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Trichomonas vaginalis: The Most Under-recognized Sexually Transmitted Infection of the 21st Century

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Overview

Trichomonas vaginalis, a protozoan parasite (Figure 1), is the etiologic agent of trichomoniasis, a sexually transmitted infection of the urogenital tract. Although vaginitis is the most common manifestation of trichomoniasis in women, it can also cause cervicitis and urethritis. It presents with malodorous, yellow-green frothy discharge and vulval irritation, and has also been associated with premature rupture of membranes and delivery in pregnant women.[8] However, it is important to recognize that not all women infected with this organism are symptomatic.

Most men infected with *T.vaginalis* are asymptomatic, however, some men may present with urethritis.

Trichomoniasis is transmitted by sexual contact, but, unlike other sexually-transmitted infections (STIs), such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, *T.vaginalis* is not reportable to the public health department so reliable data on case incidence is not available. Despite the lack of reliable data, trichomoniasis is thought to be the most common treatable STI in the world at an estimated 7.4 million new infections each year in the USA.[1,2,8]

Pathogenesis

T.vaginalis is a flagellate (Figure 1) belonging to the phylum Metamonda, class Parabasalia, order Trichomonadida and, unlike other members of this order, it infects the urogenital tract. [8] It only infects humans and only the trophozoite form is known (i.e. no cysts form). The trophozoite is pear shaped (7-23 µm long by 5-15 µm wide) and replicates by binary fission. It is motile and has a jerky, rapid movement when observed in a liquid suspension (wet preparation) by light microscopy. The trophozoite is the infectious form and is transmitted from person to person by genital contact.[8]

T.vaginalis attaches to both epithelial and immune cells in the genital mucosa of humans and secretes proteins that destroy the human cells. This elicits a local cellular immune response that leads to an intense inflammatory response. *T.vaginalis* prefers an anaerobic environment and grows best at pH 6. To achieve these conditions in the vagina, it alters the normal vaginal microbiome by phagocytosing vaginal bacteria thereby creating a more alkaline environment. The frothy, bubbly discharge is created by hydrogen gas that *T.vaginalis* stores in structures called hydrogenosomes. Trichomoniasis is a risk factor for transmission of HIV and this is thought to be because of the increased vaginal/endocervical inflammation in women and inflammation of the urethra in men. [7]

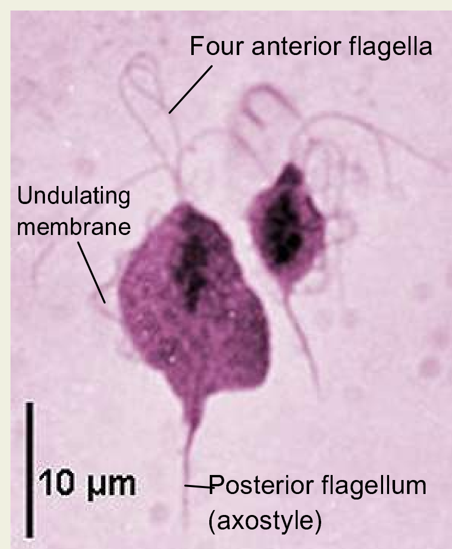

Figure 1. *Trichomonas vaginalis.* The flagella and undulating membrane are responsible for motility which is the basis of the microscopy based test methods.

Image: Guy Brugerolle Supplier: micro*scope

Epidemiology

Table 1 shows data from a recent study by Andrea *et al*[4] that reported *T.vaginalis* accounts for approximately 5% of disease in symptomatic women. Another recent study indicated that the prevalence of *T.vaginalis* in women between the ages 18 and 89 may be up to 8.7%.[6] Surprisingly, the data shown in Figure 2, which stratifies STIs by age of the symptomatic women, clearly shows that Trichomoniasis is the only STI of relevance in women over the age of 40. Similar results were reported by Ginocchio *et al.*[6] This information should help diagnostic microbiology labs to target the testing performed on vaginal specimens from older women (Note: use of the Nugent score to assess bacterial vaginosis has not been validated in post-menopausal women).

