

MTN Manual of Operational Procedures (MOP)

APPENDIX III: Laboratory Quality Control Policy

| Prepared by | Date Adopted | Supersedes Procedure # |
|--------------------------|--------------|------------------------|
| Adapted from HPTN Policy | | N/A |
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1 SCOPE

This policy applies to laboratories performing clinical laboratory testing for the Microbicide Trials Network (MTN).

2 PRINCIPLE

Quality control (QC) is an important part of every lab test. Appropriate QC practices will maximize the accuracy of results reported as well as provide early information about potential problems. This procedure is intended to give a summary of the QC program to be followed in the laboratory. A detailed description of the QC procedures for individual assays is included in the QC sections of the individual procedures.

The laboratory recognizes that the institution and maintenance of a rigorous QC program can ensure the reliability of patient laboratory data. As the spectrum of the tests offered is broad, so are the QC procedures and the way in which data from various types of QC material are handled and presented.

3 PROCEDURES

The QC Program can be divided into the following main areas of focus:

- Internal QC — testing of known materials
- Parallel testing — validation of new controls and reagent lots
- Internal proficiency testing — blind or split-sample testing
- External proficiency testing programs
- QC monitoring — corrective action logs
- Quality assessment program
- QC through preventative maintenance programs
- QC — temperature monitoring
- QC — reagent water

3.1 Internal QC — Testing of Known Materials

Qualitative test systems include the following:

- QC of assay reproducibility is achieved by testing materials of known reactivity.
- Qualitative procedures are checked by at least one positive and one negative control.
- The frequency of controls is dependent on the manufacturer's recommendation as well as the laboratory confidence/experience with each method.
- The number of controls and the frequency of control runs are specified in each test procedure.
- The testing technologist is responsible for reviewing and recording the QC results on the assay worksheet (or equivalent).
- If the QC results are within the established guidelines and the patient test results appear valid, the testing technologist will sign and forward the results to the Laboratory Supervisor or designee for final review.
- If the QC results and patient test results are acceptable, the Laboratory Supervisor will sign and release the test run.
- All results (QC and patient) must be reviewed, evaluated and signed by the Laboratory Supervisor or designee before the patients' test results can be released.
- In the event that the Laboratory Supervisor or designee is unavailable and the release of results will be delayed, peer review is allowed for release of results. Peer review results must be documented by signature. The Laboratory Supervisor or designee review must be done as soon as possible and documented.
- If the QC results are not within the established guidelines or a potential problem is noted, the testing technologist will review the results with the Laboratory Supervisor or designee.
- All QC results must be documented, including any out-of-range results.
- Out-of-range results and follow-up action will be documented on the test-system, corrective action log.
- When a control result falls outside the established range or potential problems are noted, the Laboratory Supervisor or designee will make the final decision on the disposition of the run.
- If the run is considered invalid based on review of the QC results, all tests must be repeated.
- Patient results cannot be turned out until the QC is resolved and the test run is repeated, if necessary.

- The Laboratory Supervisor or designee will review and sign off on the corrective action logs once per month. If potential problems exist, the QC results will be reviewed more frequently.
- The Laboratory Supervisor or Director may increase the number or frequency of controls or request outside testing to resolve potential problems.

Quantitative test systems include the following:

- Quantitative procedures are checked by a low-to-high range of two to three controls, depending on the procedure.
- The frequency of controls is dependent on the manufacturer's recommendation as well as the laboratory's confidence/experience with each method.
- The number of controls and the frequency of control runs are specified in each test procedure.
- For commercial QC material, the manufacturer's ranges are used until a minimum of 20 determinations are made to establish an in-house mean $\pm 1, 2$ and 3 standard deviations or 15 percent from the mean.
- The testing technologist is responsible for reviewing and recording the QC results on appropriate QC logs. The minimum requirement will include a control log. Levy-Jennings charts are required for chemistry, hematology and potentially other testing areas.
- If the test system has an automated QC record function, the control logs and Levy-Jennings charts must be checked each time the controls are run.
- Patient samples should not be run before the controls are reviewed and found to be acceptable.
- Patient samples that are included with the control run will not be reported if the controls are unacceptable.
- If the QC results are within the established guidelines and no shifts, trends, or potential problems are noted on the Levy-Jennings charts, the testing technologist will forward the patient results to the Laboratory Supervisor or designee for final review.
- If the QC results and patient test results are acceptable, the Laboratory Supervisor will sign and release the test run. Generally, patient results are considered acceptable if all QC materials fall within the established two standard deviation ranges, or 15 percent from the mean.
- All results (that is, QC and patient) must be reviewed, evaluated and signed by the Laboratory Supervisor or designee before patient test results can be released.
- In the event that the Laboratory Supervisor or designee is unavailable and result release will be delayed, peer review is allowed for release of results. Peer review results must be documented by signature. The Laboratory Supervisor or designee review must be done as soon as possible and documented.
- If the QC results are not within the expected ranges and guidelines, the testing technologist will review the results with the Laboratory Supervisor or designee.
- All QC results must be documented, including any out-of-range results.
- Any shifts or trends must be reported to the Laboratory Supervisor. Any shifts or trends must be examined.
- Out-of-range results and follow-up action will be documented on the test-system, corrective action log.
- When a control result falls outside the established range or potential problems are noted, the Laboratory Supervisor or Director will make the final decision on the disposition of the run.
- Results may be considered acceptable after review by the laboratory supervisor or director.

