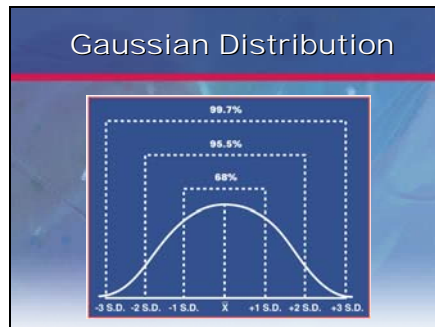
Gaussian distribution implies normal dispersal of data points on either side of the mean. In such a distribution, 68% of all values fall within one standard deviation of the mean. The ± 2 S.D. range includes 95.5% of all the data generated, and ± 3 S.D. includes 99.7% of all data generated. When plotted graphically, Gaussian distribution of data points is bell-shaped in appearance.

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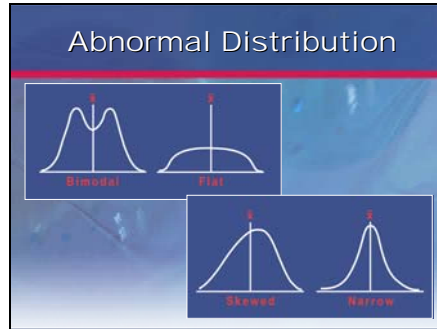
If the bell-shaped curve of Gaussian distribution is considered normal, then what does abnormal distribution look like?

Bimodal distribution shows two symmetrical curves centered on either side of the mean, each curve with approximately equal numbers of data points. For the most part, this type of distribution can be attributed to technique: one technologist blows out a pipette and the other doesn't, one shift uses a two-step pipettor and the other doesn't.

A flat curve reflecting a wide range of distribution can indicate a lack of sensitivity in the procedure.

A skewed distribution results from a greater number of values on one side of the mean complemented by a greater range of values on the other side of the mean. Skewed distribution can occur as a result of reagent deterioration or lack of linearity of the procedure.

A narrow peak reflects a disproportionate number of data points centered around the mean. This can indicate a bias such as selective retention of data.
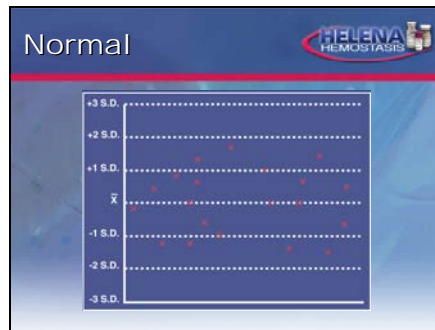
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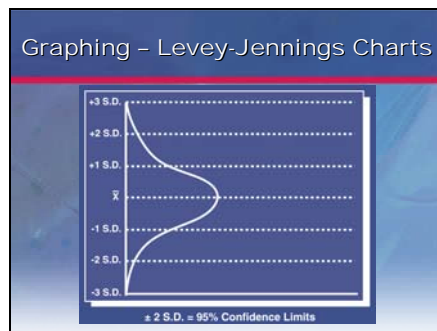
Levey-Jennings charts are frequently used to plot the distribution of quality control data. In effect, the Levey-Jennings chart is a representation of the Gaussian distribution turned at a 90° angle. The confidence limit most commonly accepted is ± 2 S.D., which, by definition, should include 95% of all the control data generated.

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Normal distribution of quality control data reflects approximately equal numbers of values above and below the mean, ranging from on the mean to almost ± 2 standard deviations.

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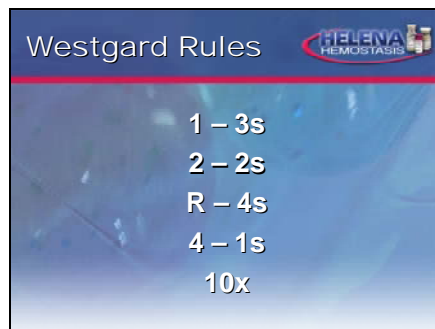
Since the 1970s, Dr. James Westgard has published theoretical models of statistical review. These models are widely used in the clinical laboratory to establish decision rules for determining out-of-control situations. The five rules we will review today are 1-3s, 2-2s, R-4s, 4-1s, and 10x.