T.vaginalis does infect the urethra of men and thus, treatment of sex partners of patients with *T.vaginalis* is recommended. Most recurrent *T.vaginalis* infections are thought to result from having sex with an untreated partner. The need to make the diagnosis of Trichomoniasis in male partners of women with this infection has been shown to be a key step in the effective control of the spread of this infection.[1]

Treatment

The 2010 MMWR guideline[1] for STIs indicates that the recommended treatment regi-

Table 1. Genital infections in symptomatic women using Affirm and APTIMA NAAT tests

Genital pathogen detected	Diagnostic test used	% of cases
Bacterial vaginosis (*Gardnerella vaginalis*)	Affirm PCR	41.3%
Candida albicans	Affirm PCR	14.8%
Trichomonas vaginalis	APTIMA TMA	5.1%
Chlamydia trachomatis	APTIMA TMA	3.4%
Neisseria gonorrhoeae	APTIMA TMA	0.7%
No pathogen detected	Affirm PCR and APTIMA TMA	34.9%

Data extracted from Andrea *et al* 2011[4]

men for *T.vaginalis* infection is metronidazole or tinidazole 2 g orally in a single dose (an alternative regimen is metronidazole 500 mg orally twice a day for 7 days). Low level resistance to metronidazole has only been reported in 2-5% of cases and high level resistance is rare.[1]

Diagnostic Testing

The diagnostic tests currently available for *T.vaginalis* are summarized in Table 2. Testing of oral or rectal specimens for *T.vaginalis* is not recommended as this parasite has not been found in oral sites and rectal prevalence is low. In women, vaginal specimens are most commonly submitted and for men, urethral swab is the preferred sample.

As shown in Table 2, microscopy is a substandard test for both men's and women's specimens. Since microscopy is done using the "hanging drop" or "wet prep" method, it must be read immediately (within 1 hour of preparation) [8] thus, it cannot be used for specimens in transport media.

Culture (e.g. vaginal sample inoculated into Trichosel media) is a reasonable test for vaginal and male urethral specimens, but it requires the presence of viable trophozoites in the specimen. The use of commercial culture systems (e.g. InPouchTV, BioMed Diagnostics, San Jose, CA) allow for direct inoculation, transport, and microscopic examination of specimens. [8] However, culture is a very labour intensive test as it requires up to 7 days incubation with daily assessments by microscopy to determine if *T.vaginalis* is present.

The antigen detection test is superior to both microscopy and culture (table 2), as it does not require viable trophozoites thereby accommodating specimens that must be transported for up to 48 hours. The sensitivity and specificity of this antigen detection test are better than the Affirm VPIII direct DNA probe test. Unfortunately, since the rapid antigen test has only been validated for use with vaginal samples, it is not an appropriate test to use on endocervical specimens, urethral swabs, or urine specimens.

The newer nucleic acid amplification tests (NAATs) include both PCR and transcription-mediated-amplification (TMA) approaches (Table 2). The report of Huppert *et al* [5] indicated that for symptomatic women, the rapid antigen test (OSOM, Sekisui Chemical Co) compares favorably with the APTIMA TMA test (92.5% sensitivity vs. 97.5% sensitivity, respectively). The NAATs have been found to have superior sensitivity for diagnosing Trichomoniasis in men. Another advantage of the NAAT tests is the wide range of specimen types that can be tested including urethral and urine specimens from men as well as vaginal, endocervical, and urine specimens from women. The NAAT data presented by Ginocchio et al.[6] suggests that *T.vaginalis* should also be tested in all women who are being screened for *N.gonorrhoeae* and *C.trachomatis*.

At present (to the best of the author's knowledge), there are no external proficiency test (EPT) programs that evaluate the competency of diagnostic staff with respect to the detection of viable trophozoites for microscopy testing of *T.vaginalis*. This is likely due to the difficulty in ensuring the trophozoites remain viable in the EPT challenge. Although it is likely that EPT programs will be developed for *T.vaginalis* NAAT tests, these are not currently available.

There are EPT programs for antigen based detection of *T.vaginalis* (e.g. CMPT in Canada).

