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Provider-Performed Microscopy Testing; Approved Guideline

This guideline provides information on specimen collection, test methodologies, procedural steps, reporting of results, and the quality assurance aspects of provider-performed microscopy.

A guideline for global application developed through the NCCLS consensus process.

NCCLS

NCCLS...

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Healthcare professionals in all specialties are urged to volunteer for participation in NCCLS projects. Please contact the NCCLS Executive Offices for additional information on committee participation.

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Abstract

NCCLS document HS2-A— *Provider-Performed Microscopy Testing*; *Approved Guideline* provides information, instructions, and performance criteria to assist providers who perform microscopy procedures (provider-performed microscopy [PPM]), with accurate reporting of diagnostic information from their observations.

These are appropriate procedures for the examining room, emergency room, or clinic environment as an adjunct to traditional clinical laboratory testing. This testing may also provide for a rapid diagnosis of the patient condition. The guideline relates information concerning specimen collection, methodologies, procedural steps, reporting of results, and the quality assurance aspects of PPM.

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Contents

Contents (Continued)

Contents (Continued)

The Quality System Approach

NCCLS subscribes to a quality system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents through a gap analysis. The approach is based on the model presented in the most current edition of NCCLS HS1—*A Quality System Model for Health Care.* The quality system approach applies a core set of "quality system essentials (QSEs)," basic to any organization, to all operations in any healthcare service's path of workflow. The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The quality system essentials (QSEs) are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Service & Satisfaction
Personnel	Process Control	Assessment	Facilities & Safety

HS2-A addresses the quality system essentials (QSEs) indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section at the end of the document.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
X GP2-A4		X GP21-A	X		X						GP5-A2 M29-A2

Adapted from NCCLS document HS1— *A Quality System Model for Health Care.*

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, GP26-A2 defines a clinical laboratory path of workflow which consists of three sequential processes: preanalytical, analytical, and postanalytical. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

HS2-A addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section at the end of the document.

Preanalytic					**Analytic**		**Postanalytic**	
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpretation	Results Report	Post-test Specimen Management
		X GP16-A2	X GP16-A2	X GP16-A2	X GP16-A2	X	X	

Adapted from NCCLS document HS1— *A Quality System Model for Health Care.*

Foreword

Provider-performed microscopy (PPM) is a testing modality that requires the use of a microscope and is performed by a clinical practitioner (referred to as a "provider" in this guideline) during a patient encounter. Specimens utilized in PPM testing are considered labile or unstable after a very short period of time. PPM testing permits providers to render a rapid diagnosis that can, in turn, facilitate the rapid initiation of treatment.

It has always been recognized that providers use certain microscopic procedures to supplement their physical examinations in the diagnosis of patients. Unfortunately, many nonpathologist providers were not afforded adequate training time to fully comprehend good laboratory principles that ensure accurate results. Accurate results come from following standardized practices for the preanalytical (prior to testing), analytical (testing), and postanalytical (reporting) phases of testing.

The purpose of this guideline is to present critical aspects of the preanalytical, analytical, and postanalytical processes that contribute to accurate test results. This includes specific guidance to assist providers with specimen collection and handling, competency assessment, testing procedures and interpretation, proficiency testing, quality control, quality assurance, and recommended documentation. This document is not intended as a recipe for complying with any particular federal laboratory laws, local laws, or accrediting organization requirements, but is intended to assist clinical practitioners by providing information that will increase the reliability and utility of microscopic testing done during the course of a client visit.

For those clinical practitioners who become involved in the testing arena, many will need external resources to supplement their education. This guideline is not only an excellent resource but, if adhered to, will reduce the potential liability of performing nonstandardized PPM procedures.

This document addresses certain characteristics of the diagnosis and management of patients in the clinical office setting. The subcommittee requests reviewers comment on this aspect of the document, i.e., appropriateness and necessity of clinical points to the utility of the guideline.

Also, it is the subcommittee's intention to include, as appropriate, photomicrographs of various constituents commonly found in microscopic examinations for the tests described herein. Reviewers are especially encouraged to submit suggestions for photomicrographs (including source information) to be included in this guideline.

Key Words

Analytic, microscopy, postanalytic, preanalytic, provider

Provider-Performed Microscopy Testing; Approved Guideline

1 Introduction

Provider-performed microscopy (PPM), as carried out by trained providers, produces rapid, reliable results intended to be used by the provider to immediately impact patient care decisions.[a] This type of testing requires that the performing individual be responsible for all aspects of the testing process, including:

- appropriate use of the procedure (i.e., ordering tests which correspond to patient history and symptoms, and ensuring correlation between results and clinical picture);
- acceptable patient preparation;
- proper specimen procurement and handling;
- correct selection, use, and maintenance of the microscope;
- test methodologies in a procedure manual;
- accurate interpretation of observed elements;
- quality assurance and competency assessment; and
- documentation of results, QA and QC activities.

The appropriate uses of the described procedures may include the following:

- urine sediment examination to identify the cause of symptomatic presentations or abnormal chemical dipstick results;
- direct wet mount preparations to detect the presence of bacterial, fungal, or parasitic organisms, and other cellular elements indicative of pathologic conditions;
- potassium hydroxide (KOH) preparations to detect yeast and fungal elements;
- pinworm examinations to detect infestation by the *Enterobius vermicularis* parasite;
- fern tests to detect the presence of amniotic fluid in vaginal secretions indicating rupture of the amniotic sac;
- postcoital, direct, qualitative examinations of cervical mucus to investigate infertility;
- qualitative semen analysis to test for effective vasectomy or to investigate infertility;
- nasal smear for granulocytes to identify an allergic etiology for upper respiratory symptoms; and
- fecal leukocyte examinations to suggest or exclude the diagnosis of a number of pathologic conditions.

[a] In the U.S., according to Clinical Laboratory Improvement Amendments (CLIA), it is the designated laboratory director's overall responsibility to assure the accuracy and reliability of the testing performed.

2 Scope

The scope of this guideline is limited to procedures that require the use of microscopic observation with minimum specimen preparation, typically performed by a provider in near-patient testing environments. It does not include procedures which require the use of stains to determine the results of the testing.[b]

It is intended to be used in providers' offices, outpatient clinics, public health clinics, health maintenance organizations, and medical training programs. Providers may include physicians, nurse practitioners, physicians' assistants, or nurse midwives.

3 Safety

Since the anticipated location of PPM testing is the medical office examining room, emergency room, or clinic environment, there may be written protocols required by regional regulatory bodies[c] for worker safety. A safety manual with specific policies should be available for the facility where testing is located. Since these locations for direct patient care may be diverse and the provider may be the only one involved in the testing process, recognition should be made to provide safe work conditions which meet applicable regional requirements, determine appropriate personal protective equipment, and provide safe disposal of biohazardous waste.

3.1 Standard Precautions

Because it is often impossible to know what might be infectious, all human body specimens are to be treated as infectious and handled according to "standard precautions." Standard precautions are new guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. Vol 17;1:53-80.), [MMWR 1987;36(suppl 2S):2S-18S] and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure, refer to the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections.*

The European Union has similarly addressed the precautions of handling human specimens to reduce the risks associated with exposure to biological agents by passage of a directive (Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work; 18 September 2000).

3.2 Personal Protective Equipment

Personal protective equipment should be utilized in the following situations:

- When the performing provider's duties involve occupational exposure, appropriate use of gloves, gowns, protective coats, face shields or masks, and eye protection is recommended.

[b] In the U.S., the CLIA certification for provider-performed microscopy (PPM) described a set of procedures that may be performed under that designation.

[c] In the U.S., for example, the U.S. Occupational Safety and Health Administration (OSHA).

- Gloves must be worn whenever collecting, handling, and testing specimens and to provide barrier protection for cuts, scratches, or breaks in the skin against potential pathogens. Glove use should be task specific, and be removed to avoid unnecessarily contaminating the microscope.

- Protective body clothing (gown, apron, or laboratory coat which is resistive or impervious to liquids) should be worn whenever collecting, handling, or testing specimens. This clothing should not be worn into other work areas that are considered free of potentially infectious substances.

3.3 Hand Washing

Hand washing is recognized as the most important deterrent in infection control. Hands should be thoroughly washed for 20 to 30 seconds under running water either with soap or antiseptic soap as soon as feasible:

- after removing gloves;
- after completing work and/or before leaving the work area to eat, drink, smoke, apply cosmetics, handling contact lenses, and using restroom facilities;
- before touching eyes, nose, mouth, glasses;
- immediately after contamination with a specimen or reagent; and
- after contact with each patient or before contact with another.[d]

Alternatively, an alcohol-based, waterless, rapid-kill gel can be rubbed thoroughly over the surface of the hands.

For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure, refer to NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections* or ISO/DIS 15190 – *Medical Laboratories – Requirements for Safety.*

3.4 Food, Drink, Cigarettes, and Cosmetics

Eating, drinking, smoking, and applying cosmetics should not take place within the testing environment, due to possible contamination of the hands with infectious organisms.

3.5 Routine Cleaning

The work area should be kept clean to prevent contamination from infectious organisms from specimens to the performing provider or other persons utilizing the area. A 1:10 solution of household bleach and water (one part bleach plus nine parts water to result in a 0.5% aqueous solution of sodium hypochlorite) is an effective disinfectant for both bacterial and viral organisms. All work surfaces should be wiped and allowed to soak a minimum of 15 minutes. To retain potency and effectiveness, the 1:10 solution must be prepared daily. **NOTE:** Aluminum and stainless steel are corroded by sodium hypochlorite, and, therefore other disinfectants may be preferred. (See the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections* or ISO/DIS 15190 – *Medical Laboratories – Requirements for Safety* for additional information.)

If blood or other biohazardous material contaminates equipment such as the microscope, carefully wipe the area with bleach solution. Care should be taken not to spill solutions onto the working mechanisms of the microscope, computers, and other equipment.

[d] In the U.S., employees must wash their hands with soap and running water as soon as feasible, as recommended above.

3.6 Infectious Waste Disposal

Disposal of medical waste in general trash exposes sanitation workers to unknown hazards and may create a risk of legal liability to the facility or organization. Therefore, handling and disposal of medical waste should be by trained personnel, utilizing materials and procedures which protect the employees, patients, and the environment. Infectious waste (biohazard) containers should be conveniently located and of sufficient volume to accommodate the infectious waste generated at the site. Containers must be labeled to warn of biohazardous material. The containers should be constructed of materials appropriate to the type of waste generated and to contain any fluids. Biohazard containers should be handled with gloved hands. Disposal of medical and infectious waste must comply with all local and regional regulatory requirements.

4 Definitions[e]

Accreditation - The process by which a private, peer-level commission or association evaluates and ensures that a program of professional study or activity, such as laboratory testing, in an institution is meeting appropriate standards of organizational performance.

Accuracy - Closeness of the agreement between the result of a measurement and a true value of the measurand/analyte.

Analyte – Component indicated in the name of a measurable quantity [ISO FDIS 17511].

Analytical process - The technical process including the operation of equipment and the performance of the defined steps of a testing procedure designed to produce data.

Biohazard – A biological agent or condition that constitutes a hazard to human beings or their environment.

Competence – Demonstrated ability to apply knowledge and skills [ISO 9001] [ISO 9000, 3.9.2].

Exogenous - Developed or originating outside an organism; caused by external factors.

Immersion oil - A liquid medium, occupying the space between the object and microscope objective, used to optimize the resolution of the image being magnified.

Measurand – A particular quantity subject to measurement.

(Microscope) objective - The lens in a microscope that is nearest to the object under examination and magnifies the specimen in relation to the power of the objective.

Ocular (eyepiece) - The lens used in a microscope for magnification of the primary image; **NOTE:** The lens nearest the eye.

Postanalytical//post-examination process - All processes following laboratory examination including systematic review, formatting and interpretation, authorization for release, reporting of results, transmission of the results, and storage of the samples of the laboratory examinations [ISO FDIS 15189.2].

[e] Some of these definitions are found in NCCLS document NRSCL8—*Terminology and Definitions for Use in NCCLS Documents.* For complete definitions and detailed source information, please refer to the most current edition of that document.

Preanalytical//pre-examination process - Steps starting in chronological order from the clinician's request, including the laboratory examination requisition, preparation of the patient, collection of the primary sample, transportation to and within the laboratory and ending when the analytical examination procedure starts [ISO FDIS 15189.2].

Precision – The closeness of agreement between independent test results obtained under prescribed/ (stipulated) conditions.

Proficiency testing, PT - A program in which multiple specimens are periodically sent to members of a group of sites for analysis and/or identification; in which each site's results are compared with those of other participants in the group and/or with an assigned value, and reported to the participating facilities and others.

Provider - In the medical context, a clinical practitioner.

Qualitative - A characterization applied to laboratory tests that detect the presence or absence of a particular analyte, constituent, or condition; **NOTE:** Specific identification may be performed.

Quality assurance - All the planned and systematic activities implemented within the quality system and demonstrated as needed, to provide adequate confidence that an entity will fulfill requirements for quality [ISO 8402:94-3.5].

Reagent - A substance that produces a chemical reaction in a sample that allows an analyte to be detected and measured.

Semiquantitative tests – Those that yield results in an approximate range of values (i.e., trace, moderate, etc.).

Spinnbarkeit – A measure of tenacity (from German), i.e., the ability to form a thread, viscosity.

Stability - The capacity for a product to retain its composition, characteristics, and properties during specified conditions.

5 Equipment

5.1 Materials

Materials vary by procedure. Specific needs and recommended supplies are addressed with each procedure. Materials include:

- collection devices;
- centrifuge tubes;
- transfer pipettes;
- microscope slides and cover slip(s);
- cotton-tipped applicators;
- immersion oil;
- lens paper; and
- lens cleaning solution.

5.2 Microscope

Microscopes have various attributes and should be carefully maintained. Proficiency testing and control procedures may not be available for all microscopic examinations. 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 examinations of urine sediment, vaginal wet mounts, KOH preparations, pinworm preparations, fern tests, postcoital examinations, qualitative semen analysis, nasal smears, and fecal leukocyte preparations:

- 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; and
- basic set of objective (10x, 40x, and 100x oil immersion) and ocular (10x or 12.5x) lenses.

Prior to purchase and use, a microscope should be evaluated for the following:

- proper illumination and use of the objectives;
- adequate resolution, i.e., crisp, clear images at all magnifications; and
- convenience and comfort with respect to placement of focusing controls and the angle of viewing of the binocular tube head.

5.2.1 Parts of the Microscope (See Figure 1.)

5.2.1.1 Lenses

The objective lens magnifies the specimen 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 as each objective is rotated into the light path. After the first objective has been 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 depends 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.

5.2.1.2 Stand

Characteristics of the stand include the following:

- rests on the base of the microscope; and
- carries the arm of the microscope and the stage on which the specimen is placed.

5.2.1.3 Stage

Characteristics of the stage include the following:

- horizontal platform upon which the specimen slide is placed;
- a slide holder fitted to the top to hold the slide in position; and
- 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).

5.2.1.4 Condenser

Characteristics of the condenser include the following:

- mounted under the stage to concentrate and focus light from the light source;
- can be raised or lowered by means of a condenser knob below the stage;
- 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; and
- special purpose condensers, such as phase contrast condensers, can be used with specially constructed phase contrast objectives to make unstained material visible.

5.2.1.5 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 will lengthen the life of the bulb.

Most microscopes have a blue filter that is used to alter the quality and/or intensity of the light passing through the specimen.

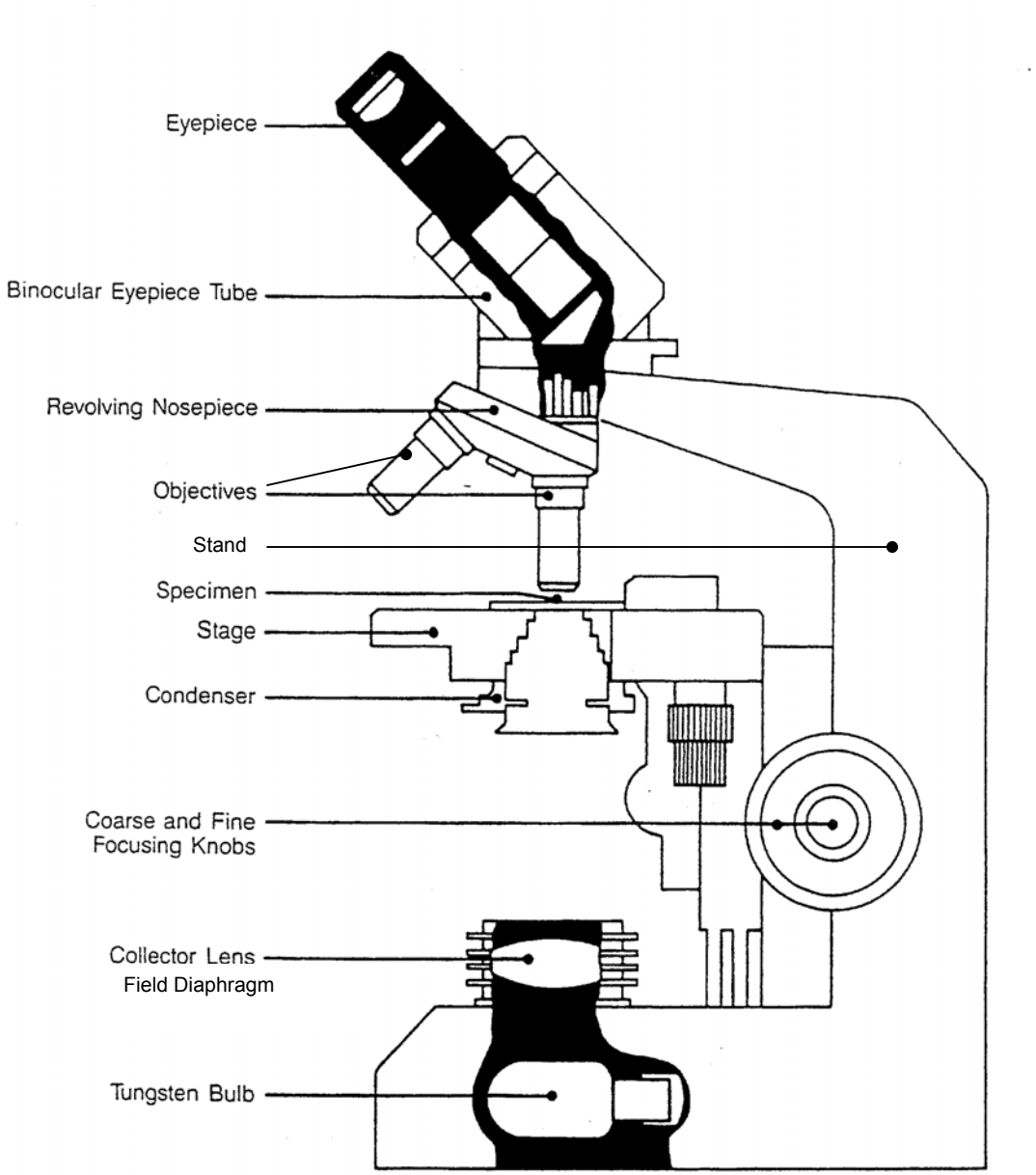

Figure 1. Standard Laboratory Microscope

5.2.2 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), i.e., 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, 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.

