original article

Application of digital slide scanning in external quality assessment program on intestinal parasites

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BACKGROUND: Intestinal parasitic infections remain prevalent in Viet Nam. Therefore, ensuring quality assurance in intestinal parasite testing is crucial for screening laboratories. The challenges associated with liquid or glass slide samples necessitate the exploration of digital slide applications, which can offer numerous benefits to program suppliers and participants.

OBJECTIVES: Compare the true and concordance rates of digital and glass slides for diagnosis.

DESIGN: Experimental research design.

MATERIALS AND METHODS: In total, 30 medical professionals from 30 hospitals participated in the trial. The sets of slides encompassed a range of densities, including negative and coinfected slides. Seven types of glass slides were selected for scanning and digital slide production.

MAIN OUTCOME MEASURES: The primary outcomes were true and concordance variables. Secondary outcomes included time sample sending and time completion. The digital slides were uploaded to a secure website for participant access while glass slides were sent individually by mail. Data collection involved participants analyzing specimens and reporting their results using a scoring method based on parasite detection and identification accuracy.

SAMPLE SIZE: 210 glass and digital slide-reading results each.

RESULTS: The mean true rate between original and glass slides diagnosis was 97.6% (range 90.0%–100%), and it slightly increased to 98.1% (range 90.0%–100%) when using digital slides. The average concordance diagnosis rate between glass and digital slides was 99.5%. Importantly, there were no differences in the diagnostic results between glass and digital slides. The findings revealed that the use of digital slides reduced the total time required by approximately 1.1 days compared with that of glass slides.

CONCLUSION: Altogether, the application of digital slides in the external quality assessment program for intestinal parasites offers convenience for users through online platforms and saves operational time process.

LIMITATIONS: The small sample size in this experimental study limited the statistical significance of the comparisons.

CONFLICT OF INTEREST: None.

Intestinal parasites are a worldwide health concern, particularly in developing tropical and subtropical regions.[1] Intestinal parasites include intestinal protozoa and intestinal helminths. According to the World Health Organization, the world population the intestinal parasitic infections are mainly the soil-transmitted helminths (geohelminths) *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Ancylostoma duodenale,* and *Necator americanus* (hookworms). Soil-transmitted helminth (STH) infections rank among the most widespread infections globally, with an estimated 1.5 billion infected people, which is about 24% of the world's population. The highest prevalence was reported in sub-Saharan Africa, China, South America, and Asia.[2] Intestinal parasite infections often cause morbidity and mortality, especially in children.[3] This study focused on intestinal helminthiasis. The major risk factors include rural areas, low socioeconomic status, inadequate sanitation, limited clean water, poor personal hygiene, overcrowded living conditions, and limited education.[3]

Several studies in Vietnam report high rates of intestinal parasite infections. In Hoa Binh (2003), 88% of stool samples contained parasite eggs or cysts, including hookworm (52%), *Trichuris trichiura* (50%), and *Ascaris lumbricoides* (45%).[4] In Hanoi (2016), had the highest prevalence of intestinal parasitic infections (30%). Suburban farmers had the highest infection rates (30%), with hookworm at 25% and *Trichuris trichiura* at 5%.[5] According to a survey conducted in 2016 on 6135 primary school students in Vietnam, the overall prevalence of intestinal worm infections in 21 provinces was 6.39%. During 6 years from 2011 to 2016, at the Institute of Malariology, Parasitology, and Entomology, Quy Nhon (Viet Nam), approximately 2.2 million patients underwent testing, and 14 thousand patients were diagnosed with intestinal parasite infections (6.34%).[6]

To date, most intestinal parasite infections in Vietnam have been detected by standard microscopic techniques using optical microscopy; these measures are still considered the gold standard for diagnosis. However, these methods are still manual and require proper technical procedures to be followed during testing, particularly because the test results depend heavily on the experience of the reader. Therefore, quality assurance during intestinal parasite testing is particularly important in screening laboratories.

The Quality Control Center for Medical Laboratory (QCC) has successfully implemented an External Quality Assessment (EQA) program for intestinal parasite testing. However, in recent years, intestinal parasite infections have decreased and become scattered, making it difficult to collect fecal samples containing intestinal parasites. Some parasites are rare and some of the most important clinical parasites cannot be grown artificially or propagated in laboratory animals. Additionally, with the increasing number of participants in the validation of intestinal parasites, the preparation of a large number of external controls in the future may be problematic. There is a risk of samples being lost, broken, or delayed during actual sample submission.