At this point in time, the ideal test for *T.vaginalis* in vaginal specimens from symptomatic women based on turn-around-time, reliability, ease of use, and existence of EPT programs would be the rapid antigen test (Table 2). However, as clinicians begin to do more testing in men and screening of asymptomatic women, it may be necessary to transition to one of the NAAT diagnostic tests that can be used reliably for the widest range of sample types from both men and women.

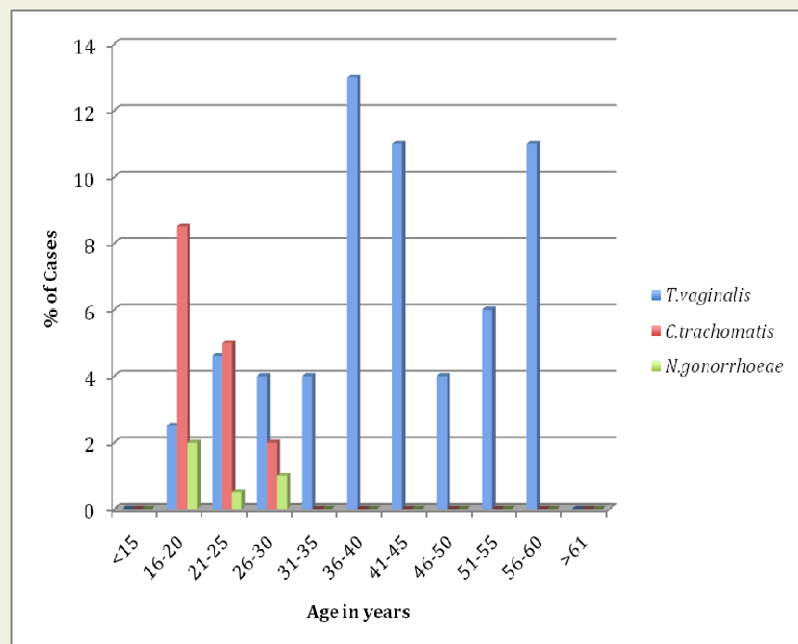

Figure 2. STI prevalence in symptomatic women. Data extracted from Andrea 2011.[4]

Table 2. Diagnostic tests for *T. vaginalis*

Test	Basis of test	Sensitivity/Specificity	Comments
1. Microscopy: Wet preparation of vaginal secretions suspended in saline.	Detection of viable *T.vaginalis* (must see actively moving trophozoites)	Sensitivity 57-69% Specificity 100%	Must be read immediately (within 1 hour) so can only be done on-site at the clinic. Poor sensitivity for men.
2. Culture: Trichosel broth, InPouch TV (BioMed Diagnostics, San Jose, CA)	Detection of viable *T.vaginalis* in culture broth (must see actively moving trophozoites)	Sensitivity 75-96% Specificity 100%	Accommodates transport time of up to 48 hours. Labour intensive as broth culture must be observed daily by light microscopy for up to 7 days of incubation.
3. Antigen detection: OSOM Trichomonas Rapid Test (Genzyme Diagnostics, Cambridge, MA)	*T.vaginalis* antigen	Sensitivity: 83-93% Specificity: 95%	Does not need viable organisms. Results in 10 minutes and is CLIA waived. Only cleared for use with vaginal samples.
4. Nucleic acid probe: Affirm VP III (Becton Dickenson, San Jose, CA)	*T.vaginalis* rRNA detected by direct DNA probe	Sensitivity: 63-83% Specificity: 99-100%	Results in 45 minutes, only cleared for use with vaginal samples.
5. Transcription mediated amplification (TMA) APTIMA TV (Gen-Probe Inc.)	*T.vaginalis* rRNA detected by TMA	Sensitivity: 89–100% Specificity: 92-100%	In addition to vaginal, endocervical, and urine specimens from women, it can be used for urethral or urine samples from men and screening of asymptomatic men and women.
6. PCR: Amplicor (Roche Diagnostic corp.)	*T.vaginalis* rRNA detected by PCR	Sensitivity: 88-97% Specificity: 98-99%	In addition to vaginal, endocervical and urine specimens from women, it can be used for urethral or urine samples from men and screening of asymptomatic men and women

Data for table extracted from Nye *et al* 2009, Huppert *et al* 2007,Andrea *et al* 2011 and MMWR 2010.

