ALABAMA COUNTY HEALTH DEPARTMENT LABORATORY SYSTEMS

POLICIES AND PROCEDURES MANUAL

CLIA #01D0665512

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Laboratory Director

Prepared by:
Bureau of Clinical Laboratories
Quality Management Division
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Policies and Procedures Manual

Alabama County Health Department Laboratory Systems

CLIA ID Number

01D0665512

For

_____ County Health Department

This manual contains policies and procedures that outline laboratory services for the Alabama County Health Department Laboratory Systems (ACHDLS) overseen by the Quality Management Division of the Bureau of Clinical Laboratories. When these policies and procedures are observed, Clinical Laboratory Improvement Amendments (CLIA) requirements are met.

All pages of this manual are to be kept together and updated per notice from the director of the ACHDLS.

The mission of the Quality Management Division of the Bureau of Clinical Laboratories is to promote excellent laboratory practice through the use of training sessions; on-site review and consultation; and competency evaluations.

Sharon P. Massingale 12/23/14

Sharon P, Massingale, Ph.D., HCLD (ABB) Date
Director of
Alabama County Health Department
Laboratory Systems

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Charlene A. Thomas, BS, MT (ASCP) Date
Manager and Technical Consultant of
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Ashley Megelin 12/23/14

Ashley Megelin, BS Date
Technical Consultant with
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12/23/14

Eric Seales, Ph.D Date
Technical Consultant with
Alabama County Health Department
Laboratory Systems

_____

Area Nursing Director Date

_____

County Clinic Nurse Supervisor Date

_____

Area STD Supervisor Date

_____

Date

ALABAMA
COUNTY HEALTH DEPARTMENT
LABORATORY SYSTEMS
(ACHDLS)

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Glossary of Laboratory Terms

GLOSSARY OF TERMS

1. **ANALYTE** - A substance or constituent for which the laboratory performs testing.

2. **ACCURACY** - State of quality of being accurate; closeness of test results to the true value and implies freedom from error; also referred to as bias.

3. **CALIBRATION** - Process by which the readings obtained from an instrument or other measuring device in an analytical process are related to known concentrations.

4. **CALIBRATOR** - A material, solution, or lyophilized preparation designed to be used in calibration. The values or concentrations of the analytes of interest in the calibration material are known within limits ascertained during its preparation and determined by the use of an analytical method of stated reliability.

5. **CLIA** - The Clinical Laboratory Improvement Amendments of 1988; the federal regulatory program governing all laboratory testing.

6. **CONTROL** - Essentially identical specimens of stable materials, usually similar in composition and physical properties to natural specimens, subjected to the same analytical process for surveillance control (monitoring) to estimate the performance characteristics (accuracy, precision) of the analytical process. The control materials are designed to be used in the quality control process and are not used for calibration purposes. The concentration of the analytes of interest in the control material is known within limits ascertained during its preparation and confirmed in use.

7. **KIT** - All components of a testing system (or unit) that are packaged together.

8. **LABORATORY** - A facility for the biological, microbiological, serological, chemical, immunohematological, hematological, biophysical, cytological, pathological, or other examination of materials derived from the human body for the purpose of providing information for the diagnosis, prevention, or treatment of any disease or impairment of, or of the assessment of the health of human beings. These examinations also include procedures to determine, measure, or otherwise describe the presence or absence of various substances or organisms in the body. Facilities only collecting and/or preparing or only serving as a mailing service and not performing testing are not considered laboratories.

9. **MEAN** - A number that represents an entire set of numbers, determined for the set in any number of ways; average.

10. **PERFORMANCE CHARACTERISTIC** - A property of a test that is used to describe the quality, e.g., accuracy, precision, analytical sensitivity, analytical specificity, reportable range, reference range, etc.

11. **PERFORMANCE SPECIFICATION** - A value or range of values for a performance characteristic established or verified by the laboratory that is used to describe the quality of patient results.

12. **PRECISION** - State or quality of being precise; freedom from inconsistency or random error; closeness with which repeated determinations agree with each other and implies freedom from variation; also referred to as reproducibility.

13. **PREVENTIVE MAINTENANCE** - A program of scheduled inspections of laboratory instruments and equipment resulting in minor adjustments or repairs for the purpose of delaying or avoiding major repairs and emergency or premature replacements.

14. **PROFICIENCY SURVEY** - A program in which specimens of quality control material are periodically sent to members of a group of laboratories for analysis and comparison of each laboratory's results with those of other laboratories in the group through some central organization. Participation in a proficiency survey does not replace the day-to-day quality control process of an individual laboratory.

15. **QUALITY MANAGEMENT** - Sum of all those activities in which the laboratory is engaged to ensure that information generated by the laboratory is correct. Quality management is not restricted to the development and retention of quality control charts but rather includes all aspects of laboratory activities that affect the results produced, from the choices of methods, to the monitoring of instruments, to the education of personnel, to the handling of specimens, and to the reporting of results. The true purpose of quality management activities is to determine how correct, or incorrect, the results emanating from the laboratory are, and to allow those managing the laboratory to determine whether or not the lab is fulfilling its function satisfactorily.

16. **QUALITY CONTROL** - Procedures performed to measure and maintain the quality of performance in the analytical laboratory through measurement of the variability against pre-established criteria specifications, correction as indicated, and documentation. The major purpose of these programs is to establish analytical goals and to assist in their achievement.

17. **REPORTABLE RANGE** - The range of test values expected for a designated population of individuals.

18. **SAMPLE** - In proficiency testing, means the material contained in a vial, on a slide, or other unit that contains material to be tested by proficiency testing program participants. When possible, samples are of human origin.

19. **STANDARD DEVIATION** - The most common measure of statistics, measuring how widely spread the values in a data set are dispersed.

20. **TARGET VALUE** - For quantitative tests, means either the mean of all participant responses after the removal of outliers (those responses greater than three (3) standard deviations from the mean) or the mean established by definitive or reference methods acceptable for use by the National Reference System for the Clinical Laboratory (NRSCL) by the National Committee for Clinical Laboratory Standards (NCCLS).

Quality Management

QUALITY MANAGEMENT

Quality management (QM) is an ongoing process encompassing all facets of the laboratory's technical and nontechnical functions. This includes patient preparation and specimen acquisition (preanalytical), test analysis or examination (analytical), and test result reporting (postanalytical). QM also extends to the laboratory's interactions with and responsibilities to patients, physicians, and the Alabama Department of Public Health.

I. General Quality Management Policies

A. Quality in the entire test system is of foremost importance.

B. All laboratory personnel must be trained properly, commensurate with their positions, duties, and responsibilities.

C. The Alabama County Health Department Laboratory Systems (ACHDLS) will maintain a quality control system to assure continued precision and accuracy of laboratory results.

D. The ACHDLS will participate in the Centers for Medicare & Medicaid Services (CMS) approved proficiency testing (PT) program.

II. The Quality Management Program

The policies and procedures of the quality management program will be approved by the laboratory director when first written, with notation of approval by signature and date. The technical consultants will review the policies and procedures on a regular basis. If a policy or procedure requires a change, a new policy or procedure will be written, approved by the laboratory director, and distributed to testing sites. The old policy or procedure will be retained in a file at the Bureau of Clinical Laboratories, County Assistance Section, for a minimum of two years.

III. Components of the ACHDLS Quality Management Program

A. Patient Test Management

The laboratory director, with the assistance of the technical consultants, will monitor and evaluate laboratory information recorded in the Complete Health Record (the patient chart used in the Alabama county health departments). A monthly "CLIA Lab Report" will be requested for the chart review. Chart review will be conducted monthly by a technical consultant and documented for the selected service area's county health departments with at least 10% of one month's charts examined. Records will be maintained in the County Assistance Section of the Quality Management Division at the Bureau of Clinical Laboratories. Any errors in documentation will be addressed and brought to the attention of the Area Nursing Directors and the Director of the Laboratory for correction.

B. Procedure Manuals

A written procedure manual containing procedures for all activities of the ACHDLS will be maintained and readily available at all times to personnel in each testing site.

The manual will be reviewed annually by the laboratory director and/or the technical consultants.

C. Quality Control (QC) Assessment

1. Quality control records will be reviewed annually by a technical consultant. This review is conducted electronically and during onsite visits to all county health department sites and is documented on the County Visit Worksheet. Documentation of these reviews will be maintained in the County Assistance Section of the Quality Management Division at the Bureau of Clinical Laboratories.

D. Training Assessment (See Quality Management Training Schedule on pages 6-7)

1. The technical consultants are responsible for assessing, performing, and documenting the training and competency of the testing staff of the ACHDLS. All laboratory testing, under CLIA, is categorized as waived or nonwaived.
   a. Waived tests performed by the county health department laboratories: Hemoglobins by *HemoCue* Method, Occult Bloods by InSure FIT, HCG by Pregnancy Test, Strep A Rapid Test, and Urine Dipstick. All tests are to be performed per product insert and no competency is required for testing staff.
   b. Nonwaived tests performed by the county health department laboratories: Darkfield Microscopy, Rapid Plasma Reagin (RPR), and Wet Prep Microscopy. Workshop trainings and competency precedes county health department laboratory staff performing nonwaived testing.
   Note: All nonwaived testing staff must provide a copy of their high school diploma per CLIA requirements.
2. New nonwaived testing personnel must have competency twice during their first year of training, then annually thereafter.
3. Nonwaived workshop trainings are presented twice a year at the Bureau of Clinical Laboratories.

E. Proficiency Testing

ACHDLS will participate in a CMS-approved PT program on regulated analytes. PT results will be reviewed by testing personnel and technical consultants, verifying that all information is correct and complete before they are mailed, faxed, or e-mailed to the PT program. Investigations of unsatisfactory PT results (performance that does not result in 100% of acceptable responses for each analyte, including graded, ungraded, and unregulated analytes) will be documented by testing personnel and reviewed by technical consultants and the laboratory director.

F. Comparison of Test Results and Method Validation

Tests performed at multiple sites will be evaluated once a year. Any test performed for which PT is not available will be verified at least once a year, and the results will be

reviewed and evaluated by the laboratory director or his/her designee. The results of these evaluations are documented and maintained in the County Management Section for each site.

G. Relationship of Patient Information to Test Results
Laboratory personnel will monitor test requests for appropriateness to the patient's age, sex, and diagnosis. If any test request or result appears inappropriate, proper consultation should be obtained.

H. Personnel Assessment
An ongoing evaluation of all testing personnel will be conducted through use of proficiency testing results, review of quality control records, Complete Health Records, observation and annual competency evaluations for nonwaived testing procedures. These include wet preps, RPRs, and darkfield microscopy. If, during competency evaluations, an employee is found to be incompetent for one or more procedures, the employee will not be able to perform the test(s). If (s)he is still not competent after reevaluation, the employee cannot perform that testing until (s)he has attended training for that procedure and deemed competent.
1. Blind Specimens - All personnel who successfully pass RPR competency will receive "blind specimens" quarterly. Blind specimens will be comprised of RPR liquid controls in screw top vials submitted to testers by Hackbarth couriers. Personnel must run the controls in the same manner as patient specimens and report the results back to QM before the end of the testing period. Specific instructions are submitted to the testers via email and results are graded either Satisfactory or Unsatisfactory. Corrective action for personnel receiving an unsatisfactory result two quarters in a row will be evaluated individually and may require retraining. To remain certified to run RPR's, staff must run these blind specimens quarterly.

I. Communications
Problems that occur as a result of breakdowns in communication between testing personnel and the authorized individual who orders or receives the results of tests will be documented. In addition, corrective actions taken to resolve problems and minimize communication breakdowns will be documented.

J. Complaint Investigations
Investigation of complaints will be made and corrective actions, when necessary, will be taken. Documentation will be maintained.

K. Quality Management Review with Staff
Quality issues (e.g., unacceptable PT results, ways to improve the quality of testing, problems identified with QC) will be brought to the attention of ACHDLS personnel through use of official memorandums and technical bulletins. Documents will also be maintained in the Quality Management Office.

L. Recordkeeping in the Laboratory

1. CLIA'88 regulations require that all laboratory records be retained for at least two years.
2. Examples of laboratory records include:
    a. Temperature charts used to document refrigerator, freezer, incubator, or room temperatures.
    b. Quality control records.
    c. Proficiency testing records.
    d. Documentation of employee competency.
    e. Equipment maintenance.
    f. Patient test results [e.g., patient logs].
    g. Quality management records.
3. Records of laboratory testing must include:
    a. Patient identification.
    b. Date of testing.
    c. Test performed.
    d. Test results, with units of measure if applicable.
    e. Time of testing (required on CHR).
    f. Initials of person performing the test (nonwaived testing only).
4. Documentation of specimens unacceptable for testing will be maintained in the patient's CHR.

M. Laboratory Log

1. The Laboratory Log is designed to assure follow-up on laboratory tests referred from the county health department to other laboratories. The Laboratory Log is also a method to track when a specimen leaves the county health department and the date the report is received. This tracking system for referred specimens is required by CLIA.
2. This tracking system will include patient name, identification number, date of service, tests referred, and date the report was received. See "Alabama County Health Department Laboratory Systems Laboratory Log".
3. Instructions for the Laboratory Log:
    a. Place the page number in the space provided.
    b. Enter the date in the space provided.
    c. Place the label (PHALCON) indicating the patient identity on the sheet. Be sure to correct the service date if labels were preprinted.
    d. Indicate the referred tests on the lines provided. Use 1 line per test (i.e., For multiple testing such as TV, indicate numbers 1, 5 and 18). If you have more than 6 tests per patient, use the next 6 lines and mark out the space for the label. A list of numbers corresponding to tests most commonly referred is printed at the bottom of the log. Any other tests should be written.
    e. Indicate the date the referred laboratory test report is received in the CHD.
    f. Have a system devised to locate any test results not received in a timely manner.

IV. Training Schedule

A. Classes

1. Rapid Plasma Reagin (RPR): One-day class for the macroscopic screen for syphilis.
2. Vaginal Wet Mount: Two-day class to include Brightfield Microscopy and identification of Vaginal Wet Mount elements.
3. Darkfield Microscopy for the Detection and Identification of *Treponema pallidum*: Two-day class to include Brightfield Microscopy, Darkfield Microscopy and Identification of *Treponema pallidum.*

B. Reminders

1. Lecture information and procedures on all of the above classes are found in the Alabama County Health Department Laboratory Systems Policies and Procedures Manual as well as on the Bureau of Clinical Laboratories'Quality Management website.
2. A competency test is administered after each training session followed by a second competency test in approximately 6 months. Thereafter, annual competency testing is administered.
3. All classes are held in the training laboratory at the Bureau of Clinical Laboratories in Montgomery.
4. Class size is limited to 12 participants per session.
5. If an employee has previous testing experience (vaginal wet mount, darkfield microscopy, and/or RPR) at another agency, a request can be made for him/her to take the competency test after reviewing the Alabama County Health Department Laboratory Systems Policies and Procedures Manual. If a score of 80% or greater for vaginal wet mount and/or darkfield microscopy is achieved, (s)he can perform the test without training from Quality Management. A score of 90% with all reactives identified is passing for RPR.
6. Classes are approved for nursing continuing education credits (CEs).

C. Faculty

1. Charlene Thomas
2. Ashley Megelin
3. Eric Seales

D. Training Schedule

Class	**Date**	**CE Hours**
RPR	Spring/Fall	5.5 contact hours
Vaginal Wet Mount	Spring/Fall	6.25 contact hours + 5.25 contact hours
Darkfield Microscopy	As Needed	6.25 contact hours + 6.25 contact hours

ALABAMA COUNTY HEALTH DEPARTMENT LABORATORY SYSTEMS LABORATORY LOG **Page_____________**

Date____________________ The Laboratory Log is designed to assure follow-up on all laboratory tests referred from the county health department to outside laboratories. This log is also a mechanism to track when a specimen leaves the CHD and if the report is received in a timely manner.

PHALCON LABEL	TESTS REFERRED	DATE RECEIVED	PHALCON LABEL	TESTS REFERRED	DATE RECEIVED

1.GC 2.PAP 3.Syphilis EIA 4.HIV 5.Chlamydia 6.MCH-FP 7.Urine C&S 8.PKU 9.Galactosemia 10.CAH 11.T4 12.TSH 13.Abn Hgb 14.Lead 15.SGOT 16.CBC 17.TB Profile 18.TV

 Any other referred test must be written in.

Laboratory Personnel in the County Health Departments

LABORATORY PERSONNEL IN COUNTY HEALTH DEPARTMENTS

The Clinical Laboratory Improvement Amendments of 1988 (CLIA'88) require the designations of laboratory director, clinical consultant, technical consultant, and testing personnel for the Alabama County Health Department Laboratory Systems. A current list of individuals functioning as the laboratory director, technical consultant, and clinical consultants is included at the end of this section. Testing personnel are persons in the local county health departments who perform any laboratory testing (e.g., nurses, clinic nurse aides, nurse practitioners, physicians, disease intervention specialists, laboratory technicians, and nutritionists).

I. Responsibilities of Laboratory Director

The laboratory director is responsible for the overall operation and administration of the laboratory, including the employment of personnel who are competent to perform test procedures, record and report test results promptly, accurately, and proficiently and for assuring compliance with the applicable regulations. Some responsibilities may be reapportioned to the technical consultants; however, the laboratory director remains responsible for assuring that all duties are properly performed.

The laboratory director must be accessible to provide onsite, telephone, or electronic consultation as needed. The laboratory director must:

A. Ensure testing systems used for each of the tests performed provide quality laboratory services for all aspects of test performance, which include preanalytic, analytic, and postanalytic phases of testing.

B. Ensure that the physical plant and environmental conditions of the laboratory are appropriate for the testing performed and provide a safe environment in which employees are protected from physical, chemical, and biological hazards.

C. Ensure that test methodologies selected have the capability of providing the quality of results required for patient care.

D. Verification procedures are adequate to determine the accuracy, precision, and other pertinent performance characteristics of the method.

E. Laboratory personnel are performing test methods as required for accurate and reproducible results. (This responsibility has been delegated to the technical consultants.)

F. Ensure that the laboratory is enrolled in a CMS approved PT program for the testing performed and that:
   1. Proficiency testing (PT) samples are tested as required by CLIA'88 regulations.
   2. Results are returned within the time frames established by the PT program.

3. All proficiency testing reports received are reviewed by the appropriate staff to evaluate the laboratory's performance and to identify any problems that require corrective action.
4. An approved corrective action plan is followed when any PT results are unacceptable or unsatisfactory. (This responsibility has been delegated to the technical consultants.)

G. Ensure that quality control and quality management programs are established and maintained to assure quality of laboratory services and to identify failures in quality as they occur. (This responsibility has been delegated to the technical consultants.)

H. Ensure the establishment and maintenance of acceptable levels of analytical performance for each test system. (This responsibility has been delegated to the technical consultants.)

I. Ensure that reports of test results include pertinent information required for interpretation. (This responsibility has been delegated to the technical consultants.)

J. Ensure consultation is available to the laboratory's clients on matters relating to the quality of the test results reported and their interpretation concerning specific patient conditions. (This responsibility has been delegated to the technical consultants.)

K. Employ a sufficient number of laboratory personnel who possess appropriate education with either experience or training to provide consultation, properly supervise and accurately perform tests and report test results in accordance with CLIA'88 regulations.

L. Ensure that prior to testing patients' specimens, all personnel have the appropriate education and experience, receive the appropriate training for the type and complexity of the services offered, and have demonstrated that they can perform all tests reliably to provide and report accurate results. (This responsibility has been delegated to the technical consultants.)

M. Ensure that policies and procedures are established for monitoring testing personnel who conduct preanalytical, analytical, and postanalytical phases of testing to assure that they are competent and maintain their competency to perform test procedures and report test results promptly and proficiently, and whenever necessary, identify needs for remedial training or continuing education to improve skills.

N. Ensure that an approved procedure manual is available to all personnel responsible for any aspect of the testing process. (This responsibility has been delegated to the technical consultants.)

O. Specify in writing the responsibilities and duties of each consultant and person engaged in the performance of the preanalytic, analytic, and postanalytic phases of testing that identifies which examinations and procedures each individual is authorized to perform, whether supervision is required for specimen processing, test performance,

or results reporting, and whether consultant or director review is required prior to reporting patient test results. (This responsibility has been delegated to the technical consultants.)

II. Responsibilities of Technical Consultant

The technical consultant is responsible for the technical and scientific oversight of the laboratory. The technical consultant is not required to be onsite at all times testing is performed; however, (s)he must be available to the laboratory on an as needed basis to provide consultation via onsite, telephone, or electronically. In addition to the laboratory director responsibilities delegated to the technical consultant, other responsibilities are:

A. Selection of test methodology appropriate for clinical use of the test results.

B. Evaluation of testing facilities at any time to ensure testing personnel and procedures, including the precision and accuracy of each test and test system, are in compliance with CLIA ’88 Regulations.

C. Enrollment and participation in a CMS approved proficiency testing program commensurate with the services offered.

D. Establishing a quality control program appropriate for the testing performed; establish parameters for acceptable levels of analytic performance and ensuring that these levels are maintained throughout the testing process from the initial receipt of the specimen through sample analysis and reporting of test results.

E. Resolving technical problems and ensure that remedial actions are taken when test systems deviate from the laboratory’s established performance specifications and ensure that test results are not reported until all corrective actions have been taken and the test system is functioning properly.

F. Identifying training needs and assuring that each individual performing tests receives regular in-service training and education appropriate for the type and complexity of the laboratory services performed.

G. Evaluating the competency of all testing personnel and assuring that the staff maintains its competency to perform test procedures and report results promptly, accurately, and proficiently. The procedures for evaluation of the competency of the staff must include, but are not limited to:
   1. Direct observation of routine test performance, including patient preparation, if applicable, specimen handling, processing and testing.
   2. Monitoring the recording and reporting of test results.
   3. Reviewing intermediate test results or worksheets, quality control records, PT results, preventive maintenance and function checks.

4. Assessment of test performance through testing previously analyzed specimens, internal blind testing of samples or external proficiency samples, and assessment of problem solving skills.

H. Evaluating and conducting the performance of employees responsible for moderate complexity testing at least semi-annually during the first year the employee tests patient specimens. Thereafter, evaluations must be performed at least annually unless test methodology or instrumentation changes, in which case, prior to reporting patient results, the employee's performance must be reevaluated to include the use of the new test methodology or instrumentation.

III. Responsibilities of Clinical Consultants

A. Be available to provide clinical consultation to the laboratory's clients.

B. Be available to assist clients in ensuring that appropriate tests are ordered.

C. Ensure that reports of test results include pertinent information for specific patient interpretation.

D. Ensure that consultation is available and communicated to clients on matters related to the quality of test results and interpretation of results concerning specific patient conditions.

IV. Responsibilities of Testing Personnel

Testing personnel performing waived and nonwaived tests are responsible for specimen processing, test performance, and for reporting test results. Employees perform only those nonwaived tests that are authorized by the laboratory director (or his/her designee) and require a degree of skill commensurate with the individual's education, training or experience and technical abilities.

Each employee performing nonwaived testing must:

A. Follow the laboratory's procedures for specimen handling and processing, test analyses, reporting and maintaining records of patient results.

B. Maintain records demonstrating that PT samples are tested in the same manner as patient samples.

C. Adhere to the laboratory's quality control policies and document all quality control activities (e.g., instrument and procedural calibrations and maintenance).

D. Follow the laboratory's established corrective action policies and procedures whenever test systems are not within the laboratory's established acceptable levels of performance.

