CLSI POCT10-A2:2011 Physician Microscopy Testing, 2nd Edition

Microscope

Microscopes are precision instruments. Achieving an accurate result with microscopic techniques requires an understanding of the operating characteristics and limitations of the equipment used. Therefore, analyzing specimens with the microscope requires training in microscopic techniques, knowledge of standard precautions, and an understanding of the capabilities, use, and care of the microscope.

A modern, high-quality microscope with the following characteristics is desirable for examination of patient samples:

- Binocular head to allow the use of both eyes when viewing the specimen
- Built-in light source with field diaphragm
- Mechanical stage to allow the easy and smooth positioning of the slide
- Basic set of objective (10x and 40x) and ocular (10x or 12.5x) lenses

Parts of the Microscope

Lenses

The objective lens magnifies the specimen by a defined amount. The objective produces the primary image and the eyepiece magnifies it. The total magnification of the image is the product of the magnification of the objective multiplied by the magnification of the eyepiece. Magnification is the relationship between the size of the image and the size of the specimen.

The objectives are important lenses in the image-forming system. They are screwed into a revolving nosepiece attached to the stand. Only one objective at a time is moved into the illumination path when the nosepiece is rotated.

The microscope and objectives are constructed so that each objective aligns with light. After the first objective is focused, only a slight adjustment of the fine adjustment knob is required to focus the specimen. Also, an object seen in the center of the field when objectives are changed should stay close to the center of the field. The clarity, sharpness, detail, and visibility needed for good performance in microscopy depend on the quality and care of the objectives.

The oculars (eyepieces) are placed in the top openings of the observation tubes of the microscope and magnify the primary image projected by the objective.

Stand

Characteristics of the stand include the following:

- Rests on the base of the microscope
- Carries the arm of the microscope and the stage on which the specimen is placed

Stage

Characteristics of the stage include the following:

- Horizontal platform on which the specimen slide is placed

• A slide holder fitted to the top to hold the slide in position
• Knobs to control the movement of the stage and the slide-holder mechanism (slight movement of the slide can carry the observed area out of view, so movements should be slow and smooth)

Condenser

Characteristics of the condenser include the following:

• Mounted under the stage to concentrate and focus light from the light source.
• It can be raised or lowered by means of a condenser knob below the stage.
• It has an aperture iris diaphragm, which can be opened or closed to control the amount of light striking the specimen.
• Centering screws center the circle of light in the viewing field.