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6.Ginocchio C, Chapin K, Smith J et al Prevalence of *Trichomonas vaginalis* and co-infection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in the USA as determined by the APTIMA *Trichomonas vaginalis* nucleic acid amplification assay Sex Transm Infect 2011; 87 Suppl 1:A72.

7.Sept 2011 Association of Public Health Laboratories Teleconference entitled: “Should we be concerned about *Trichomonas vaginalis*?”

8.Manual of Clinical Microbiology 10th edition, 2011, J. Versalovic [Editor in Chief], American Society of Microbiology Press (publisher).

Gram Smear Report - Entering Results Online

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Over the years, the CMPT committee has noted that laboratories have received unsatisfactory grades due to clerical errors such as incorrectly entering the challenge number on the on-line report form, or by not reporting results correctly or completely as requested, with the error rate greatest with the Gram stain report. This article will attempt to demystify the CMPT Gram Smear Report form for users and provide practical solutions for reporting Gram stain challenges.

Many laboratories have differing practices in how they assess, interpret, and report Gram smear results. Most laboratories assess and report organisms on oil immersion field (x1000) but there is variation on whether they observe and assess the cellular components on low power field (x100) or under oil immersion (x1000), and may have varying assessment tools for sputum, wound, fluid, or vaginal samples.

CMPT developed a report form and guideline that would allow the committee to gather data in a standardized manner, so the information could be assessed and graded without requiring laboratories to adopt one method or another. Unfortunately, the form may not reflect the practice in your laboratory, which may lead to confusion on reporting results of some components of the Gram Smear Report form.

The Gram Smear Report form is divided into 5 sections – see the table below highlighting required fields:

Section 1 – Laboratory & Challenge information

CMPT01/F/10/2009: Gram Smear Report Form

Laboratory Name: (1)	Laboratory No: (2)
Gram Smear Specimen Number: (3)	Source/Site:
Date Specimen Received:	Date Specimen Reported:

1. Enter full name of laboratory (no abbreviations).
2. Enter CMPT participant number.
3. Enter Gram smear specimen number – format **Gxxx** or **GSxxx** for Gram stain supplementary challenges.

Section 2 – Cellular results for all smears *except* sputum samples

This table is for reporting the **CELLULAR** component in **ALL** smears **except** Sputum smears

Cell Type	count per **oil immersion field** based on at least 10 fields (4)	**Interpretation: 1+, 2+, 3+, 4+** Based on laboratory's internal interpretation guidelines (5)
1. Neutrophils		
2. Epithelial cells		
3.		

4. Enter count per **oil immersion field** based on at least 10 fields (*even if your laboratory assesses and interprets cells on low power*). This average is used to assess quality and consistency of smear preparation.
5. Enter the actual value based on your laboratory's Gram stain interpretation guidelines.

Section 3 – Cellular results for sputum samples

This table is for reporting the **CELLULAR** component in **SPUTUM** smears only

Cell Type	count per **low-power field** (6)	**Interpretation: 1+, 2+, 3+, 4+** Based on laboratory's internal interpretation guidelines (include comment below, if applicable or check one of the boxes below) (7)
1. Neutrophils		
2. Epithelial cells		
3.		

Please check if your report would state: (8)
- □ "culture pending or culture results to follow" (suitable for culture)
- □ "final report – sample not suitable for culturing"

6. Enter count per **low power field** based on at least 10 fields (*even if your laboratory assesses and interprets cells on another magnification*). This average is used to assess quality and consistency of smear preparation.
7. Enter the actual value based on your laboratory's Gram stain interpretation guidelines.
8. Check box to indicate if your laboratory would accept the sample as suitable for culture, or reject due to poor quality (Q-scoring or other means of assessment may be used).