E. Be capable of identifying problems that may adversely affect test performance or reporting of test results and either must correct the problems or immediately notify the technical consultant or clinical consultant.

F. Document all corrective actions taken when test systems deviate from the laboratory's established performance specifications.

V. Laboratory Supply Evaluation

The Laboratory Supply Evaluations are used to ensure that laboratory reagents, media, test kits, and other laboratory supplies are maintained in adequate volumes and are used before the expiration dates. See "Laboratory Supply Evaluation".

A. It is the responsibility of the clinic supervisor/coordinator or designee to perform reviews and document the observations.

B. It is the responsibility of the clinic supervisor/coordinator to investigate problems.

C. "Laboratory Supply Evaluation" documentation is to be compiled and retained in the clinic by the clinic supervisor/coordinator for two years. See "ACHDLS - Quality Management Section III.L".

D. Technical consults will examine the "Laboratory Supply Evaluation" documentation during visits or may request copies be sent to their offices periodically.

VI. Monthly Laboratory Reviews

Monthly Laboratory Reviews are used to monitor and investigate potential noncompliance in the clinic's Quality Management System. See **"**Monthly Laboratory Equipment and Supply Review".

A. It is the responsibility of the clinic supervisor/coordinator or designee to perform these reviews and document observations at the end of each month.

B. It is the responsibility of the clinic supervisor/coordinator to investigate problems.

C. Monthly laboratory review documentation is to be compiled and retained in the clinic by the clinic supervisor/coordinator for two years. See "ACHDLS - Quality Management Section III.L".

D. Technical consults will examine laboratory monthly review documentation during visits or may request copies be sent to their offices periodically.

VII. Competency Evaluations

Employees responsible for nonwaived testing in the County Health Departments (CHD) will not perform testing until they have been certified by the laboratory director as being competent to perform testing. Competency will be evaluated at least semiannually during the first year the employee tests patient specimens. Thereafter, evaluations will be performed annually for each laboratory test a person performs. Competency evaluations can be performed onsite by technical consultants (TC) or in conjunction with approved workshops. Documentation of all activities will be maintained in the County Assistance Office of the Quality Management Division.

The competency evaluation process will also be used to assess training needs. As needs are identified, training will be provided to testing personnel.

VIII. Criteria Used for Competency Evaluation

A. Criteria have been defined for evaluating the competency of testing personnel.

B. All phases of laboratory testing (preanalytic, analytic, and postanalytic) will be reviewed. Evaluation methods include direct observation, record review, and testing unknown specimens.
   1. Direct observation - Personnel will be observed performing testing, QC, reporting, function checks, and maintenance to determine if written procedures are followed.
   2. Record review - Quality control and maintenance records will be reviewed for accuracy and completeness. In addition, documentation of "out of control results" and corrective actions taken to correct problems will be reviewed to assess problem solving skills. Patient records will be reviewed to determine if test reports include initials of testing personnel, date and time of testing.
   3. "Unknowns" - In addition to participation in PT programs, "unknowns" (previously analyzed specimens, internal blind testing samples or pictures)**,** will be used to evaluate testing personnel. Testing personnel should maintain a passing score of 80% or above with the **exception** being RPR which requires a score of 90% or greater and **all** reactives discerned.

C. Evaluation activities are documented on Competency Evaluation forms specific to the procedure being evaluated or completed using the Learning Content Management System (LCMS) available through the ADPH website. Completed competency evaluations are maintained for at least two years by the County Assistance Section of the Quality Management Division and are filed electronically according to the employee's Public Health Area and then by name.

D. Certificates are presented to personnel who successfully complete competency evaluations. Copies of these certificates are placed in the employee's electronic competency file.

IX. CLIA'88 Training Requirements of Testing Personnel

CLIA'88 Regulations require that individuals perform only those nonwaived tests that require a degree of skill commensurate with the individual's education, training or experience, and technical abilities. Elements included in training must ensure that each individual has the skills required for:

A. Collecting specimens properly, including patient preparation, and if applicable, labeling, handling, preservation or fixation, processing or preparation, transportation and storage of specimens.

B. Implementing all standard laboratory procedures.

C. Performing each test method and properly using instruments.

D. Performing preventive maintenance, troubleshooting and calibration procedures related to each test performed.

E. Working knowledge of reagent stability and storage.

F. Being aware of the factors that influence test results.

G. Assessing and verifying the validity of patient results through the evaluation of QC sample values prior to reporting patient test results.

X. Delegation of Responsibilities

A. The Laboratory Director of the Alabama CHDLS is Sharon P. Massingale, Ph.D.

B. Technical Consultants are:
   1. Charlene Thomas
   2. Ashley Megelin
   3. Eric Seales

C. The Clinical Consultant is Mary McIntyre, Ph.D.

LABORATORY SUPPLY EVALUATION

CIRCLE YOUR RESPONSE

COUNTY _____ LOCATION_____ YEAR _____

HAVE EXPIRATION DATES OF LABORATORY SUPPLIES SUCH AS MEDIA, REAGENTS AND TEST KITS BEEN CHECKED?

MONTH	***JAN***	***FEB***	***MAR***	***APR***	***MAY***	***JUN***	***JUL***	***AUG***	***SEP***	***OCT***	***NOV***	***DEC***
	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
DATE												
INITIALS												

HAVE EXPIRED LABORATORY SUPPLIES (MEDIA, REAGENTS AND TEST KITS) BEEN DISCARDED?

MONTH	***JAN***	***FEB***	***MAR***	***APR***	***MAY***	***JUN***	***JUL***	***AUG***	***SEP***	***OCT***	***NOV***	***DEC***
	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
DATE												
INITIALS												

N/A = No Expired Supplies, nothing to discard

COMMENTS: _____

_____

_____

_____

_____

_____

_____

Monthly Laboratory Equipment and Supply Review

County Health Department_____ Year _____

By signing and initialing this form, the staff member attests that he/she has reviewed all equipment according to the requirements stated in the *Alabama County Health Department Laboratory Systems Policies and Procedures Manual*. Make use of the back of this form to document problems, noncompliance issues, and comments.

Printed Name: _____ Signature: _____ Initials: _____

Printed Name: _____ Signature: _____ Initials: _____

Printed Name: _____ Signature: _____ Initials: _____

Printed Name: _____ Signature: _____ Initials: _____

Printed Name: _____ Signature: _____ Initials: _____

Laboratory Supply Evaluation: *Laboratory Supply Evaluation* has been started and all laboratory testing kits and supplies are within acceptable dates.

Lab Supply Evaluation	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE
Date / Initials						
	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
Date / Initials						

Refrigerators: *Annual Temperature Chart* has been started for the current calendar year with temperatures documented every day the clinic is open. Temperatures are within the acceptable range, corrective action has been performed & documented, and no food is present.

Refrigerators	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE
Date / Initials						
	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
Date / Initials						

HemoCue: *HemoCue Maintenance Chart* and *Annual Temperature Chart* have been started for the current calendar year with maintenance/temperatures documented each day of use. Daily & monthly cleanings have been documented. Corrective action has been performed & documented when instrument(s) malfunction.

HemoCue	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE
Date / Initials						
	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
Date / Initials						

Monthly Laboratory Equipment and Supply Review

County Health Department_____ Year _____

Centrifuge: *Centrifuge Preventive Maintenance* chart has been started for the current calendar year, a cover for the centrifuge is provided, and monthly cleanings have been documented.

Centrifuge	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE
Date / Initials						
	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
Date / Initials						

Wet Mount Microscope: *Microscope Maintenance Chart* has been started for the current calendar year, a cover is provided, and daily & monthly cleanings have been documented. Wet Mount supplies (KOH, saline, ph paper, lens paper and cleaner) are present and not contaminated. The last annual cleaning date has been documented.

WM Microscope	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE
Date / Initials						
	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
Date / Initials						

RPR Rotator: *RPR Rotator Preventive Maintenance* has been started for the current calendar year. All daily, monthly, and quarterly cleanings & checks have been documented.

RPR Rotator	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE
Date / Initials						
	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
Date / Initials						

Darkfield Microscope: *Microscope Maintenance Chart* has been started for the current calendar year, a cover is provided, and daily & monthly cleanings have been documented. Darkfield supplies (immersion oil, lens paper and cleaner) are present and not contaminated. The last annual cleaning date has been documented.

Darkfield Microscope	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE
Date / Initials						
	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
Date / Initials						

Incubator: *Incubator Preventive Maintenance* and *Annual Temperature Chart* have been started for the current calendar year with maintenance & temperatures documented each day of use. Temperatures are within the acceptable range and corrective action has been performed & documented. Climate control bags are present.

Incubator	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE
Date / Initials						
	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
Date / Initials						

Monthly Laboratory Equipment and Supply Review

County Health Department_____ Year _____

County Clinic Personnel

Date: _____

CHD: _____ Site: _____

Clinic Contact: _____

List ALL personnel performing laboratory testing, including: nurses, clinic aides, nutritionists, and disease intervention specialists. When changes occur, please update the form and send a copy to the Quality Management Office, Bureau of Clinical Laboratories in Montgomery.

Name	Title

PLEASE PRINT OR TYPE NAMES AND TITLES ON THIS FORM FOR EASIER READING

Laboratory Safety

LABORATORY SAFETY

Laboratory workers will be aware of safety at all times and protect themselves and others. Further information may be found in the recent publications of the Alabama Department of Public Health's *Infection Control Guidelines Manual* and *Bloodborne Pathogens Exposure Control Plan.*

I. Biological Hazards

A. Use protective coverings when handling blood or other body fluids which may contain blood or mucous membranes. For example:
   1. Cover street clothes with a lab coat.
   2. Protect eyes, nose, and mouth with a full face shield or safety goggles and mask.
   3. Wear gloves.
   4. Wear closed toed shoes.

B. Wash hands thoroughly after removing gloves, handling laboratory specimens, and before leaving the laboratory area.

C. Remove protective clothing and gloves before leaving the lab area to prevent contamination of other work areas.

D. Do not eat, drink, smoke, chew gum, apply cosmetics or handle contact lenses in the laboratory area.

E. Avoid hand contact to mouth, nose and eyes while working in the laboratory area. Keep pencils and instruments away from mouth.

F. Keep immunizations and skin tests up to date: Hepatitis B vaccine, tuberculin skin test (chest x-ray as indicated), etc.

G. Treat all specimens as potentially infectious.
   1. Take care not to spill specimens or create aerosols.
   2. Use extreme care when removing rubber stoppers to avoid spatters and aerosols.

H. Handle infectious waste separately from non-infectious waste.
   1. Dispose of all sharp objects properly into a puncture resistant container. Do not recap, break, or otherwise manipulate contaminated needles.
   2. Seal tightly and properly dispose of full puncture-resistant containers.
   3. Disinfect contaminated disposables before discarding. (e.g., Soak in 1:10 dilution of household bleach before discarding).
   4. The best solution to the disposal of medical waste is to contract with a medical waste management company.

I. Practice centrifuge safety.

1. Never open the lid while the centrifuge is in operation. Allow the centrifuge to completely stop before opening to avoid contact with broken sample tubes and to avoid injuring hands/arms.
2. Clean up all spills immediately with an appropriate disinfectant.

J. Clean up biological spills as soon as possible. Follow the steps listed below.
   1. Use puncture resistant gloves for sharp objects involved in the spill.
   2. Absorb the spill with paper towels or other appropriate absorbent material. Using two pieces of stiff cardboard as shovels, carefully scoop the soaked towels and debris into an appropriate biohazard waste container.
   3. Clean the site with soap and water until all visible material has been removed.
   4. Disinfect the area with a freshly prepared 1:10 bleach solution or other appropriate disinfectant. This may be done by covering the area with absorbent paper towels and flooding the towels until they are "glistening wet." Allow the disinfectant to set briefly, then blot up the disinfectant soaked towels and place into an appropriate waste container.
      Note: Bleach solution should be prepared fresh each day of use because it loses its germicidal effect upon prolonged sitting.
   5. Rinse the area well with water.

II. Chemical Hazards

A. Know the chemical with which you will be working. Consult the Safety Data Sheet (SDS) from the manufacturer before you begin to use the chemical to determine the physical and health hazards associated with it. For example:
   1. Does it contain hazardous ingredients?
   2. What are its physical/chemical characteristics?
   3. Are there fire and explosion hazards?
   4. Are there health hazards?
   5. What are the first aid procedures?
   6. What should be done if the chemical is spilled?
   7. How should the chemical be stored?

B. Know how to protect yourself when using chemicals.
   1. Contain the chemical within the work area.
      a. Cover the work area with absorbent paper towels.
      b. Choose a work area with adequate ventilation.
   2. Maintain a barrier between the potentially hazardous chemical and yourself by wearing protective clothing such as a lab coat, safety goggles, gloves, etc.
   3. Be prepared to safely clean up accidental spills. (Consult the SDS.)
   4. Assess the size of the spill, e.g., What is the exposure potential during clean up? Large spills may have an added risk of serious inhalation exposure.

C. Large spill example: A gallon jug of bleach was dropped and the side split, spilling the entire contents on the floor.

1. This spill could create enough toxic vapors to possibly damage the lungs of the individual exposed long enough to clean up the spill.
2. Evacuate the area.
3. Open the doors and windows to ventilate the area.
4. Limit the exposure time of each individual by having several people take turns containing and absorbing the spill.

D. Small spill example: A small puddle of bleach landed on the counter while measuring it out for a 1:10 solution.
   1. The spill is very small and easy to manage. Possible skin irritation would be the hazard most likely encountered.
   2. Maintain the spill within the area where the accident occurred.
   3. Wear protective clothing as applicable.
   4. Use absorbent towels to contain, carefully wipe up, and properly dispose of the debris.
   5. Rinse the area well to remove all traces of the chemical. (In some instances, such as strong acids or bases, this would involve a neutralizing agent).

III. Mechanical Hazards

A. Use proper lifting techniques, putting the weight on the leg muscles to avoid back strain.

B. Avoid frayed electrical cords and overloaded outlets to prevent an electrical fire.

C. Any electrical equipment that produces a “tingle” when touched should be disconnected and sent for repair.

D. Keep electrical cords coiled and away from sink areas to prevent instrument displacement and electrical shock.

E. Use caution signs to prevent falls when floors are wet.

F. Keep walkways free of articles such as pens and paper clips that could cause unsure footing.

G. Avoid exposed sharp corners such as open drawers and cabinet doors.

H. Avoid carrying sharp objects without protective coverings.

I. FOLLOW ALL LOCAL FIRE AND SAFETY CODES.

IV. References

A. Balows, Alvert. Manual of Clinical Microbiology, 5th Edition, American Society of Microbiologist, Washington, D.C., 1991.

B. Baron, Ellen Jo and Finegold, Sydney M. Bailey and Scott's Diagnostic Microbiology, 8th Edition, Mosby Co., St. Louis Co.

C. Kent, P.T. and Kubica G.P. Public Health Mycobacteriology: A Guide for the Level III Lab., Centers for Disease Control PHS, HHS, Atlanta, GA, 1985.

D. Kentucky Department for Health Services, Division of Laboratory Services. Recommended Laboratory Safety Measures for County Health Departments/Centers, 1987.

E. Kentucky Labor Cabinet, Division for Education and Training. Hazard Communication Program, 1988.

F. National Committee for Clinic Laboratory Standards. Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue, Volume 9, Number 1, 1989.

Miscellaneous Equipment

MISCELLANEOUS EQUIPMENT

NOTE: ANY EQUIPMENT NOT IN USE IS TO BE PLACED IN STORAGE.

I. General Purpose Centrifuges

A. In general, centrifuges are used as filtration and/or packing devices for the separation of components within a liquid or from a liquid medium, e.g., separating plasma or serum from the cellular components of blood. Adequate and proper separation without damage to the components depends upon proper spin (centrifugal force produced by the speed and length of the radial arm and the time the material is spun). Proper balance, lubrication, and rotor function are also important in producing the desired result. Ensuring proper instrument function with regular maintenance is absolutely essential.

B. General purpose centrifuges place a centrifugal force upon fluids to separate out cellular components. The centrifugal forces increase with the radial arm length and increasing speed. Usually the radial arm length is fixed, so most centrifuges increase centrifugal forces by increasing the speed. Speeds of 5,000 revolutions per minute (RPMs) are generally adequate for most laboratory purposes. Normograms for calculation of relative centrifugal force (RCF) are available. The RCF can be calculated by using the following formula:

$$RCF = 28.38\ (r)\ [n/1000]2$$

Where r = rotor radius in inches
n = revolutions per minute (RPM)
RCF is expressed in gravities (g)

The RCF is important to know because different centrifuges with different rotor radii will result in different RCFs when the RPM is equal.

C. Components of a centrifuge include:
1. The chamber which encloses the head and centrifuge tubes.
2. A cover.
3. The shaft and rotor (which turns the head).
4. The motor and drive assembly which impart the force to the shaft and rotor to create the desired centrifugal force.
5. A control panel with a power switch.

The centrifuge may also have a braking device, speed controls, a timer, or a fuse. Some larger centrifuges may be equipped with a tachometer.

When tubes of blood are subjected to the proper centrifugal force, the serum and clot separate. If a serum “separator” is in the tube, it will come to reside between the serum and the cells and cover the cells completely to prevent further cell-serum contact. This must be performed without damage to the cellular components to prevent release of materials such as potassium from the cells. For routine serum separations, clotted blood is centrifuged for 10 minutes at an RCF of 850 to 1000g.

D. Function Checks: See "Centrifuge Preventive Maintenance"
   1. RPM – RPMs will be checked with a tachometer annually and after repairs. The manufacturer's instructions should be followed when operating the tachometer. Documentation of RPM checks will be maintained.
   2. Timer – If the centrifuge is equipped with a timer, it will be checked at least annually against a standard time source. The results must be recorded and any correction factors posted on the unit. If a correction factor is necessary, the time should be checked against multiple times (e.g., 1 minute through 10 minutes) and corrective factors posted for each time. When used to check a constant packed cell volume, multiple times will be checked to the point (and beyond) where constant packing is achieved to ensure that the constant packing will always be obtained even though the timer might not be functional to the desired degree.
   3. Speed Control – If the centrifuge has a speed control, settings must be checked, verified, and performed at the same time the RPMs are checked. All regularly used speeds should be checked. The readings must be recorded along with any of the settings. If correction factors are critical, the correction factors will be posted with the settings.

E. Maintenance
   Always unplug the centrifuge from its electrical source before conducting preventive maintenance, cleaning, and/or inspection. Use only recommended cleaners.
   1. Spillage – Every spill will be cleaned up at the time of its occurrence to avoid materials getting into the mechanism. Biological substances will be handled with universal precautions. Be particularly cautious when cleaning up broken glass. See section: Laboratory Safety, I. J. of this manual.
   2. Unusual previously unnoted noises and/or vibrations – Noises and vibrations should be listened/looked for with each use of a centrifuge. Appropriate steps will be taken to determine the cause if they are noted. Damage to the centrifuge and/or the user may result if vibrations or unusual noises are allowed to continue.
   3. Cleaning the exterior and interior – The exterior should be cleaned each day of use. Any spills will be cleaned up immediately. The chamber should be cleaned monthly with soap and water followed by an adequate rinse. An appropriate germicidal and virucidal (with minimal residue) disinfectant-type solution should be used as the rinse. Gaskets must be washed and checked for wear and/or defects. When tube shields and/or cups are removed and cleaned, make certain that all rubber cushions and shields are replaced to maintain proper balance.
   4. Inspection of the head, head shaft, and coupling – Inspect these components for evidence of wear, cracks in fitting, corrosion, uneven wear, and/or signs of fatigue. Immediately replace any part found to be unacceptable.
   5. Rotor balance – Follow the manufacturer's instructions to check the rotor balance when any unusual vibrations occur.
   6. Brushes, bearings, and commutators – Brushes will be checked as indicated in the manufacturer's manual. Replacement is generally indicated if they have worn down by 5/16 to ½ inch or to within ¼ inch of spring depending upon the motor.

New brushes must slide freely in the holder but yet maintain spring tension to keep them in good contact with the commutator.

a. The commutator must be checked for scratches and/or dirt. Check for anything that impedes brush contact. In normal operation, a fine brush of sparks is seen where the brushes contact the commutator. If this is excessive, the commutator may need cleaning and/or smoothing. Seek help from an electrical service. The commutator and brush holders must be kept free of oil, dust, and dirt to prevent electrical arcing.
b. Always replace the centrifuge brushes oriented as you found them. The curved surfaces of the brush must be oriented to match the curved surface of the commutator prior to insertion. After new brushes have been installed, allow the centrifuge to operate a few hours so the brushes may get accustomed prior to routine use. Keep spare brushes available.

7. Power supply – Refer to the manufacturer's instructions for a lubrication schedule. Some centrifuges may not need further lubrication.
8. Gaskets, seals, mounts, and lubricants – Observe these for signs of excessive wear, corrosion, and/or fatigue. Rubber gaskets and mounts may be lightly coated with a light petroleum jelly.

II. Thermometers

A. Mercury column thermometers have a relatively thin-walled cylindrical bulb of mercury sealed to a length of capillary tubing. An increase in temperature causes the mercury in the bulb to expand and rise higher in the capillary. A temperature range and proper use depend on the type of thermometer available.

1. Periodically inspect each thermometer carefully for cracks in the capillary or bulb. Check the mercury column for separations. If separations are found, the column may be reunited by using one of the following methods:
   a. Immerse the thermometer bulb in an ice-salt mixture until all mercury is drawn into the bulb. Hold the thermometer in a vertical position, tap it gently to dislodge gas bubbles, and allow it to warm to room temperature.
   b. Hold the thermometer vertically in your fist so that the bulb is in the center of the palm of your hand. If the mercury begins to move, continue until column is reunited.
   c. Firmly hold the thermometer horizontally at arm's length and swing it downward in a circular motion. Do not use a snapping movement. For thermometers with ring tops, attach a string and carefully swing the thermometer in a circular motion.
   d. If all else fails, carefully warm the bulb over a low burner flame. Tilt the thermometer slowly back and forth so that the mercury is gradually forced into the expansion chamber. Set the thermometer in a vertical position for cooling. The column should recede united.
2. After successfully reuniting the mercury column, check the accuracy of the thermometer as outlined in the following procedure.

B. Calibration of thermometers
   1. When placed in service, the accuracy of each thermometer will be checked against an NIST-traceable thermometer. Mercury column thermometers should keep their calibration unless the mercury separates; after reuniting the mercury column, the thermometer should be calibrated. Correction factors should be noted on the thermometer and on the temperature chart. Recorded temperatures will be those obtained after the correction factor has been used. Thermometers with a correction factor greater than 1°C are not acceptable and will not be used.
   2. Because of the cost of an NIST thermometer, thermometers checked against an NIST will be used to calibrate laboratory thermometers. NIST-traceable thermometers may be purchased from suppliers of laboratory equipment.
      a. Obtain an NIST-traceable thermometer (formerly referred to as an NBS-traceable thermometer).
      b. Place the thermometer being calibrated and the NIST-traceable in the refrigerator or incubator in which the thermometer will be routinely used. If the thermometer is to be used for obtaining room temperatures, simply place the NIST-traceable thermometer next to the thermometer to be calibrated. Allow time for the temperature to stabilize.
      c. Read the temperature on both thermometers.
      d. Record readings on the "Thermometer Calibration Log", and make note of the correction factor, if any.
      e. If the correction factor is greater than $\pm$1°C, the thermometer will be replaced with one that reads within $\pm$1°C of the NIST-traceable.
      f. Correction factors will be noted on the thermometer and on temperature charts.
      g. When recording temperatures, the true temperature (thermometer reading $\pm$ the correction factor) will be recorded.

