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국내 검사실에서의 대변 검경 검사에 대한 내부정도관리 현황

Internal Quality Assurance Status of Stool Examination as Assessed by a Questionnaire in Korean Clinical Laboratories

권용준 · 원은정 · 기승정 · 김수현 · 신명근 · 신종희 · 서순팔
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This study aimed to survey the status of quality control (QC) assurance for stool examinations at clinical laboratories in Korea. We sent a questionnaire related to QC practices in stool examination by electronic mail to Korean clinical laboratories that performed stool examination. Overall, 20 of the 39 laboratories (51.3%) reported performing stool concentration methods, and 28 (71.8%) examined the slides using only saline. A large proportion (74.4%) of respondents did not check the internal QC because of the restriction of appropriate control materials. Only four laboratories (10.3%) checked the reactivity of the dye solution routinely. For appropriate external QC systems, QC slides (43.6%) were preferred, followed by QC materials (30.8%), virtual slides (17.9%), and a combination of the above options (7.7%). The most commonly observed parasites in stool samples at the clinical laboratories were *Clonorchis sinensis* (75%), followed by *Endolimax nana, Enterobius vermicularis*, and *Entamoeba coli*. The present study describes the difficulties in internal QC assessment due to the absence of standardized QC materials and systems. We hope the findings of this report will provide a foundation for a QC assessment program for stool examinations in the near future.

Key Words: Stool examination, Quality control, Survey

INTRODUCTION

Diarrheal disease is a worldwide problem causing significant morbidity and mortality, especially in developing countries [1]. It is common practice to request stool specimens for culture and/or parasitological examination in patients with diarrhea. Although it is common to think that parasitic diseases only occur in tropical regions, most of the intestinal infections occur in temperate regions of the world [1]. In addition to common parasitic organisms, laboratories should identify some of the less common intestinal parasites often observed in individuals that have traveled abroad. In general, the diagnosis of parasites depends on microscopic identification; thus, it is crucial to maintain the inspection ability of each laboratory. Therefore, exact identification of intestinal parasites should be based on the quality control (QC) of microscopic examinations. The Clinical and Laboratory Standards Institute (CLSI) has established guidelines for stool examination regarding collection, processing, and examination [2]. However, data on the status of QC systems in clinical laboratories performing stool examinations are not sufficient [3]. Thus, this study assessed the status of QC systems in Korean clinical laboratories performing stool examinations.

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METHODS

The study cohort consisted of clinical laboratories that perform stool examinations for parasitic infections within medical institutions (hospitals and medical centers) and in referral clinical laboratories accredited by the Korean Laboratory Accreditation Program [4]. A brief questionnaire was sent by electronic mail to the directors and clinical pathologists in charge of the laboratories in order to survey the clinical laboratory practices related to stool examinations. The questionnaire included the use/method of stool concentration, use of additional stains, reactivity testing of the dye solution, and internal and external QC systems.

RESULTS AND DISCUSSION

In total, 39 clinical laboratories (24.2%, 39/161) responded to the survey. Most of the responses were from laboratories in medical institutions with 500–1,000 beds (53.8%, 21/39), followed by seven institutions with less than 200 beds (including three referral medical laboratories), seven institutions with 200–500 beds, and four institutions with greater than 1,000 beds (Table 1). Fecal concentration is recommended to increase the chance of detecting parasitic ova, cysts, and larvae, particularly in specimens where they are present in insufficient numbers to be seen using direct microscopy [5]. Although more than a half of the laboratories (51.3%) performed stool concentration using formalin-ether or Tween 80, a third of the laboratories (30.8%) performed direct smears only. As the prevalence of intestinal parasitic infections in Korea decreases, fecal concentration should be used to increase sensitivity. Notably, more than 70% of responders (28 institutions) did not utilize additional stains, and there were no cases diagnosed as *Cryptosporidium parvum* by stool examination during a 1-year period. It is difficult to identify cysts or trophozoites without the aid of special stains or molecular modalities, especially for protozoa such as *Cryptosporidium* species [6-8]. The diagnosis rate for protozoa may be underestimated because most laboratories typically perform only wet-mount preparations without additional special staining [9].