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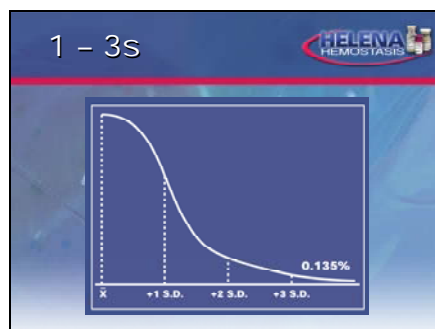
The first of the Westgard rules is 1-3s, or 1 control value outside of the 3 S.D. range. A 3 S.D. outlier can indicate significant random error. Statistically, 99.73% of all data will fall within 3 standard deviations of the mean. In other words, there's only a 0.135% chance that any given control value would fall above the 3 S.D. range or a 0.135% chance that it would fall below the 3 S.D. range. The occurrence of any 3 S.D. outlier should trigger automatic rejection of the entire run (controls and patient values).

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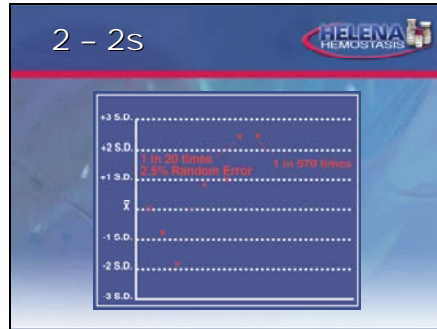
The second rule is 2-2s, or two consecutive data points outside the 2 S.D. range. Back-to-back occurrences of control values beyond two standard deviations on the same side of the mean (whether it's the same level control or two different control levels), most likely reflect systematic error and inaccuracy. Because we have chosen the 95% confidence limits, statistically there is a 5% (1 in 20) chance that a data point will fall beyond 2 S.D. The probability that two consecutive data points will fall beyond 2 S.D. on the same side of the mean is only 1 in 970.

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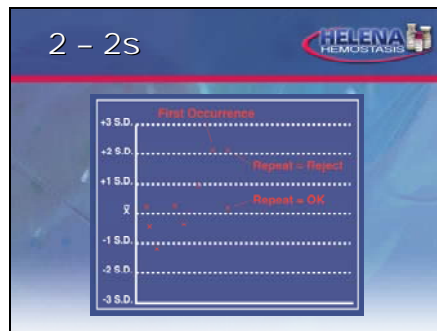
Westgard's theoretical models and most computerized quality assurance programs will accept one data point outside the 2 S.D. range (1-2s). CAP (College of American Pathologists) recommends repeat testing of any control value outside the 2 S.D. range. If the repeated result falls within the laboratory's 2 S.D. range, it is considered to be only a 1-2s failure. Patient results can be reported and both control values are used in all subsequent calculations of mean and standard deviation.

If, however, the repeated control yields a second value outside two standard deviations, the data must be considered suspect. The entire run, patients and controls, must be repeated after troubleshooting procedures have taken place.

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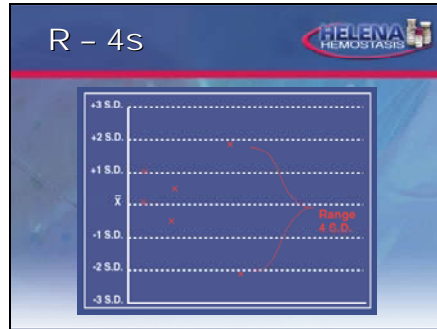
The third rule, R-4s, stands for a range of four standard deviations between two consecutive data points. This can occur with a single level control or between levels. Between levels, where one level is extremely high and the other extremely low, occurs most frequently. The run should be rejected and the source of this random error investigated. Control values of the R-4s type call into question the linearity of the procedure (a change in curve slope or curve fit has significantly lowered one value and raised the other). In enzymatic procedures, the maximum velocity of the reaction can effect low-end values; high-end values may be affected by substrate exhaustion.

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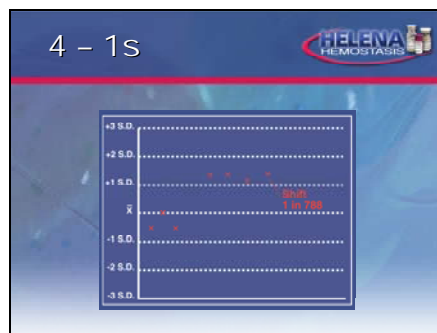
The fourth Westgard decision rule is probably the most frequently abused. The 4-1s rule addresses the occurrence of four consecutive data points outside one standard deviation, but within 2 S.D. The odds of a 4-1s phenomenon happening by pure random chance are only 1 in 788. More likely, 4-1s reflects a significant shift in the mean caused by systematic error. The 4-1s rule, also referred to as shift, applies whether the occurrence is between control levels or within a level. If a laboratory is running two levels of controls, a 4-1s shift could occur as quickly as two consecutive runs. The runs and all control values should be rejected and investigated to determine the cause – new spectrophotometer bulb, new tubing, new reagents or new calibrators. Once corrective action is taken, the run should be repeated.