To use the 100x oil immersion objective:

(1) Focus and center the specimen with the 10x objective, followed by the 40x objective; lower the stage using the coarse adjustment knobs, with the 40x objective in the light path.

(2) Place a drop of immersion oil on the cover slip of the slide being examined, directly over the light path, making certain the drop is placed so that the objective will dip into the drop when the stage is raised. Rotate the nosepiece until the 100x oil immersion objective comes into the light path.

(3) Looking peripherally at stage level and NOT through the eyepieces, raise the stage slowly until the objective makes contact with the oil drop causing a flash of light.

(4) Raise the stage slowly using the fine adjustment knobs while looking through the eyepieces; at the slightest resistance, stop, because the cover slip may be too close (at this time the specimen should come into focus).

(5) After using the oil immersion objective, it should be cleaned with a piece of lens paper dampened with lens cleaning solution. Avoid getting oil on the 40x objective. Make sure to dry the objective by either gently blowing with air or using a clean, dry sheet of lens paper.

5.2.3 Care of the Microscope

To ensure long-term quality microscope use, follow the manufacturer's guidelines for care and maintenance of the microscope. At a minimum, maintenance should be performed annually. This timeframe, however, is dependent on the number of providers and procedures performed on the microscope. Also:

- cover the microscope when not being used 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;
- wear smudge-proof make-up when using the microscope;
- wipe dust off mechanical surfaces with a soft, lint-free cloth;
- remove dust from glass surfaces with a gentle puff of air and a natural bristle brush;
- clean oil from objectives, stage, condenser, and illuminator immediately following use or spill with lens paper moistened with a commercial cleaning solution for lenses or a solution recommended by the manufacturer;
- 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;
- do not mix brands of immersion oil, and use only microscope immersion oil;
- never force open diaphragm leaves;
- schedule periodic maintenance to include professional servicing (lubrication, fine tuning, and realignment will be done at this time); and
- keep records of cleaning and other maintenance procedures.

6 Quality Assurance

Quality assurance (QA) is an ongoing process established to assure that laboratory test results reliably and accurately facilitate the diagnosis or treatment of the patient's condition for a quality outcome. Major elements in the QA operation include:

- identifying problems and errors;
- assessment of the causes;
- designing corrective actions; and
- monitoring to assure correction.

QA is an important part of a comprehensive quality system consisting of policies, procedures, and practices that regularly evaluate all aspects of laboratory testing that can affect test quality and patient outcomes. Examples include: specimen collection and handling (preanalytical); performance of the test or examination (analytical); and test reporting and documentation (postanalytical). Collectively the QA system involves review and evaluation of provider training and competency, safety, equipment function, supply selection, patient test management (ordering and reporting), procedures for test performance, mechanisms for quality control of the test, proficiency testing, test results, error detection, investigation and implementation of corrective actions, and appropriate certification and/or accreditation. This broad QA approach assures that 1) test procedures and protocols are established and followed; 2) test or examination systems function properly when patient results are produced; 3) providers perform the test or examination competently; 4) patient test results are consistent with the patient's clinical presentation; and 5) written records are available to demonstrate procedures and protocols are uniformly followed.

6.1 Microscopy Provider

6.1.1 Training

The person responsible for oversight of the testing should first establish the necessary qualifications (education and experience) required for someone performing microscopic examinations, and then train qualified individuals. Training should be developed and provided to all providers who conduct the PPM

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 same checklist may also be used for subsequent competency testing. The training protocols must include preanalytical aspects such as patient preparation and specimen collection, analytical steps in the procedure, and postanalytical aspects such as standardized recording of results and correlation with presenting symptoms. The training activity and results should be documented in provider personnel records. 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.

6.1.2 Competency Assessment

All providers performing microscopic examinations should be assessed periodically (such as twice during the first year of testing and annually thereafter) to assure that they maintain competency to perform test procedures and report test results promptly, accurately, and proficiently.[f]

Procedures for evaluating the competency 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; and
- self-assessment through optional proficiency testing surveys and split samples.

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

6.1.3 Continuing Education

Continuing education for microscopy providers is essential for maintaining the skills/knowledge required for performing PPM tests. The individual performing tests should receive regular in-service training and education appropriate for the type of services offered. Continuing education can take the form of seminars, journal clubs, and clinical rounds where current developments can be presented and discussed. Microscopy providers should be encouraged to attend meetings and seminars, and to read current literature, including textbooks and journals, and commercially available educational packages. Reference materials such as this document and others referenced here should be made readily available to microscopy providers.

Because of the unique nature of proficiency testing (PT) samples (discussed later), past PT slides and pictures can be compiled into an atlas or album and used for training purposes or used as a reference to help with identifications on patient samples.

[f] In the U.S., CLIA requires that competency testing is performed twice in the first year of testing and once per year thereafter as stated in 493.1413(b)9.

6.2 Equipment and Supply Management

6.2.1 Equipment

Accurate and reliable patient test results are achieved only when the equipment used in the testing process is properly operated and maintained. It is important to ensure that all providers follow the manufacturer's instructions in the owner/operator's manual for the operation and maintenance. Training or orientation sessions must be provided to all intended users and include the proper use and care of the equipment, as well as any function checks or adjustments that affect the accuracy of the examination.

If not provided by the equipment manufacturer, the development of preventive maintenance protocols is necessary to ensure that the equipment is operating and functioning properly. Microscopes should be inspected, cleaned, and checked on a daily basis. This assures that eyepieces, objectives, and condensers are free of fingerprints and immersion oil, and that the fine and course adjustments are smooth and accurate. Centrifuges should be inspected and cleaned daily, and require periodic routine rotor rpm and timer checks, because speed and time have a direct effect on test quality.

A qualified service technician should perform major repairs and an annual equipment performance check, preferably one recommended by the manufacturer. All maintenance activities should be documented and maintained as part of the testing site's QA system.

6.2.2 Supplies

Products to be purchased for use in testing should undergo the same evaluation as equipment for needs assessment and quality evaluation. All reagents must be labeled with the identity, storage requirements, date of receipt or preparation, the date placed in service, and the expiration date. The characteristics of the product will determine the time period in which the product can be used after opening. Many products, such as stains, may have data supplied by the manufacturer that provide details on recommended storage and useful life. Storage conditions such as temperature, light, humidity, and length of time exposed to an open environment may affect the performance of test solutions and even microscope slides. Some products may become more concentrated over time until their functional characteristics have been materially altered. It is also important to check a new reagent lot against an old reagent lot when possible with the same sample or control material to compare performance. All glassware used for PPM procedures should be single-use.

6.3 Patient Test Management

Patient test management involves all phases in the testing process, including specimen collection, test records, and the system for reporting patient results, as well as ordering, recording, and reporting activities. It is important to establish procedures that describe the process for patient test management, from the determination of the need for the test and handling of the patient's specimens, through the actual testing and reporting of the test results. Even in the environment where PPM is an infrequent occurrence, a description that shows consideration of the necessary elements is needed. The established standard of practice is that there be a written description of whatever system is used for test requests, criteria and recording of unacceptable specimens, recording of test results, and reporting patient results which meet all applicable local and regional requirements.

The significant elements of patient test management include:

- criteria for patient preparation and specimen collection, labeling, preservation and transportation, ensuring optimum patient specimen integrity and identification throughout the testing process;
- if a requisition is used, the information necessary and relevant to complete the testing;

- how confidentiality is maintained;
- complete, useful, and accurate information produced for the interpretation or utilization of test results;
- the timeliness of reporting test results;
- a reliable reporting system and maintenance of records, including a specified time period that results will be kept on file; and
- demonstrable retrieval of results showing the date of testing and who performed the test.

The results should be retained for a minimum of two years; however, retention time may vary depending on local and regional regulatory requirements and standards of practice for the medical community. The forms used for patient test management will differ from site to site, but the essential elements remain the same. Where appropriate, good laboratory practice specifies that a record should be made of the patient's name and unique identifier; ordering provider; documentation of the test request; date and time sample was collected and by whom; date and time sample was tested and by whom; and the test results. In the PPM environment, the ordering provider and person performing the microscopic examination are often the same, as are the date collected and the date tested, simplifying the elements required. All of this information can be included on one form such as the patient chart, a testing log, or a laboratory request/result form.

6.4 Procedure Manual

Of all the elements in a quality assurance program, the preparation of a comprehensive procedure manual is extremely critical.[g] The procedure manual is the compilation of individual procedures. The procedure manual is essential to provide direction with instructions for the microscopy provider. Each individual procedure should provide a standardized description with detailed instructions for performing the test. A procedure manual is also a valuable resource for training new providers, verifying provider competency (see Section 6.1.2), or troubleshooting testing problems when they arise.

As an adjunct to written procedures, these guidelines (as well as access to visual examples) are useful for learning and establishing provider competency. It is recommended that a provider review references and acquire those that can be used to develop confidence in visual recognition of the microscopic elements, as well as for future reference for less common observations.

The procedure manual should include the following information:

- Test procedures – Every test performed at the testing site should have its own unique set of instructions, including collecting and handling the specimen, testing the specimen, and reporting the test results. Information on reporting abnormal as well as normal results should be included.
- Quality assurance plan – Addresses overall quality issues such as patient test management, PT, QC, safety, and personnel policies.
- Procedural updates as necessary – Any changes to the original procedure should be documented in writing, dated, and circulated for review by testing providers.
- Provider procedure review process – All microscopy providers must review the procedure manual, including an initial approval of the procedure by the provider responsible for the testing site, who should sign each procedure. It is helpful to include a log sheet in the front of the manual where microscopy providers can sign off with date and signature that they have completed their reviews of current procedures, as well as for future revisions as they occur.

[g] In the U.S., as required by CLIA.

For more information, please refer to the most current edition of NCCLS document GP2— *Clinical Laboratory Technical Procedure Manuals*.

6.5 Quality Control

The purpose of quality control (QC) is to monitor the analytical process to determine that the test system is operating within pre-established limits, thus ensuring the accuracy of each examination or measurement performed on a patient specimen. The function of a quality control protocol is to give testing personnel, such as microscopy providers, this guidance. Testing sites that are subject to the control of regulatory agencies must obtain the specific requirements from those groups.[h]

There is a limited selection of quality control materials for this particular group of microscopic tests. Check with suppliers for information on purchasing manufactured control material. In-house control material can also be used to verify accuracy for these procedures. A patient specimen that contains multiple cell types can be used as a positive control. For example, a positive urine control could be a urine specimen with squamous epithelial cells, white blood cells, and/or red blood cells. A negative urine control could be obtained by using a patient sample that is virtually clear of any cellular material. Because the survival of positive morphological structures is limited, testing sites that choose to use fresh patient specimens for control material need to implement a program that is constantly "on the look-out" for positive materials.

This process applies to any PPM procedure. For example, additional slides can be made from patients with positive and negative nasal smears, pinworm preps, and fecal leukocyte exams for future use as controls. The slide preparations can be kept at room temperature.

The testing site must establish and follow written quality control procedures for each PPM procedure. For PPM tests there are few available manufacturers' instructions except those which may be provided with purchased reagents or control materials. The following are steps to be taken when the expected results of control materials are not achieved:

- Review all steps of the procedure to determine if any technical error has occurred. Take corrective action if necessary.
- Repeat the analysis even if no cause of error has been determined. If the new analysis produces the expected result, the test is considered satisfactory and the patient test can proceed. If on repeat, the results are still incorrect, no patient testing should be reported until the problem is identified and resolved.

The following is a list of the most common causes of unexpected control results and their corrective actions**:**

- error in technique, including anything from improper handling to using the wrong reagent. Review every step of the testing process;
- improper reconstitution or mixing of control materials. Prepare new control material;
- incorrect, deteriorated, or improperly made reagents or diluents. Acquire or prepare new reagent;
- cross-contamination by other samples, reagents, or water source. Observe when problem occurred. Change testing protocol; test one sample at a time; and
- QC documentation. Verify lot number, date in use, acceptability of results, tests performed, and QC acceptability limits.

[h] In the U.S., all PPM testing sites are subject to CLIA regulations and must comply with all applicable requirements.

A monthly assessment of recorded quality control information should include a written review of the proper performance of designated QC procedures and documentation of corrective actions. A QC checklist can be incorporated as part of a monthly QA review which includes a check off of each aspect of the quality assurance plan.

6.6 Proficiency Testing

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 proficiency testing failures. PT programs are administered by PT providers. A testing site voluntarily enrolls with a PT provider. A PT program consists of 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 PPM tests when available as part of the QA program 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 PPM provider. The grades should be reviewed and shared with all providers as part of the testing site's QA, continuing education, and competency evaluation systems.

If proficiency testing is performed on PPM tests, there are several documentation requirements that should be followed to assure good performance:

- 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.
- 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 had been sent to the PT program.
- Record the remedial actions taken for PT results that are not graded "acceptable," and use the information to improve performance.

If no PT program is available, the testing site may choose to monitor providers' proficiency by exchanging blind samples with other test sites twice each year. For more information, please refer to the most current edition of NCCLS document GP29—*Assessment of Laboratory Tests When Proficiency Testing is Not Available.*

6.7 Relationship of Patient Information to Patient Test Results

It is a vital part of the quality assurance postanalytical 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 to 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 preanalytical phase of testing must also be examined (as well as the analytical phase) to determine if the patient sample was correctly 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 (i.e., correlation of urine microscopic bacteria results with

urine culture results). Such comparisons provide additional opportunities to examine concerns with all phases of provider-performed microscopy testing. Quality assurance 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.

6.8 Accreditation

Accreditation is a voluntary process by which a testing site chooses to be inspected and guided by a professional peer group. It is not a mandated, regulated requirement, but is intended to improve quality and verify that a defined standard of practice exists when accreditation is achieved. An accreditation body will provide and require adherence to standards of performance including specific requirements for the testing site's quality assurance plan. Accreditation also offers opportunities for continuing education, operational assessment, and performance improvement. [i]

7 Urine Sediment Examinations

7.1 Principle

The study of urine is one of the oldest testing procedures utilized. It yields a great deal of information quickly and economically. Urine tests need to be carefully performed and properly controlled.

Examination of the urine may be considered from two general standpoints: 1) diagnosis and management of renal or urinary tract disease; and 2) the detection of metabolic or systemic diseases not directly related to the kidney.[1]

7.1.1 The Urine Sediment Microscopic Examination

The microscopic examination of urine is the most common testing procedure utilized for the detection of renal and/or urinary tract disease. Interpretation of urine sediment requires time, skill, training, and experience acquired through the use of various microscopic methods and pathophysiologic correlation of the sediment findings with the macroscopic results and clinical status of the patient. In order to practice with competency, microscopy providers should be knowledgeable of numerous morphologic entities, e.g., organisms, hematopoietic and epithelial cells, and casts. Also, providers should be alert regarding the clinical relevance of urine findings, as well as the chemical abnormalities associated with microscopic interpretations.[1] A review of available information, including physicochemical results, is essential before reporting the microscopic examination, if the information is available. The data contained in these reports should substantiate the microscopic results and vice-versa; e.g., calcium oxalate crystals are associated with a neutral to acidic pH, not an alkaline pH. Any discrepancies should be resolved before the final results are reported.

[i] Please note that in the U.S., accreditation is a voluntary process. However, in the U.S., laboratory certification is mandated by CLIA. U.S. laboratories may choose to obtain a certificate for PPM procedures if only PPM and CLIA-waived testing is performed. A certificate of compliance or a certificate of accreditation is required for the performance of moderate and/or high complexity testing. If a certificate of accreditation is obtained, the site must be accredited by an accrediting organization approved by the Centers for Medicare and Medicaid Services (CMS). Other accreditation may be voluntarily obtained outside of the CLIA requirements. Laboratories seeking accreditation by a CMS-approved organization to meet CLIA requirements are mandated to meet the organization's standards which have been determined to be equivalent to or more stringent than CLIA requirements.

7.1.2 Formed Elements of Urine

Centrifuged urine sediment contains all the insoluble materials (commonly referred to as "formed elements") that have accumulated in the urine in the process of glomerular filtration and during passage of fluid through the tubules of the kidney and lower urinary tract. Cells found in urine come from two sources: 1) desquamation or spontaneous exfoliation of epithelial cells lining the upper (kidney) and lower urinary tract and adjacent structures; and 2) cells from the circulating blood (leukocytes and erythrocytes). Casts formed in the renal tubules and collecting ducts are the other formed elements frequently seen.

Organisms (bacteria, fungi, viral inclusion cells, parasites) and neoplastic cells represent elements foreign to the urinary system, and proper identification of these elements may provide important diagnostic clues as to the etiology of certain urinary system disorders.

7.2 Materials

7.2.1 Supplies

The primary collection container and transport container, tubes, and slides should be labeled in a way that ensures proper patient identification. All materials used to perform a urine microscopic examination should be particle-free. All glassware should be specified as single-use.

7.2.1.1 Collection Devices

Please see the current edition of NCCLS document GP16—*Routine Urinalysis and Collection, Transportation, and Preservation of Urine Specimens* for a description of proper containers for urine collection and transportation, and other related information.