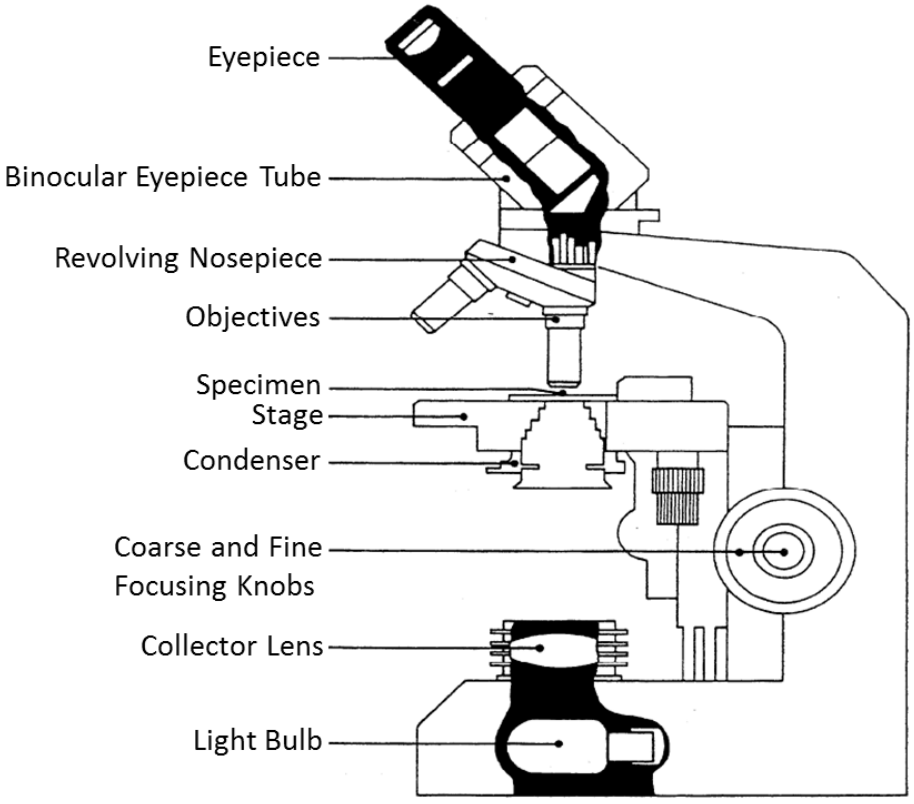
Illumination

A built-in light source, usually a tungsten bulb or tungsten-halogen bulb, works by plugging the microscope cord into an electrical socket and using the on-off switch to turn the bulb on.

Better microscopes have a field diaphragm on the collector lens. The adjustment of this diaphragm increases or decreases the circle of light in the viewing field.

A switch or dimmer may be used to control the intensity of the light. Lowering the intensity before turning off the switch lengthens the life of the bulb.

Figure 1. Standard Laboratory Microscope



Operation

Before using the microscope, read the manufacturer's instructions to become familiarized with the features of the specific microscope. The optimal conditions for the observations discussed in these procedures require that steps be followed to focus the image and adjust the illumination.

To focus a specimen and adjust the microscope for bright, even light and good contrast:

1) Open the diaphragm(s), ie, the condenser's aperture and the field diaphragm; if appropriate, lower the condenser, and turn on the illuminator to low power.
2) Using the low-power (10x) objective, focus on a slide by raising the stage with the coarse adjustment knobs, observing the slide from the side, until the slide comes close to the objective. Looking through the objectives, focus slowly with the coarse and/or fine focusing knobs until the image is sharpest.
3) Close the field diaphragm almost completely and raise the condenser until the edges of the diaphragm are sharply focused (the condenser is usually at about its highest position). Open the field diaphragm slowly, stopping just as it disappears from view.
4) Open and close the aperture diaphragm to optimize contrast. Contrast is increased by closing the aperture. If more light intensity is needed, increase the illuminator.

The microscope is now optimally adjusted for the objective; other objectives will require some readjustment.

To use the high (40x) objective:

1) Focus and center the specimen with the 10x objective, and rotate the nosepiece slowly to bring the 40x objective into the light path.
2) Move the fine adjustment knob slightly to bring the specimen into focus. If it is not clear, refocus with the 10x, making sure the specimen is in the center of the field; switch back to the 40x objective. NOTE: Never raise the stage with the coarse adjustment knobs when using the 40x lens. This may cause the lens to break by hitting it with the slide.

Care of the Microscope

Microscopes should be inspected, cleaned, and checked each day of use or a minimum of once a week. This ensures eyepieces, objectives, and condensers are free of fingerprints, and that the fine and course adjustments are operating smoothly and correctly for maximum clarity during use. To guarantee long-term quality microscope use, follow the manufacturer's guidelines for care and maintenance of the microscope. At a minimum, professionally performed maintenance should be completed annually. However, this timeframe depends on the number of persons using the microscope and the volume of testing performed. Also:

- Cover the microscope when it is not in use and leave the 10x objective in position.
- Do not expose the microscope to extreme heat, cold, or temperature changes.
- Keep all parts clean; clean external optical surfaces only (bottom of objectives, top of condenser and illuminator, top eyepiece lenses).
- Remove excess lotions, powders, or emollients from hands before use.
- Wipe dust off mechanical surfaces with a soft, lint-free cloth.
- Clean objectives, stage, condenser, and illuminator with lens paper moistened with a commercial cleaning solution for lenses or a manufacturer-recommended solution.

- Never use cleaning fluid on the underside lenses of the objective.
- Use only the bulb and illuminator as specified by the manufacturer to avoid excess heat.
- Keep a spare bulb in a convenient place.
- Never force open diaphragm leaves.
- Schedule periodic maintenance to include professional servicing (lubrication, fine tuning, and realignment will be done at this time).

Quality Assurance

QA is an ongoing process established to ensure that laboratory test results are accurate and reliable. Accurate and reliable laboratory results assist the provider in diagnosis and/or treatment of a patient's condition for a quality outcome.