The digital slide-scanning technique creates a high-resolution scanned image of the entire microscope slide, saving costs and eliminating limitations of physical slides.[7-9] In Vietnam, there are no studies on the implementation of digital slides in intestinal parasites external quality assurance currently.

The objective of this study was to compare the true and concordance rates of digital and glass slides in the EQA program for diagnosing intestinal parasite infections. The findings of this study may be applied to intestinal parasite testing with scarce, insufficient, and lacking samples of difficult parasitic agents. Additionally, these findings may support the traditional method of stool sample submission.

MATERIALS AND METHODS

This experimental research design study included participants certified to have undergone medical testing by the Ministry of Health. The participants performing EQA for intestinal parasites were proficient, meaning they had been granted a medical laboratory license by the Ministry of Health and had worked for >3 years. In total, 30 medical professionals from 30 hospitals participated in the trial. The technicians reading the results, who were unfamiliar with the digital slides and had never used them before, were trained on reading the results in at least 10 cases before participating in the study. The participants were laboratories that qualified and agreed to participate with varying levels of experience in capturing the effective landscape of digital slides.

Ensuring universality, the slide set containing intestinal parasites was designed with densities ranging from negative (−) to high positive (+++). The scale was as follows: negative (−) indicated no eggs/larvae per whole slide; (+) represented 1-2 eggs/larvae per whole slide; (++) denoted 3-5 eggs/larvae per whole slide; and high positive (+++) indicated ≥ 6 eggs/larvae per whole slide. Additionally, there was one slide co-infected with two intestinal parasites (**Table 1**).

In total, seven types of glass slides were prepared (**Table 1**); with 30 slides for each type, a total of 210 glass slides were prepared. One slide of each type was

chosen to create a set of seven glass slides with codes P01-01–P01-07 and divided the slides according to the type of helminth infection. After preparation, glass slides may not have been scanned immediately, nor can the slides be used in teaching continuously; therefore, it is necessary to evaluate the stability and quality of the slide production method by ISO standards.

Quality assessment of the prepared samples was based on testing uniformity and stability according to the provisions of ISO GUIDE 35:2006.[10] and ISO 13528:2005.[11] Because the sample prepared was qualitative, the evaluation was focused on the presence of helminth eggs/larvae for uniformity and changes in shape for stability across different time points.

Production of digital slides

A set of seven glass slides containing intestinal parasites was assigned codes P01-01–P01-07 and then scanned to create a set of seven digital slides assigned with codes P02-01–P02-07 (**Table 1**).

This set of seven glass slides was scanned using a Canon E200 microscope and a Nikon DS-Fi3 microscope camera, and the control software used was Nikon's Photodocumentation/Clinical NIS-Elements Package to create seven digital slides with coded file names. Because of the limitations of digital templates, such as very large file sizes need for specialized software for access, and relatively powerful computer configuration requirements, this set of digital templates was then uploaded to the website. The website was specially designed for external testing of standardization centers and secured using the login account of the members, file-viewing feature, and direct result reply.

The P01 glass slide sample set was sent individually to participants in phase 1. The sample set was packed in a paper box with a fixed slide position, and the paper box was sealed, kept in a sealed plastic box, and again sealed with plastic wrapping, accompanied by instructions for the use and implementation of techniques. The samples were then sent through the mail.

After a blinding period of 60 days, the sample set P02, with digital slide templates and codes P02-01–P02-07, was sent to the participants. The digital templates were saved as a file, which was then transferred to the participating laboratory using the website platform. This method presents various advantages in implementation and easy customization and upgrading of functions.

Results were collected from participants according to the form provided by the program. The participants in the pilot program read the digital specimens directly on the website of QCC. After completing the analysis, the laboratory compiled the results in a report form on the website.

The method used to score stool screening results for intestinal parasites was adapted from the United Kingdom National External Quality Assessment Service's scoring system,[12] in agreement with parasite experts at QCC. For screening stool samples for intestinal parasites, the scores varied based on how accurately the diagnostic results identified parasites in the samples. The grades were determined according to the accuracy of these results:

- Detection and identification of each parasite type: 2 points
- Incorrect reporting of unexpected parasite results: −2 points
- Reporting the remaining parasites of each type:

Table 1. Glass and digital slides sets of seven slides of each type arranged in density from negative to high positive.