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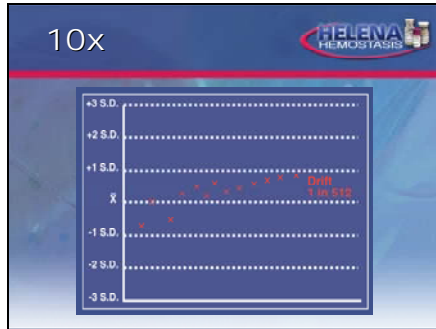
The fifth rule is 10x. Ten consecutive data points falling on one side of the mean, without having failed the 4-1s rule, is also referred to as drift. This occurrence indicates constant systematic error such as slow deterioration of reagents, calibrators, tubing, lamp source, etc. There's no dramatic change, nonetheless the cause must be identified and necessary corrective action taken.

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Beyond implementing a system of daily review and decision rules, laboratories should participate in a program for retrospective peer review. Peer review permits the laboratory to see how their values compare to other laboratories using the same instruments and reagents. Peer review reports can also be an aid in the selection of instruments and reagents. First, by simply looking at the number of users reporting results for any particular method, you get a feel for what your peer laboratories are using. Second, the mean for each method can provide an indication of the sensitivity of the method. And third, comparing the Standard Deviation Index (SDI) for various methods can indicate the precision of the method.

There are a number of peer review programs available through state and government agencies, commercial manufacturers, and agencies such as the College of American Pathologists (CAP). Data for peer review (this can include daily Q.C. data and proficiency testing data) are submitted to a computer processing center, usually on a monthly basis. The data are then analyzed. The term "retrospective" implies that analysis of data will occur with some time delay between active on-lone testing and actual review.

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SDI-Standard Deviation Index

$$\left(\frac{\text{Lab Current Mean} - \text{Group Cumulative Weighted Mean}}{\text{Group Cumulative Weighted S.D.}}\right)$$

The most valuable calculation generated through retrospective peer review is a measurement of peer precision known as the Standard Deviation Index (SDI). The SDI allows comparison of your laboratory's values to a group of laboratories using the same reagents and instrumentation. Comparison of SDIs are available for both proficiency survey specimens and routine quality control data. For routine Q.C., the laboratory's current mean is compared to the mean and S.D. of the group. In the case of a CAP survey, the laboratory's result is subtracted from the group's cumulative weighted mean and divided by the group's cumulative weighted standard deviation.

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SDI Interpretation

- Like Signs
- Spread of 1 SDI
- Reproducibility

The SDI calculation should produce a number listed as a plus (+) or minus (-) value, and ranging from 0 to 3 S.D.
How is this number interpreted? The first parameter is the plus (+) or minus (-) sign. Having like signs for different control levels, such as +0.5 SDI and +0.7 SDI, indicates that this laboratory's system is slightly higher than the group mean. Unlike signs are acceptable as long as the SDI values are within a one SDI spread. For example, a +0.25 SDI and a -0.30 are okay since the spread is only 0.55. However, +0.80 and -0.80 would be unacceptable since the spread is 1.60. Even with like signs, a laboratory that has a spread of greater than 1 SDI, i.e. +0.20 and +1.60, would indicate some sort of linearity problem. All laboratories would like to be in the 0 to 1 SDI range, however it must be kept in mind that statistics show us approximately 27% of all data falls in the 1 S.D. to 2 S.D. range. Therfore it is to be expected that 1 out of every 4 laboratories will fall in

the 1 S.D. to 2 S.D. range. Another criteria is the reproducibility of the SDI value from month to month. When compared to the same basic peer group, a laboratory with an SDI value +1.5 one month and -1.5 the next month should suspect problems with reproducibility.

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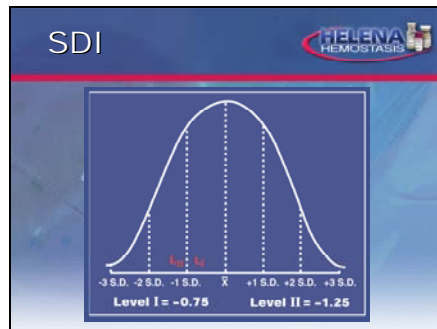
This is an example of how a SDI value might look charted. For those who are statisticians, the SDI is statistically equivalent to a Z score. In this case, the laboratory's Level I and Level II controls are in good control compared to their peer group for either proficiency testing or daily Q.C.

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"E = mc2" is an easy way to remember what our quality assurance goals should be and how we can reach these goals. Excellence is what we want from our quality assurance programs. To reach that goal, we must define and adhere to acceptable standards for methods, controls and calculations.