Section 4 – Bacterial component for all smears

This table is for reporting the **BACTERIAL** component in **ALL** smears		
Bacterial Gram Morphology □ no organisms seen	count per **oil immersion field** based on at least 10 fields	**Interpretation: 1+, 2+, 3+, 4+** Based on laboratory's internal interpretation guidelines (include comment below, if applicable
1.		
2.		
3.		
4.		

9. If no organisms are present, check the box, otherwise list the morphotype(s) observed. If your laboratory reports out morphologies suggestive of certain organisms, eg. gram positive cocci in chains, suggestive of streptococci, record this in the box provided.
10. Enter count per **oil immersion field** based on at least 10 fields (*even if your laboratory assesses and interprets cells on low power*). This average is used to assess quality and consistency of smear preparation.
11. Enter the actual value based on your laboratory's Gram stain interpretation guidelines.

Section 5 – Interpretative Comments

COMMENTS- this is a free text box. Please provide the EXACT wording and include all interpretative comments as it would appear on your clinical Gram stain report. Please attach an additional page if necessary.	
Signature:	**Date:**

12. Enter your final Gram smear result and include all interpretive comments as they would appear on your laboratory's Gram smear report.

When a Gram smear challenge arrives in your laboratory, provide a copy of the CMPT Gram smear report form to accompany the smear for recording observations – this provides the technologist reading the smear with the criteria CMPT requires. The smear can be read and reported as per your laboratory's interpretative guidelines and the requested data (counts per field – low power or oil immersion, depending on sample type and component) will be gathered simultaneously. Laboratories that utilize the challenge for competency may have several technologists read the smear and report their findings on the report form, then review the results and determine a final result for CMPT.

Care must be taken when entering results on the CMPT website. Each challenge has a specified report form associated with it, as indicated in the online data entry instructions included with the samples. It is advantageous and a good practice to enter all the results, print out the results and review for accuracy and completeness. A final check should be made to ensure that the correct challenge is associated with the specified report form before submitting the results as final data as errors such as entering the mixing up the results of challenges will not be corrected by CMPT.

Readers can refer to the CMPT website under programs for the Gram Smear Interpretive Criteria - Suggested Guideline (http://www.cmpt.ca/programs_clinbact/clin_bact_gram_protocol.htm) for further information and in CMPT Connections (Winter 2005 Volume 9 Number 4) on reporting cellular components on Gram smears.

ONLINE REPORTING

When entering results using the online forms participants MUST use the forms assigned to the specific challenge.

CMPT frequently receives results reported on the wrong report forms. This is not acceptable as it is considered a transcription error and will be graded as zero.

Participants are encouraged to call CMPT, if they are unsure as to where to report results. Alternatively, hard copies can be submitted instead.

There have been situations where two organisms have been isolated from one specimen or two different strains of the same organism have been isolated. If this is the case, and there is only one available report form specified, please report all results for that challenge by hard copy (fax).

If more information is to be added, please use the "Comments" section of the report or print out the appropriate form from the website, complete and fax or email the report form to CMPT.

Gram Staining in the Smaller Laboratory

Michael A. Noble MD FRCPC, Chair, Clinical Microbiology Proficiency Testing program, Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver BC

Gram stains can provide information including possible or probable identification of microorganisms, tissue load of microorganisms, likely host response, clues that can influence empiric antimicrobial selection.

Rapid Gram stain service can provide valuable early access to information for a variety of clinical conditions commonly seen in physician offices, emergency departments, wards, and operating rooms.

In some situations, the information may provide insights into diagnosis, for example, by allowing for the differentiation between infectious and inflammatory arthritis, documentation of bacterial meningitis, or early diagnosis of pneumonia. This information may be particularly valuable for facilities in isolated communities.

In the past, there was a general belief that preparing, reading, and interpreting Gram stains were skills that once attained, were retained forever. This would be wonderful if only it were true.

Each year, a compilation of CMPT's Gram stain challenges reveals the number of samples that laboratorians have had difficulties staining, reading or interpreting (Figure 1).

Reading of Gram stains is a skill that requires training and regular competency assessment. In small laboratories where the number of samples analyzed is infrequent or rare the use of quality control or quality assessment slides can be beneficial.