7.2.1.2 Centrifugation Tubes

A proper tube for urine centrifugation has the following features:

- clear plastic or single-use glass to permit enough strength to avoid breakage during centrifugation;
- volume graduations to ensure a standardized volume of urine;
- closures to reduce hazards from spillage and centrifuge aerosols;
- conical or constricted bottom to concentrate sediment;
- freedom from interfering chemicals; and
- labels to ensure proper identification.

NOTE: Do not reuse centrifugation tubes.

7.2.1.3 Transfer Pipettes

Disposable transfer pipettes are recommended to reduce the biohazards associated with resuspending and transferring the sediment; their reuse is not recommended. Transfer pipettes should be clean and particle-free. Single-use, glass transfer pipettes may be used.

7.2.2 Equipment

The equipment found in the testing site requires routine quality control checks and preventive maintenance. Maintenance should be documented on forms kept at the testing site.

7.2.2.1 Microscope Slides/Viewing Devices

Commercially available, disposable, standardized microscope slides or viewing devices with calibrated chambers are preferred. Single-use glass microscope slides and cover slips are acceptable.
NOTE: It is not recommended to reuse slides.

7.2.2.2 Centrifuge

A centrifuge with a self-locking lid (when rotor is spinning) and an ambient temperature (15 to 25 °C) is desirable for urine sedimentation.

Calibrate centrifuges to provide a relative centrifugal force (RCF) of 400*g*. Consult the manufacturer's instructions for the recommended protocol. Calibration should be performed at periodic intervals determined by the testing site depending on its use. At a minimum, centrifuge calibration should be performed annually. Relative centrifugal force (RCF or G) is calculated by using a rotor with a known radius (R in cm) at a given speed (N is revolutions per minute) where $G = 0.00001188RN^2$.

7.2.2.3 Microscope

A microscope with 10x and 40x bright-field objectives, or a microscope equipped with phase objectives and condenser, is recommended.

7.2.3 Reagents

All reagents must be properly labeled, dated with expiration date, and stored. When new reagents are opened or prepared, positive and negative control checks should be performed to ensure proper performance characteristics of the reagent. Results of the quality control checks with lot numbers and expiration dates of the reagents should be recorded. Reagents shall not be used after their expiration dates.

7.3 Specimen Collection

7.3.1 Specimen Type

The type and quality of the urine specimen greatly affects the results of the microscopic examination. The specimen of choice for a microscopic evaluation is the first morning urine. This urine will be the most concentrated, which maximizes recovery of sediment elements. It is extremely important that information about the type of specimen, the time of collection, and the physical/chemical results be noted when the need for a microscopic examination of urine is determined by the provider.

A minimally sufficient quantity of urine to permit both macroscopic and microscopic evaluation is usually considered to be 12 mL (50 mL is preferred). Urine specimens from infants may necessitate the use of smaller volumes.

Urine specimens that are held unrefrigerated for more than two hours are not acceptable for microscopic examination. Casts and red and white blood cells are especially susceptible to lysis in urine specimens with a low specific gravity (<1.010) and in urine specimens with alkaline pH (>7.0).

The urine specimen should be collected in a clean, leakproof, disposable container. The specimen should be free of fecal contamination and contain no bathroom tissue or other foreign materials. If these criteria are not met, the provider performing the test should seek another specimen that meets the criteria.

7.3.2 Specimen Handling

The accuracy of a urinalysis is dependent on the quality of the specimen submitted; therefore, care should be taken to submit a properly collected and transported urine specimen. For more detailed information, see the most current edition of NCCLS document GP16—*Routine Urinalysis and Collection, Transportation, and Preservation of Urine Specimens*.

To ensure suitability for an analysis, the urine specimen should be inspected as soon as possible (i.e., as part of the patient examination) following collection. Consider the following points when ensuring the suitability of the specimen:

- the specimen is labeled with the patient's name;
- acceptability of the elapsed time between collecting the specimen and its receipt at the testing site (i.e., less than two hours);
- suitability of the container and its condition (e.g., closure in place);
- adequate volume and absence of contaminating materials; and
- presence or absence of chemical preservatives consistent with intended specimen use.

7.3.3 Documentation

Documentation should indicate the type of urine specimen collected, the date and time of collection, and an indication of specimen refrigeration prior to testing.

The documentation, on a form or in the patient's chart, should also include any specific situations that might influence the results of the analysis (e.g., preservatives for specimens; medications, such as aspirin, vitamins, or antibiotics; presence of menstrual period; strenuous exercise prior to specimen collection; additional pertinent clinical information).

7.3.3.1 Label

The container should be designed to accept a label that will adhere during refrigeration. The label should include sufficient space to include the patient's full name, unique identification number, date and time of specimen collection, and the name of the preservative in the container, if applicable. To ensure proper specimen identification, place labels on the container, not on the closure.

7.4 Testing Procedure

(1) Pour 12 mL of well-mixed urine specimen into a graduated, disposable centrifuge tube.

(2) Spin tube of urine at 400 g for 5 minutes.

> **NOTE:** Higher RCF and longer centrifugation times, although useful in recovering cells, are apt to break up cellular casts.[1]

(3) Carefully decant the supernatant. The final volume used to resuspend the sediment may vary, but should remain a constant within any given testing site.

(4) Gently resuspend the sediment in the remaining supernatant.

(5) A drop of resuspended sediment is placed on a glass slide and cover-slipped for examination. Allow urine to settle for 30 to 60 seconds before examining.

(6) Examine with low- and high-power objectives. Subdued light (achieved by lowering illumination intensity) or phase-contrast illumination will be required to detect sediment entities with a low refractive index. The fine focus should be varied continuously while scanning, systematically progressing around the entire examination area while being careful to examine along the edges for casts.[1]

(7) The urine sediment should be examined microscopically on low power for crystals, casts, and epithelial cells. Ten fields should be counted and the average number of casts and epithelial cells reported per low-power field (e.g., two hyaline casts/lpf). The type and number of crystals per low-power field should also be reported (e.g., few calcium oxalate crystals/lpf, rare uric acid crystals/lpf).

(8) The urine sediment should be examined microscopically on high power for red cells, white cells, and renal tubular cells. Ten fields should be counted and the average number of cells per high-power field (e.g., 20 RBC/hpf) reported.

(9) The presence of bacteria, yeast, trichomonads, and mucus should also be noted during high-power examination.

(10) Review the entire report, including physical, chemical, and microscopic data and correlate with available clinical information. Discrepancies should be resolved before releasing the report. Although values will vary depending on the standardization system used,[1] a normal value for the procedure used should be provided for interpretation of results.

Consistency in all aspects of the microscopic examination is essential to the production of meaningful results. A properly written and maintained procedure manual helps ensure that all microscopy providers perform the microscopic examination in the same manner. Each provider should evaluate the sediment using the same procedure, look for the presence of the same sediment entities, and use the same criteria for identification.

7.4.1 Methods for Examining Urine Sediment

7.4.1.1 Bright-field Microscopy of Unstained Urine

Subdued light is needed to delineate the more translucent, formed elements of the urine such as hyaline casts, crystals, and mucous threads. Identification of leukocytes (neutrophils, eosinophils, lymphocytes), histiocytes, renal epithelial cells, viral inclusion cells, neoplastic cells, and cellular casts may be very difficult in unstained preparations. Phase-contrast microscopy is strongly recommended for the detection of casts.[1]

7.4.1.2 Phase-Contrast Microscopy

Many microscopy providers prefer to use phase-contrast microscopy for the detection of more translucent, formed elements of the urinary sediment. Such elements, notably casts (but also mucous threads and bacilli), may escape detection using ordinary bright-field microscopy. Phase-contrast microscopy has the advantage of enhancing the outlines of formed elements, making detection simple.[2] A microscope equipped with 10x and 40x phase objectives plus a 40x bright-field objective and the appropriate rotation phase/bright-field condenser is most useful. Scanning time is decreased, and the yield is increased.

7.4.1.3 Stains

Stains such as Sternheimer stain to facilitate identification of epithelial cells and casts and the Sudan III stain to assist in identifying oval fat bodies, fat droplets, and fatty casts have gained popularity in many laboratories. Administer the stain according to manufacturer's instructions.[j]

7.4.2 Examination of Urine Sediment

Sediment entities that should be identifiable using a urine microscopic examination include those listed in Table 1.[1]

NOTE: Advanced microscopy skills can be required for the identification of other elements.

See Appendix A for detailed descriptions of the formed elements to assist in their identification in the urine sediment.

NOTE: Reference values for the procedure outlined in Section 7.4 are cited in the reference given. Testing sites using standardized systems (described in Section 7.4.3) should refer to the information provided by the manufacturer.

"Normal" or reference values for formed elements will vary from one site to another because of 1) the variation in concentration of random voided urine specimens; and 2) the different methods used to concentrate the sediment by centrifugation. Individual testing sites should establish their own reference values.

7.4.3 Standardized Systems

Standardized urinalysis systems are available commercially. All systems provide a capped centrifuge tube, transfer pipettes that will retain a specific volume of sediment, supravital stain[j], and choice of standardized slides that will hold a specific volume of concentrated urine sediment. Each manufacturer also markets a urinalysis control. All systems utilize acrylic plastics that have excellent optical properties. When choosing a standardized system, each testing site will have to make evaluations about the patient population and degree of sensitivity desired. Other factors to consider include ease of loading, the number of focal planes, settling properties, and the optical properties of the slide.[1]

The importance of standardization in microscopic urinalysis cannot be overemphasized. Standardization is essential for precision in urinalysis, providing a consistent basis on which the provider can make interpretive decisions.

7.5 Quality Control

Users must adhere to all applicable regulatory requirements and manufacturer's instructions. Maintenance of the microscope is a quality control activity and should be performed at a frequency proportional to usage (see Section 5.2.3).

7.5.1 Microscopic Examination Quality Control

Quality control of the microscopic examination has been lacking if not totally overlooked. Controls used to ensure centrifuge speed and timing are appropriate to obtain an adequate sample for urinalysis. Commercial solutions are available that provide either stabilized or simulated erythrocytes and leukocytes. At the time of this printing there are no commercially available products that contain epithelial cells and casts.

[j] In the U.S., stains are not indicated for the microscopic analysis of urine sediment in PPM procedures under CLIA.

Duplicate urine testing can be used as a precision check for the identification of casts, renal cells, and other formed elements.

The use of in-house urine specimens as "known controls" on a daily basis is strongly suggested.[1]

If there is a disagreement about the presence or quantity of a microscopic element, the examination should be repeated. If necessary, referral to a reference laboratory should resolve any discrepancy. Each testing site should establish criteria for reviewing abnormal sediment results. Each testing site will also have to establish its own acceptable range of performance. This is difficult when so much of urine microscopy involves the interpretative description of microscopic elements.

It is also recommended that reference texts, atlases, charts, and posters are available for reference.

7.5.2 Recording Quality Control

All providers should use the same terminology and report results in a standard format. Unexpected control results should be identified, and appropriate corrective action should be taken and documented.

In establishing a quality assurance program the provider should ensure that the following information is documented:

- specimen collection consistently results in the correct specimen type, adequate specimen volume for the requested analysis, the use of suitable containers, and proper labeling;
- specimen transport to the testing area is timely and in accordance with recommended procedures; and
- specimen processing ensures prompt inspection and correct storage procedures (e.g., refrigeration and protection from heat and light).

7.6 Reporting Results

All providers at a testing site who perform microscopic examinations should use the same terminology, reporting format, and reference values. The individual provider should make decisions about which formed elements should be reported and quantitated. Documentation should include name, identification number, date of testing, and initials of person doing the test.

8 Direct Wet Mount Preparations and Potassium Hydroxide (KOH) Preparations

8.1 Principle

Microscopic observation of unfixed "wet mounts" of clinical specimens, either stained or unstained, can be useful for the rapid detection of the presence of bacterial, fungal, and parasitic organisms. Presumptive identification can be made, based on morphology and motility. The presence or absence of white blood cells and "clue cells" may also be demonstrated, and a number of well-recognized pathologic conditions may be identified.

Table 1. Interpretation of Microscopic Urinalysis

Finding	Abnormal Due To Systemic Disease	Abnormal Due To Genitourinary Disease	Reference Values
Crystals	Rarely: Refer to Appendix A	Rarely	None present Usually not significant
Red Blood Cells (RBCs)	Rarely: Sickle cell trait Bleeding disorders Anticoagulant use Trauma	Glomerulonephritis Tumors Stones Rarely: Infection (with WBCs)	Less than 5/hpf More with menstrual period
White Blood Cells (WBCs)	Rarely: Viral infections Radiation therapy Chronic inflammation	Infection of bladder or urinary tract Kidney infection (with WBC casts) Renal transplant rejection (especially large lymphocytes)	Less than 5/hpf
Renal Tubular Epithelial Cells	Rarely: Cytomegalovirus disease	Acute tubular necrosis Nephrotic syndrome	Rare
Transitional Epithelial Cells	Rarely: Radiation therapy Urethral/Ureteral catheterization	Infection Urinary tract lesions	Small numbers
Squamous Epithelial Cells	Vaginal exudate (with bacteria and/or yeast)	No	1-2/lpf More with vaginitis
Hyaline Casts	Rarely: Dehydration Heavy exercise Psychological stress	Proteinuria Acute tubular necrosis	Rare
Granular Casts	Rarely: Severe dehydration Prerenal azotemia Viral infection Lead poisoning	Glomerulonephritis, interstitial Pyelonephritis	None
Cellular Casts	No	RBC: nephrotic syndrome WBC: Pyelonephritis Renal Tubular Epithelial: Acute tubular necrosis, transplant rejection Fatty: Nephrotic syndrome Waxy: Progressive renal disease	None
Microorganisms	No	100,000/mL: always infection*	None

* Observation of microorganisms, indicating infection, should be confirmed by culture.

8.2 Materials

8.2.1 Supplies

Supplies used for the procedure include the following:

- frosted-end, glass microscope slides;
- cover slips;
- cotton-tipped swabs;
- capped tubes; and
- pH paper.

8.2.2 Equipment

Equipment used for the procedure includes the following:

- microscope equipped with 10x and 40x objectives;
- ocular micrometer (optional); and
- slide warmer.

8.2.3 Reagents

Reagents used for the procedure include the following:

- Sterile physiologic saline (i.e., sodium chloride, 9 grams/L [0.9%]); and
- potassium hydroxide, 10 grams/dL (10%).

NOTE: Concentrated KOH is a highly corrosive liquid that must be used with appropriate personal protective equipment including gloves and eye/face protection. It is recommended that for use in the PPM environment, 10% KOH be purchased commercially or obtained from pharmacy/laboratory sources.

8.3 Specimen Collection

8.3.1 Conditions for Patient Preparation

Specimens for direct wet mount are usually collected during an internal examination of the female genital tract. Specimens from other sources such as skin scrapings, hair, and nail analyses are collected during an appropriate exam.

8.3.2 Specimen Type

Specimen types include swabs of the vaginal mucosa and vaginal pool or scraping of suspected skin area.

8.3.3 Specimen Handling

Swabs used to collect specimens should be placed into tubes containing approximately 0.5 mL sterile physiologic saline, then resealed. To preserve the motility of *Trichomonas*, specimens should not be refrigerated and should be examined as soon as possible following collection. All tubes must be properly labeled with the patient's name and identification number.

Specimens from the vaginal tract should be tested with pH paper before being placed in saline. A pH greater than 4.5 is associated with bacterial vaginosis or trichomoniasis. The pH should be documented on the report.

8.4 Testing Procedure

(1) Place one drop of saline on one slide, and one drop of 10% KOH on the second slide.

(2) Add specimen to each slide.

(3) Check the KOH slide immediately for a "fishy," amine odor. (Note odor on report.)

(4) Cover each specimen with a cover slip to exclude air bubbles.

(5) Examine the saline wet mount with the 10x objective for epithelial cells and any budding yeast or pseudohyphae.

(6) Examine the saline wet mount with the 40x objective and quantitate organisms and cells per high-power field (hpf), using the following scheme:

QUANTITATION, DIRECT EXAMS	
Rare	Less than 10 organisms or cells/slide
Occasional	Less than 1 organism or cell/10 hpf
1+ (Very Scanty)	Less than 1 organism or cell/hpf
2+ (Scanty)	1 to 5 organisms or cells/hpf
3+ (Moderate)	6 to 30 organisms or cells/hpf
4+ (Profuse)	Greater than 30 organisms or cells/hpf

(7) Yeast/fungi can best be demonstrated on a KOH mount. The addition of 10% KOH to the clinical specimen will dissolve tissue cells and keratinized material to allow better visualization of fungal elements. Heating is helpful to speed the dissolving process, especially for skin and nail specimens. Any gentle heat source is usable.

(8) Examine the KOH preparation under low power for yeast pseudohyphae and under high power (40x) for smaller blastospores. Refer to Appendix B for descriptions to assist in the presumptive identification of fungi.[k]

8.4.1 Microscopic Examination of Direct Wet Mount Specimens

Cells and organisms that should be identifiable in a microscopic examination of a direct wet mount specimen of vaginal fluid and the interpretation of those findings are listed in Table 2. See Appendix B for a more detailed description of components found in vaginal fluid and KOH preparations.

[k] This level of identification is not included in the wet mount exam under the PPM subcategory of moderate complexity in CLIA, nor does it include enumeration of elements seen microscopically. Under CLIA, the PPM wet mount includes only direct wet mount preparations for the "presence or absence" of bacteria, fungi, parasites, and human cellular elements.

Table 2. Interpretation of Microscopic Observations of Vaginal Fluid[3]

Finding	Abnormal Due To Bacterial Vaginosis With Gardnerella	Abnormal Due To Vaginal Candidiasis	Abnormal Due To *Trichomonas* Vaginitis	Abnormal Due To Desquamative Inflammatory Vaginitis	Reference Values
White Blood Cells	Rare	3+ to 4+	2+ to 4+	3+ to 4+	2+
Lactobacilli	Rare	Present	Present/Absent	Reduced/ Absent	Predominant
"Clue Cells"	Occasional to 4+	Absent	Absent/Present		Absent
Other Cells		Large clumps of epithelial cells		Occasional Parabasal/ Basal cells	Absent (except RBCs during menses)
Other Organisms	Predominant Gardnerella morphotypes	Budding Yeast Pseudohyphae	*Trichomonas* Frequently associated with other organisms	2+ bacteria	Other lactobacilli subgroups Occasional yeast
Other Findings	pH > 4.5 Amine Positive "Lava Flow" on KOH preparation		pH > 4.5 Any finding associated with other vaginitis-causing organisms	pH > 4.5 Vaginal erythema	pH < 4.5

8.5 Quality Control

Commercial controls are not currently available for wet mount preparations. Multiple examiners of the same specimen can help determine accuracy of the test results.