C. Placement of thermometers
   Refrigerators and incubators may have "cold spots" or "hot spots". Windows and air vents may cause these same conditions in rooms. Therefore, it is important to place thermometers in locations which reflect the most consistent temperature of the area. Periodically changing locations of thermometers for short periods of time is useful in locating "cold spots" and "hot spots" so that these areas can be avoided.

III. Room Temperature: See "Annual Temperature Chart"

When performing RPR testing, the room temperature should be 23°C - 29°C (74°F - 84°F). The Hemocue requires a room temperature of 18°C to 30°C (64°F to 86°F). The room temperature of areas where reagents are stored will be maintained with the temperature range defined by the reagent manufacturer. This range is noted on the package insert.

IV. Laboratory Refrigerators

Biological or chemical materials in conjunction with food and drinks designed for human consumption will not be stored in the same refrigerator.

Note: Frost-free refrigerators will not be used if reagents or materials are temperature sensitive.

A. Function Checks
   1. Read and record internal temperature – The desired temperature range is 2°C to 8°C (36°F-46°F).
      a. A thermometer should be placed inside the refrigerator in a location where it is protected and easily read without being disturbed.
      b. A temperature chart can be attached to the front or side of each refrigerator or in a log book.
      c. Temperatures will be checked and recorded on the appropriate chart each day the clinic is open.
      d. If the temperature is not within the range of 2°C to 8°C, actions will be taken to remedy the problem (e.g., adjust the thermostat).
         i. If the thermostat is adjusted, recheck the temperature.
         ii. If the temperature is still not within the acceptable range and adjusting the thermostat has not solved the problem, move the contents of the refrigerator to another refrigerator and contact a repairman.
         iii. Dispose of materials which could have been damaged (e.g., items which were frozen or were stored for a long period of time at temperatures above 8°C).
         iv. Record corrective actions on the temperature chart.
   2. Check door gasket – Ensure that the door gasket seals properly when the door is closed. If the door does not seal properly and gasket appears to be adequate, the refrigerator may not be level.

B. Maintenance
   1. Defrost unit – If the refrigerator is not frost-free, defrost the unit approximately every three months. If frost buildup is a problem, defrosting may be required more frequently. Follow the manufacturer's instructions for defrosting.
   2. Periodic cleaning – The interior should be decontaminated periodically. The exterior will be cleaned, when necessary, with a damp cloth and mild soap.

V. Microbiology Incubators

The purpose of a microbiology incubator is to promote the growth of microorganisms such as *Neisseria gonorrhoeae*. It is constructed to maintain a temperature of 35°C – 37°C (95°F – 98.6°F).

A. Function Checks: See "Annual Temperature Chart and Incubator Preventive Maintenance"
   1. Read and record internal temperature:
      a. A suspended thermometer is to be placed inside the incubator in a location where it is protected and easily read without being disturbed.
      b. A temperature chart will be posted on the incubator or kept in a log book.

c. Temperatures will be checked and recorded on the temperature chart each day that the clinic is open and testing materials/specimens are in the incubator.
d. If the temperature is not within the range of 35°C to 37°C, actions will be taken to remedy the problem (e.g., adjust the thermostat).
   i. If the thermostat is adjusted, recheck the temperature.
   ii. After allowing time for the temperature to change, read the temperature again.
   iii. Continue adjusting the thermostat and monitoring the temperature until an acceptable temperature is obtained and maintained.
   iv. If this cannot be accomplished, contact a repair person.
   v. Document all corrective actions on the temperature chart.
   vi. If necessary, relocate the contents of the incubator to protect them from extremely high temperatures.

2. Check door gasket – Ensure that the door gasket seals properly when the door is closed.

B. Maintenance

Clean interior of incubator – The interior of the unit will be cleaned at least monthly.

1. Empty the incubator, and clean the interior with a warm solution of water and appropriate disinfectant.
2. Rinse the interior with clean water and dry thoroughly.
3. Removable parts may be washed in warm, sudsy water. Avoid soap-filled pads or metal scouring pads.
4. Return parts and contents to the incubator.

VI. References

A. College of American Pathologist. Laboratory Instrument Evaluation Verification and Maintenance Manual, 4th edition (ISBN 0-930304-35-7), 1989.

B. National Committee for Clinic Laboratory Standards. Temperature calibration of water baths, instruments, and temperature sensors – second edition; Approved Standard. NCCLS Document 12-A2. Villanova, Pa.: NCCLS, 1990.

Centrifuge Preventive Maintenance

County _____ **Year** _____

Site _____ **State Serial #**_____

Monthly maintenance consists of unplugging the centrifuge, cleaning the exterior and interior of the centrifuge with a suitable disinfectant, and drying thoroughly. After cleaning, plug the centrifuge back into the power source. If the centrifuge is not in use, monthly cleaning is not necessary. Centrifuge must have a cover in use during operation. Document on the chart when the centrifuge is not in use.

MONTHLY	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC
Clean Exterior and Interior												
Date												
Initials												

Function Checks below will be performed by the Technical Consultant annually.

RPM's _____ Acceptable _____ Unacceptable _____

Time on centrifuge _____

Time on stopwatch _____ Acceptable _____ Unacceptable _____ (3 minutes ± 30 seconds)

Date _____

Technical Consultant Signature _____

CORRECTIVE ACTION LOG:

Date	Problem	Resolution	Initials

Write NIU when not in use.

County: _____ Site: _____ **Annual Temperature Chart** Room/Equipment #:_____ Year: _____

Check scale used (°F) __ or (°C) __

Circle equipment and acceptable range used: Incubator 95°F -99°F (35°C - 37°C) Refrigerator 35°F - 46°F (2 - 8°C) Freezer 5°F or colder (-15°C or colder) or Room 64°F - 86°F (18°C - 30°C)

Date	Jan	Intls	Feb	Intls	Mar	Intls	Apr	Intls	May	Intls	June	Intls	July	Intls	Aug	Intls	Sept	Intls	Oct	Intls	Nov	Intls	Dec	Intls
1																								
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Corrective Action Log on Back

Legend: S = Saturday/Sunday, H = Holiday, NIU = Not In Use

FOOD OR BEVERAGE IS PROHIBITED FROM STORAGE IN CLINIC REFRIGERATORS/FREEZERS AT ALL TIMES!

Temperature Corrective Action Log

Date	Temp Adj	Intls	Recheck Temperature (°C)
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DATE	COMMENT

Incubator Preventive Maintenance

County _____ **Year** _____

Site _____ **State Serial #** _____

MONTHLY	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC
Clean Exterior and Interior												
Check Door Gasket for Proper Seal												
Insure Latch Secures Properly												
Date												
Initials												

Climate control bags must be properly sealed when used in the incubator. Please note on the "Corrective Action Log" below if problems occur. No response recorded indicates that there have been no climate control bag issues.

CORRECTIVE ACTION LOG:

Date	Problem	Resolution	Initials

Write NIU when not in use.

Waived Tests

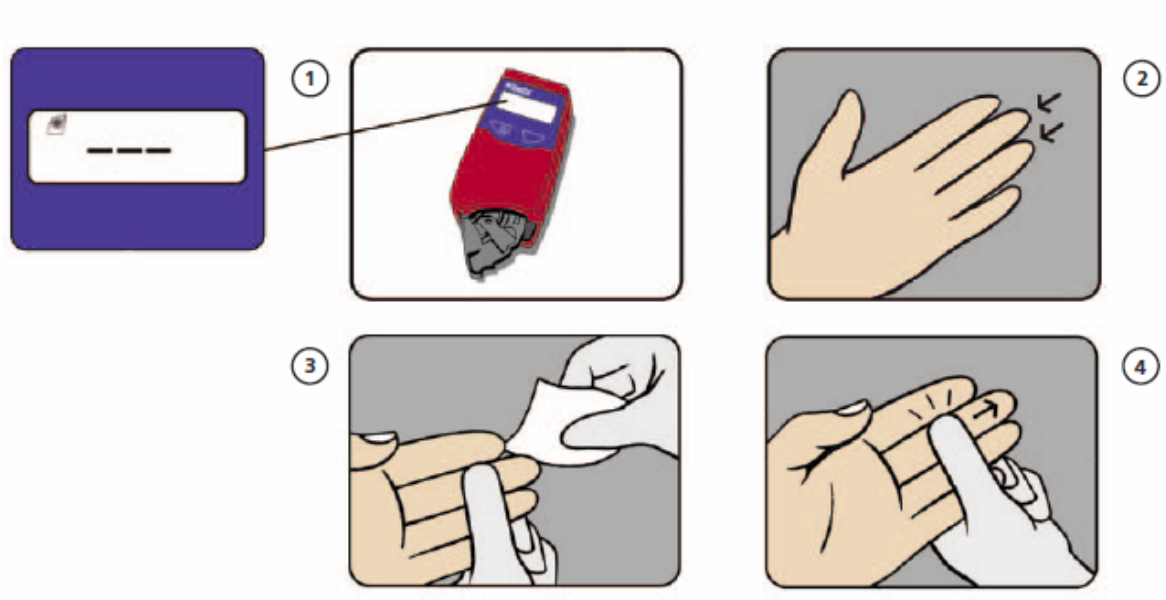
WAIVED TESTS
PERFORMED BY ALABAMA COUNTY HEALTH DEPARTMENT LABORATORY SYSTEMS

I. Hemoglobin detection with the HemoCue Hb 201+ Analyzer

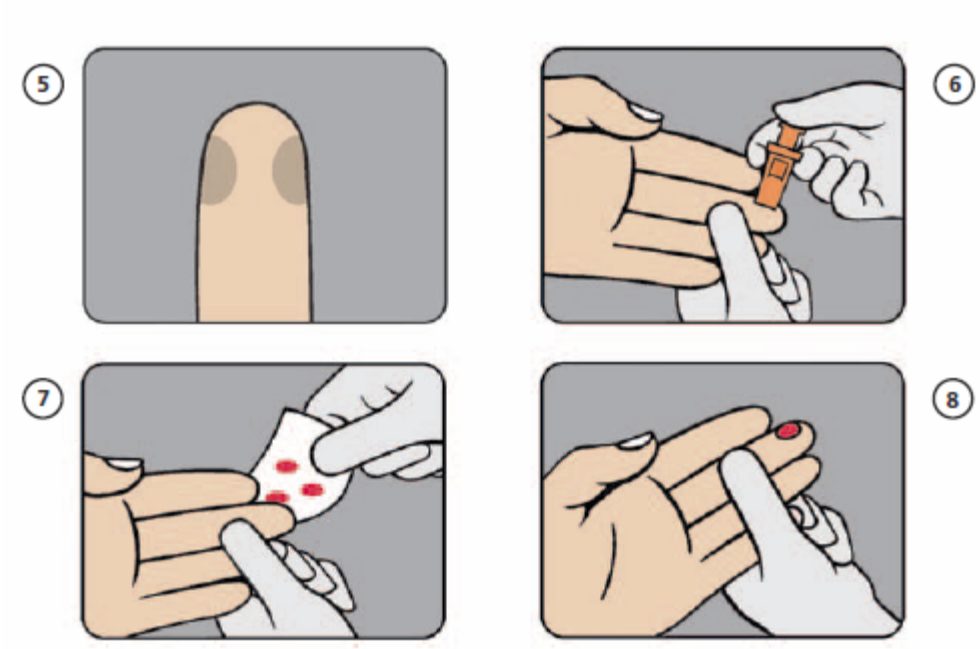
A. Provides determination of hemoglobin quickly, easily and with quality results. Capillary, venous, or arterial whole blood may be used. Refer to your HemoCue 201+ Manual for the basic instructions as well as technical specifications. Manuals and instructions below are provided by HemoCue, Inc.

B. Specimen Collection

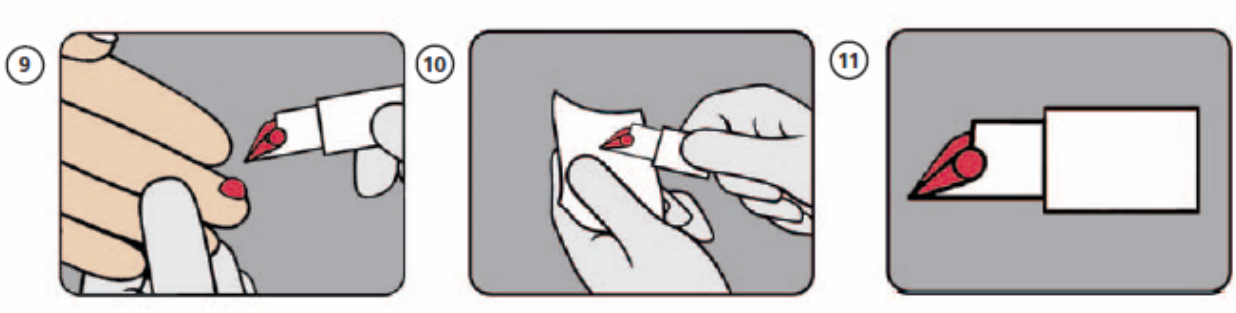
1. To perform a test using capillary blood, the cuvette holder should be in its loading position. The display will show three flashing dashes and the HemoCue symbol.
2. Make sure the patient's hand is warm and relaxed. Use only the middle or ring finger for sampling. Avoid fingers on which rings are present.
3. Clean the finger with alcohol or a suitable disinfectant and allow it to dry or wipe it off with a dry, lint-free wipe.
4. Using your thumb, lightly press the finger from the top of the knuckle towards the tip. This stimulates the blood flow towards the sampling point.



5. For best blood flow and least pain, sample at the side of the fingertip, not in the center.
6. While applying light pressure towards the fingertip, puncture the finger using a lancet.
7. Wipe away the first 2 or 3 drops of blood.
8. Re-apply light pressure towards the fingertip until another drop of blood appears.

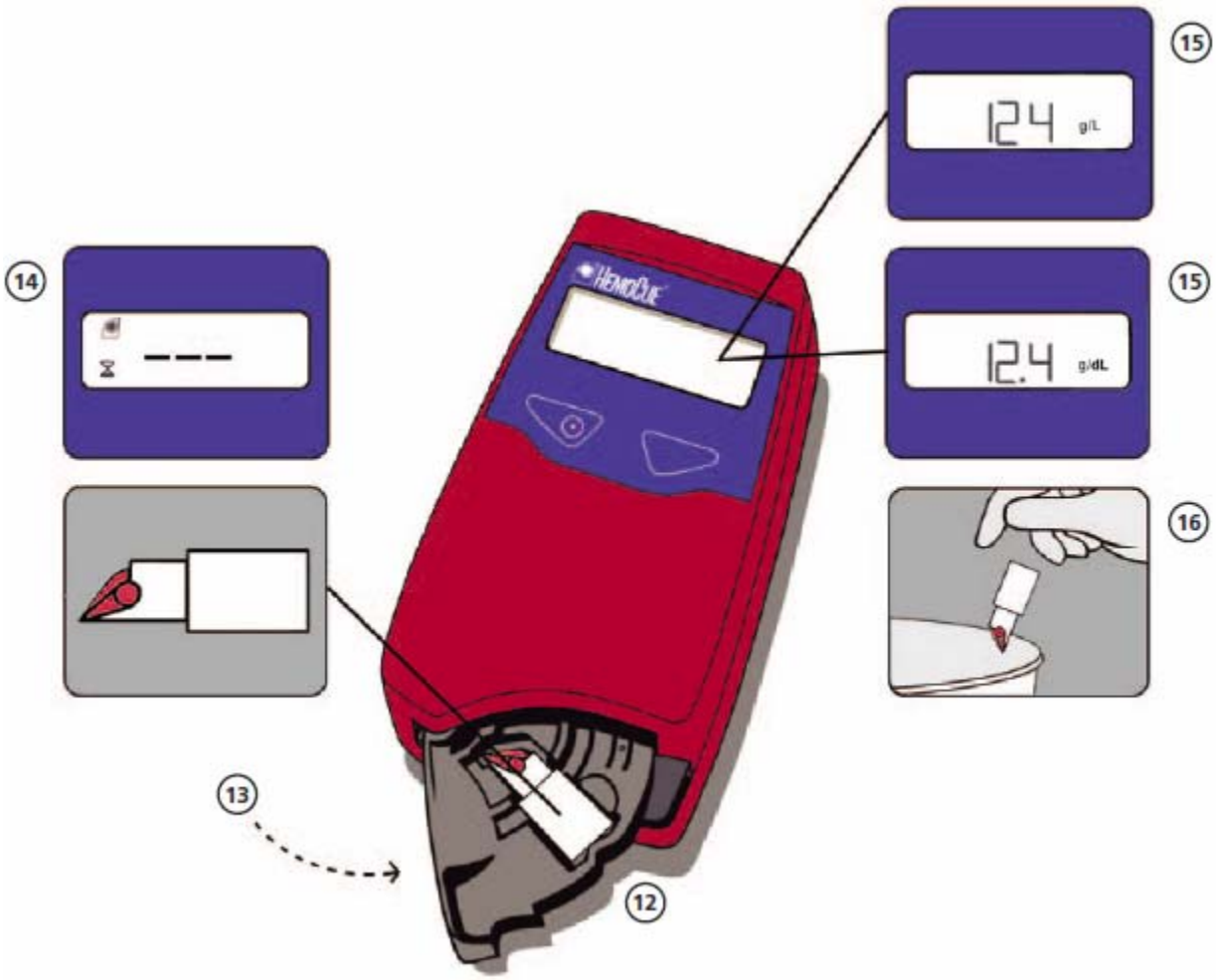


9. When the blood drop is large enough, fill the microcuvette in one continuous process. Do NOT refill!
10. Wipe off excess blood from the outside of the microcuvette with a clean, lint-free wipe being careful not to touch the open end of the microcuvette, which could result in blood being drawn out of it.
11. Look for air bubbles in the filled microcuvette. If present, discard the microcuvette, and fill a new one from a new drop of blood. Small bubbles around the edge can be ignored.



12. Place the filled microcuvette in the cuvette holder. This must be performed within 10 minutes after filling the microcuvette!
13. Gently slide the cuvette holder to the measuring position.
14. During the measurement, an hourglass and three fixed dashes will be shown on the display.
15. After 15-60 seconds, the hemoglobin value of the sample is displayed. The result will remain on the display as long as the cuvette holder is in the measuring position. When operating on battery power, the analyzer will automatically turn off after approximately 5 minutes.

16. Always handle blood specimens with care; they might be infectious. Dispose of used microcuvettes into a red biohazard (sharps) container.



C. Report results on CHR-11.

See "HemoCue Maintenance Chart"
See "Annual Temperature Chart"
See "HemoCue Trouble Shooting Chart" (also found in HemoCue 201+ Manual)

II. Occult Blood Test - Fecal Immunochemical Testing (InSure® FIT™)

A. This product is an in-home, patient sampled, fecal globin by immunochemistry test that tests for occult (hidden) blood in a fecal sample which can help in the early detection of colorectal cancer.

B. Please refer to the package inserts of the specific kit you are using.

C. Report results on CHR-11.

Note: A package insert of the test kit should be placed in a sheet protector and placed in this manual immediately after this section.

III. Strep A (Rapid Test)

A. An Enzyme Immunoassay for the Rapid Detection and Confirmation of Group A *Streptococci*.

B. Please refer to the package inserts of the specific kit you are using.

C. Report results on CHR-11.

Note: A package insert of the test kit should be placed in a sheet protector and placed in this manual immediately after this section.

IV. Urinalysis - Dipstick Method

A. The chemical analysis of urine is performed using one of many different urine reagent strips (dipsticks) available from a variety of manufacturers. Reagent strips for urinalysis are firm plastic strips to which are affixed several separate reagent pads. A color reaction develops upon contact of the urine with the reagent pads. The following are commonly available on urine dipsticks:
   1. Protein.
   2. Glucose.
   3. Ketone bodies.
   4. Bilirubin.
   5. Leukocyte esterase.
   6. pH.
   7. Occult blood/hemoglobin.
   8. Urobilinogen.
   9. Nitrite.
   10. Specific gravity.

B. Refer to the package inserts for the specific reagent areas on the product you are using.

C. Report results on CHR-11.

Note: A package insert of the test kit should be placed in a sheet protector and placed in this manual immediately after this section.

V. Urine Pregnancy Test

A. Urine pregnancy test measures the presence of human chorionic gonadotrophin (HCG) in urine for the early detection of pregnancy.

B. Refer to the package inserts of the specific kit you are using.

C. Report results on CHR-11 or CHR-12c.

Note: A package insert of the test kit should be placed in a sheet protector and placed in this manual immediately after this section.

County _____ **HemoCue Maintenance Chart** **State Serial #** _____

Site _____ **Write P (pass) or F (fail) for internal control function.** **Year** _____

January	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean Cuvette Holder																															
Check Internal Control																															
Initials																															
February	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
Clean Cuvette Holder																															
Check Internal Control																															
Initials																															
March	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean Cuvette Holder																															
Check Internal Control																															
Initials																															
April	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Clean Cuvette Holder																															
Check Internal Control																															
Initials																															
May	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean Cuvette Holder																															
Check Internal Control																															
Initials																															
June	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Clean Cuvette Holder																															
Check Internal Control																															
Initials																															
July	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean Cuvette Holder																															
Check Internal Control																															
Initials																															

Monthly Maintenance	**January**	**February**	**March**	**April**	**May**	**June**	**July**
Optical Lens Monthly Cleaning							
Date/Initials							

Legend: NIU = not in use; S = Saturday/Sunday; H = Holiday

County _____ **HemoCue Maintenance Chart** **State Serial #** _____

Site _____ **Write P (pass) or F (fail) for internal control function.** **Year** _____

August	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean Cuvette Holder																															
Check Internal Control																															
Initials																															
September	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Clean Cuvette Holder																															
Check Internal Control																															
Initials																															
October	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean Cuvette Holder																															
Check Internal Control																															
Initials																															
November	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Clean Cuvette Holder																															
Check Internal Control																															
Initials																															
December	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean Cuvette Holder																															
Check Internal Control																															
Initials																															

Monthly Maintenance	**August**	**September**	**October**	**November**	**December**
Optical Lens Monthly Cleaning					
Date/Initials					

CORRECTIVE ACTION LOG: **Date / Problem / Corrective Action / Initials**

Legend: NIU = not in use; S = Saturday/Sunday; H = Holiday

County: _____ Site: _____ **Annual Temperature Chart** Room/Equipment #:_____ Year: _____

Check scale used (°F) __ or (°C) __

Circle equipment and acceptable range used: Incubator 95°F -99°F (35°C - 37°C) Refrigerator 35°F - 46°F (2 - 8°C) Freezer 5°F or colder (-15°C or colder) or Room 64°F - 86°F (18°C - 30°C)

Date	Jan	Intls	Feb	Intls	Mar	Intls	Apr	Intls	May	Intls	June	Intls	July	Intls	Aug	Intls	Sept	Intls	Oct	Intls	Nov	Intls	Dec	Intls
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Corrective Action Log on Back

Legend: S = Saturday/Sunday, H = Holiday, NIU = Not In Use

FOOD OR BEVERAGE IS PROHIBITED FROM STORAGE IN CLINIC REFRIGERATORS/FREEZERS AT ALL TIMES!

Temperature Corrective Action Log

Date	Temp Adj	Intls	Recheck Temperature (°C)
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DATE	COMMENT

HemoCue Hb 201+ Trouble Shooting Guide

If you are unable to resolve the problem by following this Trouble Shooting Guide, please contact HemoCue, Inc. The analyzer has no serviceable parts.

