Am. J. Trop. Med. Hyg., 62(2)S, 2000, pp. 14–16

QUALITY CONTROL FOR PARASITOLOGIC DATA

B. L. CLINE, M. HABIB, F. GAMIL, F. ABDEL-AZIZ, AND M. D. LITTLE

Qalyub Center for Field and Applied Research, Qalyub Governorate, Ministry of Health, Egypt; Center for International Community-Based Studies, Department of Tropical Medicine, Tulane University, New Orleans, Louisiana

Abstract. Accuracy of data is of paramount concern for all research. The task of providing objective assurances of accuracy of parasitologic data for a large, multi-center epidemiologic research project in Egypt (Epidemiology 1, 2, 3 [EPI 1, 2, 3]) presented a unique set of challenges undertaken jointly by the Ministry of Health's Qalyub Center for Field and Applied Research with technical assistance from Tulane University (New Orleans, LA). The EPI 1, 2, 3 project was part of large bilateral research program, the Schistosomiasis Research Project, undertaken jointly by the governments of Egypt and the United States. This paper describes the nature of the quality control system developed to accomplish this task, presents results and discusses the findings.

The number of *Schistosoma mansoni* and *S. haematobium* eggs found in stool and urine specimens is influenced by many factors in addition to the actual number of eggs released into the intestine or bladder. One can predict in a reasonable manner the degree to which biologic, methodologic, and observer variations contribute to variation in egg counts. Unlike quality control (QC) of tests such as blood chemistries determined by an autoanalyzer, QC for identifying and quantifying parasite eggs presents special problems. First, it is exceedingly difficult to blindly introduce known standards (of eggs in stool or urine specimens). Second, inter-observer variation must be considered because several technicians may be used in each collaborating laboratory. Third, significant variation may occur between aliquots of the same specimen. Fourth, variation is introduced by the manner in which slides are prepared for examination. Finally, slides deteriorate over time (rapidly in a dry climate), affecting egg counts from slides re-examined after prolonged storage/transportation to the reference laboratory.

The QC system we developed considered these factors and also attempted to be practical, logistically feasible, and acceptable to principal investigators, laboratory directors, and project managers alike.

METHODS

Standardization of techniques. A modified Kato-Katz thick smear technique[1] was selected for fecal examination and Nuclepore (Pleasanton, CA) filtration for examination of urine.[2] A Tulane University parasitologist (MDL) developed detailed technical instructions and illustrations to facilitate training and to maximize standardization, and conducted the initial training. Each set of instructions listed materials required, described stepwise procedures, and identified potential sources of error to avoid. After translation into Arabic, these materials were used as the basis for training sessions for all of the technicians in the collaborating laboratories supporting Epidemiology 1, 2, 3 (EPI 1, 2, 3) project activities in Egypt carried out by 7 teams in 9 Governorates. The training was held at the Ministry of Health's Qalyub Center for Field and Applied Research (CFAR) reference laboratory in Warraq, and the parasitologist who conducted the training visited most of the laboratories to observe the use of the standardized techniques, and to suggest measures to insure consistency in their use. The CFAR laboratory, in addition to serving as the reference laboratory, also performed the routine parasitologic work for the Qalyub component of EPI 1, 2, 3. Tulane University served as the reference laboratory for the CFAR parasitology laboratory.

Initial QC activities. Before a routine, operational QC system was established, 2 initial activities were undertaken to assess variation within and between laboratories: 1) examination of a set of 252 slides by each technician in each laboratory, and 2) distribution of 5 preserved stool specimens to each of the collaborating laboratories. In the CFAR reference laboratory, 5 fecal specimens (4 positive and 1 negative) were selected for preservation and distribution to each project laboratory and to the Tulane reference laboratory. For each of the 5 specimens, 100 mg of sodium azide[3] were mixed thoroughly with 50 grams of feces. Sets of 3 aliquots were prepared by placing approximately 2 grams of preserved feces into capped 4-ml autoanalyzer tubes. Each laboratory performed egg counts on 3 aliquots from the 5 specimens for a total of 15 egg counts.

Operational QC system. Agreement was reached among involved parties on a ''Manual for Quality Control of Parasitologic Aspects of EPI 1, 2, 3''. The manual, which provided detailed instructions to each laboratory director, included 2 components, internal and external quality control measures.

Internal QC. Using a random method, each laboratory director selected 2% of all slides for re-examination by a second (senior) technician. In addition, the laboratory director selected each month a minimum of 10 test slides that were examined blindly by each technician in the laboratory. At least 4 of these 10 slides were negative. Explicit criteria were established regarding the allowable degree of variability between test and reference egg counts: 1) for egg counts 10 or less, 2 eggs, and 2) for egg count more than 10, a 20% difference. When variability was detected beyond this level, the laboratory director had the option to arrange for appropriate review or retraining for a technician. Permanent records of the internal QC data were retained by the principal investigator.

External QC. Using a random method, 10% of the slides examined during the previous month were selected by each laboratory director and delivered to the CFAR reference laboratory. The CFAR was obligated to examine, within 10 working days of receiving the specimens, 10% (of the 10%) using a systematic sampling method. This yielded an effective 1% examination of all specimens by the CFAR reference laboratory. The CFAR then provided the results to the Schis-

TABLE 1

Results of preserved specimens for quality control: mean* *Schistosoma mansoni* egg counts, by laboratory and specimen

Specimen	Tulane	CFAR†	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
1	18	34	16	24	13	8	24	17
2	19	14	14	18	7	6	21	37
3	2	5	6	3	3	4	3	8
4	3	2	7	3	4	2	4	3
5	0	0	3	0	0	0	0	0

* Based on 3 aliquots of feces examined by the Kato-Katz thick smear (50 mg) technique expressed as the arithmetic mean egg count per slide.
† CFAR = Qalyub Center for Field and Applied Research.

