Parallel Testing and Reagent Lot Validation Guidelines

Background Information:

Clinical laboratory reagents and control materials are exposed to many variables due to conditions during transportation and storage environments in different laboratory settings. The validation of new reagents kits with old reagent kits is performed to ensure that, in spite of varying environmental conditions, there are no clinically significant differences in the results obtained when different lot numbers of reagents are used.

Control materials are parallel tested to ensure that the mean of the values obtained are within the ranges specified by each manufacturer. The data gained during parallel testing should then be utilized to establish QC ranges for each individual laboratory.

The procedure outlines the parallel testing and reagent lot validation testing required for different sections of the laboratory including chemistry, hematology, coagulation, flow cytometry and HIV viral load testing.

Purpose:

The purpose of this procedure is to provide a procedural template for use when developing a program for parallel testing. The procedure is intended to be used by laboratories as a guide while developing their own parallel testing procedures.

Responsible Personnel:

Responsible personnel may vary according to location but should include the following positions or their equivalents:

- Laboratory Manager or Director
- QC/QC Coordinator
- Department/Section Heads or Chief Technologist
- Staff Technologist/Technicians

Precautions:

Standard precautions should be followed when conducting parallel testing and reagent lot validation (refer to appropriate safety SOP). In addition, personnel performing the testing should use Personal Protective Equipment (PPE) that is appropriate for the task and follow all safety rules established for their institution.

Parallel Testing of Controls:

The requirements for parallel testing of controls can vary according to the test being performed. Follow the guidelines for different testing systems as outlined below. Always refer to manufacturer's and laboratory-specific recommendations and guidelines.

CBC Controls:

1. The new lot of controls should ideally be run in parallel with the old lot of controls 2-3 times for 5-8 days before the old lot # expires.

2. The mean for the new control and standard deviation for the new lot of the controls will be approved by the Laboratory Supervisor or QC/QA coordinator before the new control

is used. Labs may have other ways of establishing QC ranges see Appendix A: Establishing Quantitative QC Ranges.

3. The laboratory Director or QC/QA coordinator should review and sign off on the QC parallel testing data before the new control is put into operation.

Chemistry Controls:

1. The new control lot number should be run in parallel with the old lot number before it expires. The new control should be run a minimum of 20 times over 3-5 days or over a longer time period if possible.

2. The mean for the new control and standard deviation for the new lot of the controls should be approved by the Laboratory Supervisor or QC/QA coordinator before the new control is put into use. Labs may have other ways for establishing QC ranges see Appendix A: Establishing Quantitative QC Ranges.

3. The laboratory Director or QC/QA coordinator should review and sign off on the QC parallel testing data before the new control is put into operation.

Coagulation Controls:

1. The procedure for the parallel testing of coagulation controls is very similar to the procedure for other quantitative testing. The new control lot number should be run in parallel with the old lot number before it expires. The new control should be run a minimum of 20 times over 3-5 days but a longer period of time is recommended.

2. The mean for the new control and standard deviation for the new lot of the controls should be approved by the Laboratory Supervisor or QC/QA coordinator before the new control is put into use.

Parallel Testing of New Lots of Reagents:

All new lots of reagents should be validated by running them in parallel with the old lot numbers as indicated and the results obtained should be within the acceptability range defined.

Qualitative Immunoassays:

1. A minimum of 3 patient samples (negative, low positive and high positive if available) tested in parallel with QC on both the old and the new lot numbers.

2. The QC and patient results should be reproducible between the two lots (reproducibility includes both the OD readings and the interpretations).

3. The Laboratory Director, QA/QC Coordinator or designated technologist is responsible for defining acceptability limits for parallel testing. (Example: OD variance of 1SD or less and agreement on the interpretation).

4. Develop a form for the documentation of parallel testing that includes appropriate space for entering the following data:

- Lot numbers (old and new lot numbers) and expirations dates
- Results obtained from the old and new lots.
- Criteria for acceptance and space to indicate if the results obtained on the new lots were acceptable.
- QC values on both runs (include QC lot numbers).
- Space for the person completing the parallel testing to sign and date the form and a place for a reviewer to sign and enter the date.

Qualitative Molecular Assays (such as CT/NG):

1. A minimum of 3 patient samples should be run in parallel on both the old and the new lots.

2. The QC and patient results should be reproducible between the two lots.

3. The Laboratory Director, QA/QC Coordinator or designated technologist reviews the results and confirms that there is agreement between the results for the two kits (i.e. negative results are negative on the new kit and positive results are positive).

4. Develop a form for the documentation of reagent lot validation that includes appropriate space for entering the following data:

    - Lot numbers (old and new lot numbers) and expirations dates
    - Results obtained from the old and new lots.
    - Criteria for acceptance and space to indicate if the results obtained on the new lots were acceptable.
    - QC values on both runs (include QC lot numbers).
    - Space for the person completing the parallel testing to sign and date the form and a place for a reviewer to sign and enter the date.

Chemistry Assays:

1. New lot numbers of chemistry reagents are run in parallel with the old lot to check the performance of the new reagent.
2. A minimum of 3 patient samples should be run on the old and new lot number.
3. The QC and patient results should be reproducible between the two lots.
4. The Laboratory Director and QA/QC Coordinator or designated technologists are responsible for defining the acceptability limits for reagent parallel testing. (Suggested acceptability limits are within +/- 1SD or within+/-10%).
5. Develop a form for the documentation of chemistry reagent lot validation that includes the following data:

    - Lot numbers (old and new lot numbers) and expirations dates.
    - Results obtained from the old and new lots.