Symptom	Explanation	Action
The analyzer shows an error code.	May be a temporary fault	Turn off the analyzer and turn it on again after 30 seconds. Take a new microcuvette and repeat the measurement. If the problem continues, see specific error code.
E00	No stable endpoint is found within the time range. 1. The cuvette is faulty. 2. The circuit board is out of order.	1a. Check the expiration date of the microcuvettes. 1b. Use a new microcuvette and repeat the measurement. 2. The analyzer needs service. Contact HemoCue, Inc.
E01-E05	1. Dirty optronic unit, faulty electronic or optronic unit.	1a. Turn off the analyzer and clean the optronic unit as described in the maintenance section. 1b. The analyzer needs service. Contact HemoCue, Inc.
E06	1. Unstable blank value. The analyzer might be cold.	1. Turn off the analyzer and allow it to reach room temperature. If the problem continues, the analyzer needs service. Contact HemoCue, Inc.
E07	1. The battery power is too low.	1a. The batteries need to be replaced. Turn off the analyzer and replace the batteries (four type AA). 1b. Use the power adapter.
E08	The absorbance is too high. 1. An item is blocking the light in the cuvette holder.	1a. Check that the analyzer and microcuvetts are being used according to the HemoCue Hb 201+ operating manual and instructions for use. 1b. The analyzer needs service. Contact HemoCue, Inc.
E09-E30	1. Dirty optronic unit, faulty electronic or optronic unit.	1a. Turn off the analyzer and clean the optronic unit as described in the maintenance section. 1b. The analyzer needs service. Contact HemoCue, Inc.

Symptom	Explanation	Action
HHH	1. Measured value exceeds 25.6 g/dl (256 g/L, 15.9 mmol/L)	1. Consult Nursing Protocol for action.
No characters on the display	1. The analyzer is not receiving power. 2. If on battery power, the batteries need to be replaced. 3. The display is out of order.	1a. Check that the power adaptor is connected to the AC power supply. 1b. Check that the power adaptor is securely connected to the analyzer. 1c. Check that the cable is not damaged. 2. Turn off the analyzer and replace the batteries (four type AA). 3. The analyzer needs service. Contact HemoCue, Inc.
The display gives erroneous characters.	1. The display is out of order. 2. The microprocessor is out of order.	1. The analyzer needs service. Contact HemoCue, Inc. 2. The analyzer needs service. Contact HemoCue, Inc.
The display shows a “partially shaded” battery.	1. The batteries need to be replaced. 2. If on AC power, the power adaptor or the circuit board is out of order.	1. Turn off the analyzer and replace the batteries (four type AA). 2a. Check that the power adaptor is properly connected and working. 2b. The analyzer needs service. Contact HemoCue, Inc.
The display does not switch to the three flashing dashes (ready for measuring).	1. The magnet in the cuvette holder may be missing. 2. The magnetic sensor is out of order.	1. The analyzer needs service. Contact HemoCue, Inc. 2. The analyzer needs service. Contact HemoCue, Inc.
Measurements on control materials are out of range - too high or too low.	1. The microcuvettes are beyond their expiration date, damaged or have been improperly stored. 2. The optical eye of the microcuvette is contaminated. 3. The control has not been mixed properly and/or is not at room temperature. 4. Air bubbles in the microcuvette. 5. The optronic unit is dirty. 6. The control is not suitable	1. Check the expiration date and the storage conditions of the microcuvettes. 2. Measure the sample with a new microcuvette. 3. Make sure that the control is mixed properly and at room temperature. 4. Check the microcuvette for air bubbles. Measure the sample with a new microcuvette. 5. Clean the optronic unit as described in the maintenance section.

Symptom	Explanation	Action
	for use with the HemoCue Hb 201+ system. 7. The calibration of the analyzer has been changed. 8. The controls are beyond their expiration dates or have been improperly stored.	6. Only use controls intended for the HemoCue Hb 201+ system. Contact HemoCue, Inc. for control information. 7. The analyzer needs service. Contact HemoCue, Inc. 8. Check the expiration dates and storage conditions of the controls. Take a new microcuvette and repeat the measurement from a new vial/bottle of control.
Measurements on patient samples are higher or lower than anticipated.	1. Improper sampling technique. 2. The microcuvettes are beyond their expiration date, damaged or have been improperly stored. 3. The optical eye of the microcuvette is contaminated. 4. Air bubbles in the microcuvette. 5. The optronic unit is dirty. 6. The calibration of the analyzer has changed.	1. See pages 8-17 in the HemoCue Hb 201+ manual. 2. Check the expiration dates and storage conditions of the microcuvettes. Check the entire system with a commercial control. 3. Measure the sample with a new microcuvette. 4. Check the microcuvette for air bubbles. Measure the sample with a new microcuvette. 5. Clean the optronic unit as described in the maintenance section. 6. The analyzer needs service. Contact HemoCue, Inc.

Brightfield Microscopy

BRIGHTFIELD MICROSCOPY

I. Principle

A. The microscope is perhaps the piece of equipment that receives the most use and, unfortunately, misuse in the clinical laboratory. Microscopy is necessary to see microbial cells and to determine morphological characteristics. It is a basic part of work in many areas of the laboratory such as hematology, urinalysis, and microbiology. Because the microscope is such an important piece of equipment and a precise instrument, it must be kept in excellent condition, optically and mechanically. It must be kept clean and aligned.

B. In simple terms, a microscope is a magnifying glass. The compound light, or brightfield microscope (used in most clinical laboratories), consists of two magnifying lenses: the objective and the eyepiece (ocular). It is used to magnify an object so it can be seen with the human eye.

C. The total magnification observed is the product of the magnifications of these two lenses. In other words, the magnification of the objective times the magnification of the ocular equals the total magnification. The magnitude of magnification is inscribed on each objective as a number. These magnification units are in terms of diameters (10x, 40x, and 100x). Thus, 10x means that the diameter of an object is magnified 10 times its original size.

D. Because of the manner in which light travels through the brightfield microscope, the image that is seen is upside down and reversed. The right side appears as the left, the top as the bottom, and vice versa. This should be kept in mind when moving the slide (or object) being observed.

II. Glossary of Terms

A. Aperture diaphragm - either a rotating disk or an iris diaphragm on the condenser used to direct a cone of light to the specimen and objective. It should never be used to regulate brightness. Resolution, control, and depth of field depend on the correct setting of the aperture diaphragm.

B. Arm – for holding the microscope while carrying it.

C. Coarse adjustment knob – for rapid focusing of the specimen.

D. Compound microscope – a microscope made up of two lens systems: eyepiece and objective.

E. Condenser – the lens system beneath the microscope stage positioned to concentrate light correctly on the specimen and direct light rays into the objective. When the condenser is used at a lowered position, the resolving power is reduced.

F. Depth of field – distance just above and below the focal plane (area being examined) that can be focused clearly.

G. Eyepiece – lens system of the microscope nearest to the eyes.

H. Field diaphragm – an aperture diaphragm which restricts the area of illumination.

I. Fine adjustment knob – focuses the lens in small increments.

J. Immersion oil – oil with the same refractive index as glass, 1.515; used between the cover glass and an oil immersion objective to prevent scattering of light in air.

K. Interpupillary distance – the distance between the eyes. The eyepieces of a binocular scope must be adjusted so that left and right images merge into one.

L. Koehler illumination – optical illumination providing bright, evenly dispersed, glare-free light with good contrast and resolution.

M. Nosepiece – a revolving plate that holds the objectives.

N. Ocular – eyepiece (lens system of the microscope nearest to the eyes).

O. Parcentric – the ability to center a specimen in the field of view for one objective and have almost the same field in place when rotating to another objective.

P. Parfocal – the objectives are constructed so that only slight refocusing with the fine adjustment knob is needed after rotating to another objective.

Q. Resolution – the ability of a microscope to reveal fine detail in a specimen. The better the resolving power of a microscope, the closer two objects can be and still be distinguished as two objects.

R. Stage – the platform on which the microscope slide is placed.

S. Working distance – distance between the coverslip of a slide and the tip of an objective. The low power objective has the greatest working distance. The oil immersion objective has a very small working distance.

III. Parts of the Microscope

A. The basic structures of compound microscopes are categorized into 4 groups (see "Microscope Diagram"): the framework, the illumination system, the magnification system and the adjustment system.

1. Framework – consists of several units
    a. The base – a firm, horseshoe-shaped foot on which the microscope rests.

b. The arm – the structure that supports the magnifying and adjusting systems. It is also the handle by which the microscope can be carried without damaging the delicate parts.
c. The stage – the horizontal platform, or shelf, on which the object being observed is placed. Most microscopes have a mechanical stage, which makes it easier to manipulate the object being observed.

2. Illumination – Good microscope work cannot be accomplished without proper illumination.
   a. Light source (or bulb) – The illumination system begins with a source of light.
      - Is often built in the microscope.
      - This illumination system has a control to regulate light intensity, ensuring adequate illumination and comfort for the employee.
      - Is located at the base of the microscope, and the light is directed up through the condenser system.
      - It is important that the bulb is positioned correctly for proper alignment throughout the microscope.
      - Modern microscopes are designed so that the light bulb filament will be centered if the bulb is installed properly.
   b. Condenser
      - Microscopes generally use a substage "Abbe" condenser.
      - It directs and focuses the beam of light from the bulb onto the material under examination.
      - Its position is adjustable; it can be raised or lowered beneath the stage by an adjustment knob.
      - The iris in the condenser can also be opened or closed by an adjustment knob.
      - The condenser must be adjusted with each objective used in order to maximize the light focus and the resolving power of the microscope. This adjustment is often necessary in the clinical laboratory when observing wet, unstained preparations such as vaginal preps.
   c. Field diaphragm
      - It controls the amount of light passing through the material under observation.
      - It is located at the bottom of the microscope.
      - It contains an iris that can be opened or closed to adjust the intensity of the light by a lever.
      - Proper illumination techniques involve a combination of proper light intensity regulation, light source position, condenser position, and field size regulation.
   d. Koehler Illumination

      For optimum results in light microscopy, it is crucial that the light path be set properly before the light reaches the specimen. In 1893, German scientist August Koehler introduced this technique for applying exact control of the light path in the illuminating beam.

      i. Turn the lamp to the lowest setting.

ii. Raise the condenser to the highest setting.
iii. Open the condenser all the way and close the field diaphragm all the way.
iv. Adjust eyepieces so only one circle of light is observed.
v. Place prepared slide in slide brackets, centering it over the light well.
vi. Starting with the 10x objective and the stage closest together, focus on the specimen. This procedure can be done without using a slide.
vii. A small circle of light should be visible in the eyepieces.
viii. While viewing, lower the condenser slightly until the image is in focus (a polygonal outline with a pink-reddish tinge). You may need to close the condenser diaphragm.
ix. Center the polygonal image in the field of view using the condenser centering screws.
x. Open the field diaphragm to the edge of the field of view. **Note:** If needed, re-center the image by adjusting the condenser centering screws.
xi. After it appears centered, open the field diaphragm until the image just clears the field of view.

Note: According to which manufacturer's microscope is in the county health department, it may not be possible to set Koehler Illumination. Some manufacturers align the optics during manufacturing so that it does not have to be done on a daily basis. The microscope must have a field diaphragm in order to set Koehler Illumination. In addition, if one person uses the microscope, it is not necessary to set Koehler Illumination daily. If there are questions concerning optical alignment, perform Koehler Illumination if possible.

3. Magnification System – contains important parts and plays a vital role in the use of the microscope.
   - The ocular (eyepiece) is a lens that magnifies the image formed by the objective.
   - The usual magnification of the ocular is 10x.
   - Most microscopes have two oculars and are called binocular microscopes.
   - Some microscopes only have one ocular and are called monocular microscopes.
   - The magnification produced by the ocular, when multiplied by the magnification produced by the objective, gives the total magnification of the object being viewed.
   - There are usually three objectives on each microscope, with magnifying powers of 10x, 40x, and 100x. They are mounted on the nosepiece - a pivot enabling a quick change of the objectives. They are described or rated according to focal length (inscribed on the outside of the objective). The focal length of a lens is very close to the working distance. The greater the magnifying power of a lens, the smaller the focal length or the working distance.
     a. Low-Power Objective:
        i. Is usually a 10x magnification lens.
        ii. Is used for the initial scanning and observation in most microscope work.
        iii. Is employed for the initial focusing and light adjustment of the microscope.

iv. There are two terms often associated with a microscope: parfocal and parcentric.
    - Parfocal means if one objective is in focus and a switch is made to another objective, the focus will not be lost. Thus, the microscope can be focused under low power and then switched to the high power or oil immersion objective, and it will still be in focus except for fine adjustment.
    - Parcentric means that when an object is centered in the field of view using low power and then switched to high power or oil immersion, the object will still be in view and centered with only minor adjustments needed.

b. High-Power Objective (High-Dry Objective):
   i. Is usually a 40x magnification lens.
   ii. Is used for more detailed study, as the total magnification with a 10x eyepiece is 400x rather than the 100x of the low-power system.
   iii. Is used to study wet preparations in more detail.

c. Oil-Immersion Objective:
   i. Is generally a 100x lens. The objective almost rests on the microscope slide when in use.
   ii. An oil-immersion lens requires a special grade of oil (immersion oil – used to increase the resolving power of the objective), to be placed between the objective and the slide or coverglass.
   iii. The oil-immersion lens, with a total magnification of 1000x when used with a 10x eyepiece, is generally the limit of magnification with the brightfield microscope. It is routinely used for morphologic examination of microbes. The short working distance requires dry films, so wet preparations cannot be examined under an oil-immersion lens.

4. Adjusting System
   - The body tube is the part of the microscope through which the light passes to the ocular.
   - The tube length from the eyepiece to the objective lens is generally 160 mm.
   - This is the tube that actually conducts the image.
   - The adjustment system enables the body tube to move up or down for focusing the objectives.
   - This system usually consists of two adjustments, one coarse and the other fine.
     - Coarse adjustment - gives rapid movement over a wide range and is used to obtain an approximate focus. This adjustment should only be used with the 10x objective.
     - Fine adjustment - gives very slow movement over a limited range and is used to obtain exact focus after prior coarse adjustment. This adjustment should only be used with the 40x and 100x objectives.

IV. Maintenance

See “Microscope Maintenance Chart”

A. Daily Maintenance
   1. Clean all optical surfaces with lens cleaner and cotton-tipped applicators and/or lens paper.
      a. Eyepieces, objectives, and condenser should be cleaned using a cotton-tipped applicator.
      b. The light source should be cleaned using lens paper.
   2. Protect the microscope with a cover (or trash can liner if a cover is unavailable) when not in use.
   3. Initial that "1" and "2" have been completed.

B. Monthly Maintenance
   1. Clean all nonoptical surfaces with mild detergent, and rinse with warm water.
   2. Check the Potassium Hydroxide (KOH) solution for contamination.
      a. If contaminated or flocculation has occurred, replace with in-date KOH.
      b. If transferring KOH from a stock bottle to a working bottle, label the working bottle with name of reagent, % strength, and expiration date which should be one year from being placed into the working bottle unless this date exceeds the manufacturer's expiration on the stock bottle. In this case, the manufacturer's expiration date supersedes a one year expiration from placement in the working bottle. If the expiration date given is written as the month and year only, the solution expires on the last day of the printed month.
      c. KOH may not last one full year contamination free. If the KOH becomes flaky or cloudy before a year has passed it must be replaced.
   3. Check saline solution for contamination.
      a. If contaminated, replace with in-date saline.
      b. If transferring saline from a stock bottle to a working bottle, label the working bottle with name of reagent, % strength, and expiration date which should be one year from being placed into the working bottle unless this date exceeds the manufacturer's expiration on the stock bottle. In this case, the manufacturer's expiration date supersedes a one year expiration from placement in the working bottle. If the expiration date given is written as the month and year only, the solution expires on the last day of the printed month.
      c. Saline may not last one full year contamination free. If the Saline becomes cloudy or otherwise contaminated before a year has passed it must be replaced.
   4. Check the appearance of the pH paper according to the pH paper package insert.
   5. Initial that all duties listed above have been completed.

C. Annual Professional Microscope Maintenance
   1. Microscopes should be serviced annually by a professional.
   2. Document the company performing the annual cleaning.
   3. Document the date the annual cleaning was performed. When starting this form at the beginning of the year, place the last year's cleaning date on this line; then, when the new cleaning occurs, write the new cleaning date next to the previous year's date.

V. Corrective Action Log

See "Microscope Maintenance Chart"

A. Document any problems encountered with the microscope.

B. Document date of the problem.

C. Document corrective action taken.

D. Document initials of employee performing corrective action.

VI. Additional Information

A. Turn lamp voltage down and then off if the microscope has a rheostat.

B. If slideways and gears on mechanical stage become difficult to move, lubricate with machine oil or light grease. Document in the corrective action section of the maintenance chart.

C. Keep at least one extra bulb available in case the bulb in the microscope blows. Contact your technical consultant for ordering information. Follow the manufacturer's instructions for ordering and replacing bulbs, or check with your technical consultant.

D. If your microscope requires a halogen bulb, **DO NOT TOUCH** the new bulb with your fingers or gloves. Install it with a lint-free tissue.

E. Avoid exposure to corrosive fumes, extreme heat or cold, or sudden drastic temperature changes. When moving from one temperature extreme to another, allow optical parts to equilibrate until all moisture has evaporated.

F. If immersion oil is required, use only chemically inert, low fluorescent, PCB-free immersion oil. Low viscosity is acceptable. **DO NOT USE CEDAR WOOD OIL.** Different brands of immersion oil are incompatible and should not be mixed.

G. Use a small amount of oil in a frequently-cleaned application bottle to minimize growth of fungi which may reflect erroneously on specimen content.

VII. Helpful Hints

A. Turn on the light source.

B. Be sure low objective is in position.

C. Rack down, then up until field is in focus.

D. When moving from one objective to another, use fine adjustment knob to refocus.

E. Never drag high-dry objective through oil.

F. The higher the magnification, the more light you will need.

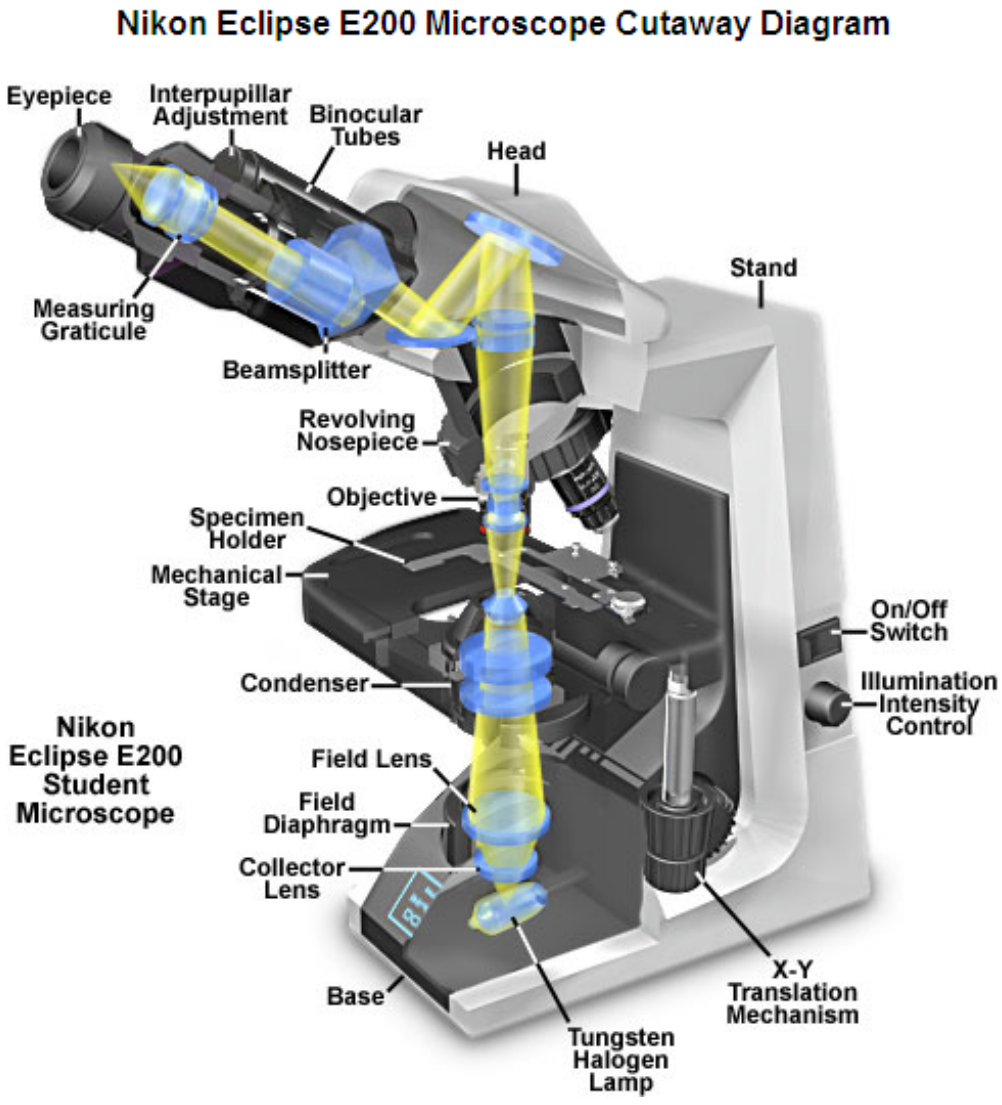
G. Always clean oil from objective and/or condenser at the end of the day.

H. Be sure the open position on the objective revolving nosepiece is in the working position at the end of the day. If your microscope does not have an open position on the revolving nosepiece, ensure the lowest power objective is in the working position at the end of the day.

I. Cover the microscope when you have finished for the day.

J. Try not to place the microscope on the same work bench as a centrifuge to avoid the microscope and specimen from vibrating. If they will be on the same bench, secure a mat that absorbs vibrations to place under the microscope.

Microscope Diagram



VIII.Troubleshooting

Several conditions can affect good function of the microscope.

1. The brightness of the viewing field is poor.

Problem	Solution
The condenser is too low.	Raise the condenser to correct its position.
The condenser iris diaphragm is closed.	Open the diaphragm properly.

2. There are dark shadows in the field which move as the eyepiece is turned.

Problem	Solution
The surface of the eyepiece has scratches.	Replace the eyepiece.
The eyepiece is dirty.	Clean the eyepiece.

3. The image with the high power objective is not clear.

Problem	Solution
The slide is upside down.	Turn the slide over.
There is an air bubble in the oil.	Move 100x lens quickly from side to side.
There is dirt on the objective.	Clean the lens.
The oil is too sticky.	Use specified immersion oil.

4. The image with the low power objective is not clear.

Problem	Solution
There is debris/oil on the lens.	Clean the lens.

5. Objective lens hits specimen before achieving focus.

Problem	Solution
Objective not screwed in nosepiece securely.	Securely tighten objective in nosepiece.
Stage mounted too high.	Call Annual Maintenance Personnel

IX. References

A. Alabama Department of Public Health. Wet Prep Competency; Microscope Teaching Video Series, 2000.

B. Olympus. BX41 System Microscope Instruction Manual, 2003.

C. Olympus Video Library. The Basic Microscope - Use & Care.

Microscope Maintenance Chart

County: _____ **Year:** _____ **Manufacturer:** _____

Location: _____ **State #:** _____ **Model #:** _____

January	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															
February	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
Clean optical surfaces																															
Cover when not in use																															
Initials																															
March	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															
April	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Clean optical surfaces																															
Cover when not in use																															
Initials																															
May	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															
June	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Clean optical surfaces																															
Cover when not in use																															
Initials																															

Monthly Maintenance	**January**	**February**	**March**	**April**	**May**	**June**
Clean nonoptical surfaces						
Check KOH solution						
Check Saline						
Check pH paper for color change						
Date/Initials						

Annual Professional Microscope Cleaning Company _____

Annual Professional Microscope Cleaning Service Date _____ / _____

Previous Year Current Year

"NIU" indicates not in use.