8.6 Reporting Results

Providers should use the same equipment, procedure, reporting format, terminology, and reference values. The individual provider should make decisions about which elements should be reported and quantitated.

See Appendix B for more detailed descriptions of constituents found in vaginal fluid.

8.7 Limitations of the Procedure

Limitations of the procedure include the following:

- Many intravaginal medications will leave oil droplets which can make interpretation of direct wet mounts difficult. It is often useful in such situations to perform a gram stain.

- Many vaginal infections are uncomplicated and can be diagnosed using wet mount/KOH alone. If there are unusual circumstances, a gram stain can be a very helpful aid.[1]

9 Pinworm Examinations

9.1 Principle

Enterobiasis or pinworm infestation is the most common intestinal nematode in humans and quite common among school-aged children. It is very contagious by direct ingestion or inhalation of the ova.

Because only a small percentage of infected persons have demonstrable eggs in their stools, the standard laboratory examination for ova and parasites is rarely diagnostic. A direct specimen collected from the perianal region can contain the eggs of *Enterobius vermicularis,* as it is to this area that the gravid female migrates and lays large numbers of eggs while the host is resting.

The most common test to detect ova is the cellulose tape test or cellulose acetate cellophane tape test developed by Graham et al.[4]

The identification of the adult worms is done by the collection of fecal material by digital rectal examination.

9.2 Detection of Ova: Cellophane Tape Test

This method consists of applying an adhesive tape onto the perianal surface to capture the eggs. The adhesive surface is mounted onto a glass slide and then examined under the microscope for identification of the eggs. The tape captures no adult forms.

The eggs are ovoid, elongated, smooth and transparent, measuring approximately 50 x 30 µm. Under the microscope, eggs appear asymmetric and flattened on one side. (See Figure 2.) Ova are transparent, and many contain well-developed larvae.

9.2.1 Materials

9.2.1.1 Supplies

Supplies used for the procedure include the following:

- adhesive, clear cellophane tape of ½- or 1-inch width;
- wooden applicator/tongue depressor;
- clean container/specimen cup; and
- microscope slide.

9.2.1.2 Equipment

Equipment used for the procedure includes a microscope with 10x and 40x objectives.

[1] In the U.S., under CLIA, gram stains cannot be performed as a PPM procedure.

9.2.1.3 Reagents

Reagents are not required for the identification of ova via the cellophane tape test.

9.2.2 Specimen Collection

9.2.2.1 Conditions for Patient Preparation

The specimen should be collected from the anal verge in the morning, right after the person wakes and before moving around or washing. Written instructions should be provided to the person assisting in collection, including a precaution to wash hands after collection and handling. Specimens should be obtained before therapy has begun.

9.2.2.2 Specimen Type

Specimens are collected by adherence of the eggs to the sticky surface of the tape. Commercial “paddles” with a sticky surface mounted in a capped tube are also available for specimen collection.

For collecting the eggs with cellophane, one-and-a-half to two inches of transparent, adhesive cellophane tape are rolled and fixed at the end of a tongue depressor or wooden stick with tape sticky side out.

With gloved hands, spread the anal folds apart and firmly press the adhesive tape or paddle onto the patient’s anal skin in several regions for 10 to 15 seconds, and then remove gently.

9.2.2.3 Specimen Handling

The tongue depressor or paddle should be placed in a capped container for transport. The test is to be performed the same day of collection. Specimens may be kept at room temperature. Pinworm eggs are very infectious; caution should be used to avoid cross-contaminating individuals involved in handling the specimen.

9.2.3 Testing Procedure

(1) After collecting, the cellophane tape is mounted onto a glass slide or kept at room temperature rolled with sticky side out in a covered, clean container or specimen cup. Label slide with the patient's name and identification number.

(2) Examination of the mounted glass slide is performed in conventional bright-field or phase-contrast microscopy using a 10x to 40x dry objective.

(3) The eggs are distinguishable by their characteristic shape, size, and transparent appearance. (See Figure 2.)

Figure 2. Egg. From the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand. Reprinted with permission.

9.2.4 Quality Control

No direct controls are available except for reference illustrations of *E. vermicularis* eggs and differentiating features from other intestinal nematodes.[5]

9.2.5 Reporting Results

Report positive or negative for pinworm ova. Report adult pinworms if found.

9.2.6 Limitations of the Procedure

Multiple specimens, collected over several days, may be necessary before confirming diagnosis. Only five to ten percent of infected patients pass demonstrable eggs into their stools. Because the female worms may not lay eggs every night, three to six consecutive, daily specimens are recommended.

9.3 Detection of Adult Parasites

For this procedure, a wet preparation is made in normal saline by applying the fecal material with a wooden stick onto a slide.

The adult *Enterobius vermicularis* is a whitish roundworm, approximately 2 to 5 mm and 8 to 13 mm long for the male and female, respectively. Under microscopy, it can be recognized by a cuticular, wing-like alar expansion, "caudal alae" at the anterior portion, a ventrally curved posterior end for the male, and a long, pointed tail for the female. The worm has a complete digestive system with a well-developed esophagus. (See Figure 3.)

9.3.1 Materials

9.3.1.1 Supplies

Supplies used for the procedure include the following:

- sterile surgical gloves;
- wooden applicator;
- glass slide and cover slip; and
- nail polish or mounting medium (optional).

9.3.1.2 Equipment

Equipment used for the procedure includes a microscope with 10x and 40x objectives.

9.3.1.3 Reagents

The reagent used for the procedure is saline solution.

9.3.2 Specimen Collection

9.3.2.1 Condition for Patient Preparation

Female pinworms can be observed directly on the perianal area at night when they migrate to deposit eggs.

A parent can be instructed to examine a potentially infected child with a flashlight while the child is sleeping. Alternately, the provider examines the patient in the practitioner's office. The patient is positioned lying on his or her side, while gowned, and drawing the knees forward.

9.3.2.2 Specimen Type

The fecal material is collected by inserting a gloved finger into the anus and removing fecal material from the anus inside rim. Care must be taken to observe the potential for contamination when obtaining a specimen in this fashion.

9.3.2.3 Specimen Handling

After collecting the fecal material, the provider should put it in a small container and maintain the specimen at room temperature until examined.

9.3.3 Testing Procedure

(1) Make a wet preparation with a drop of saline on a microscope slide labeled with the patient's name, using a wooden stick. It is recommended that the preparation be covered with a cover slip and sealed with nail polish.

(2) The specimen should be examined soon after collection before drying.

(3) The examination of the wet preparation is performed under conventional bright-field or phase-contrast microscopy using 10x and 40x dry objectives.

The adult female *E. vermicularis* of up to 13 mm is distinguished by its round shape, whitish transparent appearance, and the marked distention due to egg development. (See Figure 3.) Parasites are easily disrupted and occasionally surrounded by eggs.

Female

Male

Figure 3. Adult Worms. From the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand. Reprinted with permission.

9.3.4 Quality Control

No direct controls are available except for reference illustrations of *E. vermicularis* adult worms and differentiating features from other intestinal nematodes.[5]

9.3.5 Reporting Results

Report positive or negative for pinworms.

9.3.6 Limitations of the Procedure

A negative result does not preclude pinworm infestation. The cellophane tape test for detection of the ova should be pursued if clinically indicated. See also Section 9.2.6.

10 Fern Test

10.1 Principle

The fern 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, vaginal fluid pH of 7.0 or greater, and a positive fern (crystallization) test in deciding whether the amniotic sac has ruptured. The test is based on the ability of amniotic fluid to form a fern pattern when air-dried on a glass slide. See Section 11 for postcoital evaluation of cervical mucus.

10.2 Materials

10.2.1 Supplies

Supplies used for the procedure include the following:

- glass microscope slides; and
- transfer pipette.

10.2.2 Equipment

Equipment used for the procedure includes a microscope with a 40x objective.

10.2.3 Reagents

Reagents are not required for the fern test.

10.3 Specimen Collection

10.3.1 Conditions for Patient Preparation

The patient is examined in the dorsal lithotomy position.

10.3.2 Specimen Type

A vaginal liquid pool specimen is collected by transfer pipette after insertion of a sterile speculum into the vaginal vault.

10.3.3 Specimen Handling

Specimens are tested immediately. Once the specimen on the slide is dry, it does not need to be read immediately.

10.4 Testing Procedure

(1) Obtain a few drops of vaginal fluid in a clean transfer pipette.

(2) Place a drop of fluid onto a clean glass slide labeled with the patient's name.

(3) Place the slide on a flat surface.

(4) Allow the slide to dry completely in room air.

(5) Examine the slide under the microscope at 40x magnification.

10.5 Quality Control

No direct controls are available for the fern test.

10.6 Reporting Results

Amniotic fluid will form a tree-like or fern pattern when air-dried. A positive fern test exhibits this characteristic pattern, indicative of the presence of amniotic fluid and therefore rupture of fetal membranes. A negative test is reported when no fern pattern is seen on the slide.

10.7 Limitations of the Procedure

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

The greatest accuracy in identifying released amniotic fluid is achieved by Papanicolaou staining and cytologic evaluation.

Erroneous results may be obtained when the slide is examined before it is completely dried; if the slide is dried under a circulating air current (near or under a fan); if dirty or detergent-contaminated slides, cover slips and/or pipettes are used; if the slide is heat fixed; or if cytologic fixatives or preservatives are used on the slide.

11 Postcoital, Direct, Qualitative Examinations of Cervical Mucus

11.1 Principle

The postcoital test (PCT), also known as the Sims-Huhner Test,[6] is part of an infertility evaluation to determine the receptivity of cervical mucus and the ability of sperm to penetrate the cervical mucus and to maintain activity.

The infertility assessment may identify contributing factors by the male (identified by a semen analysis) or by the female (primarily a failure to ovulate or other hormone abnormalities, tubal pathology, or a cervical factor).

11.2 Materials

11.2.1 Supplies

Recommended supplies used for the procedure include the following:

- two glass slides (minimum);
- cover slips;
- cotton swabs;
- transfer pipette;
- sterile vaginal speculum;
- 14-gauge catheter or similar suction device;
- 12cc syringe;
- forceps; and
- Petri dish.

11.2.2 Equipment

Equipment used for the procedure includes a microscope with 40x objective.

11.2.3 Reagents

Reagents are not required for postcoital testing.

11.3 Specimen Collection

11.3.1 Conditions for Patient Preparation

Patients who are to have postcoital testing performed should be using some form of ovulation determination (BBTs, LH surge testing, etc.).

When the patient determines an ovulation is occurring, she and her partner should have intercourse, timing the act in such a way that the testing can be performed within 2 to 12 hours following intercourse.

On arrival to the provider, a history should be taken to record the time since intercourse and the length of time since last previous intercourse.

11.3.2 Specimen Type

The patient is examined in the dorsal lithotomy position. A sterile vaginal speculum, lubricated with only water, is inserted into the vagina and the cervix visualized. Excess vaginal secretions are gently removed with a cotton swab. A 14-gauge catheter attached to a 12cc syringe, or similar suction device, is then placed into the cervical os and the cervical mucus is removed by pulling back on the syringe plunger to create suction in the catheter.

Cervical mucus is the specimen used for this procedure.

11.3.3 Specimen Handling

The specimen is tested as soon as possible after collection.

11.4 Testing Procedure

(1) The volume of mucus is measured in the syringe.

(2) The mucus is discharged into a Petri dish.

(3) The amount of mucus is then classified as scant, moderate, or profuse.

(4) The color and clarity are noted.

(5) The amount of tenacity (Spinnbarkeit) is then tested by grasping a portion of the mucus with forceps and noting the distance (cm) which it can be drawn before breaking (6 to 10 cm desirable).

(6) A drop of mucus is placed onto a microscope slide.

(7) A cover slip is placed over a portion of the mucus.

(8) The mucus is immediately evaluated using high-dry 40x objective. The number and motility of sperm are then recorded.

11.5 Quality Control

No direct controls are available for postcoital testing.

11.6 Reporting Results

Report volume, color, clarity, Spinnbarkeit, sperm per high-power field, and percentage of mobile sperm. Any other cellular elements or organisms should be noted.

The amount of mucus is classified as scant, moderate, or profuse, based on the provider's estimation.

11.6.1 Interpretation

Midcycle mucus should be clear, watery, and profuse. Good Spinnbarkeit at midcycle is at least 10 cm. What constitutes a normal number of sperm in a PCT has been a matter of dispute. Within six to eight hours after coitus, at least five to ten motile sperm should be present per high-power field.[1]

Greater than 20 sperm/hpf has been positively correlated with pregnancy; no sperm suggests faulty sperm production in the male. While the former gives a better prognosis for pregnancy, a substantial number of pregnancies occur even when no sperm are found in the postcoital test.[6] A poor result on the postcoital test can raise a suspicion of an immunologic or other problem and can be of value in determining therapy.

11.7 Limitations of the Procedure

The limitations of the procedure include:

- procedure not performed during the midcycle ovulatory stage (the mucus is thick and opaque instead of thin and clear);
- use of dirty or contaminated materials;
- patient examined too long after intercourse (greater than 12 hours);
- specimen was contaminated by excess vaginal secretions due to failure to wipe the area with a cotton swab prior to the collection of cervical mucous from the os, causing any sperm present to be inadequately evaluated due to overlying vaginal debris;
- examining the cervical mucous after drying, killing active spermatozoa.

12 Qualitative Semen Analysis

12.1 Principle

Presence and/or motility of sperm are direct microscopic procedures used to evaluate semen samples. Active motility of spermatozoa is necessary for penetration of the cervical mucous and subsequent migration up the fallopian tubes to fertilize the ovum. Seminal fluid contains not only spermatozoa, but also prostatic and other glandular secretions.

Seminal fluid examinations for the presence or absence of spermatozoa are of value in substantiating the effectiveness of a vasectomy and should be done with great care. To reliably conclude that no sperm are present, examination should use a concentrated (centrifuged) specimen, and conclusions should not be

based on results from a single specimen. These cautions are especially important, considering the potential for detailed procedural review that may occur in a medico-legal case.

Microscopic examination of seminal fluid for presence and motility of spermatozoa is also utilized in evaluating couples for infertility. Many cases of male infertility are detectable in seminal fluid samples using routine methodologies beyond the scope of PPM.

12.2 Materials

12.2.1 Supplies

Supplies used for the procedure include:

- glass or plastic container for collection;
- glass microscope slides;
- cover slips; and
- transfer pipette.

12.2.2 Equipment

Equipment used for the procedure includes a microscope with 40x objective.

12.2.3 Reagents

Reagents are not necessary for qualitative semen analysis.

12.3 Specimen Collection

12.3.1 Conditions for Patient Preparation

The patient, following a 48- to 72-hour period of abstinence from sexual activity, collects freshly ejaculated semen. The most satisfactory collection method is obtained by masturbation.

12.3.2 Specimen Type

Seminal fluid is the specimen used for this procedure.

12.3.3 Handling Conditions

The specimen should be collected in a clean glass jar or suitable plastic or polyethylene container free of preservatives or other chemical additives. The specimen container should be warm, at least to room temperature if not body temperature, prior to collection. The specimen should be labeled with the name of the patient and the time of collection.

The specimen should be evaluated as soon as possible after ejaculation, within 30 minutes if possible. The specimen should not be evaluated if it is more than two hours old. If collected away from the testing site, the specimen should be transported to the testing site immediately after collection, avoiding temperature changes during transport and keeping the specimen at body temperature until self-liquefaction is complete (15 to 30 minutes). The sample should then be mixed thoroughly before testing.

12.4 Testing Procedure

(1) If the sample has been produced outside of the test site, it should be warmed to 37 °C for five to ten minutes before examination.

(2) Using a transfer pipette, place one drop of well-mixed seminal fluid on a clean glass slide and cover with a cover slip.

(3) Examine the slide immediately using the high-dry 40x objective. Presence or absence of sperm is recorded. **NOTE:** If examining for azoospermia, centrifuge a portion of the specimen following the directions for urine microscopic testing and reexamine for the presence of sperm.

(4) Examine at least 200 spermatozoa for motility. **NOTE**: Do not use fields close to cover glass edges.

(5) Estimate the number of motile forms, using the high-dry objective. Only forward motion of the spermatozoa is considered as true motility. Circular or sluggish motion or just motion of the tail is not considered true motility. Count all forms of motility in the calculation and comment on type observed, i.e., "sluggish," "little or no forward progression."

(6) If RBCs are present, indicate the number per high-power field. Note other cells and miscellaneous or exogenous components observed.

12.5 Quality Control

Commercially available controls have recently been introduced with varying levels of sperm concentration for quantitative analysis. Currently, there are no quality control materials available for motility testing.

12.6 Reporting Results

(1) Record presence or absence of spermatozoa. It is not unusual to find viable sperm in a post-vasectomy patient. Instruction 3 above is advised to assure a thorough analysis.

(2) Report percent of motile spermatozoa calculated using the following equation:

$$\frac{\textit{No. of Motile Sperm}}{\textit{Total No. of Sperm}\ (100)}$$

Comment on any motility that is other than forward progression.

(3) Report red blood cells per high-power field. Report other cells or debris present.

12.6.1 Interpretation[7]

Reference Range: >50% motile spermatozoa

Azoospermia: less than one sperm per high-power field

While erythrocytes are not normally found in semen, a few can be present without indicating pathology. Some debris is typical, as are epithelial cells in low numbers. **NOTE:** Advanced microscopy skills can be required for the identification of abnormal morphology.

Complete sterilization can be determined as two consecutive monthly centrifuged specimens showing no spermatozoa.