- The review and consideration will be documented on the assay sheet and the corrective action log.
- If the run is considered invalid based on review of the QC results, all tests must be repeated.
- Patient results cannot be released until the QC is resolved and the test run is repeated, if necessary.
- The Laboratory Supervisor or designee will review and sign off on the QC data and corrective action logs once per month. If potential problems exist, the QC results will be reviewed more frequently.
- The Laboratory Supervisor or Director may increase the number or frequency of controls or request outside testing to resolve potential problems.

Other Test Systems include the following:

- Gram Stain:
 - Gram stain reagent and procedure will be quality-controlled each day of use by including a control slide containing gram-positive and gram-negative organisms such as *E. coli* and *Staphylococcus aureus* or equivalents.
 - These control slides may be made in-house from known cultures.
 - Acceptance criteria for the gram stain slides will be defined.
 - The slide control results will be documented on a gram stain QC log.
 - The control log will be initialed and dated by the technologist performing the QC.
 - The control log will be reviewed and signed at least once per month by the Laboratory Supervisor or designee.
 - If the control slide stain is not acceptable, check both the staining technique and the stain. Document any problems and corrective action on the gram stain corrective action log.
- Differential and/or Malaria Stain:
 - The differential stain will be checked each day of staining.
 - The first slide read after staining will be reviewed for correct color formation for the white blood cells (WBC) and red blood cells (RBC) along with excessive background debris.
 - Acceptance criteria for the differential stain will be defined and documented on the control log.
 - The control log will be initialed and dated by the technologist performing the QC.
 - The control log will be reviewed and signed at least once per month by the Laboratory Supervisor or designee.
 - If the control slide stain is not acceptable, both the staining technique and the stain will be checked. Document any problems and corrective action on the differential stain corrective action log.

3.1 Parallel Testing — Validation of New Controls and Reagent Lots

Reagent kits and controls that the laboratory uses have a limited shelf life. It is important to ensure that test kits and reagents are not used beyond their expiration date. Parallel testing of reagents or controls is done to validate the lot-to-lot variability. Contact the MTN LC for guidance on alternate procedures in cases of reagent shortages or short outdates.

HIV RNA PCR Quantitative Assay: To validate lot-to-lot variability, three patient samples (not detected, a mid-range viral load and a high viral load) are assayed on the old and the new lot

number. The Laboratory Supervisor or Director will sign off on the validity check. These results will be recorded in chart form and filed with the QC records for this assay by the Laboratory Supervisor. Any variation greater than three-fold needs to be investigated and documented.

NAAT (Trichomonas, GC, Chlamydia) Qualitative Assay: To validate lot-to-lot variability, a minimum of three patient samples (negative, low positive and high positive) are run in parallel. The patient results should be reproducible between the old and new lots. The Laboratory Supervisor or Director will sign off on the validity check. The patient samples will be marked as validation samples and filled with the other NAAT runs.

GeneXpert (GC/ Chlamydia) Qualitative Assay: To validate lot-to-lot variability, a minimum of two patient samples (negative, positive) are run using the in-use lot and new lot of reagent/kit. The patient results should be reproducible between the two lots. The Laboratory Supervisor or Director will sign off on the validity check. The patient samples will be marked as validation samples in the GeneXpert specimen log.