S=Saturday/Sunday, H=Holiday

Microscope Maintenance Chart

County: _____ **Year:** _____ **Manufacturer:** _____

Location: _____ **State #:** _____ **Model #:** _____

July	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															
August	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															
September	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Clean optical surfaces																															
Cover when not in use																															
Initials																															
October	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															
November	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Clean optical surfaces																															
Cover when not in use																															
Initials																															
December	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															

Monthly Maintenance	**July**	**August**	**September**	**October**	**November**	**December**
Clean nonoptical surfaces						
Check KOH solution						
Check Saline						
Check pH paper for color change						
Date/Initials						

CORRECTIVE ACTION LOG:	**Date / Problem / Corrective Action / Initials**

Vaginal Wet Mount

VAGINAL WET MOUNT

I. Principle

The direct microscopic examination of a saline suspension of material taken from an area of vaginal inflammation can provide a quick presumptive diagnosis for *Gardnerella vaginalis* and *Candida albicans* and/or a definitive diagnosis for *Trichomonas vaginalis.* The addition of Potassium Hydroxide (KOH) to a portion of the suspension helps clear cellular debris, allowing better visualization of *C. albicans* (yeast).

II. Specimen Collection and Handling

A. Specimens from the human body may be infectious. Use universal precautions.

B. Patient preparation and collection procedures should be performed only by qualified personnel. The procedure for inserting a speculum and performing vaginal examinations are found in the ADPH Nurse Orientation Manual: Vaginal Examination and Specimen Collection Education Module.

C. Collect the specimen by rolling a cotton or Dacron® swab in vaginal discharge pooled in the posterior fornix or in excess discharge on the speculum blade. COLLECTION ORDER: Wet smears are collected first followed by the pap smear, *Gonorrhea /Chlamydia* specimen, then the pelvic bi-manual examination.

D. There is no way to preserve the specimen, so immediately examine the slide preparation under the microscope since trophozoites rapidly lose their motility.

E. Process the specimen by preparing 1 or 2 slides as described in the test procedure.

III. Specimen Rejection

A. Do not test specimens that:
   1. Exceed the time limit (10 minutes) for immediate examination.
   2. Are not properly labeled or have questionable identity.
   3. Are collected after the use of a lubricant.

B. If the specimen does not meet the criteria for acceptability, collect a new sample.

IV. Reagent, Supplies, and Equipment

A. Normal saline (0.9% sodium chloride)

B. Potassium hydroxide (KOH) - 10% solution in a dropper bottle

C. Speculum

D. Cotton swabs

E. Microscope slides - 1" x 3" - frosted end

F. Cover slips 22 x 22 mm

G. Microscope with mechanical stage, low power (10x) and high power (40x) objectives

V. Reagent Storage and Usage

A. Store reagents at room temperature.

B. Solutions should be clear. Observe for turbidity, and discard if cloudiness is noted.

VI. Calibration

Calibration is not applicable.

VII. Quality Control

Quality control materials are not available.

VIII. Preliminary Procedures and Precautions

A. Set or verify Koehler Illumination on the microscope each day of use. This may not be possible on some microscopes due to illumination being permanently set at the factory.

B. Personnel who perform this procedure must be adequately trained as follows:
   1. Know proper technique for preparing the specimen on a slide.
   2. Have thorough knowledge of the proper use and care of a microscope.
   3. Have thorough knowledge of the morphology of organisms and other microscopic structures.
   4. Have interpretive skills to determine the significance of the microscopic elements observed.
   5. Have the ability to distinguish medically significant organisms and structures from background debris and artifacts.

IX. Procedure

A. Preparation of slides
   1. Prepare slide by placing one drop of normal saline on one end of the slide and one drop of KOH on the other end of the slide.
   2. On the slide, mix each solution separately using a swab. Dip the swab in saline first, **then** in KOH, or use two different swabs.

Note: Separate slides (One for the saline and one for the KOH) may be used if necessary. Remember to dip the swab in the saline first, then KOH.

3. Apply a cover slip to each smear by placing it at a 45° angle at the edge of the smear and easing it down gently to avoid air bubbles. Blot excess liquid from the edges carefully being sure to not draw liquid out from under the slide.
   Note: Cover slips often stick together. Carefully separate them so that only one is placed on the smear.
4. Label the frosted end of the slide with the patient's name.

B. Detection of inadequate slides

If an inadequate slide is detected, prepare a new one. A separate slide may be used for saline and KOH. Any of the following conditions would produce an inadequate slide:

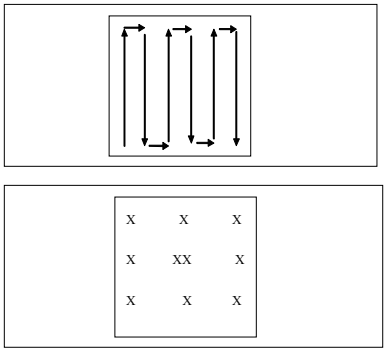
1. Too much saline or KOH on slide, causing material to move too rapidly.
2. Preparation is too thick or too thin.
3. The drop of saline and drop of KOH were accidentally mixed on the slide.
4. The swab was mixed with KOH before saline.

C. Microscopic examination of the slide

1. Place the slide on the microscope stage.
2. Use low power to find and focus on the specimen using reduced light.
3. Turn to high power and scan 10 fields on the saline side examining for *Trichomonas,* "clue cells," yeast, white blood cells (WBCs), bacteria, and normal epithelial cells.
4. For WBC reporting, average the fields using the formula on page 43 & 44 of this manual under section XII. Calculations.
5. A systematic pattern of examining the specimen is to examine three sites on each edge of the cover slip and two sites in the center.

Systematic Pattern of Exam

Examine @ 10X & 40X



6. Move the stage so the KOH specimen is under the high power objective. Examine 10 high power fields for yeast cells (other elements are usually destroyed by KOH).
   Note: Because there is little contrast between yeast or pseudohyphae and background material, you may not observe these cells if the light is too bright.
7. A fishy odor (amine) may be noticed by performing the whiff test when KOH is added. This is often noted when clue cells and/or *Trichomonas* are present in the specimen. This odor is recorded as "Whiff Test – Positive."

X. Interpretation of Results

A. ***Trichomonas vaginalis***
   1. Characteristics of the affected discharge:
      a. Yellowish to gray-green
      b. Frothy
      c. Copious
      d. Foul odor
   2. Microscopic description using 10x objective:
      a. Tiny
      b. Round
      c. Transparent
      d. The size of white cells
      e. Rapid movements (jerks and loops)
   3. Microscopic description using 40x objective:
      a. Approximately 15 mm (10-20 mm)
      b. Round
      c. Ovoid
      d. Globular
      e. Oval nucleus
      f. Motility: Whirls, jerks, turns, seeming to vibrate
      g. Undulating membrane on 1 side only, like the fin of a fish, the main impression is movement.
   4. Flagella:
      a. Four flagella at the anterior end
      b. Whip-like
      c. Very motile
      d. Slightly larger than white cells
      e. Can be easily identified by their undulating swimming motion
      f. Distinguish the trichomonad from the white cell by using the 40x objective to detect the beating flagella.
      g. These organisms are called "friendly white cells" because the flagella appear to wave.

B. ***Candida albicans*** **(yeast) – (pseudohyphae and hyphae)**
   1. Characteristics of the affected discharge:
      a. Thick

b. White (occasionally yellow or colorless)
c. Curd-like
d. Cheesy
e. Musty odor

2. Microscopic description using 40x objective:
   a. ***Candida albicans***
      - Vary in size (2-6 μm)
      - Round or oval
      - Non-motile
      - Some show budding
   b. **Pseudohyphae, hyphae, and Mycelia filaments**
      - Vary in length (20-100 μm) & breadth (2-4 μm)
      - Filaments with rounded ends
      - Distinguishing from other elements:
        - Debris from epithelial cells or mucus may be mistaken for or may obscure yeast cells.
        - Examine for yeast on the KOH specimen since other elements are usually destroyed by the KOH.

C. ***Gardnerella vaginalis***

1. Characteristics of the affected discharge:
   a. Thin
   b. Homogenous
   c. Grayish
   d. Adherent
   e. Foul odor
2. Microscopic description:
   a. Small
   b. Thin
   c. Gram-negative rod
   d. Bacterium
   e. Parasitizes the surface of vaginal epithelial cells
3. Microscopic description of "clue cells":
   a. Squamous, vaginal epithelial cells covered with many bacteria, giving them a stippled or granular appearance.
   b. Borders are obscured or fuzzy because of the adherence of the bacteria.
   c. So many organisms may attach to a vaginal epithelial cell that its entire border is obscured.
   d. Absence of a distinct border, rather than the granular appearance of the cytoplasmic or nuclear portion, identifies the clue cell.
   e. Look for sheets of epithelial cells that are studded with bacteria.
4. Other elements seen in the presence of clue cells:
   a. On the saline preparation trichomonads may be seen with clue cells, but mycelia and clue cells are almost never found together.
5. Diagnosis of *Gardnerella*:

a. At least 20% of the epithelial cells present must be clue cells to establish the diagnosis of *Gardnerella*.
b. A fishy odor (amine) may be noticed by performing the whiff test when KOH is added if clue cells are present in the specimen. Record this fishy odor as "Whiff Test - Positive".

XI. Reporting of Results

Note: Report all results on CHR-11 or CHR-12c with the date and time procedure was performed along with initials and signature of person performing test.

A. Reporting of Organisms
1. *Trichomonas vaginalis:* Observation of a single motile trichomonad establishes a diagnosis.
   a. When *Trichomonas* are observed, report as "Present".
   b. When no *Trichomonas* are observed, report as "Absent".

   Note: The use of signs (e.g. "-", "+", "0") and words (e.g. "positive", "negative", "zero", "none") must be avoided as they can be misinterpreted. No documentation means the test was not performed.
2. Yeast: Observation of yeast, pseudohyphae, hyphae or buds establishes a diagnosis.
   a. When yeast, pseudohyphae, hyphae or buds are observed, report as "Present".
   b. When <u>no</u> yeast, pseudohyphae, hyphae or buds are observed, report as "Absent".

   Note: The use of signs (e.g. "-", "+", "0") and words (e.g. "positive", "negative", "zero", "none") must be avoided as they can be misinterpreted. No documentation means the test was not performed.
3. Clue Cells: Epithelial cells obliterated with bacteria.
   a. Clue cells are significant only when symptoms are present.
   b. <u>Twenty percent of total epithelial cells must be clued to establish bacterial vaginosis.</u>
   c. When clue cells are observed, report as "Present".
   d. When no clue cells are observed, report as "Absent".

   Note: The use of signs (e.g. "-", "+", "0") and words (e.g. "positive", "negative", "zero", "none") must be avoided as they can be misinterpreted. No documentation means the test was not performed.
4. White Blood Cells (WBCs):
   a. Must be enumerated and reported by indicating low and high numbers determined per high power field (hpf).
   b. WBCs $\geq$ 10/hpf is significant in vaginal wet mount observation.
   c. WBC quantity is determined by the following method:
      i. Ten high powered fields are viewed microscopically using the systematic pattern of exam (See Section IX.C.5).
      ii. The averaged total of the 10 high powered fields is determined (See Section XII) and reported using the following range:

Absent or None = 0 /hpf
Rare = 0-1/hpf
Few = 2-5/hpf
Moderate = 6-20/hpf
Many = 21-35/hpf
Numerous = > 35/hpf

Note: The range wordage (Absent/None, Rare, Few, Moderate, Many, Numerous) may be used instead of the numbers. Do not use abbreviations. The use of signs (e.g. "-", "+", "⦸") and words (e.g. "positive", "negative", "zero") must be avoided as they can be misinterpreted. No documentation means the test was not performed.

5. "Amine Test" or "Whiff test"- a tool (along with other test observations and patient symptoms) to assist the clinician in establishing bacterial vaginosis.
   a. The test is performed by placing KOH on the wet mount slide and noting the presence or absence of a "fishy odor".
   b. When a fishy odor is detected, report as "Positive".
   c. When no fishy odor is detected, report as "Negative".

   Note: The use of signs (e.g. "-", "+", "0") must be avoided as they can be misinterpreted. If the test was not performed the "Not Done" box should be checked on the CHR-12c or write in the words "Not Done" on the CHR-11. Do not leave this space blank.
6. Other findings that may be documented in the "Other" space:
   1. Bacteria: Quantitate as 1+, 2+, 3+ or 4+.
   2. Red Blood Cells (RBCs)
      a. When RBCs are observed, report as "RBCs".
   3. pH
   4. When no other findings are to be reported, fill the "Other" space with the word "None."

XII. Calculations

C/10 = A where

C = (Cellular total of 10 high power fields)
A = (Average cellular total /high power field)

Example: Craig uses the systematic pattern of exam to observe a wet mount slide. After 10 high power field observations, he notes the following:
HPF One = 2, HPF Two = 3, HPF Three = 1, HPF Four = 6, HPF Five = 1, HPF Six = 1, HPF Seven = 2, HPF Eight = 1, HPF Nine = 3, HPF Ten = 3

C= 2+3+1+6+1+1+2+1+3+3
C = 23 Craig determines the total cellular elements.
A = 23/10 Craig determines the average total by dividing by 10 hpfs.
A = 2.3/hpf Craig determines the average to be 2.3/hpf.

Based on the range chart (See Section XI.4.c.ii), Craig's average (2.3/hpf) is in the "Few" "2-5/hpf" range. He may report his observation as "2-5/hpf" **or** "Few".

XIII. Troubleshooting

A. If patient results do not agree with clinical symptoms, an error may have occurred.

B. Review the "Sources of Error" for specific problems which may affect testing and attempt to determine the cause of the problem.

C. If the cause of the problem is not easily determined, follow a systematic approach to troubleshoot the situation.

D. Double check the specimen identification to ensure that the testing is being performed on a properly collected and labeled specimen on the correct patient.

E. Compare the procedure as you performed it with each step in the written procedure. Make sure the written procedure was followed exactly as it is written.

F. Examine reagents, solutions, and/or materials used for:
   1. The right color.
   2. Turbidity or precipitation.
   3. Expiration dates.

G. Check the equipment.
   1. Have function verification checks been performed?
   2. Are function check results within acceptable limits?
   3. Has preventive maintenance been performed at proper intervals?

H. Does the testing personnel have adequate training and experience to properly perform the procedure?

I. An abrupt change in patient results that coincides with a change in reagents, materials, equipment, or testing personnel can easily indicate the source of the problem.

XIV. Sources of Error

A. Specimen errors

   1. Specimen not collected prior to use of lubricant.
   2. Specimen not examined immediately, causing a loss of motility.
   3. Preparation too thick or thin.

B. Reagent errors

1. Reagents not stored properly.
2. Reagents are contaminated.
3. Reagents are not the correct strength.
4. KOH accidentally mixed with saline.
5. Too much saline on slide, causing material to move rapidly across the field.

C. Equipment errors

1. Microscope condenser, objectives, eyepieces, etc., not clean.
2. Microscope not set at optimum Koehler Illumination.
3. Microscope not properly focused.

D. Procedure Errors

1. Exam not performed using a thorough and systematic review of the slides.
2. Testing personnel not able to correctly recognize and/or differentiate microscopic findings.
3. Testing personnel not able to correctly use the microscope.
4. Failure to read slide using reduced light.

XV. Remedial Action

A. Once the potential source of the problem is discovered, take proper actions such as:
   1. Collect new specimen.
   2. Use new reagents/materials.
   3. Correct the equipment problems or replace the equipment.
   4. Follow written procedure exactly.
   5. Change testing personnel until additional training can be conducted.

B. Retest the patient specimens and reevaluate results to determine if they are acceptable.

C. If results are still unacceptable, continue to troubleshoot to determine the problem.

1. Do not report any patient results.
2. Call an outside source to help troubleshoot.

D. If the patient results are now acceptable, the problem is resolved.

E. Once the problem has been resolved, report patient results on CHR-11 or CHR-12c, including time, date, and initials of analyst.

F. Document the problem and the corrective action taken on the appropriate laboratory record form.

XVI. Normal Values

A. A normal patient value is negative for *Trichomonas vaginalis, Candida albicans and Gardnerella vaginalis*.

B. A normal patient may have 1-3 WBCs and/or epithelial cells per high power field.

C. Few, if any, WBCs are usually seen with *Gardnerella vaginalis.* If increased numbers of WBCs are seen with clue cells, consider other infections.

XVII. Reportable Range

Not applicable.

XVIII. Test Limitations

Take care in interpreting apparent results: artifacts are common in KOH preps as a result of cell degeneration, air bubbles, crystallization, and glycerol.

XIX. Course of Action if Test System Becomes Inoperable

A. If the test cannot be performed due to the absence of materials or inadequate materials, if possible, borrow from an appropriate source the materials, reagents, equipment, etc. needed, and run the test.

B. Since the microscopic examination must be performed immediately after the specimen is collected, do not store the specimen or send it to another laboratory for examination.

C. If the problem cannot be resolved, reschedule the patient to return at a later date or send the patient to another location equipped to perform the examination.

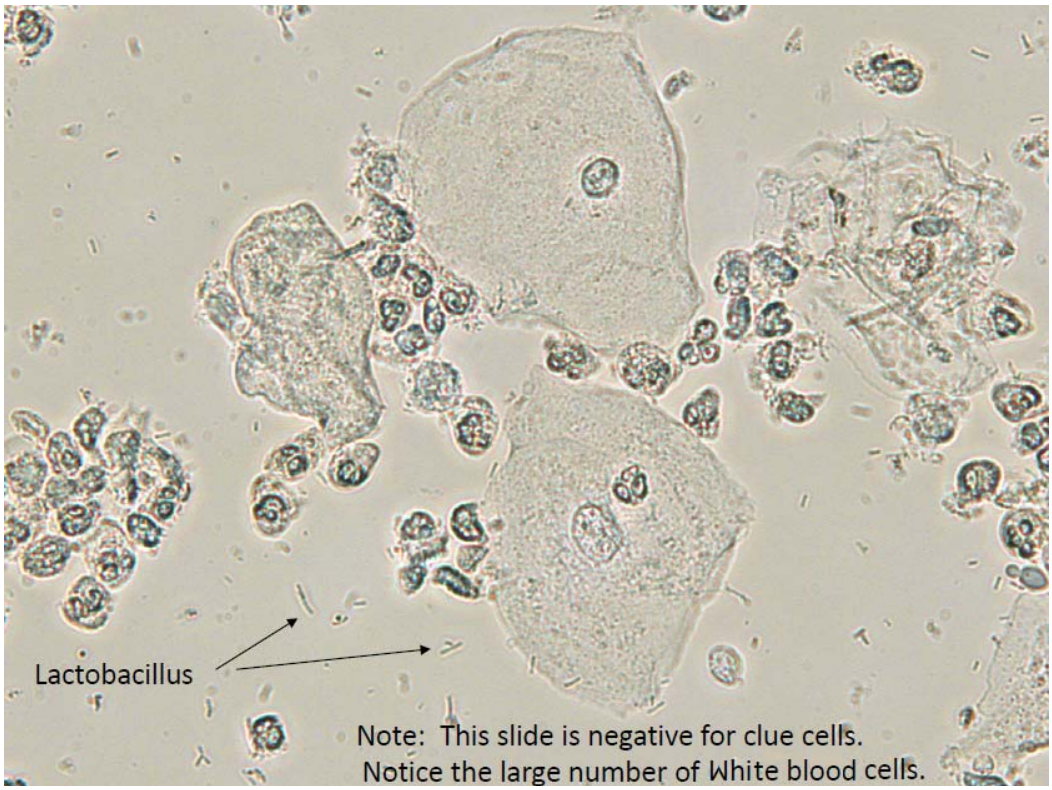
XX. References

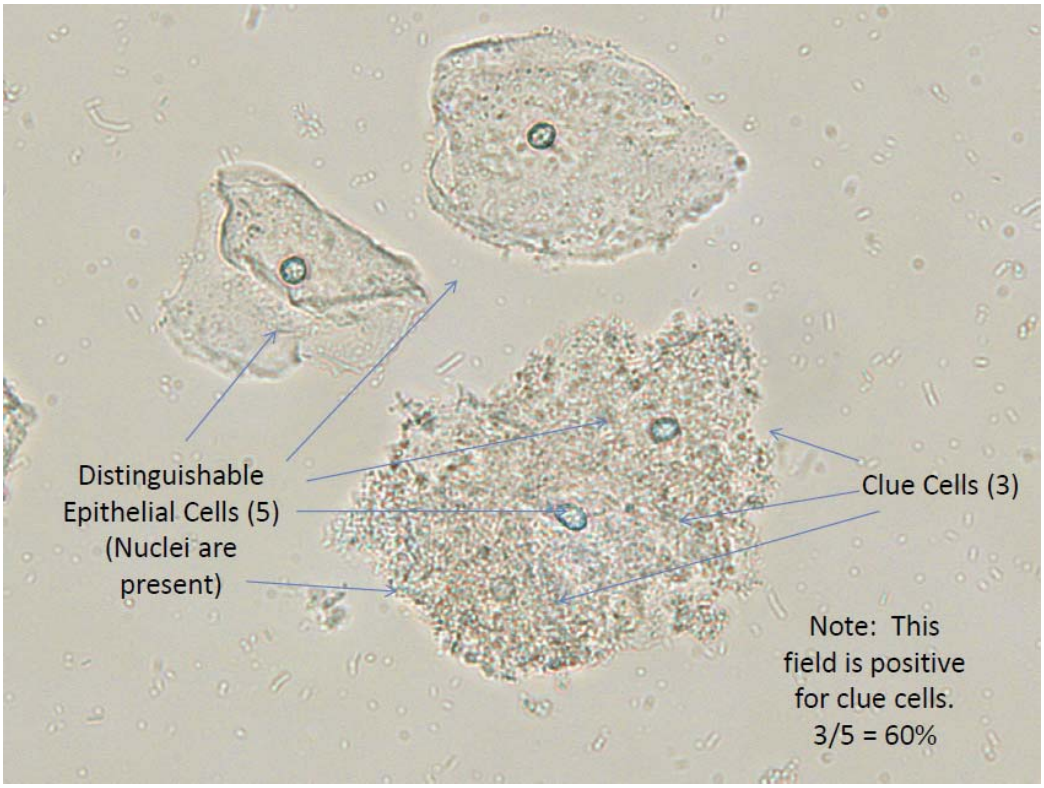
A. Alabama Department of Public Health. Wet Prep Competency; Microscope Teaching Video Series, 2000.

