



## Clinical Parasitology—Quality Control and Quality Assurance: Our Emphasis on Investigating Discrepant Results and Our Blind Quality Control Program

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Quality control and quality assurance are essential elements in any clinical analytical setting and many areas need to be included in quality planning.

It takes a well-trained, experienced eye to pick parasites out of the mix of debris and artifacts that are so abundant in stool specimens. After a thorough training program, the practices of investigating discrepant results, as described below, and our blind quality control give the technologists the immediate feedback that helps develop and hone these skills. The discussion that follows outlines our approach to quality with emphasis on how to investigate discrepancies and utilize a blind quality control program to ensure the reporting of accurate results.

**Methodologies** The Modified Iron Haematoxylin Stain combined with Carbol Fuchsin and Formalin-Ethyl Acetate Sedimentation Concentration technique are used to process an average of 70 specimens per day. Seasonal variation can increase the daily specimen numbers into the nineties or lower them to the mid-forties in low volume periods.

### Quality Control and Quality Assurance Program Elements include:

1. Demonstration of expected reactivity with known organisms for all stains and reagents. Control parasites include: *Dientamoeba fragilis* to check iron haematoxylin staining, *Cryptosporidium* species for the Kinyoun's acid-fast stain, and *Iodamoeba busch-III* for the iodine used with concentrated specimens.
2. Proper storage conditions and regular changing of stains and reagents.
3. Specimen acceptance protocols, for example, (a) un-preserved specimens are rejected (as time and reagents will be wasted on specimens that may not give reliable information), (b) if two specimens are collected from a patient with the same collection date, then one is not processed.
4. Comprehensive atlases and reference text books are available<sup>1,2</sup>.
5. Competency program based on the laboratory procedure manual.
6. Concentrates and stained smears on the same specimen are read by different technologists.
7. Investigation of discrepant results between: (a) concentrate and stained smears on the same specimen, and (b) between two specimens from the same patient.

8. Established criteria for reviewing or reprocessing specimens with: (a) rare organisms where morphology may be atypical and, or (b) a rare organism found on a slide that has been processed adjacent to another that shows a heavy amount of the same organism (to rule out possible contamination).
9. Documentation (reason for repeat and outcome) and monthly review of all repeated stained smears.
10. A blind quality control program that covers all areas of specimen handling from initial processing to reporting results into the laboratory information system (LIS). Documentation includes expected results, actual results, LIS entry errors, and any investigations of discrepant results.
11. Monthly review of the blind QC program for potential concerns which need to be addressed, as well as, demonstration of competency of the analytical personnel.
12. External quality assessment (proficiency testing.)

**Documentation, documentation,  
documentation!**

**Investigating Discrepant Results** Concentrates and stained smears give complementary information and both components are required for a complete ova and parasite examination. If necessary, additional slides are examined before a final report is issued.

Since trophozoites are more often found on stained smears while cysts, oocysts, and ova, will be more abundant in the concentrate, we have established the following protocols to keep the additional work relevant:

1. Between the concentrate and stained smear on the same specimen, check for the presence of pathogenic or non-pathogenic cysts, oocysts, and *Blastocystis hominis*. Investigate all discrepancies with pathogenic organisms regardless of numbers, however, do not investigate non-pathogens if seen in numbers of three or less. Note: *Giardia* cysts may be trapped in the ethyl acetate plug of the concentrate and therefore, may not concentrate well. *Giardia* cysts may occasionally only be observed in the stained smear and not in the concentrate even on re-examination of the concentrated stool.
2. **Helminths** are more likely to be found in the

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concentrate. Even if helminths are found in the stained smear, they are identified and quantified from the concentrated preparation. Stains can distort helminth morphology. Do not re-examine a stained smear for missing helminths.