The problems associated with inadequate Gram stain reporting are listed below.

From the experience of CMPT, the most common errors appear to be:

1. Incorrect reporting of host cellular components
2. Poor slide handling
3. Slide contamination
4. Incorrect interpretation of microbial forms found on the slide.

Unfortunately, the decision to provide urgent Gram stain analysis in laboratories with limited resources is an economic reality found across Canada and indeed, around the world. In some areas, this situation is the result of consolidation of services, which has left some laboratories providing a very small number of examinations.

A recent survey indicated that some laboratories perform as few as four Gram stain examinations in a year. Arguably, the number is sufficiently low that it would be an unreasonable expectation for laboratories to maintain expertise under these conditions. Although not studied, it is within the likely realms of possibility that some laboratories are not providing a sufficiently reliable and consistent service, which may have a negative impact on patient care.

The following is put forward as recommendations to allow laboratories progress towards a more consistent performance.

1. Ensure that there are enough samples available so that at least one sample is being examined every month.

a) It is unreasonable to expect that staff will retain their expertise at preparing, reading, and interpreting samples if they see slides infrequently at best. Sufficient number of slides can and must be made available at least quarterly if not more frequently. This could be arranged through formal liaison programs with other laboratories or through proficiency testing programs such as Clinical Microbiology Proficiency Testing (CMPT).

b) The slides should address both microbial and host materials in a clinical context where some slides, by design, have certain elements missing. Negative information from Gram staining is as clinically valuable as positive information.

c) Laboratories with poor performance need to be offered refresher programs to ensure they meet better performance parameters.

2. Ensure that more than one sufficiently experienced person is always available.

a) Small laboratories have in the past commonly identified single individuals as subject specialist so that when a difficult situation arises, there is a "go-to" person. Unfortunately, that person is not always available because of days off, illness, or vacation. If a laboratory recognizes the value of providing on-site microscopic service, ensuring that a capable and competent person is always reasonably available, then at least two people will need to be trained and ready.

3. Ensure that the microscope is in its best condition possible.

a) Reducing services through centralization may be necessary, however, this does not mean that laboratories outside the central area should have sub-optimal equipment. While microscopes can be relatively expensive instruments, their working life can be expected to be very long.

b) Small laboratories, especially those in remote areas, have a greater need for a precision microscope because they have more limited backup than larger laboratories. Microscopes should be equipped with lenses that make reading of samples as rigorous as possible. Ideally, they should be equipped with eye-pieces for wide fields and with objectives with the least spherical (plan) and color (achromatic or apochromatic) distortion.

c) Microscopes should be in environments that take ergonomic factors into consideration. Ideally they should be on low, vibration-free benches so that the reader can sit comfortably with his/her feet on the ground. Eyepieces and units should be adjustable to the reader, rather than making the reader conform to a fixed configuration. Readers should be able to read with reduced glare and be free of distractions and noise where possible.

4. There should be regular quality and competence assessment.

a) All laboratory personnel understand that having been trained once does not mean being trained and capable forever. It is common place for quick, time-saving measures to get subtly introduced at the price of reduced reliability, which over time, may result in flaws and routine bad habits.

b) Common errors include poor application of sample to slides with insufficient drying time and poor staining, slides that get read too quickly or inadequate numbers of fields read. The solution to this

is ensuring that people regularly participate in competency assessment, including proficiency testing.

c) In this regard, it is insufficient to focus on reading and interpreting photographic images competency alone. At a minimum, full competency should include the process of slide preparation and staining as well.