Complete Blood Count/Full Blood Count (CBC/FBC) Controls: To validate new CBC/FBC controls, the new lot of controls will be run in parallel with the old lot of controls for three to five days, when possible. The Laboratory Supervisor or Director will sign off on the validity check before the old lot is finished.

Chemistry Controls: To validate new chemistry controls, the new lot of controls will be run in parallel with the old lot of controls until the mean and standard deviation is obtained for the new lot of controls. The mean and standard deviations for the new lot of controls will be reviewed and signed off by the Laboratory Supervisor or Director before being put into use.

CD4/CD8 Assay: To validate lot-to-lot variability of reagents, a minimum of two patients (one with CD4/CD8 ratio <1.0, and one with CD4/CD8 ratio >1.0) are run in parallel. The patient results should be reproducible (that is, based on the manufacturer guidelines for sample-to-sample, lot-to-lot variation) between the old and new lots. The patient samples will be marked as validation samples and filled with the other CD4/CD8 runs. The Laboratory Supervisor or Director should sign off on the validity check. The patient samples will be marked as validation samples and filled with the other Flow Cytometry runs. It is also important to check expiration dates and perform lot testing on primary and secondary antibodies used for this purpose.

Chemistry and Hematology— New Reagent Lot Check In: New lot numbers of reagent must be validated before being introduced into routine use. QC should be acceptable for old and new lots. Samples should be assayed by both lots within a time period in which there has been no loss of integrity to the sample or analyte. Results should be compared to the old lot. Acceptability criteria should be set by the Laboratory Director.

3.2 Internal Proficiency Testing — Blind or Split-Sample Testing

As part of the laboratory's internal proficiency testing program, personnel proficiency testing is done periodically during the year. Coded samples, blind samples or split samples may be given to the technologists to assess the reproducibility of the assays as well as the technologist-to-technologist variability and accuracy. The Laboratory Supervisor or designee will be responsible for assigning the samples, documenting the results and reviewing the results. The acceptable range of reproducibility will be determined by test and documented on the result form.

The documentation will include the results by technologist and whether the results compared acceptability for accuracy and reproducibility. The Laboratory Supervisor and Director will sign off on the results. The results will be filed as Internal Proficiency Testing records.

3.3 External Proficiency — Testing Programs

The laboratory will participate in external proficiency panels/surveys, which are blind assessments of the laboratory's performance. Where possible, the laboratory will participate in a proficiency program for each test performed. For testing where no external proficiency program samples are available, other methods of proficiency checks will be used, if possible. Proficiency samples are tested in the same manner as any routine specimen submitted to the laboratory. All staff involved in patient testing should rotate testing proficiency samples.

The Laboratory Supervisor or designee will prepare the final result forms and send it to the testing agency in a timely manner. A copy of the final results form will be kept in the External Proficiency Testing file. When the survey results are returned, the Laboratory Supervisor and Director will review and sign the results. The Laboratory Supervisor and Director will investigate any noted deficiencies.

A written report of the findings and corrective action will be written. The Laboratory Supervisor and Director will sign this report. The report will be sent to the Laboratory Center for review. A copy of the response will be filed with the survey results.

3.4 QC Monitoring — Corrective Action Logs

Corrective action logs are maintained for each test and instrument. The logs are used to document QC results that fall outside the established ranges and inconsistency in results or problems with the test system (for example, reagents, controls, instruments or equipment). The testing technologist is responsible for documenting any problems and corrective action taken on the corrective action log for that test system. The Laboratory Supervisor or designee is to be notified immediately of any problems and will review the corrective action. The logs provide valuable information for troubleshooting test methods or instrument problems. The Laboratory Supervisor or designee will review and sign off on the corrective action logs once per month.

3.5 Quality Assessment Program

The main purpose of the Quality Assessment Program (QAP) is to evaluate the quality of work provided by each section of the laboratory. The QAP is another tool for monitoring potential problem areas of the laboratory that might not be detected by the Quality Control Program. Refer to the Quality Assessment Policy Procedure for more details.

3.6 QC through Preventive Maintenance Program

Instrument Maintenance: All instruments used in the laboratory follow a preventive maintenance program based on the manufacturer's recommendations. Documentation of the instrument maintenance, calibration, service, and corrective action logs is generally found in the equipment logbooks in each area. The bench technologist maintains these records. These records are reviewed and signed monthly by the Laboratory Supervisor or designee.