### 3. Trophozoites

i. re-examine the stained smear if trophozoites are seen in the concentrate but missing from the stained smear. Do not re-examine a concentrate for missing trophozoites.

ii. *Dientamoeba fragilis* is reported only from the stained smear where the typical fragmented nuclei can be properly seen. As the organism is shed cyclically with wide ranging numbers, discrepant results between samples from the same patient are not rechecked.

iii. All **amoebic trophozoites** are identified from the stained smear.

4. **Cryptosporidium oocysts** need to be seen in both the concentrate (for morphology and typical lack of staining with iodine) and the stained smear for acid-fast confirmation. Typically, many more oocysts will be seen in the concentrated specimen than in the stained smear. Acid-fast organisms resembling *Cryptosporidium* found on the stained smear are confirmed by finding the oocysts in the concentrate, where the morphology of coccidian oocysts is often better. Conversely, organisms in the concentrate with typical morphology of *Cryptosporidium* are confirmed by finding the acid-fast stained organisms in the stained smear. The more formed the stool specimen, the more likely artifact material will be present, including yeast which may also stain acid-fast. Yeast and *Cryptosporidium* can be differentiated in the concentrate by the yeast's ability to stain with iodine. *Cryptosporidium* appears clear and bright in contrast to the darker iodine-stained yeast.

### Advantages of investigating discrepant results:

- Ensures correct identification of a parasite.
- Gives the technologist immediate feedback if an organism was missed.
- Builds expertise by providing an opportunity for review of typical or atypical morphology.

### Blind Quality Control Program

1. Sample selection, assessment of results, and investigations of discrepant results is done by bench technologists on a rotation basis. Each sample has accompanying documentation describing the expected result, the actual result, and any follow-up investigations performed.

2. Each sample is divided into two portions, then both are put through our entire system from LIS patient entry, processing, staining, reading, to final result entry. Once the specimens are in the system, they are indistinguishable from routine patient samples.

3. Finalized printed reports are then compared to each other for the recovery of the same organisms and quantification, where appropriate.

4. If discrepancies between results occur, it is the technologist who originally read the concentrate and/or the

stained smear who re-examines the samples.

5. A report is compiled from the Blind QC specimens each month and the results are reviewed with the technologists at a monthly staff meeting and posted as one of the monitored Quality Indicators.

### Advantages of Blind QC Program:

- Systematic review of all the involved processes such as set-up and reporting procedures. It provides an opportunity to learn from and document outcomes for the pre-analytical, analytical, and post-analytical processes of two paired specimens per week (104 pairs or 208 individual specimens per year).
- Monthly review of the previous group of results provides useful information for improvement in areas such as result reporting or the identification of particular organisms. The selection of organisms for the next set of QC samples can come out of these discussions. Also, if recommendations are made as a result of the review, these can be assessed by monitoring subsequent samples.
- The process demonstrates and documents competency with on-going individual feed-back to technologists including:
  1. Finding and identifying parasites.
  2. Reporting results and LIS entry.
  3. Accuracy of recognition, reporting, and consistency of quantification of *Blastocystis hominis* and non-parasitic elements, such as fecal leukocytes.

### References:

1. Garcia, Lynn S. 2001. *Diagnostic Medical Parasitology*. 4<sup>th</sup> ed. American Society for Microbiology, Washington, D.C.
2. CLSI. 2005. Procedures for the Recovery and Identification of Parasites from the Intestinal Tract; Approved Guideline. 2<sup>nd</sup> ed. M28-A2 Vol. 25 No. 16, Pennsylvania, Pa.

[Ed. Notes: 1. **Tara Bonham** is a member of the **CMPT Enteric Parasitology Committee**.

2. Internal and external monitoring of practices are both valuable, especially when laboratories don't defeat the advantages of monitor-samples, regardless of source, by giving them special treatment. (CMPT Connections 'on-line' Summer 2005 9:2 CMPT Satisfaction Survey 2005 - Part 2: CMPT Materials.)

3. The 5th edition of Garcia's *Diagnostic Medical Parasitology* was published November 2006 by ASM Press.]