Major elements in the QA process include:

- Identifying problems and errors
- Assessment of the causes
- Designing corrective actions
- Monitoring to ensure correction

QA is an important part of a comprehensive quality system, consisting of policies, procedures, and practices that identify and regularly evaluate all aspects of the examination process that can affect test quality and patient outcomes. Aspects include specimen collection and handling (preexamination), performance of the test (examination), and test reporting and documentation (postexamination).

Collectively, the QA system involves review and evaluation of provider training and competence, safety, equipment function, patient test management (ordering and reporting), procedures for test performance, mechanisms for QC of the test, proficiency testing (PT), test results, error detection, investigation and implementation of corrective actions, and appropriate certification and/or accreditation. This broad QA approach ensures that:

- Test procedures and protocols are established and followed.
- Test or examination systems function properly when patient results are produced.
- Providers perform the test or examination competently.
- Patient test results are consistent with the patient's clinical presentation.
- Written records are available to demonstrate that procedures and protocols are uniformly followed.

Microscopy Provider

Training

The person responsible for oversight of the testing should first establish the necessary qualifications (education and experience) required for those performing microscopic examinations, and then train qualified individuals. Training should be developed and provided to all providers who conduct the microscopy testing. The scope of the position in the workplace should be documented by a position description or contractual documents to comply with any regulatory agencies or standards of practice in the medical community.

It is helpful to use a training checklist for each procedure to be performed so that each critical step in the process is understood and can be performed correctly before testing patient specimens. The training protocols must include preexamination aspects such as patient preparation and specimen collection, examination steps in the procedure, and postexamination aspects such as standardized recording of results and correlation with presenting symptoms. The training activity and results should be documented in provider personnel records prior to performing any patient testing. As part of the training, it is important that providers read and sign all procedure manuals, safety procedures, policies, and equipment manuals that are associated with microscopic examinations.

Competence Assessment

All providers performing microscopic examinations must have a competency assessment conducted prior to performing any patient testing. Providers should be assessed twice during the first year of testing and annually thereafter to ensure that they maintain competence to perform test procedures and report test results promptly, accurately, and proficiently.

Procedures for evaluating the competence of the testing provider may include a documented system of:

- Direct observation of patient test performance by another experienced provider
- Monitoring of the recorded results of testing by a consultant or experienced provider, with consideration for the correlation of patient information to patient test results
- Assessment through review of testing of previously analyzed specimens or proficiency test samples by the provider responsible for the testing location
- Assessment examinations using microscopy images in atlases or textbooks, photographs, or computer software
- Assessment through proficiency testing surveys

The occurrences of errors detected through the QA system offer another opportunity to assess competence. Such detected errors should be investigated, analyzed, and corrected according to QA protocols, with appropriate documentation and follow-up to prevent recurrence.

Proficiency Testing

PT programs were developed to investigate the variation in results of testing among different testing sites, assess the ongoing accuracy of test results, and facilitate education through the resolution of problems causing PT failures. PT providers administer external or commercial PT programs. A testing site voluntarily enrolls with a PT provider and pays an enrollment fee. A PT program provides stable specimens sent to subscribing testing sites, which submit their independently derived results to the program provider for assessment. Examination of the peer group data gives a guide to accuracy and methodology comparison. PT should be performed on all microscopy tests, when available, to verify and assess accuracy, measure precision, and detect errors. PT samples should be tested in the same manner as patient specimens; therefore, the handling, preparation, processing, examination, and documentation of results should be performed by those providers who routinely perform the patient testing. The PT provider grades the results as “pass” or “fail” and then sends the grades back to the testing site. The grades should be reviewed and shared with all providers as part of the testing site’s QA, continuing education, and competence evaluation systems.

When PT is performed for microscopy tests, there are several steps to follow to ensure good performance:

1) Where available, commercial PT program surveys are used for every microscopy test in order to have an external, independent evaluation and comparison to peer testing facilities.
2) The individual testing site is responsible for ensuring that a system for receipt, handling, distribution, and analysis of the material, and return of results by the stated deadline, is in place.
3) Following approved laboratory procedures, PT samples are handled in the same manner as patient specimens. Most microscopy PT challenges are in the form of photographs or slides. It is imperative to adhere to the following guidelines:
    a. Integrate samples within the routine laboratory workload (with attention to any directions regarding time to completion), including performance on off-shifts.
    b. Perform testing and rotate it among personnel who routinely perform testing.
    c. Give no special treatment to the performance of testing.
    d. Do not communicate with other laboratories regarding results before submission of results is confused.
    e. Do not refer PT samples to another laboratory or location for testing.
4) Before submission, external PT results should be reviewed for clerical errors. Results must be recorded accurately on the PT program's result form. Transcription errors are causes for PT failure. The significance of this type of error is apparent if one considers the consequences of transcribing patient results incorrectly.
5) The testing personnel and director or designee must sign the included attestation statements before submission.
6) A copy of all documentation must be retained for two years. Evaluation by the source agency, peer evaluation information, and records of problem samples or exception reports must also be retained for two years. The entire result form should be copied and retained. When a failure occurs, it is a good idea to verify that results were entered correctly by the PT program by comparing the evaluation report to those results documented on the copy of the result form that was sent to the PT program.
7) There must be proof of review of each external program evaluation. Such proof is provided by the signature of the testing site director on the evaluation form. Review of external PT program evaluation should occur within recommendations of the commercial PT supplier or accreditation body.
8) For most external PT programs, an 80% pass rate is required on PT results; however, it is a better practice to investigate all failures and document them on a PT Exception Response Form. The original should be retained with the PT records for a minimum of two years.
9) Documentation of corrective action should include investigation into the following:
    a. Methodological or technical problems
    b. Clerical error
    c. Problems with PT sample (eg, matrix)
    d. Assessment as to whether performance impacted patient care
    e. The results of the investigation (these should be shared with the person who performed the initial analysis, and re-education or demonstration of competence should be demonstrated)
    f. Record of the remedial actions taken for PT results that are not graded "acceptable," and use of the information to improve performance
10) The results of PT may be used to cover or complement competence assessment of testing personnel.

Documentation of Patient Testing Results

It is a vital part of the QA postexamination process to consider the relationship of known patient information to the produced patient test results. In addition to the assessment of the accuracy and reliability of the testing process, a review of test results must include assessing the logic of the relationship of test results to the patient's age, sex, diagnosis, and other test results. Since it is intended that the practitioner performing the test is the same as that assessing the patient's condition, it may appear obvious that the results would be evaluated for consistency with relevant clinical information. When results appear inconsistent or absurd, the preexamination phase of testing must also be examined (as well as the examination phase) to determine if the patient sample was collected, identified, and tested properly.

The consideration of the expected results with the actual results may also include later comparisons with data obtained through alternate testing methods. Such comparisons provide additional opportunities to examine concerns with all phases of PPM testing. QA reviews should include assessments as to whether or not the inconsistencies have been resolved after implementing solutions. Unresolved problems should prompt retesting, if possible, or alternate diagnostic procedures to confirm or rule out the suggested diagnosis.

Ferning Test

Principle

The ferning test is used to evaluate the pregnant female for the presence of amniotic fluid in vaginal secretions. The provider can rely on a combination of patient history and a positive ferning (crystallization) test to decide whether the amniotic sac has ruptured. Due to the protein and sodium chloride content, amniotic fluid crystallizes when allowed to air-dry on a microscopic slide. The test is based on the ability of amniotic fluid to form a fern pattern.

Materials

Supplies
Supplies used for the procedure include the following:

- Glass slides
- Sterile vaginal speculum
- Sterile cotton-tipped swab

Equipment
Equipment used for the procedure is a microscope with a 10x and 40x objective.

Reagents
Reagents are not required for the fern test.

Specimen Collection

Conditions for Patient Preparation
The patient is examined in the dorsal lithotomy position. Avoid use of any lubricants or antiseptics, because these may interfere with this test.

Specimen Handling and Slide Preparation

Swab Procedure

1) Label a clean glass slide with the patient's name and unique identifier.
2) Saturate a sterile swab with vaginal fluid obtained from the posterior vaginal pool. Do not touch the mucous plug while sampling.
3) Roll the saturated swab tip across the slide while applying slight pressure to express the fluid from the slide. Spread the specimen so that a thin smear is formed.
4) Place the slide on a flat surface avoiding air currents.
5) Allow the slide to dry completely in room air. This will require approximately five to ten minutes; do not blow air or heat the slide to shorten dry time. Do not apply a coverslip.

Testing Procedure

Once the sample is air-dried, examine the slide under the microscope at 10x and 40x magnification and interpret findings.