Equipment Maintenance: Routine maintenance on laboratory equipment is performed according to the manufacturer's recommendations. The technologist performing the maintenance documents the maintenance and results. The Laboratory Supervisor or designee reviews and signs off on the maintenance records monthly. Documentation of the equipment maintenance is generally found in the laboratory Maintenance Manual.

Preventive maintenance, monitoring or calibration, at minimum, covers the following equipment:

- Precision pipette calibration
- Centrifuge calibration (for example, rpm, timer and temperature, if applicable)
- Thermometers
- Timers
- Plate washers
- Plate readers
- Thermocyclers
- Incubators/water baths
- Biological/fume hoods

3.7 QC — Temperature Monitoring

All temperature-sensitive equipment, such as freezers, refrigerators, water baths and incubators, must be monitored on a daily basis. All test work areas and reagent storage areas must be monitored on a daily basis (that is, room temperature monitoring where equipment and testing is done, as well as where room temperature reagents are stored). Temperature charts must include the name of the equipment (if applicable), the location, the acceptable temperature range, space to record the actual temperature and the initials of the person recording the temperature and date. The temperature chart may include a comments/corrective action section. The charts should be reviewed on a monthly basis by the Laboratory Supervisor or designee.

3.8 QC — Reagent Water

The following procedures and specifications are for the testing of water that has been purified for clinical laboratory use. There are three grades of water recognized, with the minimum specifications for bacterial count for each.

Type I: Used for the preparations of solutions and reagents (EIA testing) requiring minimum interference and maximum precision and accuracy (10cfu/ml)

Type II: Used for general laboratory testing other than described above

Type III: Used for glassware washing, but not final rinsing, and for feed water for the production of higher-grade water

The preferred water is Type I, distilled, deionized water. If this is not available, distilled water can be used and sterilized, if necessary. Refer to the Water Procedure in the Maintenance Manual for details.

ATTACHMENTS

A: Quality Control Testing Summary

B: Corrective Action Log

ATTACHMENT A: QUALITY CONTROL TESTING SUMMARY

| Test | Quality Control | | Proficiency Program | | Parallel Testing | Comments |
|--|-----------------------------|------------|---------------------|---------|------------------|---|
| | Material | Frequency | CAP | UKNEQAS | | |
| CBC | Low, Normal, High | Daily | X | | Overlap Controls | Calibrate per manufactures instructions or every 6 mos. |
| Differential | Stain Check | Daily | X | | | |
| ESR | Low/High | Daily | X | | | |
| Malaria Smear | Stain Check | Daily | X | | | |
| CD4/CD8 | Manufacturer Controls | Daily | X | X | Reagents | |
| Chemistry | Minimum 2 levels | Daily | X | | Overlap Controls | Calibrate per manufacturer instructions or every 6 mos. |
| HIV-1/2 EIA | Kit controls | Lot | X | | Reagents | |
| HIV-1/2 Rapid | Commercial or In-House | Run | X | | | |
| HIV-1 Western Blot | Kit: Neg/Weak to Strong Pos | Run | X | | | |
| HIV Viral Culture | | Buffy Coat | | | | |
| HIV-1 P24 Ag | Kit controls | Run | X | | Reagents | |
| Urinalysis | Commercial, Normal/Abn | Daily | X | | | |
| Urine Microscopic | Commercial, Normal/Abn | Daily | X | | | |
| Urine Pregnancy | Commercial, Neg/Pos | Kit | X | | | |
| HIV RNA PCR QT Kit Controls, Neg/L-H Pos | | Run | X | | Reagents | |
| HIV RNA PCR QL | Kit Controls, Neg/Pos | Run | | | Reagents | VQA |

| | | | | | | |
|---------------------|-----------------------------|--------------|---|--|----------|--|
| GC, Chl PCR QL | Kit Controls, Neg/Pos | Run | X | | Reagents | |
| Bacteriology | In-house Organisms/Reag. | Weekly | X | | | |
| Gram Stain | Stain Check | Daily | X | | | |
| Media | Media Check | Per Lot | | | | |
| | | | | | | |
| Storage-Pla, Ser | Self Audit | As Needed | | | | |

ATTACHMENT B: CORRECTIVE ACTION LOG

CORRECTIVE ACTION/REMARKS LOG FOR INSTRUMENT/TEST SYSTEM

| Date | Problem/Comments | Initials | Corrective Action/Comments | Initials | Date |
|------|------------------|----------|----------------------------|----------|------|
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