The current study showed that the majority of laboratories (74.4%) did not perform internal QC testing for stool examinations. When asked why positive and negative control materials were not included before testing the patient samples, 75.9% of the laboratories indicated it was difficult to secure adequate positive and negative control materials. According to CLSI guidelines [2], stool samples used for QC can be fixed stool specimens that contain protozoa or polyvinyl alcohol (PVA)-preserved negative stool samples to which buffy coat cells have been added. The CLSI recommends that a QC slide should be included in each run of stained slides; however, this step is not mandatory, and the exact QC assessment can be adjusted at the laboratory's discretion [2]. A few laboratories (10.3%) checked the reactivity of the dye solution at least once every month. We found a gap between the laboratory protocols and the CLSI recommendation that fixative should be checked weekly or when using a new lot number [2]. Only three laboratories compared the reactivity of the staining reagent lot by lot and included positive control materials. A QC smear prepared with a PVA-preserved stool or buffy coat cells should be used when a new stain is prepared or at least once every month according to CLSI guidelines [2]. For external QC systems, the use of a QC slide (43.6%) was preferred, followed by QC materials (30.8%), virtual slides (17.9%), and a combination of the above options (7.7%). Generally, for external quality assessment programs, manufactured stool materials or slides have been used [10]. Liebman et al. suggested that pooling pairs of stool specimens for microscopy is likely to be more cost effective than commercial QC slides [10]. In order to obtain an adequate supply of pooling materials representing common and educationally important parasites, however, it might be necessary to survey endemic regions of parasitic disease around the world in addition to domestic multicenters. Moreover, the recently introduced Web Microscope for Parasitology could be an alternative tool [11].

In this study, 74.4% of respondents diagnosed protozoan infections without the aid of a special stain; however, 90.6% of respondents stated that special stains were necessary for the diagnosis of a protozoan infection. Furthermore, 81.1% of respondents indicated that additional enzyme-linked immunosorbent assays (ELISAs) could be necessary for the diagnoses of *C. parvum, Giardia lamblia*, and *Entamoeba histolytica* infections. These protozoan infections are monitored by the government, as they are pathogens relevant to public health. Over the last few years, several alternative diagnostic methods such as direct immunofluorescence staining or ELISAs have been developed and commercialized [12]. Previous researchers found that ELISAs were superior to conventional parasitological microscopy for the detection of protozoa, and they suggested that ELISAs should be used more routinely for

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Table 1. Current status of quality control systems for stool examination in Korea

Items	No. (%)
No. of respondents	39
No. of beds in the health care institute	
> 1,000	4 (10.3)
500–1,000	21 (53.8)
200–500	7 (17.9)
< 200	7 (17.9)
Stool examination method	
Concentration using formalin-ether or Tween 80	20 (51.3)
Direct smear only	12 (30.8)
Cellophane thick smear only	4 (10.3)
Direct smear and cellophane thick smear	3 (7.7)
Stain used for stool examinations	
Unnecessary (in the case of cellophane thick smear only)	4 (10.3)
Saline only	28 (71.8)
Iodine or trichrome stain	7 (17.9)
Internal quality control system	
Test patient samples without checking positive/negative controls	29 (74.4)
Test patient samples after examining negative and positive controls	7 (17.9)
Test patient samples after examining the positive control	3 (7.7)
Patient samples are tested without control materials due to:	
Difficulty in obtaining adequate positive/negative control materials	22 (75.9)
Lack of necessity of internal quality control materials for stool examinations	4 (13.8)
No answer	3 (10.3)
Reactivity check of the dye solution	
Do not check	35 (89.7)
Mix stool and fixation solution	4 (10.3)
Inspection cycle for the reactivity check of the dye solution	
Do not check/no response	35 (89.7)
At every test	2 (5.1)
At least once per month	1 (2.6)
At least once per week	1 (2.6)
Management of the dye solution	
Lot-to-lot variation check, Yes	3 (7.7)
Use of a positive control, Yes	3 (7.7)
Preference of external quality control system for stool examination	
Quality control slides for stool examination	17 (43.6)
Positive/negative control materials	12 (30.8)
Virtual slide photo	7 (17.9)
Other (combination of the above options)	3 (7.7)
Diagnostic methods used for protozoa	
Direct smear, cellophane thick smear or formalin-ether concentration without special stains	29 (74.4)
Special stain after direct smear or formalin-ether concentration	10 (25.6)
Opinion regarding special staining for the diagnosis of protozoan infection	
Special staining should be performed only if a protozoan infection is suspected	20 (62.5)
Special staining should be performed for diarrhea specimens, even if a protozoan infection is not suspected	6 (18.8)
Special staining should be performed for stool examinations in general	3 (9.4)
Special staining is not necessary for the diagnosis of a protozoan infection	3 (9.4)
Opinion regarding the usefulness of enzyme-linked immunosorbent assays for the diagnosis of *Cryptosporidium parvum*, *Giardia lamblia*, and *Entamoeba histolytica* infections	
Useful for more sensitive and economical diagnosis	30 (81.1)
Unnecessary	7 (18.9)