5. **Ensure that there are readily available, high quality reagents.**

a) The most common problems that plague Gram stain results are poorly maintained staining reagents. Crystal violet is particularly problematic because it tends to separate out crystals. Counterstains, safranin and basic fuchsin become contaminated with forms resembling bacteria or yeast. Many problems occur because the reagents have sat too long or are contaminated by the practice of filling and re-filling staining bottles.

b) Anomalous forms can cause problems even for experienced readers, but they are especially problematic in the low volume laboratory. The easiest way to reduce risk is to purchase staining materials in small amounts, avoid filling and re-filling bottles, and filtering out crystal violet crystals.

c) Unclean “Gram stain” sinks and racks can transfer off contaminants to the slide. A heavily stained sink can also be a hazard to laboratory personnel.

d) Immersion oils reduce the refraction of the light stream between the light source, the condenser, the sample, and the objective lens. Oils designed for that purpose have a specific refraction index (1.515) and other oils should not be used. Immersion oils come in a variety of viscosities. Although high viscosity oils are less likely to make a mess because they do not migrate into the lens, they can trap air bubbles which make microscopy more difficult. For this reason, most laboratories tend to use low viscosity immersion oil.

e) As with staining reagents, filling and re-filling oil immersion bottles increases the risk of contamination. Smaller laboratories should purchase oil in the smallest container and discard when empty.

6. **Ensure that the microscope is always free of dust and oil and that there is always a replacement bulb available for the light source.**

a) Microscopes need to be properly maintained and stored for maximum functioning and durability. Allowing the objective lenses to sit with oil, dust, or with the light source always on gradually decreases its performance. This is critical for instruments that sit for an extended period of time without regular maintenance or use. The laboratories’ regular internal audit process should ensure that unused microscopes are under a dust cover, with the objective lens up, without a slide on the stage, and the light source off.

b) Care of the oil immersion lens requires gently removing the oil after use. This can be done using any lens quality papers. Most facial tissues contain fibres that can scratch the lens and should not be used. If the oil has sat for a long time on/in the lens, cleaning can be done using a lens paper damped with very dilute liquid detergent or with xylene. Storing xylene in the laboratory, however, can be problematic because of its fumes, flammability, and toxicity.

c) Lens cleaners used for cameras generally contain acetone or alcohol, neither of which is particularly useful for oil immersion lenses.

d) A replacement bulb should always be available in case the one in use burns out.

e) The laboratory should consider a preventive maintenance program either yearly or every two years even when a microscope is not used on a regular basis.

7. **Ensure that the microscope is always in optimal illumination configuration.**

a) Microscopes get moved about to adjust for different readers and sometimes they get jostled, too. This can allow for subtle changes in the alignment of the condenser, the iris, and the light source. In the laboratory, where samples are not regularly read, proper realignment should be built into the lab’s routine to ensure that the microscope is in Köhler alignment at the beginning of every reading session.

8. **Develop a reading, interpretation, and reporting style that provides appropriate contextual clinical information without overreaching**.

a) A common error observed in CMPT challenges is the reporting the presence of epithelial cells that are not present or the misidentification of monocytes or lymphocytes as neutrophils. These are errors usually caused by reading cell types on too low a power. This may represent an under appreciation of the value of reporting on cell types. Cells are commonly enumerated and reported as cells per low power field (X10 objective), but their morphology should always be confirmed using a higher power lens (X50 or X100 oil). Laboratories may not readily be able to distinguish lymphocytes from basophils, eosinophils, or monocytes, but all of them can be easily distinguished from neutrophils or epithelial cells. Reporting should routinely avoid the term WBC (white blood cell) and replace it with terms such as PMN or polys or lymphocytes.

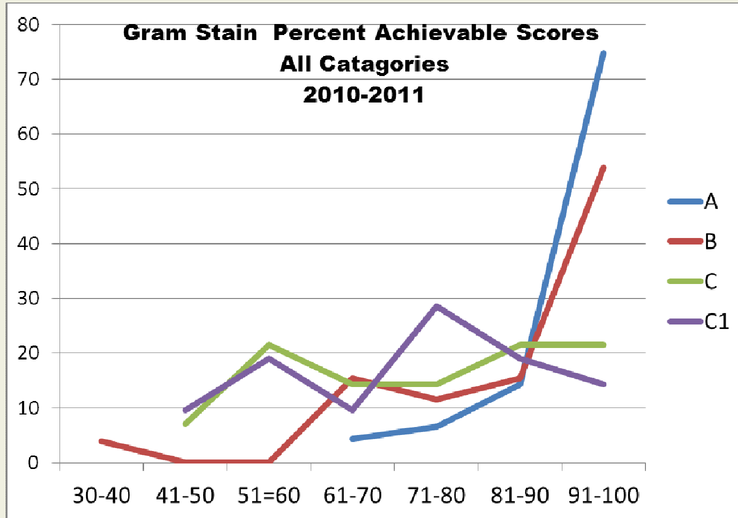
Interpretation and reporting of slides should be done in a manner that provides useful and relevant information. To the extent possible, laboratories that provide clinically relevant information should consider limiting jargon. The reporting of bacteria at the genus level based on morphology may be imperfect, but can be applied reasonably.