12.7 Limitations of the Procedure

The limitations of the procedure include:

- delayed (greater than two hours) examination of the specimen;
- collection in improper container, such as condoms or other receptacles which may be contaminated with spermicides, lubricants, detergents, or powders;
- exposure of the specimen to temperature extremes during transport;
- abnormally low sperm count allowing for evaluation of less than 200 spermatozoa; and
- use of dirty or contaminated supplies.

Testing should be repeated to confirm azoospermia on a subsequent specimen.

13 Nasal Smears for Inflammatory Cells

13.1 Principle

Testing of a nasal discharge for presence of white blood cells by direct smear. May also be referred to as "nasal smear for eosinophils" or "nasal WBCs."

Examination of a direct smear is useful in distinguishing the nature of a nasal discharge. In nasal smears, the identification of eosinophils is correlated with allergic rhinitis. The procedure is useful to provide a differential diagnosis. When the condition is due to allergy, the predominant cell is the eosinophil. When the condition is due to nonallergic causes, the discharge will show a predominance of neutrophils or acellular mucus. Infectious processes show a predominance of neutrophils.

13.2 Materials

13.2.1 Supplies

Supplies used for the procedure include:

- nonabsorbent collection material;
- microscope slide; and
- cotton-tipped swab.

13.2.2 Equipment

Equipment used for the procedure includes a microscope.

13.2.3 Reagents

Commercially prepared Wright-Giemsa or Hansel stain is the reagent used for the procedure.

13.3 Specimen Collection

13.3.1 Conditions for Patient Preparation

There is no special preparation for the patient except the ability to produce the symptomatic discharge. Therefore some withdrawal of antihistamines or decongestants for a brief period may be helpful. The patient should be present to produce the specimen.

13.3.2 Specimen Type

Have the patient discharge their nasal passages into a nonabsorbent material. Paper with a smooth finish, such as waxed paper, plastic wrap, or an unused laboratory specimen transport bag is acceptable.

13.3.3 Handling Conditions

The specimen should be maintained at room temperature until examined. To prevent drying, assemble all necessary equipment to examine the discharge before initiating the specimen collection.

13.4 Testing Procedure

(1) Smears are prepared by transferring a sample of the produced mucus with a cotton swab onto a glass microscope slide.

(2) A thin smear is essential. A simple test is to check whether standard print can be read through the smeared material. Identifying elements of cellular detail will be difficult to determine if the smear is too thick.

(3) The smears should be allowed to air dry.

(4) Stain the smears using either a commercially prepared Wright-Giemsa stain or a Hansel stain. **NOTE:** Commercial kits are readily available for the rapid Wright-Giemsa stain technique and are provided with instructions and references.

(5) Neutrophils are recognized by their segmented or lobulated (two to five lobes) nuclei connected by a thin filament of chromatin. The abundant cytoplasm is pale pink or colorless and contains many fine, lilac-colored, neutrophilic granules. Eosinophils are recognized by their bright orange-red, spherical granules. There is typically a bilobed nucleus separated by a thin filament, but occasionally more than two lobes may be seen. The granules are larger than neutrophilic granules.

(6) Wright-Giemsa bloodstains may yield bluish granules in eosinophils, while the granules will appear bright red using a Hansel stain, and the neutrophils and mucus debris will have a blue color.

(7) With low-power scanning, make rough qualitative counts by approximating the average number of polymorphonuclear cells. Determine whether the leukocytes seen are neutrophils or eosinophils.

13.5 Quality Control

No commercially prepared direct controls are currently available; however, positive patient slides may be used for comparison.

13.6 Reporting Results

Presence or absence of eosinophils and neutrophils.

13.7 Limitations of the Procedure

Poor staining can cause false-negative recognition of eosinophils; therefore, staining of known material for quality control purposes is essential.

Artifacts, cellular distortion, and cellular degeneration are common in smears, making recognition of the cell type difficult. Reliance on staining characteristics provides the specificity required.

14 Fecal Leukocyte Examination

14.1 Principle

Testing for fecal leukocytes may also be referred to as "stool WBCs," and includes evaluation of fecal material for presence of white blood cells by direct smear.

A positive stained smear for polymorphonuclear leukocytes has a high sensitivity and specificity for bacterial diarrhea. Combined with a history of abrupt onset, greater than four stools per day, and no vomiting before the onset of diarrhea, the identification of leukocytes is an effective presumptive diagnostic test for bacterial diarrhea. Conditions associated with numerous fecal leukocytes, blood, and mucus include diffuse antibiotic-associated colitis, ulcerative colitis, shigellosis, salmonellosis, *Campylobacter,* and *Yersinia* infection. *Salmonella typhi* may evoke a monocyte response. Conditions associated with modest numbers of fecal leukocytes include early shigellosis involving small bowel, antibiotic-associated colitis, and amebiasis. Conditions associated with an absence of fecal leukocytes include toxigenic bacterial infection, giardiasis, and viral infections.

14.2 Materials

14.2.1 Supplies

Supplies used for this procedure include:

- plastic specimen container;
- microscope slide;
- wooden applicator stick; and
- cover slips.

14.2.2 Equipment

Equipment used for this procedure includes a microscope.

14.2.3 Reagents

Commercially prepared Loeffler's methylene blue or Wright-Giemsa stain is the reagent used for this procedure.

14.3 Specimen Collection

14.3.1 Conditions for Patient Preparation

If the patient is asked to bring a specimen from home, the uninstructed patient can exhibit considerable ingenuity in collecting a stool specimen. Therefore, a few simple instructions are likely to provide for a

more satisfactory specimen. If the patient does not have a bedpan, a clean glass jar is a satisfactory alternative. A plastic bag cut halfway down each side seam and taped to each side of the top of the toilet bowl is a suitable alternative collection device. Patients should be warned against passing urine at the same time into the container. Tongue depressors or pieces of cardboard are reasonable instruments for transferring the stool from the bedpan or other collection device to a transport container, e.g., a plastic, hard paper, or glass container.

14.3.2 Specimen Type

At least one gram of a naturally passed fecal specimen should be placed in a plastic specimen container. Polyvinyl alcohol (PVA) is a recommended preservative. A ratio of one volume of feces to five volumes PVA is recommended.

14.3.3 Handling Conditions

The specimen should be maintained at room temperature until examined. Because gas frequently accumulates in unfixed stool specimens, care should be taken to gradually loosen the cap of a transport container. Failure to observe this simple precaution can have unpleasant consequences.

14.4 Testing Procedure

(1) Place a small speck of mucus or a drop of liquid stool on a glass microscope slide with a wooden applicator stick.

(2) Add two drops of commercially prepared Loeffler's methylene blue, taking care not to contaminate the dropper with the specimen. The stain can be placed on the slide first to avoid this possibility.

(3) Mix thoroughly and carefully.

(4) Place a cover slip on the mixture.

(5) Let stand for two or three minutes for good nuclear staining.

(6) With low-power scanning, make a qualitative estimate of the number of leukocytes.

Alternatively, smears are prepared by selecting flecks of mucus from fecal material with a cotton swab that is then rolled across a glass slide. The smear is allowed to air-dry and is stained with a commercially prepared Wright-Giemsa stain.

The neutrophil demonstrates a nucleus that is often segmented or lobulated (two to five lobes) and is connected by a thin filament of chromatin. The abundant pale pink or colorless cytoplasm contains many fine, lilac-colored, neutrophilic granules with a Wright-Giemsa stain.

Artifacts, cellular distortion, and cellular degeneration are common in smears. The nuclear lobes may appear eccentric, and the cytoplasm may contain toxic granules or be vacuolated. Neutrophils may show morphologic changes due to autolysis, including nuclear pyknosis and fragmentation, making recognition of the cell type difficult.

14.5 Quality Control

No direct controls are currently available for fecal leukocyte testing; however, positive patient slides may be used for comparison. Proper storage and use of the stain is necessary for accurate interpretation.

14.6 Reporting Results

Report results in terms of the presence or absence of WBCs.

14.7 Limitations of the Procedure

Ten to fifteen percent of stools that yield a bacterial pathogen lack fecal leukocytes. The results may not be sufficiently specific to preclude the use of culture.

References

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Website:

http://www.vh.org/Providers/CME/CLIA/UrineAnalysis

Appendix A. Microscopic Components in Urine Sediment

A1 Red Blood Cells (Erythrocytes)

Under high power, unstained red blood cells in wet preparations appear as pale yellow-orange discs. They vary in size but are usually about 8μ in diameter. In old or hypotonic specimens, cells may appear as faint, colorless circles or ghosts, since the hemoglobin has dissolved out. Dysmorphic RBCs (acanthocytes, donut shaped, etc.) may be clinically meaningful in supporting the diagnosis of glomerular bleeding. Red blood cells may become crenated in hypertonic urine and appear as small, rough cells with irregular edges and surfaces. Smooth, folded, shrunken, and crenated cells may all be seen in the same urine specimen.

Red blood cells may be confused with oil droplets or yeast cells. Oil droplets (mineral oil or vaginal creams) show a great variation in size and are usually refractile. Yeast cells are oval to round, generally smaller than erythrocytes, and often show budding. Surface crenations on red blood cells resemble granules, and the cells may be confused with small granulocytes.

A2 White Blood Cells (Leukocytes, Neutrophilic)

Under high power, neutrophilic leukocytes in wet preparations appear as colorless, granular cells about two to three times the size of a red cell. It is not unusual to see dense, granular neutrophils not much larger than a red cell and swollen, large neutrophils in the same specimen. Occasionally, ingested bacteria or yeast are seen in the cytoplasm of leukocytes, crowding the nucleus and enlarging the cell two to three times.

In freshly voided urine, nuclear detail is well defined. With cellular degeneration, nuclear segments fuse into a single, round nucleus and granules are lost from the cytoplasm, making distinction from renal tubular cells difficult or impossible. In dilute or hypotonic urine, neutrophils swell. There also may be small intracytoplasmic vacuoles and loss of nuclear segmentation. Cytoplasmic granules wiggle or "dance" due to Brownian movement. Neutrophils containing these refractile "dancing" granules are called "glitter" cells.

A3 Epithelial Cells

A3.1 Renal Tubular Epithelial Cells

Renal tubular epithelial cells are derived from the epithelial lining of all segments of the nephron. They vary in size from approximately three to five times the size of a red cell, up to twice as large as a neutrophil. Typically, they are polyhedral in shape and elongated or ovoid with granular cytoplasm. The single nucleus is round and sometimes eccentric. Renal tubular cells originating from the proximal tubule may show a microvillus border, which is visible with bright-field microscopy. Disintegrating RTE cells become swollen and frayed, and the cytoplasm is often indistinct. In wet preparations, RTE cells may be difficult to distinguish from degenerating neutrophils, mononuclear leukocytes, or transitional epithelial cells.

A3.1.1 Oval Fat Bodies

Oval fat bodies are renal tubular epithelial cells containing fat globules. Lipid droplets containing cholesterol are anisotropic in polarized light, but show up brightly against a dark field and appear to be divided into four quadrants. Their appearance resembles a Maltese cross.

A3.1.2 Inclusion Bearing Cells

Inclusions such as those produced by cytomegalovirus, herpes simplex virus, and polyoma virus may occasionally be detected in the urine; these cells are excreted intermittently, and multiple samples should be examined. Due to the rapid degeneration of these cells, the urine must be processed as soon as possible after collection.

A3.1.3 Atypical Urothelial Cells

Urothelial neoplasms shed varying numbers of malignant cells into the urine. The presence of papillary fragments or urothelial cells with increased nuclear cytoplasmic ratios and cytomegaly should be noted, and cytologic follow up should be recommended.

A3.2 Transitional Epithelial Cells

Transitional epithelial cells (urothelial cells) vary in size, averaging about four to six times the size of a red cell. The nucleus is well defined, oval or round, usually central. Binucleate cells may occur. Transitional epithelial cells can occur singly, in pairs, or in small groups (syncytia). In wet preparations, they appear smaller and plumper than squamous epithelial cells and have a well-defined cell border. They may be spherical, ovoid, or polyhedral. The smaller cells resemble renal tubular epithelial cells. Some, called ”tadpole cells,” have elongated cytoplasmic processes, indicating a direct attachment to the basement membrane. Small vacuoles and/or cytoplasmic inclusions may be present in degenerating cells.

A3.3 Squamous Epithelial Cells

These large, flat cells are derived from the lining of the female urethra, the distal male urethra, or from external skin. In wet preparations, squamous cells are about five to seven times as large as a red cell and larger than most transitional epithelial cells. A single small, condensed, round, polygonal or oval central nucleus about the size of a small lymphocyte is seen in flat, round, or rectangular cells. Degenerating squamous cells have granular swollen cytoplasm with a frayed cell border and a pyknotic nucleus.

A4 Spermatozoa

In wet preparations, the sperm head is about 4 to 6µ in length, usually tapering anteriorly. It is smaller and narrower than a red cell. Slender tails are about 40 to 60µ in length. The head may be separated from the tail, making identification more difficult.

A5 Casts

Casts are cylindrical protein bodies that form in the distal tubules and collecting ducts of the kidney. They are subclassified on the basis of their morphologic appearance and composition. They have delicate parallel sides, rounded or tapered ends, and may be straight, curved, or convoluted. True casts should not be confused with long mucus threads, rolled-up squamous epithelial cells, cellulose, or other fibers.

A5.1 Hyaline Casts

Hyaline casts are colorless, homogeneous, translucent, and have a low refractive index. They have a smooth or finely wrinkled surface and may appear tortuous or coiled. Inclusion granules may occasionally be seen in the cast matrix.

A5.2 Granular Casts

Granular casts may contain many fine or coarse granules that are most often evenly dispersed over the cast, but may be confined to one area or loosely scattered. They may also include degenerating cell remnants. Distinction between coarsely and finely granular casts has no clinical relevance.

A5.3 White Blood Cell Casts

The casts may be crowded with cells, or have only a few clearly defined cells present in the matrix, often at one end. They contain predominantly intact segmented neutrophils, with cell membranes and nuclei clearly visible in most of the cells. The nucleus of the segmented neutrophil may be degenerated and rounded, precluding categorization of the cell.

A5.4 Red Blood Cell Casts

The predominant cells are intact erythrocytes, densely or loosely covering the hyaline or granular matrix. The red cells may be shrunken or crenated when compared with those in the surrounding urine. A yellow or red-brown color is seen when a large number of red cells fill the cast. Red cells are of uniform size within the cast, as opposed to fat globules, which vary in size.

A5.5 Cellular Cast, Renal Tubular Epithelial (RTE)

These casts contain RTE cells within their matrix that are usually intact and irregularly dispersed over the surface. However, in some RTE casts, the cells may be "lined up" in columns or rows, indicating sloughing of the epithelium of an entire tubule. RTE cells have a large, single, central nucleus and relatively sparse agranular cytoplasm. As RTE cells degenerate, their nuclei become pyknotic and dense. The cast matrix may contain granules thought to arise from degenerating RTE cells. While the cast matrix may be scant or difficult to visualize due to overlying RTE cells, it must be present in order to identify a cast.

A5.6 Fatty Casts

Fatty casts contain large numbers of spherical, highly refractile fat droplets of varying size in the cast matrix or within oval fat bodies in the cast. Casts may appear pale yellow, but fat droplets appear black with focusing under low power. Fat may be stained with commercially prepared Sudan stain or examined with polarized light to demonstrate the birefringent "Maltese-cross" pattern of cholesterol esters.

A5.7 Waxy Casts

Waxy casts are usually broad and stubby with blunt ends that may appear "broken off." They have well-defined parallel margins that may be serrated or notched. The colorless or waxy yellow interior is dense and homogenous. They are thought to arise from the degeneration of cellular casts.

A6 Crystals

Crystals in the urine are of limited clinical significance. Phosphates, urates, and oxalates are especially common and occur in normal urine sediment. A prerequisite for the positive identification of crystals is knowledge of the urinary pH. This helps with the preliminary separation.

A6.1 At Acid pH

- **Cystine** - A clear, colorless, hexagonal crystal sometimes pitted and occasionally twinned or laminated. There may be a wide variation in size in the same specimen from about the size of a red cell to that of a squamous epithelial cell. Cystine may be classified as a pathogenic crystal.

- **Uric acid -** These crystals appear at low-acid pH. They are usually colored yellow to brown. Common forms are four-sided, flat, and show a large variation in size and shape. Twinned, thick, brown rosettes and thick, yellow wedge shapes also occur.

- **Sulfonamides -** These crystals precipitate at low-acid pH. They are colorless to yellow-brown or green-brown in color. Sulfonamide crystals appear as bundles of long needles with eccentric binding resembling stacked wheat sheaves. They are also seen as spherical clumps with radiating spikes and fan shapes. Sulfamethoxazole crystals are dark brown, divided or fractured spheres.

- **Ampicillin -** Following large, intravenous doses it appears in urine as long, slender, colorless crystals that aggregate into irregular sheaves after refrigeration.

- **Amorphous urates** - These crystals are often referred to as "brick dust." These colorless or red-brown aggregates of granular material must be distinguished from bacteria. Amorphous urates are generally more pleomorphic than bacteria, and are not associated with increased white blood cells. A gram stain may be needed for further evaluation.[m]

A6.2 At Neutral-to-Acid pH

- **Calcium oxalate -** These crystals vary in size and may be much smaller than red blood cells. The dihydrate form appears as small, colorless octahedrons that resemble "stars" or "envelopes." They are sometimes described as two pyramids joined at the base. Larger crystals sometimes clump together. Oval, elliptical, or dumbbell monohydrate forms are less commonly seen. All calcium oxalate crystals are birefringent.

- **Cholesterol** - These crystals are large, flat, clear, colorless rectangular plates or rhomboids, that often have one notched corner. They are frequently accompanied by fatty casts and oval fat bodies. They may be confused with radiographic contrast media, but are not associated with a high urinary specific gravity.

- **Tyrosine -** This crystal is rarely seen. Silky, fine needles appear colorless to black under low power depending on focusing. Clumps or sheaves form after refrigeration. Tyrosine may be classified as a pathogenic crystal.

- **Leucine -** These highly refractive, brown, spherical crystals have a central nidus and "spoke-like" striations extending to the periphery. Leucine spherules are birefringent. Leucine may be classified as a pathogenic crystal.