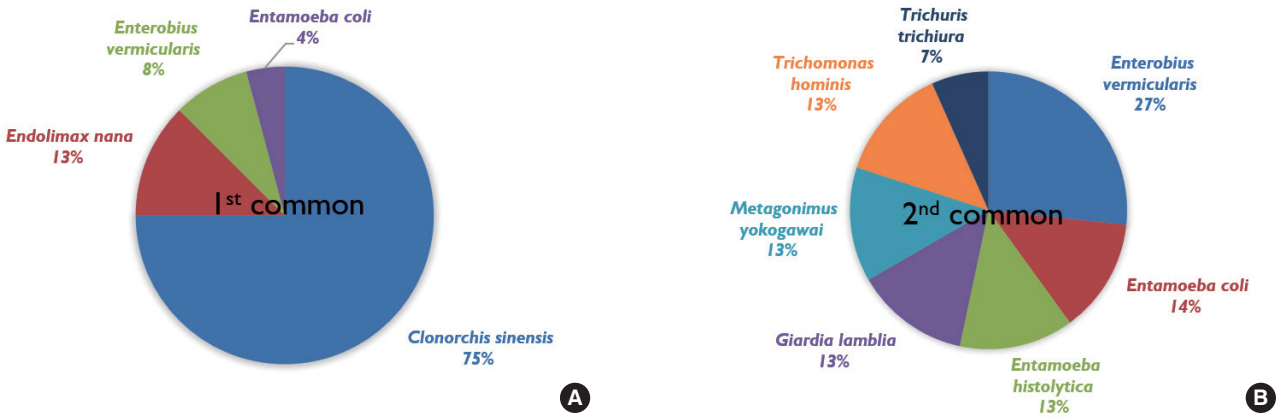

Fig. 1. The most commonly observed (A) and the second-most commonly observed (B) parasites during stool examinations in Korean clinical laboratories participating in this study.

diagnosis [13]. Special tests should be requested for patients who are suspected of having giardia, ameba, or cryptosporidium infections instead of conventional microscopic examinations for ova and parasites. An expanded parasite-screening repertoire that allows for more patient-specific options should be provided to clinicians. Although there are no available ELISA kits authorized by the Korea Centers for Disease Control and Prevention yet, for testing for protozoa, the introduction of ELISAs in the future could be useful to meet expanding clinical demands [13].

The current study demonstrates that *Clonorchis sinensis, Enterobius vermicularis*, and *Endolimax nana* are the most frequently observed parasite ova or protozoan cysts in stool samples (Fig. 1). This finding is consistent with a recent nationwide survey that showed large increases in the egg-positive rates of *C. sinensis* [14]. Importantly, the rate of positive stool tests differed markedly according to the laboratory performing the testing (ranging from 0.0% to 6.7%; data not shown). Previously, Manser et al. also demonstrated that variations in the procedures for stool examinations could reduce the recovery of parasites at different stages, particularly if present in small numbers [5]. We suggest that the standardization of stool examinations in regards to the overall methodology and QC is needed.

To our knowledge, this is the first report to assess the current status of QC systems in Korean clinical laboratories performing stool examinations. We have found that many laboratories have inadequate internal QC systems, mostly due to limitations in obtaining appropriate positive control materials. This study highlights that it is crucial to support the development of adequate QC materials for the establishment of a QC system in the field of stool examination.

요 약

본 연구의 목적은 국내 임상 검사실에서 대변 검사의 내부정도관리 현황을 파악하기 위한 것이다. 대변 검경 검사를 시행하고 있는 국내 임상 검사실을 대상으로 하여 대변 검사의 정도관리 수행에 관한 전자우편 설문을 시행하였다. 설문에 응답한 총 39개 기관 중 20개 기관(51.3%)에서 대변 농축법을 통한 검사를 수행한다고 답변하였으며, 28개 기관(71.8%)에서 생리식염수법을 이용한 슬라이드 검경만 하고 있다고 답변하였다. 응답한 기관 중 대부분(74.4%)이 적절한 정도관리 물질을 확보하기 어려워 내부정도관리를 시행하지 못하고 있다고 응답하였다. 오직 4개 기관(10.3%)이 정기적으로 염색약의 반응도를 점검하고 있었다. 적절한 외부정도관리법으로 선호하는 방법으로는 정도관리 슬라이드의 배포(43.6%)가 가장 많았고, 다음으로 정도관리 물질 자체의 배포(30.8%)나 가상 슬라이드(17.9%), 또는 이들의 조합(7.7%) 순이었다. 국내 검사실에서 대변 검경 시 흔하게 관찰되는 기생충은 간흡충(75%), 왜소아메바, 요충, 대장아메바 순이었다. 본 연구를 통해 국내 검사실에서 대변 검경 검사의 내부정도관리가 어려운 것은 표준화된 정도관리 물질과 체계의 부재에서 기인함을 알 수 있었다. 본 연구 결과가 향후 대변 검경 검사의 적절한 정도관리 체계의 구축에 기반이 되리라 기대한다.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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