Gram positive cocci can, with a reasonable level of confidence, be reported as either gram positive cocci, suggestive of staphylococci or suggestive of streptococci or enterococci. Gram negative bacteria can, with confidence, be reported as suggestive of coliforms, pseudomonads, or *Haemophilus* species. In proper context, reporting of gram negative diplococci suggestive of *Neisseria* species is more valuable than the reporting of gram negative cocci. This is especially true in laboratories where the transport of the sample to another laboratory for culture may take an additional 24 hours.

In summary, Gram stains are still a central component of laboratory diagnosis in infectious diseases. Used as a modern tool, they can function as a rapid biopsy-like examination of host tissues and inflammatory reaction and provide insights into infectious pathogenesis. Some laboratories today are put in a difficult position to provide that service because of limited resources and suboptimal practices.

At some time, remote reading through the use of digital microscopy may be a solution, but in the meantime, laboratory personnel will need to apply more remedial actions to improve their services.

Figure 1: Histogram analysis of performance on Gram stain challenges by CMPT laboratories.



Values across the bottom of the chart are the percent of total achievable scores attainable. Values on the left indicate the amount percent achieved by each of the laboratory categories. Larger laboratories (categories A and B) have their predominance of laboratories achieving between 80-100 percent of their achievable score. Smaller laboratories (categories C and C1) have their predominance of laboratories achieving lesser scores.

Table 1: Factors that adversely affect gram stain performance

Challenges that need to be addressed by all laboratories
Inappropriate sample application and fixation to the slide
Excess thickness
Insufficient air drying time
Excess heat fixation
Stain contamination
Refilling bottles
Environmental contamination
Problematic staining technique
Over decolourization
Under decolourization
Microscope suboptimal to the required task
Insufficient lens quality
Soiled lens
Light path misalignment
Poor reading practices
Reading using more than a single lens?
Insufficient number of fields examined
Insufficient examination of cellular elements
Nuclei
Intracellular bacteria
Insufficient examination of microbial elements
Gram reaction
Bacterial morphology – typical or atypical
Polymicrobial samples – mixed or uniform?
Insufficient reporting results in absence of contextual conditions

Get Connected

Upcoming events

March 2012

22nd European Congress of Clinical Microbiology and Infectious Diseases ECCMID

March 31 - April 3, 2012 London, UK

More information: http://www.congrex.ch/eccmid2012/

May 2012

CACMID – AMMI Canada 2012 Annual Conference (Vancouver)

May 3 - 5, 2012 Vancouver, British Columbia

More information: http://www.cacmid.ca/2011/08/cacmid-ammi-canada-2012-annual-conference-vancouver/

30th Annual Meeting of the European Society for Paediatric Infectious Diseases

May 8 - 12, 2012 Thessaloniki, Greece

More information: http://www2.kenes.com/espid/pages/home.aspx

June 2012

15th International Congress on Infectious Diseases (ICID)

June 13-16, 2012, Bangkok, Thailand

More information: http://www.isid.org/icid/index.shtml

CSM 62nd Annual Conference

June 20 - 23, 2012, Vancouver, British Columbia

More information: http://www.csm-scm.org/english/conf_upcoming.asp

Anaerobe 2012

June 27 - July 1, San Francisco, California

More information: http://www.anaerobe.org/2012/anaerobe2012.html

August 2012

The 30th World Congress of Biomedical Laboratory Science

August 18 - 22, Berlin, Germany

More information: http://www.ifbls-dvta2012.com/

About Connections

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