- **Bilirubin -** Occasionally seen in urine with large amounts of bilirubin, and usually accompanies bile-stained cells. Small, brown needles cluster in spheres or clumps or on cells or hyaline casts.

[m] In the U.S., under CLIA, gram stains cannot be performed as a PPM procedure.

A6.3 At Neutral-to-Alkaline pH

- **Amorphous phosphates -** These crystals form colorless or brown granular aggregates. They are similar in appearance to amorphous urates, but occur in alkaline urine.

- **Triple phosphates (ammonium magnesium phosphate) -** These are typically colorless, often large crystals with a "coffin-lid" appearance. They exhibit birefringence and are often accompanied by amorphous phosphates and bacteria.

- **Ammonium biurate -** Biurates appear as crystalline, yellow-brown, smooth spheres with radial or concentric striations. The "thorn apple" variety has projecting thorns. These should not be confused with sulfonamide crystals.

A7 Bacteria

Most commonly, gram-negative enteric organisms are identified in wet mounts as rod-shaped organisms of medium size. Large, longer bacilli seen in urine are likely to be the gram-positive lactobacilli from vaginal or fecal contamination. Coccoid forms are more difficult to identify in wet urine sediments and must be distinguished from amorphous phosphates and amorphous urates.

A8 Yeasts

Yeasts are seen as colorless, oval, thick-walled cells, slightly smaller than a red blood cell. A cell with a single bud is characteristic. The cells stain poorly with aqueous stains in wet preparations but are strongly gram-positive with gram staining. Candida species form elongated cells up to about 50µ long, resembling mycelia. These are branched and have terminal budding forms.

A9 Parasites

A9.1 Protozoa

Trichomonas vaginalis **primarily** causes vaginal infection but is also capable of infecting the urethra, periurethral glands, bladder, and prostate. It ranges up to 30µ in length, is oval to pear-shaped with a single anterior nucleus, four anterior flagellae, and a posterior protruding sharp axostyle. Under high power, living forms show a short, undulating membrane and jerky movement. Degenerating forms resemble large, oval cells without visible flagellae and few distinguishing features and may easily be confused with neutrophils or other leukocytes.

A9.2 Helminths

Enterobius vermicularis is an intestinal nematode or roundworm known as pinworm. Females are white and threadlike and 5 mm to 13 mm long with a pointed (pin-like) posterior end. Eggs are about 50 to 60µ long and about half as wide, forming an elongated, well-defined oval with one flat side. These usually contain an embryo. Eggs laid in the perianal skin may occasionally be seen in the urine. Adult worms are rarely seen in urine.

A10 Miscellaneous/Exogenous

A10.1 Fat Droplets

Free, refractile droplets are seen as dark spherules under low power and clear spheres of varying size under high power. Fat droplets may represent endogenous triglycerides, neutral fats, cholesterol esters, or

combinations of all three. Exogenous mineral oil, catheter lubricant, or vaginal cream also appear as fat globules sometimes assuming large, amorphous, irregular shapes.

A10.2 Fecal Contamination

This may be the result of a fistula between the colon and urinary tract or due to contamination of the urine specimen with feces at the time of collection. There is an overall yellow-brown color. Plant structures, muscle fibers, and microorganisms are seen.

A10.3 Fibers

Hair, synthetic and natural fibers from clothing, cotton balls, dressings, and disposable diapers are all found in urine specimens. Most fibers are long, sometimes twisted and large. Short cellulose fibers from disposable diapers resemble large, broad, waxy casts. They are well defined, flat, refractile, and colorless and have fissures, pits, or cross-striations.

A10.4 Mucus

Mucus strands or threads frequently are found in urinary sediments arising from glands in the lower urinary and vaginal tracts. Translucent, delicate strands are seen in long, wavy, intertwined aggregates. They are usually in the background of the field and are more obvious with phase microscopy than bright field.

The material in mucus strands resembles that in hyaline casts. Casts are shorter and cylindrical with parallel, rounded edges. Casts may appear frayed and fibrillar with degeneration, but these strands are not as long as the usual mucus strand.

A10.5 Pollen Grains

These contaminate urine and urine containers on a seasonal basis. They are usually large, about 20µ or more in diameter and tend to be rounded or regularly shaped with a well-defined, thick cell wall. Some resemble worm ova. They may be winged or have short, regular, thorny projections. Some are yellowish-tan.

A10.6 Starch Granules

They are commonly found in surgical gloves and are a frequent contaminant of body fluids. Size varies from that of a red cell to four to six times larger. The usual form is colorless and irregularly rounded with a central slit or indentation.

A10.7 Stain[n]

Crystal violet-safranin or similar stains used for wet urinary sediments crystallize, especially at alkaline pH, and form brown-to-purple, needle-shaped crystals. These stains are available from various commercial suppliers.

[n] In the U.S., stains are not indicated for the microscopic analysis of urine sediment in PPM procedures under CLIA.

References for Appendix A

College of American Pathologists. Glossary of terms for urine sediment. *1999 Surveys Hematology Glossary*. 1999;27-36.

Henry JB. *Clinical Diagnosis and Management by Laboratory Methods*. 19th ed. Philadelphia: WB Saunders Company; 1996.

Appendix B. Microscopic Components in Vaginal Fluid and KOH Preparations

B1 Squamous Cells

Squamous cells measure 25 to 70 μm and demonstrate a polygonal "flagstone" appearance. These cells are almost always present in vaginal fluid.

B2 White Blood Cells

White blood cells measure 14 to 16 μm and exhibit a granular cytoplasm. White blood cells (with characteristic multilobed nucleus) are usually present in vaginal specimens in rare to scanty numbers. If they appear to be 3+, abnormal flora may be suspected. Conditions usually associated with 3+ white blood cells include *Trichomonas*, Vaginal Candidiasis, *N. gonorrhoeae*, herpes simplex, and severe atrophic vaginitis.

B3 Red Blood Cells

Red blood cells appear as biconcave discs measuring 7 to 8 μm in diameter. They are normally smooth, but may be greatly distorted in vaginal and urine specimens. The cytoplasm is clear and does not contain a nucleus. Red blood cells may be confused with yeast. They will lyse with addition of KOH, which is useful in distinguishing between RBCs and yeast. RBCs may be present in vaginal fluid as a result of current or recent menses, or due to desquamative inflammatory process.

B4 "Clue Cells"

Diagnostic "clue cells" should have small gram-negative, curved gram-negative, or gram-variable rods, covering not only on the surface of the cell, but also spread out past the cell boundaries, obscuring the cytoplasmic margins. The entire cell need not be covered with bacteria, but cells with organisms simply sticking to the surface without extending past the cytoplasmic margins should not be considered "clue cells." "Clue cells" may be described as "shaggy" in appearance. Of note is a phenomenon that occurs when KOH is added to a slide with moderate to large numbers of "clue cells." The lysis of the epithelial cell membranes causes a massive release of bacteria into the surrounding liquid, creating a "lava flow" appearance.

B5 Parabasal Cells

Parabasal cells measure 16 to 40 μm in diameter and appear oval to round in shape. They have a nucleus to cytoplasm ratio of 1:1 to 1:2. Less mature epithelial cells may be found in increased numbers at the time of menstruation and postmenopause. Parabasal cells, if present with large numbers of WBCs and altered flora in vaginal fluid, are suggestive of desquamative inflammatory vaginitis.

B6 Basal Cells

Basal cells measure 10 to 16 μm and appear round. They have a nucleus to cytoplasm ratio of 1:2. Similar in size to WBCs, they are distinguished by round rather than lobed nucleus. Basal cells are deep tissue cells, and their presence with large numbers of WBCs and altered flora in vaginal fluid strongly suggests desquamative inflammatory vaginitis.

B7 Bacteria and Parasites[o]

B7.1 *Gardnerella vaginalis*

Gardnerella vaginalis appears as small, nonmotile, coccobacillus that attaches to the epithelial cell (gram-negative/gram-variable, facultative anaerobe). It is one of the major bacterial species associated with bacterial vaginosis (see Section B4, "Clue Cells"). It produces a characteristic "amine odor" when KOH is added to the specimen.

B7.2 *Mobiluncus spp.*

Mobiluncus spp. appears as thin, curved, gram-negative anaerobic bacilli, demonstrating corkscrew motility in wet preparations. An "amine odor" is produced with addition of KOH. This bacterium is best demonstrated on gram stain. If the specimen is amine-positive and "clue cell"-negative, a gram stain may identify *Mobiluncus*.

B7.3 *Trichomonas vaginalis*

The typical *Trichomonas vaginalis* trophozoite measures approximately 7 x 15 μm and is oval in shape. Organisms can be twice this size, and the shape may vary greatly. Other features include a supporting axostyle that bisects the trophozoite longitudinally and protrudes from the posterior end; and five anterior flagella, one of which bends back along the outer edge of the undulating membrane creating "jerky motility." The pointed tip may be instrumental in attachment and may also be responsible for tissue damage. It should not be confused with sperm that have a single tail, much smaller head, and no axostyle. *T. vaginalis* may be found in vaginal specimens and urine specimens (especially those contaminated with vaginal secretions), as well as secretions from the urethra, prostate, and epididymis.

B7.4 Lactobacilli

Lactobacilli are present as normal flora in the vaginal fluid of postpubescent females and produce lactic acid. It is this metabolic waste which helps maintain the pH in the acidic range of 3.8 to 4.2 in the normal vaginal environment. They appear as relatively large, nonmotile rods (gram-positive on gram stain). Hydrogen peroxide-producing strains are thought to be responsible for protection against pathogenic organisms. If lactobacilli are absent or rare relative to squamous cells, abnormal flora may be suspected.

B8 Yeast and Fungi[j]

The two basic structures used to confirm the presence of yeast and fungi are:

1) Hyphae – long filaments which grow and form a mat (mycelium); and

2) Blastospores – buds formed during the reproductive process of yeasts.

Multiple buds that do not detach can form chains known as pseudohyphae.

Some fungi will produce both forms (although usually not both at the same time) in tissue depending upon environmental conditions — these are the dimorphic fungi; others will produce only one form or the other. Most pathogenic fungi are dimorphic.

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[o] In the U.S., identification of bacteria or parasites is considered high-complexity testing, and is not included in PPM under CLIA regulations. The general groups of bacteria, fungi, or parasites can be detected as part of the moderate-complexity procedures in this subcategory of tests.

Fungal hyphae, spores, and other fungal forms can be distorted in clinical specimens by the host's inflammatory response.

Reference to Appendix B

Koneman EW, Allen SA, Janda WM, Schereckenberger PC, Winn WC. Parasitology. In: *Color Atlas and Textbook of Diagnostic Microbiology.* 5th ed. Philadelphia: Lippincott. 1997.

NCCLS consensus procedures include an appeals process that is described in detail in Section 9 of the Administrative Procedures. For further information, contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Subcommittee Responses

HS2-P: *Provider-Performed Microscopy Testing; Proposed Guideline*

General

1. The tone of this document is somewhat colloquial. I have indicated examples of text I suggest be removed or rephrased. I emphasize this, because I anticipate that this guideline, when finalized, will be widely cited. The revision, to a more stringent editorial style, will establish the tone that quality assurance in PPM testing is not optional, but essential. Also the words "*should"* and "*must,"* must be carefully chosen. If the authors determine that an activity is essential, the word *must is* to be used. If the authors are making a recommendation that may or may not be adapted, then *should is* to be used.

- **Full consideration was given to the suggestion that the document reflect a firmer tone with regard to essential quality criteria. The text has been modified, where appropriate, to reflect the suggested change.**

2. The document does not cite the need for quality assurance plan under U.S. federal regulation CLIA '88. Although the document is intended for use outside the U.S. also, certainly the need to conform to regulatory agencies should be addressed. In fact, the document does use OSHA regulations as an example of workplace safety regulations.

- **Where appropriate, the document has been modified to cite regulations such as CLIA and credentialing and accreditation requirements as examples for conformance to quality standards.**

3. The model procedures in this guideline do not confirm totally to NCCLS document GP2-A3. The outline of each PPM should be the same as for the test procedure. Have you considered each procedure in NCCLS format as an appendix, which could be more easily updated than the final guideline? I suggest using the body of the guideline to identify essential quality assurance practices for each test. For example, quality control for urine sediments should include not only the description of sources of QC materials but also the frequency of performing the QC testing, documentation (log sheets), how and who must do a QC material review.

- **NCCLS document GP2-A3—*Clinical Laboratory Technical Procedure Manuals—Third Edition; Approved Guideline* is currently being revised. By their nature, PPM procedures are not as analytical as the next complexity level of laboratory procedures, and fewer portions of GP2 are applicable. Future reviews of the PPM guideline can consider recommended formats for the procedures when those formats have been revised.**

4. Identification of adult *Enterobius vermicularis* worms requires a level of technical expertise, which may not be available in the typical pediatrician's office or local health department setting. I suggest that CLIA program be consulted to determine if this examination falls under the subspecialty of parasitology. I would consider removing this section for issues related to technical expertise and regulatory compliance.

- **Pinworm examinations by light microscopy have been determined by the CLIA advisory committee to be within the scope of PPM procedures and, therefore, are the basis for the procedures included in this guideline.**

5. Throughout the document there are several references made to the economics of testing (choice of equipment) and the use of an adequate QA program to decrease the burden of liability. Are these issues consistent with the NCCLS mission? If the goal of this guideline is to promote quality testing, this side, albeit valid, interest should be omitted.

- **Value is an equation of quality given cost. Attention was paid to the practicality of the recommendations made for the expected setting. These procedures were given separate regulatory status, because they have value in patient care. The purpose of the document is to set forth uniform practices that will ensure accurate results. Medical decisions made with inaccurate results do carry the risk of increased liability for the practitioner.**

6. I suggest that this document also be reviewed by accrediting agencies like COLA, regulatory programs like CLIA, and subject matter experts.

- **Accrediting and regulatory agencies are members of NCCLS and have the opportunity to participate in all NCCLS document reviews.**

Foreword

7. I disagree with the statement that market demands are leading to more office-based testing. The number of POL certifications has decreased since the implementation of CLIA '88, in part due to what providers consider adequate reimbursement for the additional activities required under a comprehensive QA program.

- **The text has been modified as suggested. However, a recent national survey by the U.S. Centers for Medicare and Medicaid Services (CMS) notes that 74% of the CLIA-certified laboratories perform only waived or waived and microscopy testing, primarily in physicians' offices. Therefore, office-based testing represents a significant portion of the test market. Perhaps they are more focused on the lesser complex tests, but nevertheless the numbers are substantial enough to warrant attention.**

8. My first comment is on what is not in the guideline. As I went through the index, I noted there was no mention of gram stain. This was not clarified until the footnote on page 49. For international users of the document, it may be of value to comment in the Foreword, Introduction, or Scope on why gram stains are excluded from this guideline.

- **An additional line of clarification has been added to the Scope to reflect the types of specimens and procedures that are within the scope of the guideline. The following text and footnote have been added after the first sentence: "It does not include procedures which require the use of stains to determine the results of the testing. In the U.S., the CLIA certification for provider-performed microscopy (PPM) described a set of procedures that may be performed under that designation.**

Introduction

9. The second line of the first sentence should be rewritten to read, "…reliable results intended to be used by the provider to immediately impact patient care decisions."

- **The text has been modified as suggested.**

10. It is not recommended that nurse practitioners, physicians' assistants, and nurse midwives read KOH preparations for fungal elements or methylene blue preparations for fecal leukocytes. It is recommended that only physicians that are properly trained read these preparations.

- **The proposal for the document identified a lack of proper training in physician's office laboratory procedures in medical residency programs. It was also noted that changes in scope of practice expanded the need to other levels of providers. Clinical practitioners need resources such as this guideline to make accurate and appropriate use of these procedures. Recognizing changes in scope of practice, the PPM CLIA category was changed from "physician-performed microscopy" to "provider-performed microscopy." In making this change, DHHS specifically noted that in rural areas, among low-income populations, and in other areas where there is a shortage of physicians, the midlevel practitioners are the only available healthcare providers.**

Section 3.1

11. In the first sentence, reference to “all human body fluid specimens” should have the term “fluid” removed. Universal safety applies to fluid and nonfluid specimens, such as fecal samples.

- **The text has been modified as suggested.**

Section 3.2

12. Personal Protective Equipment: Under the bullet point "recommendations" include gloves. I encourage the authors to indicate that certain practices are required if the testing site is to operate under the laboratory industry standards of practice. The heading indicates that the following bullet points are situations when PPE should be used, but the last point recommends availability, not actually the use, of face masks. Recommending availability is weak. These high-risk aerosol procedures usually do not apply to PPM procedures and could be omitted.

- **The text has been modified as suggested.**

13. One of the infection control challenges of people wearing gloves is that they often touch charts, light switches, and microscope controls before they take their gloves off, thereby contaminating everything. We prefer that glove use is "task-specific," that they are taken off as soon as they are not required. With that in mind, while I support the need for gloves in sample preparation, I challenge their need while examining the sample under the microscope. While this section doesn't specifically say to wear gloves during microscopy, the second bullet can be interpreted in that manner. Would you consider adding a bullet or phrase such as "Glove use should be task specific, and be removed to avoid unnecessarily contaminating the microscope”?

- **The subcommittee agrees with the comment. The recommended text has been added to the second bullet point to address the commenter’s concerns.**

14. The second bullet should read: "Gloves must be worn whenever collecting, handling, and testing specimens, to provide barrier protection for cuts, scratches, or breaks in the skin against potential pathogens, and to provide barrier protection for the samples under investigation (i.e., protection of the integrity of the sample, as well as the individual performing the test)."

- **See response to Comment 13.**

15. Rewrite third bullet to read, “…gown, apron, or laboratory coat which is resistive or impervious to liquids…”

- **The text has been modified as suggested.**

Section 3.3

16. The use of *should* in the hand washing and basic laboratory safety considerations is too weak.

- **The following statement has been added as a footnote to Section 3.3: "In the U.S., employees must wash their hands with soap and running water as soon as feasible, as recommended above." Also, a reference to NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections*, has been included for completeness.**

17. From a laboratory biosafety perspective the single most important safety procedure is hand washing. It is very helpful that you have included this section. Would you consider expanding it slightly by including a brief description on hand washing? "Hands should preferably be thoroughly washed for 20 to 30 seconds under running water either with soap or antiseptic soap. Alternatively, an alcohol-based, waterless, rapid-kill gel can be rubbed thoroughly over the surface of the hands."