Quality Control

No commercially prepared controls are available for the fern test. Photographic examples of positive ferning and negative ferning should be available to providers for comparison.

Reporting Results

Amniotic fluid forms a tree-like or fern pattern when air-dried. Report fern test positive when this characteristic pattern is present (see Figure 2). This indicates the presence of amniotic fluid, and therefore, rupture of fetal membranes. Report fern test negative when no fern pattern is seen on the slide (see Figure 3).

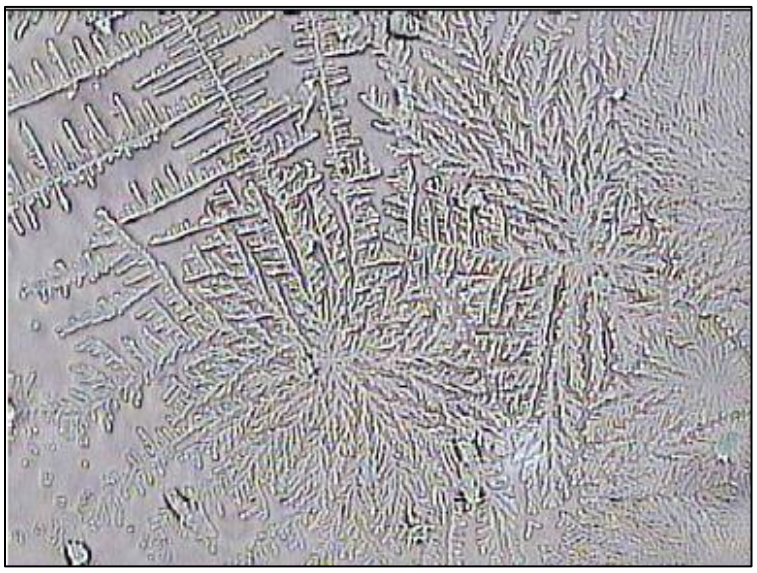


Figure 2. Positive Fern Test (10x) (Courtesy of Dr. Mohammed Hanafy)

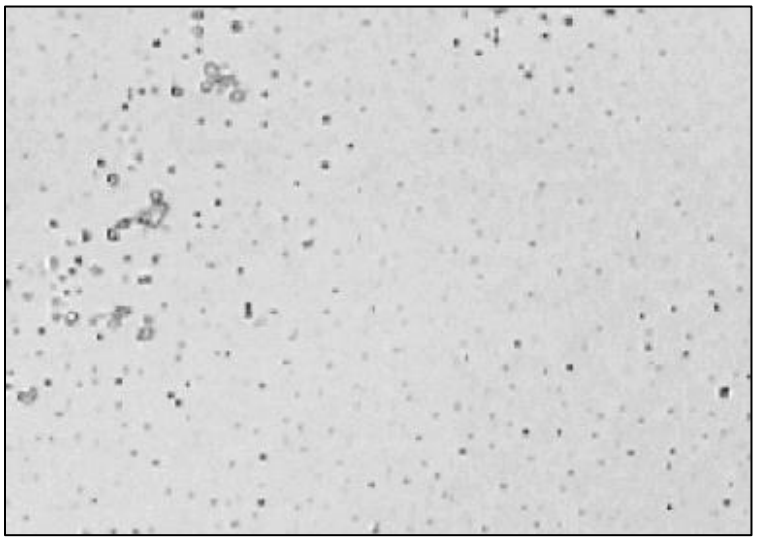


Figure 3. Example of a Negative Fern Test (Courtesy of University of Texas Medical Branch, Galveston, Texas, USA)

Limitations of the Procedure

A positive fern test should be used in conjunction with the patient's clinical history and presentation when deciding whether or not fetal membrane rupture has occurred.

The presence of blood, urine, or cervical mucus may result in a false-positive fern test.

False negatives may result during prolonged rupture of membranes (longer than 24 hours) or if only a small quantity of amniotic fluid has leaked.

Erroneous results may be obtained when:

- The slide is examined before it is completely dried.
- The slide is dried under a circulating air current (near or under a fan).
- Dirty or detergent-contaminated slides and/or pipettes are used.
- The slide is heat fixed.
- Cytological fixatives or preservatives are used on the slide.

Slides should be maintained in a covered box when not in use to minimize contaminates, which could lead to erroneous results.