- Criteria for acceptance and space to indicate if the results obtained on the new lots were acceptable.
- QC values on both runs (include QC lot numbers).
- Space for the person completing the parallel testing to sign and date the form and a place for a reviewer to sign and enter the date

CBC Analyzer Reagents

1. Comparison of materials of known value prior to and following changing or priming of new lots or shipments is required.
2. Comparison studies must be performed before or at the same time that new reagent lots are placed in service
3. Material of known value may include a patient samples or control material.
4. Background checks must be performed on inert materials such as diluent to ensure that new lots do not interfere with patient results.
5. Develop forms for the documentation of reagent lot validation and diluent background checks that include:
    - Lot numbers (old and new lot numbers) and expirations dates.
    - Results obtained from the old and new lots.
    - Criteria for acceptance and space to indicate if the results obtained on the new lots were acceptable.
    - Space for the person completing the comparison testing to sign and date the form and a place for a reviewer to sign and enter the date
    - A place for background counts to be recorded.

Coagulation Reagents:

PT Reagents:

1. Parallel testing of a new lot of PT reagent should be completed well in advance of the expiration date of the old lot. Parallel testing of new lots of PT reagents also includes verifying the reference range, geometric mean and programming the correct ISI (international Sensitivity Index) into the coagulation analyzer.
2. To verify the reference range and geometric mean it is necessary to collect specimens from 20 “normal” patients and to run a PT with the new lot of thromboplastin reagent. 90% of the samples must fall within the current range in order to verify the range and geometric mean. If they do not, a new reference range study must be conducted to determine them. Microsoft Excel or other appropriate clinical reference range software must be used to calculate the new range and geometric mean.
3. Perform comparison studies between the old and new lot number to verify the consistency of patient results and controls. The R value for the correlation study should be >0.97.
4. Validate the PT reference range with 20 specimens. If the reference range does not validate perform a new reference range study using at least 60 specimens.
5. Finally, perform a manual check of the INR and compare with the instrument generated INR result.

PTT Reagents:

1. Parallel testing of PTT reagents should be conducted well in advance of the expiration of the old reagent.
2. Perform comparison studies between the old and new lot number using patient samples and controls. The R value for the correlation study should be R=>0.97.
3. To verify the PTT reference range it is necessary to collect specimens from 20 “normal” patients and to run a PTT with the new lot of reagent. 90% of the samples must fall within the current range in order to verify the range. If they do not, a new reference range study must be conducted to determine them. Microsoft Excel or other appropriate clinical reference range software must be used to calculate the new range.
4. Please note that if you monitor patients on heparin therapy you should perform a new heparin curve with each change of reagent lot.
5. Develop a form for the documentation of PT/PTT reagent lot validation that includes the following data:

    - ❑ Lot numbers (old and new lot numbers) and expirations dates.
    - ❑ Results obtained from the old and new lots.
    - ❑ Criteria for acceptance and space to indicate if the results obtained on the new lots were acceptable.
    - ❑ QC values on both runs
    - ❑ Space for the person completing the reagent validation testing to sign and date the form and a place for a reviewer to sign and enter the date

Semi Quantitative Tests (such as Urinalysis dipsticks):

1. A minimum of 3 patient samples are run in parallel on both the old and the new lots (The three samples should demonstrate varying results across the range for different strip analytes).
2. The QC and patient results should be reproducible between the two lots.
3. Acceptance ranges should be established by the Laboratory Director, QC/QA coordinator or designated technologist. (Generally negative results should remain negative, ,positive results should give the same results or be one level up or down from the original result)
4. Develop a form for the documentation of the reagent lot validation that includes appropriate space for entering the following data:

    - ❑ Lot numbers (old and new lot numbers) and expirations dates.
    - ❑ Results obtained from the old and new lots.
    - ❑ Criteria for acceptance and space to indicate if the results obtained on the new lots were acceptable.
    - ❑ QC values on both runs
    - ❑ Space for the person completing the parallel testing to sign and date the form and a place for a reviewer to sign and enter the date

Qualitative Testing (Rapid HIV test kits, urine/serum qualitative hCG etc.)

1. A minimum of 3 patient samples are run in parallel on both the old and the new lots.
2. The QC and patient results should be reproducible between the two lots.
3. The Laboratory Director, QA/QC Coordinator or designated technologist reviews the results and confirms that there is agreement between the results for the two lots (i.e. negative results are negative on the new lot and positive results are positive).
4. Develop a form for the documentation of reagent lot validation that includes appropriate space for entering the following data:
    - Lot numbers (old and new lot numbers) and expirations dates
    - Results obtained from the old and new lots.
    - Criteria for acceptance and space to indicate if the results obtained on the new lots were acceptable.
    - QC values on both runs
    - Space for the person completing the parallel testing to sign and date the form and a place for a reviewer to sign and enter the date

References

1. College of American Pathologists (CAP) 2021. Commission on Laboratory Accreditation, Laboratory Accreditation Program; All Common Checklist.
2. Clinical Laboratory Standards Institute (CLSI) User Evaluation of Acceptability of a Reagent Lot Change Implementation Guide. CLSI EQ26 ED2IG:2022 . Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087
3. DAIDS Good Clinical Laboratory Practice Guidelines. 08/16/2021
4. CLIA Corner Arthurs Grove N., Rotzoll, K., Bochmann, M.: CLIA Updates for Prothrombin Time and INR Testing. 2017
5. CLIA Corner, Second Quarter 2023. Requirements for Non-waived, Non-manual Coagulation Test Systems.