B. Olympus. BX41 System Microscope Instruction Manual, 2003.

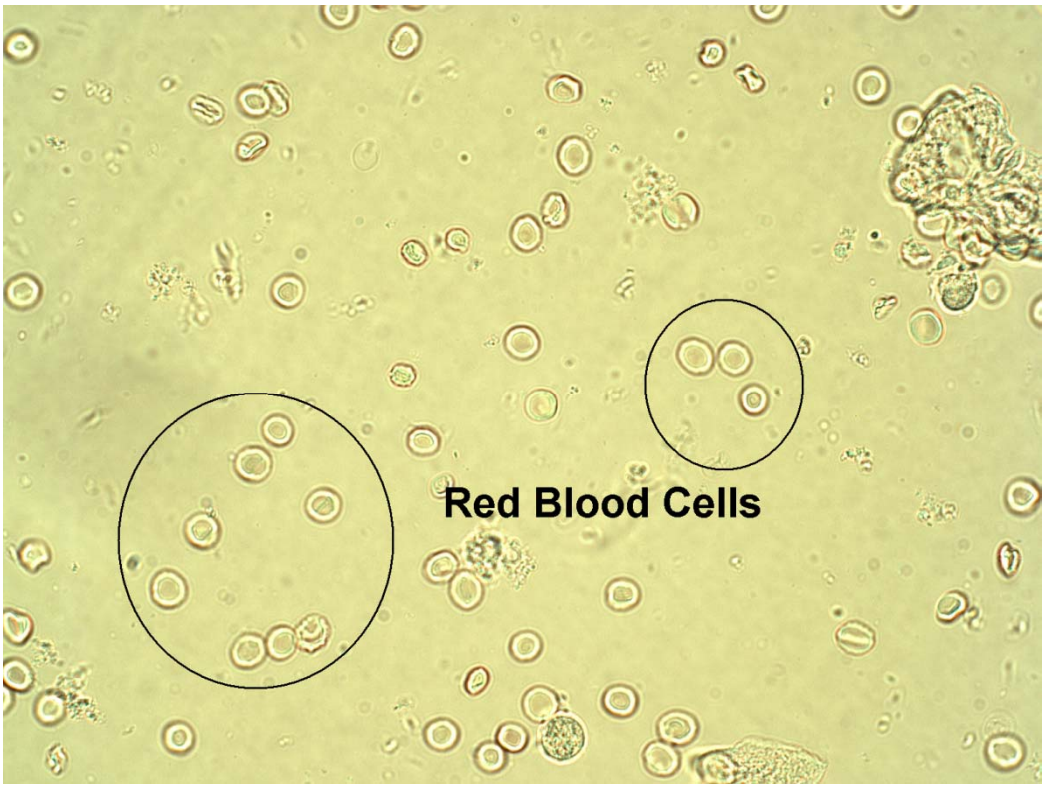
C. Olympus Video Library. The Basic Microscope - Use & Care.

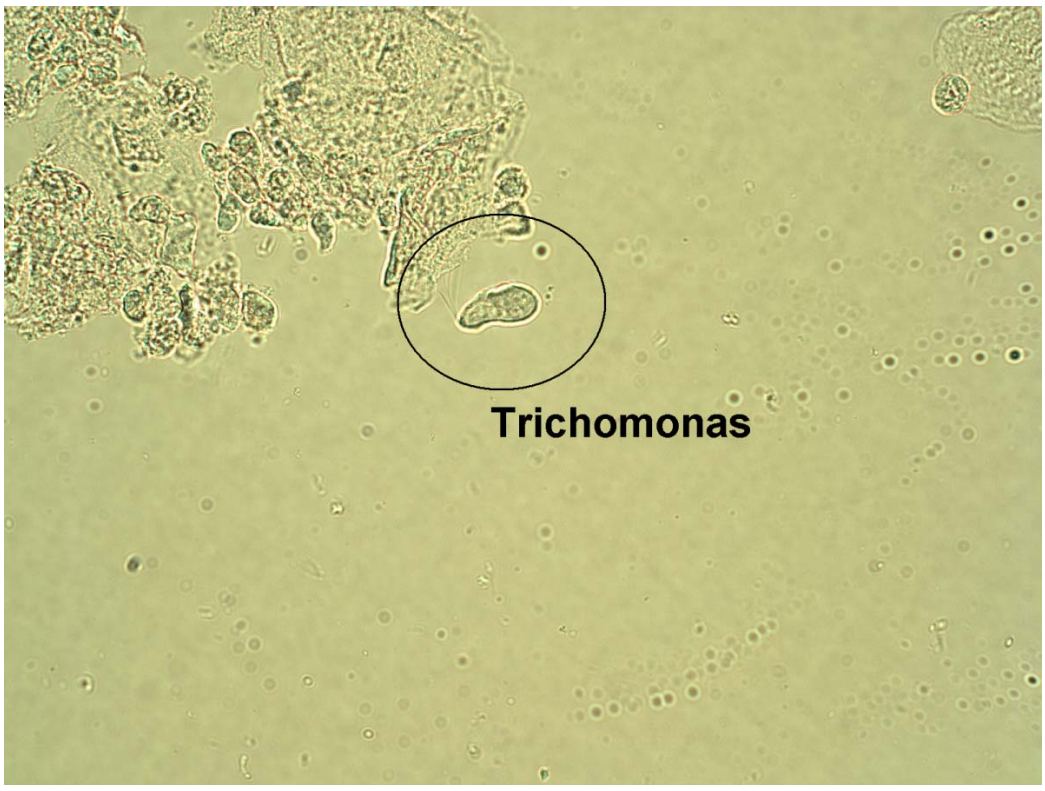
Cellular Components in Vaginal Specimens



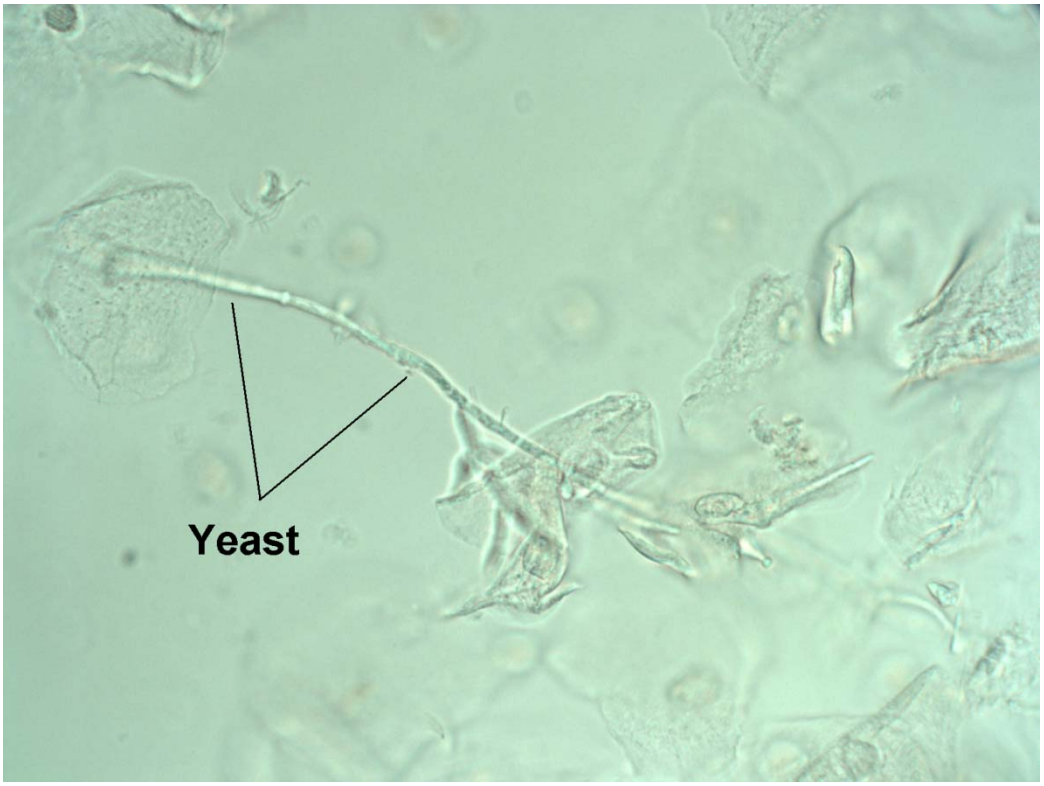


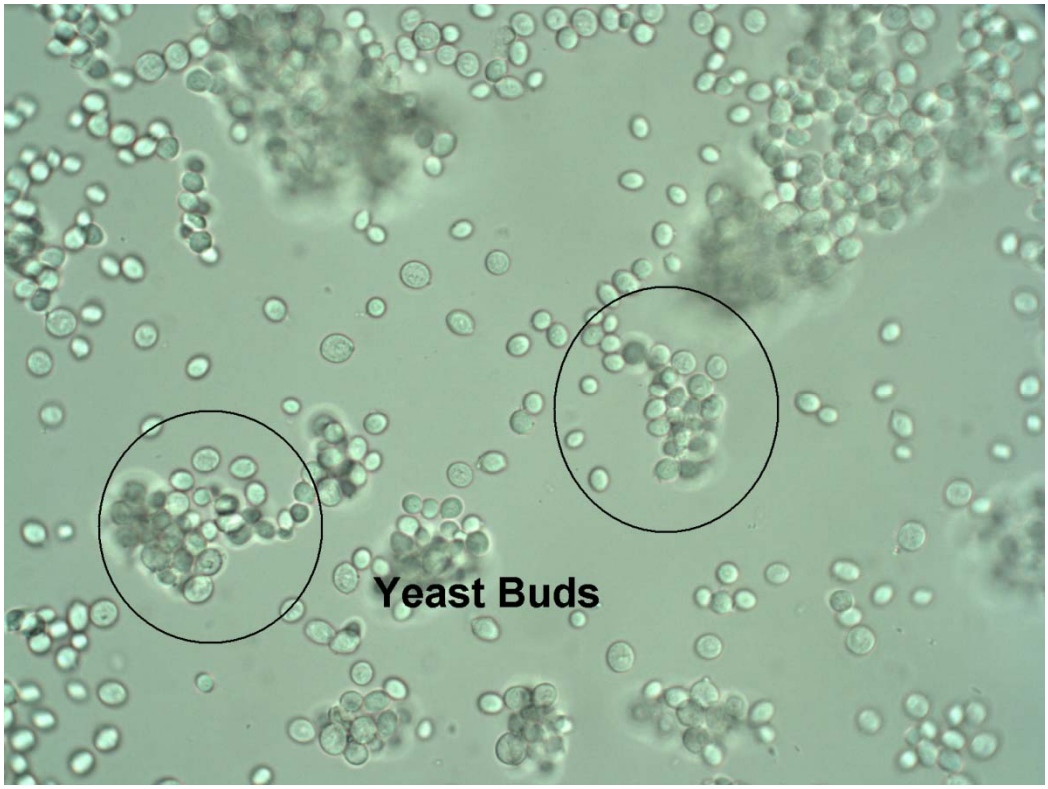
Cellular Components in Vaginal Specimens



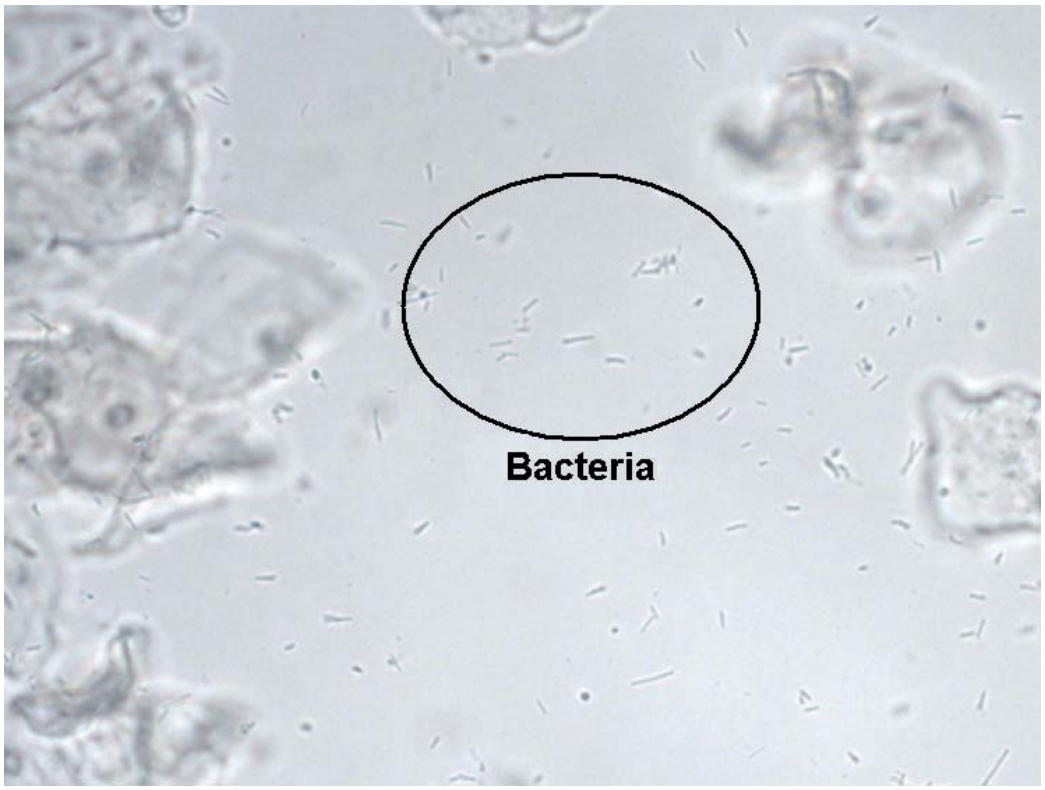


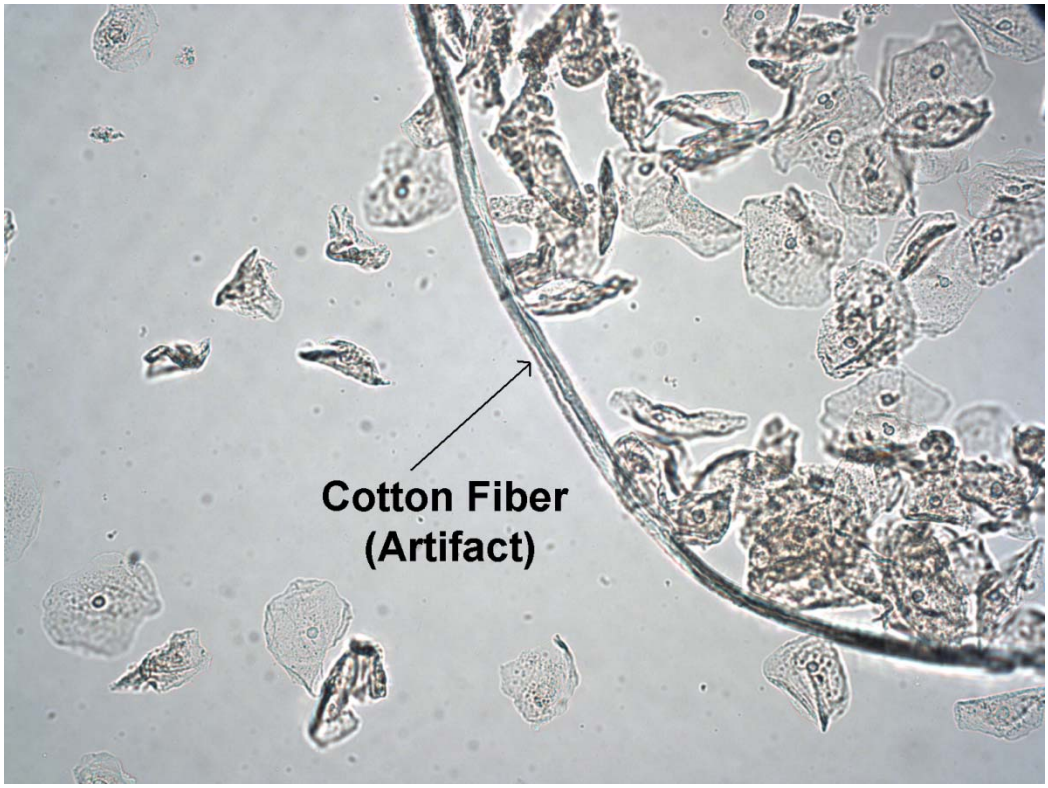
Cellular Components in Vaginal Specimens





Cellular Components in Vaginal Specimens





Rapid Plasma Reagin (RPR) 18-MM Circle Card Test

RAPID PLASMA REAGIN (RPR)
18-MM CIRCLE CARD TEST

I. Principle

The rapid plasma reagin (RPR) 18-mm circle card test is a macroscopic, nontreponemal flocculation card test used to screen for syphilis. The antigen is prepared from a modified Venereal Disease Research Laboratory (VDRL) antigen suspension containing choline chloride to eliminate the need to heat inactivate serum, ethylenediaminetetraacetic acid (EDTA) to enhance the stability of the suspension, and finely divided charcoal products as a visualizing agent. In the test, the RPR antigen is mixed with unheated or heated serum or with unheated plasma on a plastic-coated card. The RPR test measures IgM and IgG antibodies to lipoidal material released from damaged host cells as well as to lipoproteinlike material and possibly cardiolipin released from the treponemes. The antilipoidal antibodies are produced not only as a consequence of syphilis and other treponemal diseases but also in response to nontreponemal diseases of an acute and chronic nature in which tissue damage occurs. If antibodies are present, they combine with the lipid particles of the antigen causing them to agglutinate. The charcoal particles coagglutinate with the antibodies and appear as black clumps against the white card. If antibodies are not present, the test mixture is uniformly gray. Without some other evidence for the diagnosis of syphilis, a reactive nontreponemal test does not confirm *Treponema pallidum* infection.

II. Specimen Collection and Handling

A. Specimen
1. Avoid accidental infection when collecting and processing samples by observing universal precautions.
2. Serum and plasma are suitable specimens for the qualitative test. Test plasma samples within 48 hours of collection.
   An acceptable specimen does not contain particulate matter that would interfere with reading test results.
   Note: Hemolysis may be caused by transporting blood in freezing or extremely hot weather without proper insulation.

B. Collection
1. Serum – Collect whole blood into a clean, dry tube without an anticoagulant.
2. Plasma – Collect blood in a tube containing EDTA as an anticoagulant. Completely fill the tube or collect blood until the vacuum in the collection tube has been exhausted.
3. Label each specimen with patient identifier and date.

C. Handling Serum
1. Allow sufficient time (approximately 20 minutes) at room temperature for the specimen to clot.

2. Centrifuge the specimen at room temperature at 1500 - 2000 rpms for at least 5 minutes to sediment cellular elements.
3. Keep serum specimens in the original collection tube if testing will be performed within a few hours.
4. Remove serum from the clot and store at refrigerator temperature (2-8°C) if testing is to be delayed. If a delay of more than 5 days is anticipated before testing, freeze the specimen at -20°C or lower. Avoid repeated freezing/thawing of specimens.
5. Although unheated serum specimens may be used, serum may be heated at 56°C for 30 minutes without affecting test outcome.
6. Specimens must be at room temperature (23-29°C or 73-85°F) at the time of testing.

D. Handling Plasma
1. Centrifuge the specimen at room temperature at 1500 - 2000 rpms for at least 5 minutes to sediment cellular elements.
2. Plasma may need to be retained in the original collection tube if the test is to be performed immediately. If not, plasma should be removed from cellular elements.
3. Store plasma specimens at 2-8°C, and test within 48 hours. Plasma samples must be at room temperature (23-29°C or 73-85°F) at the time of testing.
4. Do not heat plasma.
5. Do not use plasma specimens for confirmatory treponemal tests.

III. Specimen Rejection

A. Specimens exceed the acceptable time limit since collection.

B. Specimens are collected in the wrong tube.

C. Specimens are not properly labeled or the identification is questionable.

D. Specimens are not stored properly after collection.

E. Specimens are grossly contaminated, hemolyzed, or lipemic.

F. Core blood and spinal fluid are unsuitable for RPR testing.

IV. Reagent, Supplies, and Equipment (See "Health Department RPR Reagent Requisition")

A. RPR antigen suspension
- RPR antigen suspension is a stabilized combination of 0.003% cardiolipin, 0.020-0.022% lecithin, 0.09% cholesterol, 10% choline chloride, 0.0125M

EDTA, 0.01875% charcoal, 0.01M Na_2HPO_4, 0.01M KH_2PO_4**,** and 0.1% thimerosal in distilled water.
- The antigen suspension is packaged in ampoules.

B. Control serum samples
- They are lyophilized reactive (R), minimally reactive (MR), and nonreactive (NR) control serum specimens on a card.

C. Disposable, calibrated 20-gauge needle without bevel, silicone treated.

D. 1 ml syringe for needle calibration (TB syringes work nicely)

E. Plastic antigen dispensing bottle, 1 dram

F. Plastic-coated RPR cards with 10 circles, each approximately 18 mm in diameter.

G. Dispenstirs®, a disposable, plastic dispensing/stirring device that delivers 50 µl.

H. Mechanical rotator, fixed-speed or adjustable to 100 ± 2 rpm, circumscribing a circle ¾ inch or 2 cm in diameter on a horizontal plane.

I. Distilled water (NOT Saline or "Sterile" Water)

J. Humidifying cover.

L. High-intensity incandescent lamp.

M. Discard containers and disinfectants.

N. Disposable gloves, safety glasses, and protective clothing.

V. Reagent Storage and Usage

A. RPR antigen suspension
- Store unopened ampoules at 2-8°C.
- Do not store the antigen in bright sunlight or in temperatures above 29°C.
- Do not freeze.
- An unopened ampoule of antigen is stable until the expiration date.
- Once placed in the dispensing bottle and refrigerated at 2-8°C (35-46°F), the antigen reactivity remains satisfactory for approximately 3 months.

B. Control serum samples
- Store control cards according to the manufacturer's directions.

C. Plastic-coated RPR cards
- Store at room temperature.

VI. Calibration

See "STD Patient Log and QC Form"
See "RPR Rotator Preventive Maintenance"

A. Needles
1. Check the calibrated needle every day of use and/or if the needle has been dropped, wiped, or when control pattern is not met to ensure the delivery of the correct volume of antigen suspension (60 drops ± 2 drops per ml; 17µl per drop).
2. Place the needle on a 1-ml syringe.
3. Fill the syringe to the 1 ml mark with RPR antigen suspension.
4. Holding the syringe in a vertical position, count the number of drops delivered in 1 ml.
5. The needle is correctly calibrated if 60 drops ± 2 drops is delivered in 1 ml.
6. Replace the needle if it does not meet this specification. Be sure to test the calibration of the replacement needle.

B. Rotator
1. Speed – For all rotators, the speed can be estimated by counting the number of rotations per minute.
   a. To count the rotations, place a pen or pencil next to the rotator and count the number of times the rotator touches the pen or pencil in 60 seconds.
   b. If the rotator is properly adjusted, the count should be 100 ± 2.
   c. The rotator's speed should be checked/calibrated each day of use.
2. Time – The rotator's timer should be checked against another laboratory timer or stopwatch quarterly. The rotator's timer should be 8 minutes ± 15 seconds.
3. Circumference – Check quarterly to make sure the rotator is producing a circle with a diameter of 2 cm or ¾ inch.
   a. Place a blank sheet of paper underneath the edge of the bottom of the rotator.
   b. With the rotator off, secure a pen, pencil or marker next to the side of the rotating platform with a clothes pin, and position it so that the writing surface is barely touching the piece of paper.
   c. Turn the rotator on for several seconds, and let the pen or pencil "draw" a circle on the piece of blank paper. This circle is the circumference of the rotating action.
   d. After stopping the machine from rotating, remove the paper and measure with a ruler the diameter of the circle drawn on the paper. If the diameter of the circle is not 2 cm or ¾ inches, call your technical consultant.

VII. Quality Control

See "STD Patient Log and QC Form"
See "RPR Rotator Preventive Maintenance"

It is the responsibility of the laboratorian to ensure that the reagents are of good quality and standard reactivity. Chemicals and distilled water should be of high quality, and solutions should be prepared according to the manufacturer's directions.