- **The text has been modified as suggested.**

18. Combine second and fourth bullet, i.e., "after completing work and/or before leaving the work area to eat, drink, smoke, apply cosmetics, handling contact lenses, and using restroom facilities."

- **The text has been modified as suggested.**

Section 3.5

19. While bleach is generally accepted as the agent of choice for cleaning up blood spills, it is not necessarily the only effective agent. Most of the samples being tested in this guideline are not bloody. Would you consider recommending use of common household, phenol-based disinfectants as an alternative to bleach? Generally they are easy to purchase, easy to use, and for many, they have a less irritating odor.

- **The subcommittee has considered other disinfectants for this section and believes the reference to NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections* addresses the issue of disinfectant options.**

20. Is bleach solution, which can be corrosive, the best disinfectant for microscopes? Other disinfectants such as pure alcohol could be better for microscopes with stainless steel components.

- **The text in Section 3.5 contains a note suggesting the use of other disinfectants that are less corrosive than sodium hypochlorite. Users are referred to NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections* for additional information.**

21. The microscope stage is the only part of the microscope we routinely disinfect with an alcohol pad (70%); all other parts are cleaned with a microscope cleaner. I haven't heard of using bleach on a microscope; perhaps it is recommended by vendors.

- **See response to Comment 20.**

Section 3.6

22. It is recommended that only designated provider-personnel handle medical waste and should never be handled by general housekeeping personnel.

- **The following text has been added to address the commenter's concern: "Therefore, handling and disposal of medical waste should be by trained personnel, utilizing materials and procedures which protect the employees, patients, and the environment."**

23. It is important to specify that infectious waste containers should be changed regularly, even if they are not full, to prevent possible contamination.

- **See response to Comment 22.**

Section 4

24. Replace "intensify" with "optimize or maximize" in the definition for immersion oil, i.e., "…used to optimize the resolution of the image being magnified."

- **The text has been modified as suggested.**

25. The definition of "precision" is not listed. Understanding of this term is essential in discussing good laboratory practice. The term is used in the document.

- **The text has been modified as suggested.**

26. The definition for “qualitative” should use the phrase "...that detect the presence or absence of a particular analyte, constituent, or condition. Specific identification may be performed."

- **The text has been modified as suggested.**

27. I understand within ISO that the phrases "preanalytic" and "postanalytic" in other countries are referred to as "preexamination" and "postexamination." Since this document is intended for an international audience, consider including these synonyms within the definitions.

- **The text has been modified as suggested.**

28. “Spinnbarkeit” should be included in the Definitions section.

- **A definition for “Spinnbarkeit” has been added to Section 4.**

Section 5.1

29. The fourth bullet should read, “…cover slip(s). “

- **The text has been modified as suggested.**

Section 5.2

30. Once a person has been trained properly to use the microscope, that knowledge and skill is very unlikely to degenerate. However, if a new technique were to be introduced to the field, then it would be beneficial to place within the provider-performed microscopy manual a mechanism to make an addendum.

- **The subcommittee agrees with the comment. The document production process has a mechanism in place to make changes or additions to the content. This is achieved through the consensus process, as stated on the inside of the cover, and during the review of the documents by the area committee, which occurs every three years or earlier if necessary.**

31. I do agree that a binocular-head microscope with a built-in light source and mechanical stage are very nice to have, but a monocular-headed microscope with a reflective light source would work just as well. Within an office setting with practitioners literally doing a test just a few times per day, a monocular scope would be adequate and would not create any environmental or ergonomic types of problems. Lastly, nothing is stated about the quality of the optics, the resolution of the optics. This characteristic is arguably the most important for a microscope.

- **The subcommittee agrees with the comment; however, the text states a binocular-head microscope is desirable and does not exclude the use of a monocular microscope.**

  With regard to the quality of the optics, the recommended equipment is a modern, high-quality microscope with the basic set of objectives and ocular lenses to assure the quality of the optics.

32. There is an extensive presentation of how microscopes work and operation of microscopes. This information is adequately cited. Is it really necessary to paraphrase references in this document? A more definite recommendation of preventative maintenance activities and intervals, documentation, and training includes information that could be presented in this document that is not stated in other references.

- **The subcommittee appreciates the comment; we believe, however, that the recommendations are useful and appropriate.**

Section 5.2.1.1

33. Please note that in Section 5.2.3 you use the phrase "back lens," but you have not included this in either the figure or this section.

- **The text has been modified to read "underside lenses."**

Section 5.2.1.4

34. Does the condenser "control the angle of light striking the specimen" or the amount of light?

- **The text has been modified to read "amount of light."**

Section 5.2.2

35. On the use of oil immersion objective, the last line of number (4) should read, "…cover slip. "

- **The text has been modified as suggested.**

36. The method of setting the condenser described here is not consistent with Koehler illumination.

- **The text has been modified to better reflect the use of Koehler illumination during operation of the microscope.**

37. Oil immersion examination is not indicated in PPM testing. This testing should be performed at 40x. This section could be omitted.

- **The subcommittee believes that the oil immersion lens is used in microscopic examination procedures and should be included for the benefit of those who would have an individual procedure that used the oil lens.**

38. The proposed guidelines have neglected to mention that the basic requirement for optical performance is the Koehler illumination. It should precede the explanations of how to focus a specimen. When we adjust the microscope, the condenser is fixed in an upright position and must not be used to control the contrast. The diaphragm controlling the condenser's aperture plays this role during examination. The diaphragm of the field aperture must be open just enough to illuminate the microscopic field. It is fixed during the adjustment of the microscope (Koehler illumination). Therefore, the first step of "how to focus a specimen" should be the actual step (3). The actual steps (1) and (2) should be deleted. (See Clancy, M. N., M. Cohen and L. S. Garcia. 1992. Bright-Field Microscope. In: H. Isenberg (ed.). Clinical Microb. Proc. Handbook, ASM, Washington, D.C. p. 12.14.1; 12.14.812.14.9.)

- **See the response to Comment 36.**

Section 5.2.3

39. Include a specific recommendation for maintenance interval for professional microscope service to be rendered.

- **The text has been modified to include the COLA requirement, at a minimum, of annual maintenance. This timeframe is dependent on the number of providers and the number of procedures performed on the microscope.**

Section 6

40. The quality assurance plan is the backbone of a testing program. This topic should be covered directly after safety issues due to its importance. This section should also clearly state in bullet points the essential

components of an adequate quality assurance plan. To the text presented in Section 6, an additional factor for error detection, investigation, correction, and documentation should be included.

- **The subcommittee appreciates the comment; however, the format of HS2 is consistent with other NCCLS documents and has been maintained for consistency.**

  The following components of a quality assurance plan have been added, as bullet points, following the first sentence: "identifying problems and errors, assessment of the causes, designing corrective actions, and monitoring to assure correction."

  The fourth sentence has been modified to include, "…error detection, investigation and implementation of corrective actions."

Section 6.1.1

41. I suggest rephrasing this section so that, according to accreditation or regulatory requirements, the person responsible for testing must determine first who can perform testing, and then identify individuals in the organization who meet these requirements.

- **To address the commenter's concerns this section has been modified to read, "The person responsible for oversight of the testing should first establish the necessary qualifications (education and experience) required for someone performing microscopic examinations, and then train qualified individuals. Training should be developed and provided to all providers who conduct the PPM testing."**

Section 6.1.2

42. The actual training of the providers occurs mostly while they are seeing patients. There are very few, if any, formalized courses with regard to training and the various testing. With regard to competency testing, once it has been ascertained that the person does know how to do the test properly, the likelihood of losing that competency is very small, if not nonexistent. Ongoing competency testing would yield very few inadequacies at a great cost in time and effort. The process would be very inefficient. On the other hand, recorded results should be monitored. Tests need to be charted properly. This would document whether the entire test is being done.

- **For a variety of reasons, individuals can lose competency and must be refreshed from time to time. Depending on frequency of test performance, adequacy of the initial training, ongoing monitoring of performance, etc., competency can be lost. The issue may be the term "testing" in the Competency Testing section. Testing sounds formal, rigid, and demanding. The intent of Section 6.1.2 is to describe possibilities for assessing competency on a periodic basis. Therefore, the subcommittee has changed the heading of Section 6.1.2 to Competency Assessment.**

43. The bulleted remarks regarding method of competency testing are reasonable. However, the list is not complete. There are many instances where collections of photos, textbooks, or educational software are used to help with competency testing. Competency assessment exams based on software, books, or photos are complementary to (not necessarily replacements for) the bulleted list of competency assessment methods listed in Section 6.1.2. It is important to include these types of competency assessment exams in the list of bulleted items, since these methods have distinct advantages over the other methods listed.

- **The subcommittee agrees with this suggestion. The following text has been added: "assessment examination using microscopy images in atlases or textbooks, photographs, or computer software."**

44. During the first year of testing, newly trained personnel must receive competency evaluation twice and annually thereafter.

- **The first sentence has been modified to include a parenthetical statement, i.e., "(such as twice during the first year of testing and annually thereafter)," to address competency evaluation intervals.**

Section 6.1.3

45. Published books and software should be mentioned as sources of continuing education. Well-tested, well-designed, accredited software is at least as good as the "homemade" PT slide/picture albums mentioned, and certainly much more organized.

- **The subcommittee agrees with this comment and has modified the text to include textbooks and journals, as well as commercially available educational packages.**

Section 6.2.1

46. Having the appropriate initial training with a new piece of equipment is prudent. If equipment requires calibration checks, those should be performed as part of the operational manuals of the equipment.

- **The text has been modified to address the commenter's concern.**

47. There are several statements that, although good, practical advice, do not address the issue of quality testing. I suggest deleting these, as indicated. (delete - last paragraph of Section 6.1.3; last part of first sentence of first paragraph in 6.2.1 beginning with "…because it is expensive"; from the end of first sentence in second paragraph in 6.2.1 ("both practical and wise") and the entire second sentence of the second paragraph; the last sentence of the second paragraph of 6.2.1; the fourth sentence of the first paragraph of 6.4; and the second sentence of the first paragraph in Section 6.5.)

- **The text has been modified as suggested.**

Section 6.2.2

48. I am having difficulty envisioning a situation where glassware used in PPM testing could be reused with a blank. Microscope slides and tubes should simply be single use.

- **In an international setting, there may be instances in the world where glass slides and other equipment are at a premium.**

49. Regarding the sentence: "If glassware is reused, a blank must be run to detect carryover." With perhaps the single exception of using a counting chamber, the reuse of glassware in microscopy is totally unacceptable. By including this alternative, you detract from the clear, unambiguous statement in the first sentence, and imply that reuse is OK if you run a blank. Since you have no reason to believe that one reused slide provides any information on another, this alternative gives no assurance. I strongly urge you to delete this sentence.

- **The text has been modified as suggested. The sentence has been deleted.**

Section 6.3

50. The difference between the test result (which is part of the patient's permanent medical record) and the quality assurance documentation used to derive that result is not clear in this section. The need to establish a document retention schedule is part of the QA plan. Under CLIA, documents must be available for two years.

- **The term "data" has been replaced with "test results."**

  With regard to document retention, the first sentence of the third paragraph has been modified to read, "The results should be retained for a minimum of two years; however, retention times may vary depending on local and regional regulatory requirements and standards of practice for the medical community."

51. First paragraph, last sentence. Reword sentence to read, "…criteria and recording of unacceptable specimens."

- **The sentence has been modified to read, "… recording of unacceptable specimens, recording of test results, and reporting…"**

Section 6.4

52. The bulleted statements beginning "Initial Approval Process" are very important. This paragraph should be emphasized with regard to competency.

- **The subcommittee agrees with the comment. The text has been modified to read, "A procedure manual is also a valuable resource for training new providers, verifying provider competency (see Section 6.1.2), or troubleshooting testing problems when they arise."**

Section 6.5

53. The purpose of QC testing is to determine that the testing system is operating within pre-established limits.

- **The subcommittee agrees with the comment. The text has been modified to read, "The purpose of quality control (QC) is to monitor the analytical process to determine that the test system is operating within pre-established limits, thus ensuring the accuracy of each examination or measurement performed on a patient specimen."**

54. This section addresses one of the challenges in quality control extremely well, i.e., the availability of control materials for microscopy. However, one aspect needs more emphasis. Collection of multiple samples from positive and negative patients is helpful, but if they remain unfixed, or if they are heat-fixed or alcohol-fixed, the survival of morphologic structures beyond a few weeks is very limited. Once the typical positive morphology has decayed, the value of the sample as a positive control is lost. Laboratories depending on fresh materials for positive controls need to implement a program that is constantly "on the look-out" for positive materials. Alternatively, commercial sources can be sought.

- **The following sentence has been added to address the commenter's concern: "Because the survival of positive morphological structures is limited, testing sites that choose to use fresh patient specimens for control material need to implement a program that is constantly 'on the look-out' for positive materials."**

55. The paragraph beginning "The most common causes of unexpected control results are:" is excellent. This provider-performed microscopy manual is an excellent training and teaching guide, and I would encourage it to be included as one of the manuals that all people should read as part of their quality control testing.

- **The subcommittee appreciates the comment.**

Section 6.6

56. It is a requirement of CLIA, ISO, and ILAC that proficiency-testing materials should duplicate clinical samples. It is fair to say that looking at pictures is nothing like testing clinical samples. While it may be beyond the scope of NCCLS, and while I would not incorporate this into this document, I strongly believe that standard format pictures are an unacceptable substitute, and that accrediting bodies do a disservice by accepting the results as a valid reflection of laboratory performance. In this situation "something" is not better than "nothing."

- **The subcommittee agrees with the comment. The text has been modified.**

57. All testing personnel should have PT testing responsibilities. There should be a schedule to assure that all testing personnel have the opportunity and responsibility to participate in PT.

- **The seventh sentence of the first paragraph has been modified to read, "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."

58. All PT results should be reviewed with testing personnel.

- **The following sentence has been added to the end of the first paragraph: "The grades should be reviewed and shared with all providers as part of the testing site's QA, continuing education, and competency evaluation systems."**

59. This section is most important with regard to initial training and evaluation. Further, the suggestions regarding documentation would be prudent to do initially. Once all of this is done, very few people would then lose competency.

- **See response to Comment 58.**

60. When PT programs are unavailable, providers should be required, just as clinical laboratories, to demonstrate proficiency by exchanging blind samples with a "neighboring" laboratory every six months.

- **The following text has been added in response to the commenter's concern: "If no PT program is available, the testing site may choose to monitor providers' proficiency by exchanging blind samples with other test sites twice each year."**

Section 6.7

61. This is a particularly difficult section, especially when the clinician, specimen processor, and microscopist are the same person. To be critical and objective about the diagnostic process in this setting may be impossible. As an alternative to trying to foster self-discipline, did the committee consider introducing the concept of voluntary internal audit as an approach to nonconformity detection?

- **The text has been revised to better clarify this step in the diagnostic process as it relates to QA.**

Section 7

62. The sentence, "Interpretation of urine sediment requires time, skill, training, and experience acquired through constant use of various microscopic methods and continuous pathophysiologic correlation of the sediment findings with the macroscopic results and clinical status of the patient" may be overstated. In this field one develops an expertise within a short time. The "learning curve" is not long, and once someone has gotten to the top or close to the top of the curve, the likelihood of losing that skill becomes small. Continuing medical education can easily fine-tune the knowledge, or tweak skills.

- **The text has been modified as suggested.**

Section 7.1.1

63. Edit the third line to read as a proactive statement. How many normal urine specimens have abnormal microscopic exams? Under what clinical circumstances would a clinician encounter this situation? Include literature citation that has documented those clinical situations having bonafide, clinically relevant abnormal microscopic exam results from properly collected, transported, and processed urine specimens having normal, physicochemical results?

- **The subcommittee is unaware of recent publications dealing with normal urine showing abnormal microscopic findings. The text has been edited to remove the second paragraph of this section, which relates to microscopic examination of urines with normal physicochemical results.**

64. The automated urine sediment analyzer should be noted, as the use of these instruments is now prevalent worldwide. Their usefulness in screening tests (as well as their limitations of performance) should be added.

- **While the subcommittee agrees that automated urine sediment analyzer use is now prevalent, discussion of automated technology is beyond the scope of this document.**

Section 7.2.1.2

65. I don't think "is not recommended" is strong enough.

- **The note has been modified to read, "Do not reuse centrifugation tubes."**

Section 7.2.2.2

66. Note that in this section the term 400g, which I think is correct, is used. In Section 7.4 (2) the term is replaced by 400-x g. Would the committee consider putting in a table that gives rotor arm length, rotor speed, and RCF? While this is available in other places, it may be convenient for the reader to have it in this guide.

- **A description of relative centrifugal force (RCF) and a formula for its calculation have been added to this section. Section 7.4 (2) has been revised for consistency.**

67. Consider recommending a "set" calibration interval, e.g., performed every four months, every six months.

- **The following recommendation has been incorporated in Section 7.2.2.2 to address the commenter's concern: "At a minimum, centrifuge calibration should be performed annually."**

Section 7.2.3

68. Last sentence: replace "should" with "shall," i.e., "reagents shall not be used after their expirations."

- **The text has been modified as suggested.**

Section 7.4

69. In Section 7.4 (6), the condenser should not be lowered to control the contrast.

- **The parenthetical statement has been corrected to read, "(achieved by lowering illumination intensity)."**

Section 7.4.1.2

70. Third line: replace "hardening" with "augmenting" or "enhancing."

- **The term "hardening" has been replaced with the term "enhancing."**

71. Sternheimer stain and Sudan III stain should be inserted in the document. In Japan, Sternheimer stain is popular for routine urine tests for the purpose of the identification of epithelial cells and casts. Sudan III is also very useful for the identification of oval fat bodies, fat droplets, and fatty casts.