TABLE 2

Percent discrepancy* between project laboratories and the CFAR† reference laboratory, by laboratory and specimen type

Laboratory	Urine	Stool
1	2.5	0
2	0	0
3	0	12
4	0	9
5	0	0
6	1	0

* For egg counts 10 or less, 5 eggs or more, for egg counts 10 or more, 50% or more.
† CFAR = Qalyub Center for Field and Applied Research.

tosomiasis Research Project (SRP) secretariat, who was responsible for comparing test and reference egg counts, and for communicating the results to the respective principle investigator. In cases of variation exceeding the accepted level, the SRP secretariat was responsible for discussing and resolving concerns with the principle investigator.

Because of logistical difficulties, many of the external QC slides were delayed as much as 8–10 months in reaching CFAR. Due to deterioration of these slides, variation was far greater than the criteria used for internal QC. For this reason, the criteria for an acceptable degree of variation for the external QC data were 1) for egg counts of 10 or less, 5 eggs and 2) for egg counts of more than 10, a 50% difference.

RESULTS

As noted, all project technicians received training at CFAR supervised by the consultant parasitologist using the manual specifically developed for training and standardization. Similar training was subsequently provided for newly employed technicians by the laboratory director. The training was well received because many technicians were not familiar with these techniques. The detailed instruction manual was found to be extremely helpful to most trainees.

The 252 slide set exchange exercise proved useful in initially identifying the laboratories that required some degree of reinforcing the standardization of parasitologic techniques. The results of the preserved specimen exercise showed good comparability even in the early pilot phase of the project (Table 1).

The internal QC system, after being instituted, led to improved performance of technicians in the opinion of most of the laboratory directors. In one laboratory, the internal QC revealed that one technician had substandard results due to serious visual problems, and he was assigned to different tasks. One measure of success of the external QC system was the degree to which project laboratories provided the requisite slides on schedule. Five of the 7 laboratories did so half or more of the time, while 2 laboratories did quite poorly in this regard. More important, however, is the degree of comparability between the project laboratories and CFAR reference laboratory. These results were quite good, as summarized in Table 2.

DISCUSSION

While many of the parasitology technicians had years of experience, few were familiar with the techniques used. Training (or retraining) was thus a critical first step towards assuring quality control. The training, based upon detailed, step-by-step, written procedures, helped create and maintain inter-laboratory standardization. Some of the laboratories instituted an explicit reward system for technicians based upon performance in the internal QC. This additional motivation appeared to improve performance and consistency.

The results of the preserved specimen exercise was reassuring in that the inter-laboratory variation was not great even in the early pilot phase of the project. Two laboratories had relatively low counts for 2 specimens, a degree of undercounting that suggested the need for further training and supervision. Distribution of preserved specimens has the advantage of including variation introduced by the preparation of slides as well as the actual examination and counting eggs on a slide. We believe this approach can be effectively used periodically throughout the life of a project, since its usefulness is not limited to initial evaluation.

The level of agreement in the external QC shown in Table 2 is, in our opinion, quite acceptable. For reasons noted above, even under ideal circumstances within the same laboratory, and with the same technician, egg counts vary.

Useful lessons learned from this approach to quality assurance are several. First, it must be viewed as a means to assist the investigators and technicians, not to threaten them. Second, the psychology operates at 2 levels: individual and collective. The individual technician is reminded of the importance of high professional standards, and the group of technicians collective accuracy is stimulated vis-a-vis the other laboratories. Third, the constant reminder of the need for high-quality work may be more important than the QC monitoring *per se* because, as previously noted, practical and theoretical considerations limit the precision in parasitology laboratories. If the demands upon the principle investigator and technicians are excessive, or viewed as unreasonable from their perspective, the system will not succeed.

In summary, the quality of the EPI 1, 2, 3 parasitology was satisfactory. Independent, objective quality assurance such as we used is essential to the credibility of any large scale, multi-laboratory field research project, and we believe the model described herein can be adapted to similar projects.

Acknowledgments: We express an immense debt of gratitude to Dr. Pat Carney for his dedicated and selfless contributions to this effort. We also thank the principal investigators, laboratory directors, and laboratory technicians who participated in such a positive manner. Additional special thanks are directed to the workers in the CFAR

reference laboratory, who took so seriously their important responsibility.

Authors' addresses: B. L. Cline, PO Box 1477, Blanco, TX 78606. M. D. Little, Department of Tropical Medicine, Tulane University, New Orleans, LA 70112. M. Habib, F. Abdel Aziz, and F. Gamil, Qalyub Center for Field and Applied Research, Qalyub Governorate, Ministry of Health, Egypt.

Reprint requests: Schistosomiasis Research Project, Medical Services Corporation International, 1716 Wilson Boulevard, Arlington, VA 22209.

REFERENCES

1. WHO, 1991. *Basic Laboratory Methods in Medical Parasitology*. Geneva: World Health Organization.
2. Peters PA, Mahmoud AAF, Warren KS, Ouma JH, Arap Siongok TK, 1976. Field studies of a rapid accurate means of quantifying *Schistosoma hematobium* eggs in urine samples. *Bull World Health Organ 54:* 159–161.
3. Ratard RC, Kouemeni LE, Ndamkou CN, 1990. Distribution and preservation of *Schistosoma mansoni* eggs in stools. *J Trop Med Hyg 93:* 413–416.