A control card should be run each day patient specimens are tested. Controls will be run before patient testing is performed. Control results must be recorded on the STD Patient Log.

A. RPR Test Antigen
   1. Test results on control serum specimens of graded reactivity must give expected results.
   2. The antigen suspension should show in tests with nonreactive serum specimens the complete dispersion of antigen particles.

B. Daily Controls
   1. Check room temperature. For accurate and reproducible test results, the RPR card antigen suspension, controls, and test specimens must be at room temperature (23-29°C or 73-85°F) when tests are performed.
   2. At each routine test run, check the expiration date of the antigen.
   3. Determine antigen suspension reactivity with control cards of graded reactivity (reactive, minimally reactive, and nonreactive) which have been reconstituted with distilled water.
   4. Use only RPR antigen that reproduces the established reactivity pattern of controls.
   5. Date and initial.

C. Monthly Controls
   1. Clean humidifier sponge.
   2. Remove dust in and/or on the rotator.
   3. Clean black rubber sheeting on top of oscillating plate.
   4. Date and initial.

D. Quarterly Controls
   1. Check rotator timer (8 min ± 15 seconds) and record data.
   2. Check rotator circumference (2 cm or ¾ inch) and record data.
   3. Date and initial.

VIII. Preliminary Procedures and Precautions

A. Check the temperature of the testing area.
B. Check the accuracy of the needle delivery.

C. Check the speed of the rotator.
D. Check humidifying cover on rotator.
E. Run the Reactive, Minimal Reactive, and Nonreactive control samples.

IX. Procedure

A. To prepare antigen for testing:
   1. Attach the hub of the dispensing needle to the fitting on the plastic dispensing bottle.
   2. Rotate the antigen ampoule from end to end gently to resuspend the particles.
   3. Open the ampoule.
   4. Squeeze the dispensing bottle to collapse it.
   5. Insert the needle into the ampoule and withdraw all the antigen suspension into the dispensing bottle.

B. Place a drop of patient serum or plasma onto an 18-mm circle of the RPR test card, using a disposable Dispenstir.

C. Using the inverted Dispenstir (closed end, paddle side), spread the serum or plasma to fill the entire circle.

D. Gently rotate or swirl the antigen dispensing bottle to resuspend the particles.

E. Holding the dispensing bottle and needle in a vertical position, dispense several drops on the edge of the card to clear the needle of air.

F. Add exactly one free-falling drop of antigen suspension to each circle containing serum or plasma. **Do not mix with Dispenstir**.

G. Place the card on the mechanical rotator under a humidifying cover.

H. Rotate the card for 8 minutes at 100 rpms ± 2 rpms.

I. Immediately remove the card from the rotator; briefly rotate and tilt the card by hand (3 or 4 to-and-fro motions) to aid in differentiating nonreactive from minimally reactive results.

J. Read the test reactions (without magnification) in the “wet” state under a high-intensity incandescent lamp.

X. Interpretation of Results

A. The RPR card test is an aid in the diagnosis of syphilis.

- Clinicians combine the RPR card test with results of other serologic tests, darkfield examinations, clinical signs and symptoms, and risk factors in arriving at a syphilis diagnosis.
- Without some other support for the diagnosis of syphilis, a reactive RPR card test is commonly unrelated to *T. pallidum* infection.
- The predictive value of a reactive RPR card test in a serologic diagnosis of syphilis is increased when combined with a reactive treponemal test, such as the fluorescent treponemal antibody absorption test or the microhemagglutination assay for antibodies to *T. Pallidum.*

B. A reactive RPR card test can suggest past or present infection with a pathogenic treponeme; however, it may also be a false-positive reaction.
- False-positive reactions can result from laboratory error as well as serum antibodies unrelated to syphilis infection.
- Technical errors are detected by a nonreactive RPR card test with a second serum specimen.
- False-positive RPR card tests from infections with a nontreponemal disease or other disease conditions are identified by an accompanying nonreactive treponemal test.

C. A nonreactive RPR card test without clinical evidence of syphilis may suggest no current infection or an effectively treated infection.
- A nonreactive RPR card test without clinical evidence of syphilis can be seen in early primary syphilis, in secondary syphilis, as a result of the prozone reaction, and in some cases of late syphilis.
- A nonreactive card test result does not rule out an incubating syphilis infection.

XI. Reporting of Results

A. Report results as follows:
1. Reactive (R) or Nonreactive (NR)
   a. See CHR-11 or CHR-12c and Instructions in the Document Library.
   b. If specimen is a field blood, document as such on the “STD Patient Log and QC Form”.

XII. Calculations

Not applicable.

XIII. Troubleshooting

A. If patient results do not agree with clinical symptoms, an error may have occurred.

B. A testing problem is usually caused by one of the following:
   1. Specimen error.
   2. Reagent error.
   3. Equipment error.
   4. Human error in reading the agglutination of the patient specimen.

C. Review the "Sources of Error" for specific problems which may affect testing, and attempt to determine the cause of the problem.

D. If the cause of the problem is not easily determined, follow a systematic approach to troubleshoot the situation.

E. Double check the specimen identification to ensure that the testing is being performed on a properly collected and labeled specimen on the correct patient.

F. Compare the procedure as you performed it with each step in the written procedure. Make sure the written procedure was followed exactly as it is written.

G. Examine reagents, solutions, and/or materials for:
   1. The right color.
   2. Turbidity or precipitation.
   3. Expiration dates.

H. Check the equipment.
   1. Have function verification checks been performed?
   2. Are function check results within acceptable limits?
   3. Has preventive maintenance been performed at proper intervals?

I. Does the testing personnel have adequate training and experience to properly perform the procedure?

J. An abrupt change in patient results that coincides with a change in reagents, materials, equipment, or testing personnel can easily indicate the source of the problem.

XIV. Sources of Error

A. If the temperatures of the sera, reagents, or testing area are less than 23°C (73°F), test reactivity decreases; if temperatures are greater than 29°C **(**85°F**),** test reactivity increases.

B. If the speed of the mechanical rotator is too fast or slow, improper antigen-antibody interaction will cause unpredictable results.

C. If the time of rotation is too long, test reactivity may be increased. If the time of rotation is too short, test reactivity may be decreased.

D. If the card is excessively rotated and tilted by hand after removal from the rotator, a false reactive may occur.

E. If lighting produces a glare on the card, the reactions may be obscured.

F. If the antigen is outdated or not adequately tested for standard reactivity, the results may be inaccurate.

G. If the serum is unevenly spread in the circle, the antigen and antibody may not mix properly.

H. If hemolyzed, contaminated, or improperly collected serum or plasma specimens are tested, the reactions may be masked.

I. If the moistened humidifying cover is not used to cover the cards as they are being rotated, proper humidity will not be maintained, and test components may dry on the card causing false reactive results.

XV. Remedial Action

A. When you think the cause of the problem is discovered, take proper actions such as:
   1. Collect a new specimen.
   2. Use new reagents/materials.
   3. Correct equipment problems or replace the equipment.
   4. Ensure the written procedure is exactly followed.
   5. Change testing personnel until additional training can be conducted.

B. Retest the patient specimens, and reevaluate to determine if results are acceptable.

C. If results are still unacceptable, continue to troubleshoot to determine the source of the problem.

   1. Do not report any patient results.

   2. Call an outside source to help troubleshoot.

D. If the patient results are now acceptable, the problem is resolved.

E. Document the problem and the corrective action on the appropriate laboratory record form.

XVI. Normal Values

A. Nonreactive

XVII. Reportable Range

Not applicable.

XVIII. Test Limitations

A. The RPR card test cannot be used to test spinal fluids.

B. A prozone reaction may be encountered occasionally.
- In a prozone reaction, complete or partial inhibition of reactivity occurs with undiluted serum (maximum reactivity is obtained only with diluted serum).
- A specimen should be tested for the prozone phenomenon when the clinician suspects syphilis, but the qualitative RPR is nonreactive.

C. The RPR card test may be reactive in persons from areas where yaws, pinta or nonvenereal syphilis is endemic. Generally, residual titer from these infections will be < 1:8.

D. Biological false-positive reactions occur occasionally with cardiolipin antigens, mainly in specimens from persons who abuse drugs, have diseases such as lupus erythematosus, mononucleosis, malaria, leprosy, or viral pneumonia, are pregnant, or who have recently been vaccinated.

E. Nontreponemal test titers of persons who have been treated in latent or late stages of syphilis or who have become reinfected do not decrease as rapidly as those of persons in the early stages of their first infection. In fact, these persons may remain "serofast," retaining a low-level reactive titer for life.

XIX. Course of Action if Test System Becomes Inoperable

A. If the test cannot be performed due to the absence of materials or inadequate materials, if possible, borrow from an appropriate source the materials, reagents, equipment, etc. needed, and run the test.

XX. Reference

A. Manual of Tests for Syphilis, 9th Edition, 1998.

COUNTY HEALTH DEPARTMENT
RPR REAGENT REQUISITION

Person certified to perform RPR testing: _____

Health Department: _____

_____ (_____)_____
Street Telephone number

_____ _____
City Zip code

Date request was sent: _____

RPR REAGENTS	QUANTITY REQUESTED	QUANTITY SENT
Testing cards (50/pkg)		
Control cards (10/pkg)		
Antigen (3/box)		
Needles (10/pkg or each)		
Dispensing bottles (10/pkg)		
Dispenstirs (500/box)		

For office use only. Order filled by: _____ Date filled: _____

Order from: Charlene Thomas, Ashley Megelin, or Eric Seales
Bureau of Clinical Laboratories / Quality Management Division
8140 AUM Drive (P.O. Box 244018)
Montgomery, AL 36117 (36124)
Telephone: 334-213-2853
Fax: 334-260-3483

Please use this form when ordering RPR supplies. Individual components should be ordered as needed. "Whole" kits are no longer in stock.

Please do not phone in orders unless absolutely necessary. Use of this order form will ensure accuracy and expediency in filling orders.

STD Patient Log and QC Form

County _____ **Date** ___/___/___
Site _____ **Page** ___ **of** ___

Patient Name/CHR	RPR	Darkfield	Wet Prep	Comments	Initials

RPR Quality Control Data

Antigen Lot Number _____
Antigen Manufacturer Expiration Date _____
Antigen Open Date _____
Control Card Lot Number _____
Control Card Expiration Date _____

Test Area Temperature (23 - 29°C) _____
Rotator Speed (98 - 102) rpm's _____
Needle Check (58 - 62 drops/ml) _____

CONTROL RESULTS

Reactive _____ (R)
Minimal Reactive _____ (MR)
Nonreactive _____ (NR)

RPR QC Performed By _____
(Signature)

Note: Label the dispensing bottle with the antigen lot number, manufacturer's expiration date, and date antigen was placed in the dispensing bottle (Open Date). Ensure the antigen is used within three months of the antigen open date not to exceed the manufacturer's date.

STD Patient Log and QC Form
Continuation Form

County _____ **Date** ___/___/___
Site _____ **Page** ___ **of** ___

Patient Name/CHR	RPR	Darkfield	Wet Prep	Comments	Initials

STD Patient Log and QC Form

County _____ **Date** ___/___/___
Site _____ **Page** ___ **of** ___

Patient Name/CHR	RPR	Darkfield	Wet Prep	Treatment	Labs	Comments	Initials

RPR Quality Control Data

Antigen Lot Number _____
Antigen Manufacturer Expiration Date _____
Antigen Open Date _____
Control Card Lot Number _____
Control Card Expiration Date _____

Test Area Temperature (23 - 29° C) _____
Rotator Speed (98 - 102) rpm's _____
Needle Check (58 - 62 drops/mL) _____

CONTROL RESULTS

Reactive _____ (R)
Minimal Reactive _____ (MR)
Nonreactive _____ (NR)

RPR QC Performed By _____
(Signature)

Note: Label the dispensing bottle with the antigen lot number, manufacturer's expiration date, and date antigen was placed in the dispensing bottle (Open Date). Ensure the antigen is used within three months of the antigen open date not to exceed the manufacturer's date.

STD Patient Log and QC Form
Continuation Form

County _____ **Date** ___/___/___
Site _____ **Page** ___ **of** ___

Patient Name/CHR	RPR	Darkfield	Wet Prep	Treatment	Labs	Comments	Initials

ADPH/BCL/QM/Rev 10-13

RPR Rotator Preventive Maintenance

County _____

Site _____

Year _____

State Serial # _____

Instructions:

1. Write in actual value, if applicable
2. If no value, use a check mark.
3. Record date and initials when performed.

MONTHLY	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC
Clean Humidifier Sponge												
Remove Dust in or on the Rotator												
Clean Black Sheeting on Oscillating Plate												
Date												
Initials												

QUARTERLY	FIRST QUARTER	SECOND QUARTER	THIRD QUARTER	FOURTH QUARTER
Check Rotator time (8 min ± 15 sec)				
Check Rotator Circumference (2 cm or ¾ inch)				
Date				
Initials				

Date	Corrective Action or Additional Comments or Notes	Initials

Write NIU when not in use.

Darkfield Microscopy

DARKFIELD MICROSCOPY

I. Principle

A. The standard brightfield microscope may be equipped for darkfield examination by replacing the brightfield condenser with either a double- or single- reflecting darkfield condenser. Illumination for darkfield microscopy is obtained when light rays strike the object in the field at an oblique angle so that no direct light rays enter the microscope objective, only the rays reflected from the object. Therefore, the object appears self-luminous against a dark background. When a fluid containing particles, including bacteria or treponemes, is placed on a slide, the oblique rays are reflected from the surfaces upward into the barrel of the microscope; these particles appear brightly illuminated against a black background. This type of illumination can be obtained by using a double-reflecting darkfield condenser or a single-reflecting darkfield condenser.

B. In the double-reflecting darkfield condenser, two reflecting surfaces produce intense illumination; however, this type of condenser requires precise focusing and accurate centering.

C. The single-reflecting condenser contains one reflecting surface which does not produce a sharp focusing of the hollow cone of rays. This characteristic makes it easier to manipulate, but less intense illumination is produced. Thus, the single-reflecting condenser is less desirable when high intensity of illumination is required.

D. Most darkfield condensers require the numerical aperture (NA) of the oil immersion objective be reduced below that of the condenser. This can be accomplished by inserting a funnel stop in the objective or by using the oil immersion objective with a built-in iris diaphragm.

II. Glossary of Terms

A. Aperture diaphragm – either a rotating disk or an iris diaphragm on the condenser used to direct a cone of light to the specimen and enter the objective. It should never be used to regulate brightness. Resolution, control, and depth of field depend on the correct setting of the aperture diaphragm.

B. Arm – for holding the microscope while carrying it.

C. Coarse adjustment knob – for rapid focusing of the specimen.

D. Compound microscope – a microscope made of two lens systems: oculars and objectives.

E. Condenser – the lens system beneath the microscope stage positioned to concentrate light correctly on the specimen and direct light rays into the objective. When the condenser is used at a lowered position, the resolving power is reduced.

F. Depth of field – distance just above and below the focal plane (area being examined) that can be focused clearly.

G. Eyepiece – lens system of the microscope nearest to the eyes.

H. Field diaphragm – an aperture diaphragm which restricts area of illumination.

I. Fine adjustment knob – focuses the lens in small increments.

J. Immersion oil – oil with the same refractive index as glass, 1.515; used between the cover glass and an oil immersion objective to prevent scattering of light in air.

K. Interpupillary distance – distance between the eyes. The eyepieces of a binocular scope must be adjusted so that left and right images merge into one.

L. Koehler illumination – optical illumination providing bright, evenly dispersed, glare-free light with good contrast and resolution.

M. Nosepiece – a revolving plate that holds the objectives.

N. Ocular – eyepiece (lens system of the microscope nearest to the eyes).

N. Parcentric – the ability to center a specimen in the field of view for one objective and have almost the same field in place when rotating to another objective.

O. Parfocal – the objectives are constructed so that only slight refocusing with the fine adjustment knob is needed after rotating to another objective.

P. Resolution – the ability of a microscope to reveal fine detail in a specimen. The better the resolving power of a microscope the closer two objects can be and still be distinguished as two objects.

Q. Stage – the platform on which the microscope slide is placed.

R. Working distance – distance between the coverslip of a slide and the tip of an objective. The low power objective has the greatest working distance. The oil immersion objective has a very small working distance.

III. Adjusting the Microscope for Correct Alignment and Illumination

Always complete the microscope adjustment and have the microscope in satisfactory working condition BEFORE collecting a darkfield specimen for examination.

Note: To complete the microscope adjustment and to verify that the microscope is in good working order before examining patient material, prepare a suspension of gingival scrapings in a drop of saline on a slide of proper thickness, mount with a coverslip, and use this as a control slide.

A. Place the control slide on the stage, and raise the substage containing the darkfield condenser. The top of the darkfield condenser should be slightly below the level of the stage, but as close to the control slide as possible without pushing it up.

B. Turn on the variable transformer to produce the maximum light intensity.

C. Lower the substage slightly and place 2-3 drops of immersion oil on the top of the condenser.

D. Center the specimen over the condenser with the mechanical slide carrier.

E. Slowly raise the substage until complete oil contact between the top of the condenser and the bottom of the slide occurs.

F. Rotate the nosepiece to center the 10x objective over the specimen.

G. Bring the specimen into focus by using the coarse adjustment knob.

H. At this point, center the light in the field by rotating the 2 centering screws at the base of the darkfield condenser.

I. Rotate the nosepiece until the high-dry (40x) objective is in place over the specimen.

J. Bring the specimen into focus by using the fine adjustment knob only.

K. If a satisfactory image is obtained, place a small drop of immersion oil on the cover glass.

L. Rotate the nosepiece until the oil immersion objective (100x) is in place over the specimen and is in contact with the oil on the cover glass.

M. Bring the specimen into focus by using the fine adjustment knob only. (Use of the coarse adjustment knob with the high-dry or oil immersion objectives may cause damage to the objectives by allowing them to come in contact with the specimen slide).

IV. Maintenance

See "Microscope Maintenance Chart"

A. Daily Maintenance
   1. Clean all optical surfaces using lens cleaner and lens paper.
   2. Eyepieces and objectives
      a. Dampen a cotton-tipped applicator with lens cleaner.
      b. Begin in the middle of the eyepiece or objective, and work in a circular motion toward the outer edges.
      c. Lightly touch the surface with lens paper to dry it.
   3. Condenser and illuminator
      a. Dampen lens paper with lens cleaner and gently clean the surface**.**
      b. Use a piece of dry lens paper to dry the surface.

4. Cover the microscope when not in use.
5. Document performance of maintenance and initials of the person performing maintenance.

B. Monthly Maintenance
1. Clean all non-optical surfaces with warm water and mild detergent.
2. Please indicate N/A on the maintenance chart for the checking of KOH.
3. Check saline for cloudiness or contamination.
4. Document the maintenance on the chart.
5. Document the initials of the person performing maintenance.

C. Annual Professional Microscope Maintenance
1. Microscopes should be serviced annually by a professional.
2. Document the company performing the cleaning.
3. Document the date the annual cleaning was performed.

V. Corrective Action Log

See "Microscope Maintenance Chart"

A. Document any problems encountered with the microscope.

B. Document date of the problem.

C. Document corrective action(s) taken.

D. Document initials of employee performing corrective action(s).

VI. Additional Information

A. Turn lamp voltage down and then off if the microscope has a rheostat.

B. If slideways and gears on the mechanical stage become difficult to move, lubricate with machine oil or light grease. Document in the corrective action section of the maintenance chart.

C. Keep at least one extra bulb available in case the bulb in the microscope blows. Contact your technical consultant for ordering information. Follow manufacturer's instructions for ordering and replacing bulbs, or check with your technical consultant.

D. If the microscope requires a halogen lamp, **DO NOT TOUCH** the new bulb with your fingers or gloves. Hold the lamp with a lint-free tissue to install it.

E. Avoid exposure to corrosive fumes, extreme heat or cold, or sudden drastic temperature change. When moving from one temperature extreme to another, allow optical parts to equilibrate until all moisture is evaporated.

F. If immersion oil is required, use only chemically inert, low fluorescent, PCB-free immersion oil. Low viscosity is acceptable. **DO NOT USE CEDAR WOOD OIL.** Different brands of immersion oil are incompatible and should not be mixed.

G. Use a small amount of oil in a frequently-cleaned application bottle to minimize growth of fungi which may reflect erroneously on specimen content.

VII. Helpful Hints

A. Turn on the light source.

B. Be sure low objective is in position.

C. Rack down, then up until field is in focus.

D. When moving from one objective to another, use fine adjustment knob to refocus.

E. Never drag high-dry objective through the oil.

F. The higher the magnification, the more light will be needed.

G. Always clean the oil from the objective and/or condenser at the end of the day.

H. Be sure low power objective is in working position at the end of the day.

I. Cover the scope when you have finished for the day.

VIII. References

A. Alabama Department of Public Health. Wet Prep Competency; Microscope Teaching Video Series, 2000.

B. Dark-Field Microscopy and Morphology of *T. pallidum.*

C. Manual of Tests for Syphilis, 9th Edition, 1998.

D. Olympus. BX41 System Microscope Instruction Manual, 2003.

E. Olympus Video Library. The Basic Microscope - Use & Care.

Microscope Maintenance Chart

County: _____ **Year:** _____ **Manufacturer:** _____

Location: _____ **State #:** _____ **Model #:** _____

January	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															
February	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
Clean optical surfaces																															
Cover when not in use																															
Initials																															
March	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															
April	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Clean optical surfaces																															
Cover when not in use																															
Initials																															
May	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															
June	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Clean optical surfaces																															
Cover when not in use																															
Initials																															

Monthly Maintenance	**January**	**February**	**March**	**April**	**May**	**June**
Clean nonoptical surfaces						
Check KOH solution						
Check Saline						
Check pH paper for color change						
Date/Initials						

Annual Professional Microscope Cleaning Company _____

Annual Professional Microscope Cleaning Service Date _____ / _____

Previous Year Current Year

"NIU" indicates not in us

S=Saturday/Sunday, H=Holida

ADPH/BCL/QM/ Rev 10-13

Microscope Maintenance Chart

County: _____ **Year:** _____ **Manufacturer:** _____

Location: _____ **State #:** _____ **Model #:** _____

July	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															
August	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															
September	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Clean optical surfaces																															
Cover when not in use																															
Initials																															
October	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															
November	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Clean optical surfaces																															
Cover when not in use																															
Initials																															
December	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean optical surfaces																															
Cover when not in use																															
Initials																															

Monthly Maintenance	**July**	**August**	**September**	**October**	**November**	**December**
Clean nonoptical surfaces						
Check KOH solution						
Check Saline						
Check pH paper for color change						
Date/Initials						

CORRECTIVE ACTION LOG:	**Date / Problem / Corrective Action / Initials**

Darkfield Microscopy for the Detection and Identification of *Treponema pallidum*

DARKFIELD MICROSCOPY FOR THE DETECTION AND IDENTIFICATION OF *TREPONEMA PALLIDUM*

I. Principle

A. A clinical diagnosis of syphilis is confirmed by using darkfield microscopy to demonstrate *Treponema pallidum* in material from suspected lesions or regional lymph nodes. A positive darkfield result is an almost certain diagnosis of primary, secondary, or early congenital syphilis. For patients with early primary syphilis or for patients with syphilitic lesions and advanced acquired immunodeficiency syndrome (AIDS), the darkfield examination may identify the etiologic agent of syphilis and help diagnose the disease even when antibodies to *T. pallidum* cannot be detected.