- **Section 7.4.1.3 has been added to include a description of Sternheimer stain and Sudan III stain.**

Section 7.4.2

72. In Table 1, the only diagnosis associated with finding of crystals is cancer. This is inaccurate and misleading. More importantly, it is inconsistent with the comments in Appendix A, Section A6. I suggest removing the term "cancer" in the table and replacing it with "refer to Appendix A."

- **The text has been modified as suggested.**

73. I note that you include antibody-coated bacteria in Table 1. Is this a procedure that is included under provider-performed microscopy? If so, labeling as being synonymous with pyelonephritis is not correct (also prostatitis, chronic infection, catheterization).

- **The text "antibody-coated: pyelonephritis" has been removed from Table 1 for clarity.**

74. In Table 1, under "Finding: Crystals," cystine and bilirubin crystals should be added. Also, the meaning of "cancer (uric acid)" is not clear.

- **See response to Comment 72.**

75. In Table 1, under "Finding: Renal Tubular Epithelial Cells," nephrotic syndrome should be included.

- **The committee agrees with the comment. "Nephrotic syndrome" has been added to the table as recommended.**

76. In Table 1, Finding: Cellular Casts, nephrotic syndrome should be included.

- **See response to Comment 75.**

77. In Table 1 under "Finding: microorganisms: 100,000/mL: always infection"—A culture is usually necessary to quantitate bacteria present in that way. By direct microscopy, the number of cells seen by high-power field or oil-immersion field is usually reported. Is it the same for bacteria?

- **The text has been modified for clarity as follows: "Observation of microorganisms, indicating infection, should be confirmed by culture."**

Section 7.4.3

78. Reword second paragraph, first sentence as follows: "The importance of standardization in microscopic urinalysis cannot be overemphasized."

- **The text has been modified as suggested.**

Section 7.5.1

79. I do not understand the sentence, "The unavailability of commercially prepared quality control solutions does not justify the neglect of microscopic quality control." I believe that once someone has learned how to identify the various types of cells or findings for a specific test, that there is actually very little further subtlety. Clearly, if someone does not have competency initially to identify certain items, then their initial competency testing should not be rated as "satisfactory." That person would need to be continually trained until he/she has demonstrated competency.

- **The second paragraph of this section has been deleted to clarify the purpose of this section.**

Section 8.2.3

80. "Sterile physiologic saline" is not "9 grams/dL" but 9 grams/L or 0.9 gm/dL.

- **The text has been corrected to reflect the proper preparation of physiological saline.**

81. I do agree that concentrated potassium hydroxide is a highly corrosive liquid, but to state specifically that one should wear gloves or eye/face protection might overstate the problem when one is using small amounts of it from a small container. We must encourage safety, but we must also encourage people to use common sense and to be responsible for their actions. Taking away a person's responsibility totally might make sense from a

medical/legal point of view or from a work/legal point of view, but to encourage workers to be sloppy or not to take responsibility for their actions would be much more dangerous.

- **The following text has been added to the Note in this section: "It is recommended that for use in the PPM environment, 10% KOH be purchased commercially or obtained from pharmacy/laboratory sources."**

Section 8.4

82. In Section 8.4 (8), the last part of the first sentence should read, "…and under high power (40x) for smaller blastospores." Furthermore, the last sentence is an incomplete repetition of the first one.

- **The text has been modified as suggested.**

Section 8.4.1

83. A typographical error appears in the second line of text. It should read, "See Appendix B for a more detailed..."

- **This typographical error has been corrected.**

84. Table 2, in row "other organisms" under column "abnormal due to desquamative inflammatory vaginitis," there appears to be an over-call for gram-positive cocci when a gram stain was not performed.

- **Table 2 was included as authored; it has been modified, to remove gram-positive cocci, in response to the comment.**

85. Table 2, last item should read, "…vaginal erythema."

- **This typographical error has been corrected.**

Section 8.7

86. A typographical error appears in the first bullet. It should read, "... which can make interpretation of direct wet mounts difficult."

- **The text has been modified as suggested.**

Section 9

87. Parasite testing: Why is only the pinworm test described in this document? Moreover, the anal cellophane tape method is more efficient for pinworm detection than fecal analysis.

- **The scope of the prescribed provider-performed microscopy procedures was limited to those in the CLIA PPM category of testing. That category specified the performance of pinworm examination, not parasite testing. While the cellophane tape method is more common, the wet preparation was not excluded.**

88. Figures 2 and 3 are hand-drawn renderings. With the availability of electronic imaging and almost universal access to authoritative texts, the use of these quality illustrations is unnecessary.

- **Better quality illustrations have been provided for Figures 2 and 3.**

Section 9.2

89. In the cellophane tape test results for medical checks of school children in Japan, the adult pinworm is sometimes found. Therefore, it is recommended that suggested reporting not be limited to "none," but should include "rare" as a reporting option.

- **The text has been modified as suggested.**

Section 9.2.2.2

90. Third paragraph, edit first sentence to read, “With gloved hands, spread the anal folds apart…”

- **The text has been modified as suggested.**

Section 9.2.3

91. Item (2) should read, "... using a 10x to 40x dry objective."

- **The text has been modified as suggested.**

92. Figure 2. This drawing is not absolutely representative. Another drawing may be more accurate. See Murray, P.R., E. J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken. 1999. Manual of Clinical Microbiology, 7th ed. ASM Press, Washington, D.C.

- **See response to Comment 88.**

93. It is not possible to avoid contamination by such procedure; moreover, it is more infectious. We recommend using the cellophane tape method.

- **It is not clear to which procedure the commenter refers. Section 9.2 is the cellophane tape method. Section 9.3 covers Detection of Adult Parasites—including the wet preparation of fecal material—and is within the scope of the document. Additional mention is included regarding precautions to avoid contamination.**

Section 9.3

94. The description would be more precise if it were presented in the following manner: "... a ventrally curved posterior end for the male, and a long, pointed tail for the female.”

- **The text has been modified as suggested.**

Section 9.3.1.2

95. For the identification of worms 2 to 13 mm in length, stereoscopic microscopy examination is more appropriate than that of ordinary bright-field microscopy.

- **Stereoscopic microscopic equipment is not generally available in the settings described; therefore, the original recommendation for (worm) identification has been maintained.**

Section 9.3.2.1

96. The cellophane tape method is sufficient and convenient. The labor-consuming procedure, described in the document, is not needed.

- **The procedure may be undertaken to confirm parental observation under some circumstances.**

Section 9.3.2.3

97. Where do we put the fecal material after collection and before examination? (Suggestion: The fecal material is then put in a small container and maintained at room temperature until examined.)

- **The text has been modified as suggested.**

Section 9.3.3

98. Figure 3. A drawing of the female worm should be added to that of the male worm illustrated as it is referred to in Section 9.3 and in Section 9.3.3. It is the most frequently seen.

- **An illustration of the female worm has been included.**

99. For the purpose of detecting worms measuring 2 to 13 mm in length, magnification 40x seems to be too high.

- **The text has been modified to clarify use of both 10x and 40x magnifications.**

Section 9.3.6

100. The rationale for the limitations of the procedure should be described (as is done in Section 9.2.6).

- **Limitations of the procedure have been incorporated. The cellophane tape test has been recommended if clinically indicated.**

Section 10.1

101. The fern test certainly is one of the tests used to determine whether there is amniotic fluid within the vagina. However, the fern test is also used to determine the level of estrogen affect when cervical mucus is being evaluated at the time that a woman is ovulating. Both of these indications should be included.

- **The procedure for postcoital testing includes evaluation of cervical mucus for estrogen effect at ovulation. A cross-reference to Section 11 has been included for clarity.**

Section 10.3.2

102. Certainly, vaginal fluid can be picked up with a transfer pipette or a cotton-tipped applicator and spread out onto a slide. Why state so specifically how the specimen is collected?

- **No prior level of technique was assumed. There is a caution that no interfering materials be introduced. Certain types of swabs may differently absorb constituents of the specimen; therefore, a simpler direction can be established with the liquid transfer pipette than specifying the type of swab.**

Section 10.3.3

103. The specimen must be dry. Drying can take some time depending on the amount of fluid. Once the specimen is dry, it does not need to be read immediately. Crystals tend to keep.

- **The text has been modified as suggested.**

Section 10.4

104. Why include the statement "confirmation of test results using the 100x oil immersion objective is acceptable"? This does not need to be done unless one enjoys looking at the crystal formation. It is not helpful to determine ferning.

- **The text has been modified. The reference to confirmation using 100x objective has been deleted.**

Section 10.6

105. Besides the presence of a fern pattern, there is also the quality or characteristics of the crystal pattern. The ferning pattern is also a function of the various cellular and chemical elements co-mingled within the vagina.

- **The quality and characteristics of the crystal pattern are not part of a PPM work-up for the presence of amniotic fluid.**

Section 11

106. More sophisticated laboratories are not using this test. We abandoned the assay over ten years ago, along with many other laboratories. A retrospective analysis of our own patient population showed that its value in patient care was next to worthless. I really don't know if the practitioner in the field is using this test. Assuming that this section is of use to the practitioner, I think that the section adequately covers the topic and points out some of the pitfalls of this test.

- **The HS2 guideline is intended for the practitioner in the field, not the sophisticated laboratory; therefore, the recommended procedure for postcoital testing has been included.**

Section 11.2.1

107. The listing of supplies might be too specific. Different people will do things differently. One might state merely that the supplies should be single-use and sterile.

- **Section 11.2.1 lists recommended supplies for use in postcoital testing procedures. If an item is essential for testing, mention of its use is included in the procedure.**

108. I prefer a transfer pipette. Why even use any of these terms? Why not state things very simply?

- **Use of a syringe has been recommended, as it "doubles" as a volume measurement device. However, if sufficient suction can be generated to remove mucus, a transfer pipette could serve as an alternative. The phrase "or similar suction device" has been added to address the commenter's concern.**

Section 11.4

109. I would add to the testing procedure the fern test that determines the quality of the estrogen effect in the cervical mucus.

- **Interpretation of the fern test as a measure of estrogen effect was not available in the references reviewed.**

Section 11.6

110. This section has similar descriptors to the ones in Section 11.4. Maybe one of these two sections could be eliminated/combined?

- **The separation of testing procedures and reporting results has been maintained for consistency.**

Section 11.6.1

111. The postcoital test is a test which is interpreted differently by different people. There is not a lot of agreement on particular numbers or what a particular number means. Some are happier with less, others are happier with more. This manual should be far more concerned about the techniques and less concerned about the interpretations. I would suggest leaving the interpretations out. Certainly if one wants to include what should be part of the interpretation, one might again incorporate this section with Section 11.6.

- **The intent of Section 11.6.1 is to provide general guidance on result interpretation. Figure 4 has been deleted, as detailed explanation and interpretation of fertility testing is beyond the scope of this document.**

112. I am not sure that I agree that this guide is the best place to be providing information about diagnostic and therapeutic approaches to infertility. While interesting, I recommend the committee re-evaluate the need for the decision tree in this section.

- **See response to Comment 111.**

Section 12

113. With regard to semen analysis, I would advise against collection of the semen sample into anything other than clean, sterile, plastic specimen cups provided by the office or clinic. Avoid the mention of glassware. There are all sorts of hazards when glassware is used (e.g., recycling, reuse).

- **There are reports of decreased motility in specimens remaining in plastic containers for more than 60 minutes. The choice of container is less of a concern with qualitative analysis for presence or absence of sperm.**

114. The recommended time for liquefaction of an ejaculate is 30 minutes (room temperature to 37 °C). I would advise the practitioner to mix the sample thoroughly after liquefaction to obtain a good sample aliquot.

- **The phrase "well-mixed" has been added to Step 2 for clarity.**

Section 12.3.1

115. Instead of "three-day period of abstinence," the World Health Organization Guidelines (1999) suggests "48 to 72 hours of sexual abstinence prior to collection."

- **The text has been modified as suggested.**

Section 12.4

116. According to WHO guidelines, all forms of motility should be included. A comment can be made concerning the type of motility, for example: "sluggish," "little to no forward progression," etc.

- **The text has been modified in accord with WHO guidelines.**

Section 12.6.1

117. The reference range for sperm motility is >50% according to WHO guidelines.

- **The text has been modified in accord with WHO guidelines.**

Section 14.1

118. Is this test still useful and significant? It seems that there is something contradictory between the principle and the limitations of the procedure. In the principle, the test has "a high sensitivity and specificity for bacterial diarrhea"; in the limitations of the procedure, however, "the method is not sufficiently sensitive or specific to preclude the use of culture."

- **The test is in frequent use in the primary care office as a rapid diagnostic tool. The limitations should lead the practitioner to use the test as an adjunct to culture, and in association with clinical symptoms to determine treatment. The text has been modified to address the commenter's concern.**

Section 14.3.2

119. In addition to PVA, is 10% buffered formalin acceptable? We use two gram stain smears made from 10% formalin for a "quick" read for preserved specimens, i.e., no fresh stool submitted. It also avoids spreading mercury chloride throughout the laboratory. The PVA, however, appeared much more sensitive when stained with our standard O&P stain. For fresh, unpreserved stools, we gram-stain them, i.e., never stained with methylene blue.

- **If the opportunity is available to provide the patient with a preservative, leukocytes will be maintained for same-day testing in any preservative compatible with cytology, tissue, or parasitology examinations (e.g., formalin).**

120. Could formalin 10% also be used as a good preservative as used for intestinal parasites (in combination with PVA)? Its use could be easier for direct examination of fecal leucocytes (a ratio of one volume of feces to three volumes of formalin 10% is usually recommended).

- **See response to Comment 119. Many "kits" for collection of O&P (ova and parasites) specimens include polyvinyl alcohol (PVA) and formalin. PVA is usually used for preserving trophozoites and 10% formalin for ova.**

Section 14.3.3

121. Gas is usually produced in unfixed specimens. The precision should be made as follows: "…because gas frequently accumulates in unfixed stool specimens…"

- **The text has been modified as suggested.**

Section 14.4

122. Methylene blue should be placed first onto the slide to prevent contamination of the dropper, i.e., step (2) should come before step (1).

- **The addition of stain should be via a dropper without coming in contact with the specimen. Text has been added to reflect that caution as well as the alternative suggested in the comment.**

123. It could be useful to briefly describe the protocol for staining with Wright-Giemsa stain in this section.

- **The protocol for Wright-Giemsa staining is described immediately following the numbered steps of the methylene blue protocol.**

References

124. References on pinworms should be updated.

- **The references have been updated as suggested.**

Appendix A

125. Section A1, Red Blood Cells (Erythrocytes), dysmorphic RBC (acanthocyte, donut shape, etc.), which are clinically meaningful for the diagnosis of glomerular bleeding, should be inserted in the document.

- **Text has been added to indicate the significance of dysmorphic red blood cells.**

126. In Section A3, subsections regarding oval fatty body, inclusion-bearing-cells, and atypical cells should be added respectively.

- **Three subsections have been added to address the recommended topics.**

127. Section A5.3 Cellular Cast, Neutrophil: The description sounds like "cellular casts are equal to epithelial casts." We recommend describing WBC casts individually.

- **The title has been changed as recommended.**

128. Bilirubin crystals should be mentioned in Section A6.2, Crystals At Neutral to Acid pH.

- **The section has been modified as recommended.**

129. Although it may be a rare case, we recommended mentioning 2, 8,-Dihydroxyadenine crystals in Section A6.3.

- **The subcommittee appreciates the input; however, due to the rarity of these crystals, they have not been included in this section.**

Appendix B

130. Table B1 may dangerously oversimplify or understate the difficulty of identifying fungal species by direct microscopy, even presumptively. Pictures may help, as well as a warning that identification of the organisms, several of which may cause life-threatening infections, based on a direct smear examination, can be extremely difficult and should be referred, whenever possible, to personnel with more extensive experience.

- **Picture references are a requirement of both CLIA and COLA and should supplement any written description. Table B1 has been deleted in response to this comment.**

131. Section B6 contains a typographical error. The statement should read: "They have a nucleus to cytoplasm ratio of 1:2."

- **This typographical error has been corrected.**

132. Table B1. There is a typographical error that should be corrected accordingly: “Blastomyces dermatitidis and **Coccidioides** immitis.”

- **Table B1 has been deleted in response to Comment 130.**

133. Appendix B, Table B1 is unrelated to the appendix and should not be included. Microscopic examination of Aspergillus species, Cryptococcus species, and especially Histoplasma, Blastomyces, and Coccidiomyces do not belong here.

- **Table B1 has been deleted in response to Comment 130.**

Summary of Delegate Comments and Subcommittee Responses

HS2-A: *Provider-Performed Microscopy Testing; Approved Guideline*

Appendix A

1. Regarding the statement that *amorphous urates* "must be distinguished from bacteria": Is it within the scope of this document to clarify that distinction? I suggest adding the following or something similarly appropriate: "Amorphous urates are generally more pleomorphic than bacteria, and are not associated with increased white blood cells. A gram stain may be needed for further evaluation."

- **The text has been modified as suggested with the addition of an applicable footnote.**

2. Radiographic crystals in urine are described in this document. The CAP Hematology & Clinical Microscopy Resource Committee, at its last meeting, deleted this definition from the Hematology Glossary, because these crystals no longer appear with new contrast media in use.

- **The text has been edited to remove the section describing radiographic media.**

Related NCCLS Publications*

GP2-A4 **Clinical Laboratory Technical Procedure Manuals; Approved Guideline—Fourth Edition (2002).** This document provides guidelines that address the design, preparation, maintenance, and use of technical procedure manuals (whether they are in paper or electronic formats) for use by the patient-testing community.

GP5-A2 **Clinical Laboratory Waste Management; Approved Guideline—Second Edition (2002).** Based on U.S. regulations, this document provides guidance on safe handling and disposal of chemical, infectious, radioactive, and multihazardous wastes generated in the clinical laboratory.

GP16-A2 **Routine Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline—Second Edition (2001).** This guideline offers descriptions of routine urinalysis test procedures that address materials and equipment, macroscopic examinations, clinical analyses, and microscopic evaluations.

GP21-A **Training Verification for Laboratory Personnel; Approved Guideline (1995).** This document provides background and recommends an infrastructure for developing a training verification program that meets quality/regulatory objectives.

M29-A2 **Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Second Edition (2002).** This document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection for laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

NOTES

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