QUALITY CONTROL FOR PARASITOLOGIC DATA

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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 technique1 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 Warqaq, 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 azide3 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, 10% 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-
fully trained employees of the laboratory. Initial training was performed by the laboratory director at the project site in most instances. The laboratory director was provided with an outstanding training manual specifically developed for training and standardization of laboratory procedures. The laboratory director was also provided with a written, step-by-step checklist for preparing slides. 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 investigator. 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.
reference laboratory, who took so seriously their important responsibility.

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