B. Proper equipment, adequately trained personnel, and the examination of several slides may be required to demonstrate the presence of *T. pallidum* in lesion material by darkfield microscopy.

II. Specimen Collection and Handling

To avoid infection when collecting specimens, observe universal precautions. Before collecting specimens, ensure that the darkfield microscope is in good working order.

A. Specimen
1. The ideal specimen for darkfield examination is a serous fluid rich in *T. pallidum* but contains few blood cells (treponemes may be obscured if many cells are present).
2. Consider every genital lesion in sexually active patients as syphilis until subjected to a darkfield examination and proven otherwise. Other lesions on the skin or mucous membranes should be examined when syphilis is suspected.
3. Darkfield examination of oral lesions is not recommended. All positive darkfield tests from mouth specimens must be confirmed by a direct fluorescent antibody test. The indigenous flora of the oral cavity frequently contains a spiral organism, *T. denticola*, which is indistinguishable from *T. pallidum*.
4. If topical antimicrobial therapy has been applied to a syphilitic lesion, it may not be possible to demonstrate motile *T. pallidum* even if several specimens are examined.

B. Collection
1. Lesions
   a. Remove any scab or crust covering the lesion.
   b. Secondary infection exudate, if any, should be removed with a sterile gauze sponge.

c. If necessary, compress the base of the lesion or apply a suction cup to it to promote the accumulation of tissue fluid on the ulcer surface.
d. Apply a glass slide to the opened lesion to transfer the fluid from the lesion to the slide.
e. Place a cover glass on the specimen and flatten or depress it evenly on the slide, using the blunt end of an applicator stick to remove air bubbles.
f. Examine the slide immediately.
g. To prevent drying, place additional slides containing specimens in a moist chamber such as a large plastic petri dish containing a moist paper towel. **Note**: The slide preparations should not contain a large volume of fluid (large volumes cause a rapid liquid flow across the field), nor should the preparation be so thin that it begins to dry before an adequate examination can be made.

2. Dry papulosquamous lesions of the skin
   a. Gently remove the superficial layer of skin with a scalpel, needle tip, or mechanical abrader.
   b. Try not to cause bleeding. If very little serous fluid appears, compress the lesion.
   c. Touch the corner of the surface of a microscope slide to the fluid to transfer the material to the slide.
   d. Material can also be collected by injecting a small drop of sterile saline into the base of the lesion and aspirating the fluid with a small-gauge needle and syringe.
3. Cervical/vaginal lesions
   a. With visualization by a bivalve speculum, remove any cervical or vaginal discharge.
   b. Clean the area around the lesion with sterile gauze.
   c. Obtain serous exudate from the lesion.
   d. Serous exudate, if necessary, can be produced from the lesion by compressing it with a Kelly clamp.
   e. Prepare slides as described in number 1 of this section.
4. Mucous patches
   a. Collect some of the mucous material and place it on a clean glass slide.
   b. Place a cover glass on the specimen, and examine it immediately.

C. Handling
   1. Label slide with patient identifier.
   2. Examine the slide within 5-20 minutes of collection, either by bringing the patient to the microscope or the microscope to the patient.

   Note: Any appreciable delay in examining a specimen may result in questionable findings because the motility of the treponemes may be reduced or completely lost.

III. Specimen Rejection

A. Specimens not properly labeled or where the identification of the sample is questionable should not be tested.

IV. Reagent, Supplies, and Equipment

A. Reagents
1. Saline, physiological (0.85%), sterile
2. Disinfectant (70% alcohol or iodine solution on swabs)

B. Equipment
1. Microscope assembly containing:
   a. Condenser – darkfield, oil immersion condenser; single- or double-reflecting type
   b. Substage – a rack-and-pinion focusing substage for holding a darkfield condenser
   c. Microscope stand with course and fine adjustment knobs and revolving nosepiece for 3 objectives
   d. Body – inclined monocular or binocular type
   e. Stage – plain stage with an attachable graduated or ungraduated mechanical slide carrier
   f. Objectives – parfocal
      - 10x – low-power used to focus on specimen and center condenser; NOT used to search specimen
      - 40x - 45x – high-power used to search the specimen
      - 90x – 100x – oil immersion fitted with a funnel stop or built-in iris diaphragm used for final identification of organisms
   g. Oculars – 10x
   h. Illuminator – should be built into the base of the microscope
      - It should not be attached to the darkfield condenser because the heat generated may cause complete loss of a critical identifying criterion such as motility.
      - The built-in base illuminator should consist of a 6.0 to 6.5 volt or equivalent, high intensity lamp with a variable transformer for regulating light intensity.
      - If a separate external illuminator is used for the microscope, it should be equipped with an iris diaphragm and a 100-watt lamp which requires the microscope to have a flat-surface mirror for reflecting the light into the darkfield condenser.
   i. Eye shields – can be obtained for the oculars of some binocular microscope models, thus eliminating the necessity of having to work in a darkened room.
2. Microscope slides – 1 x 3 inches (clean and free of scratches)
   a. Using slides of the correct thickness is very important.
   b. The thickness required by American-made microscopes is usually engraved on the top of the darkfield condensers.
   c. For foreign-made microscopes, refer to the manufacturer's literature.

3. Cover glass – size number 1, 22 x 22 mm (clean and free of scratches)
4. Oil – immersion, nondrying, Cargille type A (Cargille code 1248, R. P. Cargille, Inc., Cedar Grove, N.J.) or equivalent
5. Lens paper and lens cleaner
6. Applicator sticks
7. Surgical gloves (latex or nitrile)
8. Gauze, 2 x 2 inches, sterile
9. Scalpel
10. Pipette – sterile, disposable capillary with safety pipetting device
11. Speculum, bivalve
12. Clamp (Kelly or hemostat)
13. Syringe – 1 or 2 ml with 20-gauge needle, sterile
14. Petri dish, plastic, 150 x 15 mm
15. Containers and disinfectant for discards
16. Paper towels

V. Reagent Storage and Usage

A. Store reagents at room temperature.

VI. Calibration

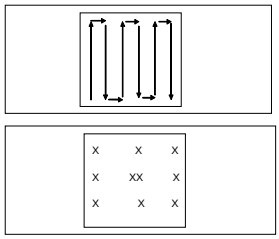
Calibration is not applicable.

VII. Quality Control

A. To complete the microscope adjustment and verify that the microscope is in good working order before examining the patient material, prepare a suspension of gingival scrapings in a drop of saline on a slide of proper thickness, then mount with a cover glass. Examine under oil immersion in a systematic manner.

B. Systematic Pattern of Exam.

Examine @ 10X & 40X



C. Treponemes observed in the patient's specimen must have characteristic morphology and motility for *T. pallidum* as described in this manual.

VIII. Preliminary Procedures and Precautions

A. Ensure that the darkfield microscope is in good working order, and employees are properly trained in its use.

IX. Procedure

A. Examination of the specimen for *T. pallidum*
   1. Place the slide to be examined on a previously adjusted darkfield microscope.
   2. Search the entire specimen methodically with the high-dry objective for spiral organisms that have the morphology and motility characteristics of *T. pallidum.* Search carefully, systematically, and exhaustively before making a negative report.
   3. If a suspected treponeme is observed, center it in the field with the slide carrier so it can be examined with the oil-immersion objective.
   4. Rotate the nosepiece halfway so that a small drop of immersion oil can be placed on the cover glass.
   5. Continue to rotate the nosepiece until the oil-immersion objective is in place over the specimen and is in contact with the oil on the cover glass.
   6. Examine the organism carefully, focusing the fine adjustment knob only.
   7. When organisms are found that have the characteristic morphology and motility of *T. pallidum,* report as positive.
   8. After examining a slide, discard it into a container of suitable bactericidal solution such as 70% alcohol or 10% hypochlorite.
   9. After examining all specimens for the day, remove the immersion oil from the stage, the darkfield condenser, and the oil-immersion objective of the microscope. Use only lens paper and lens cleaner to clean the oil-immersion objective to avoid scratching the lens.
   10. Keep the microscope free of oil and dust and in good working order at all times.

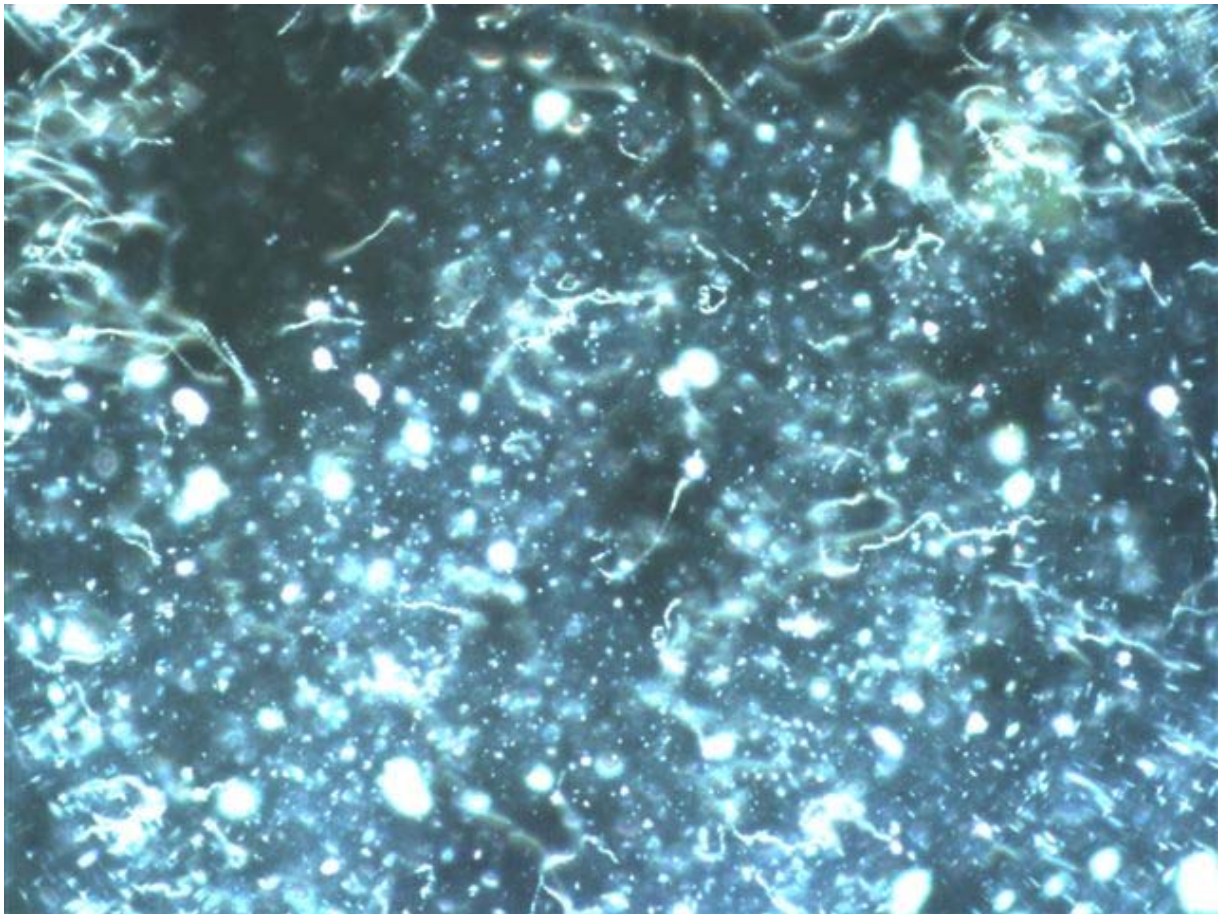
B. Differentiation of *T. pallidum* from other organisms and objects
   1. Characteristics of *T. pallidum*
      a. Morphology
         i. Shape – delicate, corkscrew-shaped organism with rigid, uniform, tightly wound, deep spirals
         ii. Length – 6-20 micrometers (µm), average length = 10 µm. The average organism is slightly longer than the diameter of a blood cell (8 µm).
         iii. Width – approximately 0.13-0.15 µm
         iv. Spiral wave length – approximately 1.0-1.5 µm
         v. Spiral depth – approximately 0.5-0.7 µm

         Note: Coil appearance is maintained despite active motility of the organism.
      b. Motility
         i. Translation (uniform movement in a straight line) – slow, may exhibit deliberate forward and backward movement with occasional erratic movement

ii. Rotation – slow to rapid rotation about the longitudinal axis (like a corkscrew); may rotate without changing place
iii. Flexion – rotation is accompanied by soft bending, twisting, or undulation from side to side, giving a shimmering, graceful effect. Bending of the organism usually occurs in the middle and is stiffly executed, like the bending of a coil spring which comes back into place when released.
iv. Distortion – may occur as a ring with ends seemingly attached or occur as tortuous convolutions. When attached to or obstructed by heavier objects, vigorous struggling distorts the coils.

2. Characteristics of *T. refringens* (formerly *Borrelia refringens*)
   a. A member of the normal genital flora
   b. Morphology
      i. Shape – spiral organisms that may appear loosely coiled, thick, and coarse
      ii. Length – approximately 5-16 µm
      iii. Width – approximately 0.2-0.3 µm
   c. Motility
      i. Translation – rapid movement across or out of the field with a writhing motion
      ii. Rotation – active serpentine and rotating motion with marked flexion. The organism may rotate so rapidly that it looks straight.
      iii. Flexion – marked bending and frequent relaxation of coils
3. If the material on the slides contains numerous artifacts or refractile objects, the untrained observer may be deceived by pieces of cellular debris, flagella, and wavy fibrin filaments which can, because of Brownian movement, be quite deceptive and must be interpreted cautiously.
4. In summary, *T. pallidum* is a thin, tightly wound, spiral organism capable of extreme contortions from which it snaps back to its original form in a coiled springlike manner. It may spin rapidly without translation, move slowly forward and backward without obvious change in direction of rotation or pitch of coils, or may move more slowly and thread its way corkscrew-fashion in viscous material. A springlike rigidity is constant, and *T. pallidum* does not move rapidly from place to place with a serpentine motion. Any coarsely wound spiral organism exhibiting great flexion and rapid movement from place to place is **NOT** *T. pallidum.*

5. Picture of ***T. refringens***



X. Interpretation of Results

A. The earliest and empirically most specific means of diagnosing syphilis (if yaws, bejel, and pinta are excluded) is by darkfield microscopy. The demonstration of treponemes with characteristic morphology and motility for *T. pallidum* constitutes a positive diagnosis of syphilis in primary, secondary, or early congenital stages, whatever the outcome of serologic testing. False-positive darkfield tests may occur with oral specimens; therefore, such positive specimens must be confirmed by direct fluorescent antibody tests specific for identification of *T. pallidum*. When patients with untreated primary syphilis are positive by darkfield microscopy but are serologically nonreactive, they usually become serologically reactive within several days to several weeks. In other stages, the patient should be seroreactive; if not, the darkfield interpretation and serologic results should be analyzed to decide whether test results may be false positives or false negatives, respectively.

B. Every genital lesion should be considered syphilitic until proven otherwise. Extragenital lesions characterized by indolence, induration, and regional lymphadenopathy should be regarded as possibly syphilitic. Failure to find the organism does not exclude a diagnosis of syphilis.

C. Negative results may be reported for the following reasons:
1. The number of organisms was insufficient for detection.
2. The patient has received antitreponenal drugs, topically or systemically.
3. The lesion is "fading" or approaching natural resolution or disappearance.
4. The lesion is one of late syphilis.
5. The lesion is not syphilitic.

D. When the darkfield examination is negative in patients suspected of having primary syphilis, repeated examination (on as many as 3 consecutive days) may be indicated. Theoretically, serologic tests for syphilis should be repeated at 1 week, 1 month, and 3 months. If nonreactive, serologic results are obtained for longer than 3 months in untreated patients, syphilis may be excluded as the cause of such lesions. Among patients suspected of having syphilis in other stages, a negative darkfield examination and nonreactive serologic tests suggest that syphilis is extremely unlikely, and follow-up tests are unnecessary.

XI. Reporting of Results

A. Reporting Laboratory Findings: See CHD-11 (County Health Record) and Instructions.

Results	**Report**
Organisms found that have characteristic morphology and motility of *T. pallidum.*	Darkfield positive

No treponemal organisms found, OR spiral organisms found but without characteristic morphology and motility of *T. pallidum.*	Darkfield negative
No *T. pallidum* found, but specimen had too many refractile elements (blood cells, air bubbles, tissue fragments) to be able to identify any treponemes or the specimen is drying.	Darkfield unsatisfactory

XII. Calculations

Calculations are not applicable.

XIII. Troubleshooting

A. Review the "Sources of Error" for specific problems which may affect testing, and attempt to determine the cause of the problem.

B. If the cause of the problem is not easily determined, follow a systematic approach to troubleshoot the situation.

C. Double check the specimen identification to ensure that the testing is being performed on a properly collected and labeled specimen on the correct patient.

D. Compare the procedure as you performed it with each step in the written procedure. Make sure the written procedure was followed exactly as it is written.

E. Examine any reagents, solutions, and/or materials for:
1. The right color.
2. Turbidity or precipitation.
3. Expiration dates.

F. Check the equipment.
1. Have function verification checks been performed?
2. Are function check results within acceptable limits?
3. Has preventive maintenance been performed at proper intervals?

G. Do testing personnel have adequate training and experience to properly perform the procedure?

H. An abrupt change in patient results that coincides with a change in reagents, materials, equipment, or testing personnel can easily indicate the source of the problem.

XIV. Sources of Error

A. Preparation errors
   1. If the specimen contains too many blood cells, air bubbles, or tissue fragments, these refractile elements can obscure the presence of *T. pallidum.*
   2. If cover glasses and slides are dirty or scratched, obtaining a good darkfield will be difficult.
   3. If slides are too thick or thin, the apex of the cone of light will not coincide with the object being studied.
   4. If the cover glass is too thick, it is impossible to focus on the specimen because of the short working distance of the oil immersion objective.
   5. If there is excessive fluid between the glass slide and cover glass, the liquid will flow rapidly across the field of vision, and it will be too deep to scan.
   6. If there is too little fluid between the glass slide and cover glass, the specimen will begin to dry, and the organisms will lose motility.

B. Microscopy errors
   1. If immersion oil is not placed between the condenser and the slide or if immersion oil with an incorrect refractory index is used, no light will reach the specimen.
   2. If slides of improper thickness are used, focusing on the lesion material and illumination of the objects on the slide will be affected.
   3. If, while using a paraboloid condenser, the concave side of the microscope mirror is used with an external light source, the light intensity in the field of view will decrease.
   4. If the darkfield condenser is not properly centered, the object will be illuminated poorly or not at all.
   5. If the darkfield condenser is not properly focused, the most intense illumination of the object will decrease.
   6. If oil and dust are on the subsurface or reflecting area of the darkfield condenser, the intensity of illumination of the object will decrease.
   7. If the high numerical aperture of the oil-immersion objective is not compensated with a funnel stop or an iris diaphragm, undiffracted direct light will enter the oil objective.
   8. If immersion oil is on the lens of the low-power (10x) or high-power (40x-45x) objectives, the picture will be hazy without sharp definition.
   9. If the specimen is inadequately illuminated, it will be impossible to differentiate *T. pallidum* from other spiral organisms.
   10. If the microscope is focused on the cover glass instead of on the specimen, a false-negative report might be issued.
   11. If the search of the specimen is inadequate or unmethodical, a false-negative report might be issued.

C. Errors in differentiating *T. pallidum* from other organisms and objects
   1. If one is unfamiliar with the morphology and motility characteristics of *T. pallidum*, a false-positive or a false-negative report might be issued.

2. If one is unfamiliar with the characteristics of nonspecific spiral organisms, tissue debris, fibrin strand, and other extraneous objects, a false-positive report might be issued.
3. If one mistakes the effects of Brownian movement on spiral objects for motility, a false-positive report might be issued.
4. If one sees occasional erratic movement of *T. pallidum* or no movement at all, too much time may have elapsed between making and examining the slide.

XV. Remedial Action

A. When you think you have discovered the cause of the problem, take proper actions such as:
   1. Collect new specimen.
   2. Use new reagents/materials.
   3. Correct equipment problems or replace the equipment.
   4. Follow written procedure exactly.
   5. Change testing personnel until additional training can be conducted.

B. Retest the patient specimens and reevaluate to see if results are acceptable.

C. If results are still unacceptable, continue to troubleshoot to determine the source of the problem.
   1. Do not report any patient results.
   2. Call an outside source to help troubleshoot.

D. If the patient results are now acceptable, the problem is resolved.

E. Document the problem and the corrective action on the appropriate laboratory record form.

XVI. Normal Values

A. A normal patient is negative for *Treponema pallidum.*

XVII. Reportable Range

Not applicable.

XVIII. Test Limitations

A. Oral lesions at or near the gingival margin are unsatisfactory for darkfield examination, as the indigenous flora in this area frequently contain a spiral organism indistinguishable from *T. pallidum.*

B. The examination of lesion material from patients who have received antitreponemal drugs topically or systemically may produce negative results.

C. Fading lesions of the skin are less likely to yield a positive darkfield because fewer treponemes are present.

XIX. Course of Action if Test System Becomes Inoperable

A. If the test cannot be performed due to the absence of materials or inadequate materials, if possible, borrow from an appropriate source the materials, reagents, equipment, etc. needed and run the test.

XX. References

A. Alabama Department of Public Health. Wet Prep Competency; Microscope Teaching Video Series, 2000.

B. Dark-Field Microscopy and Morphology of *T. pallidum.*

C. Manual of Tests for Syphilis, 9th Edition, 1998.

D. Olympus. BX41 System Microscope Instruction Manual, 2003.

E. Olympus Video Library. The Basic Microscope - Use & Care.

STD Patient Log and QC Form

County _____ **Date** ___/___/___
Site _____ **Page** ___ **of** ___

Patient Name/CHR	RPR	Darkfield	Wet Prep	Comments	Initials

RPR Quality Control Data

Antigen Lot Number _____
Antigen Manufacturer Expiration Date _____
Antigen Open Date _____
Control Card Lot Number _____
Control Card Expiration Date _____

Test Area Temperature (23 - 29°C) _____
Rotator Speed (98 - 102) rpm's _____
Needle Check (58 - 62 drops/ml) _____

CONTROL RESULTS

Reactive _____ (R)
Minimal Reactive _____ (MR)
Nonreactive _____ (NR)

RPR QC Performed By _____
(Signature)

Note: Label the dispensing bottle with the antigen lot number, manufacturer's expiration date, and date antigen was placed in the dispensing bottle (Open Date). Ensure the antigen is used within three months of the antigen open date not to exceed the manufacturer's date.

STD Patient Log and QC Form

County _____ **Date** ___/___/___
Site _____ **Page** ___ **of** ___

Patient Name/CHR	RPR	Darkfield	Wet Prep	Treatment	Labs	Comments	Initials

RPR Quality Control Data

Antigen Lot Number _____
Antigen Manufacturer Expiration Date _____
Antigen Open Date _____
Control Card Lot Number _____
Control Card Expiration Date _____

Test Area Temperature (23 - 29° C) _____
Rotator Speed (98 - 102) rpm's _____
Needle Check (58 - 62 drops/mL) _____

CONTROL RESULTS

Reactive _____ (R)
Minimal Reactive _____ (MR)
Nonreactive _____ (NR)

RPR QC Performed By _____
(Signature)

Note: Label the dispensing bottle with the antigen lot number, manufacturer's expiration date, and date antigen was placed in the dispensing bottle (Open Date). Ensure the antigen is used within three months of the antigen open date not to exceed the manufacturer's date.