Sample P01	Type	Sample P02	Type	Result	Density
P01-01	Glass slide	P02-01	Digital slide	*Ancylostoma duodenale/ Necator americanus* egg.	+++
P01-02	Glass slide	P02-02	Digital slide	*Taenia sp* egg.	++
P01-03	Glass slide	P02-03	Digital slide	*Trichuris trichiura egg*	+
P01-04	Glass slide	P02-04	Digital slide	*Ascaris lumbricoides egg* + *Ancylostoma duodenale/ Necator americanus* egg	++ +++
P01-05	Glass slide	P02-05	Digital slide	*Fasciola sp*	++
P01-06	Glass slide	P02-06	Digital slide	Negative	Negative
P01-07	Glass slide	P02-07	Digital slide	*Strongyloides stecoralis* larval stage II	+++

+ denotes positive; ++ denotes ; and+++ denotes high positive

0 points

The primary outcomes were the true and concordance diagnosis rates. "True rate" was defined as reading results (the diagnostic accuracy) between glass and digital slides to the original diagnosis. The original diagnostic results for the seven slides are listed in **Table 1**. The "concordance diagnosis rate" between glass and digital slides was compared.

The laboratory was "true" if they correctly determined the positive or negative status and the number of parasites present in the glass and digital slide samples compared to the original diagnosis. When the laboratory incorrectly read or determined the positive or negative status or numbers of parasites, it was denoted "no true." The "true rate" was defined as the number of laboratories with "true" readings divided by the total number of laboratories participating in the study.

The laboratory was considered to have "concordance" when there was "true" reading in the diagnostic accuracy using the glass and digital slides. Conversely, the laboratory was deemed to have "discordance" if there was a difference in diagnostic accuracy between the two slide types. The "concordance diagnosis rate" was defined as the number of concordant laboratories divided by the total number of laboratories participating in the study.

The secondary outcomes included the time of sample sending and overall time. The sending time of the glass slides was measured as the duration from the time the shipping unit received the parcel to the time the laboratory received the package on the software of the shipping unit. For digital slides, the sending time was determined as the interval between the email notification of the researcher regarding the EQA program and the laboratory log-in time on the web platform to initiate the test. The overall time for both methods was from sending the sample to receiving the report.

All statistical analyses were performed using STATA 14. All statistical analyses were performed at Quality Control Center for Medical Laboratory at Ho Chi Minh City using STATA 14 (StataCorp, College Station, TX, United States). The difference in the concordance percentage was tested using the exact McNemar test, with the statistical significance threshold of $P<.05$. The Mann–Whitney test was performed to find the difference in the time between sample sending to the completion of submission between glass and digital slides.

RESULTS

Diagnostic accuracy of digital and glass slides

The average true rate between the glass slides and the original diagnoses was 97.6% (range: 90.0%–100%). However, this rate was slightly higher (98.1%, range: 90.0%–100%) when digital slides were used. This represents a 0.5% difference in favor of using digital slides (**Table 2**). The average concordance diagnosis rate between the glass and digital slides was 99.5% (**Table 2**).

Evaluation of seven glass and seven digital slides between the results of participants and assigned values for true and non-true revealed that among the seven glass slides, except for samples P*1-03 (three participants) and P*1-05 (two participants), there were no true results with assigned values. The remaining samples exhibited 100% accuracy. Among the seven digital slides, sample P*1-03 (three participants) and sample P*1-05 (one participant) presented "not true" results compared with the assigned value. For the remaining sam-

Table 2. The true and concordance diagnosis rates of participants using the glass and digital slides in the external quality assessment program for intestinal parasites.

	Glass slide	Digital slide	Glass vs. digital slide
	True	True	Concordance
P*-01 (n=30)	30 (100)	30 (100)	30 (100)
P*-02 (n=30)	30 (100)	30 (100)	30 (100)
P*-03 (n=30)	27 (90)	27 (90)	29 (96.7)
P*-04 (n=30)	30 (100)	30 (100)	30 (100)
P*-05 (n=30)	28 (93.3)	29 (96.7)	30 (100)
P*-06 (n=30)	30 (100)	30 (100)	30 (100)
P*-07 (n=30)	30 (100)	30 (100)	30 (100)
Total (n=210)	205 (97.6)	206 (98.1)	209 (99.5)

Numbers in parentheses are percentages.

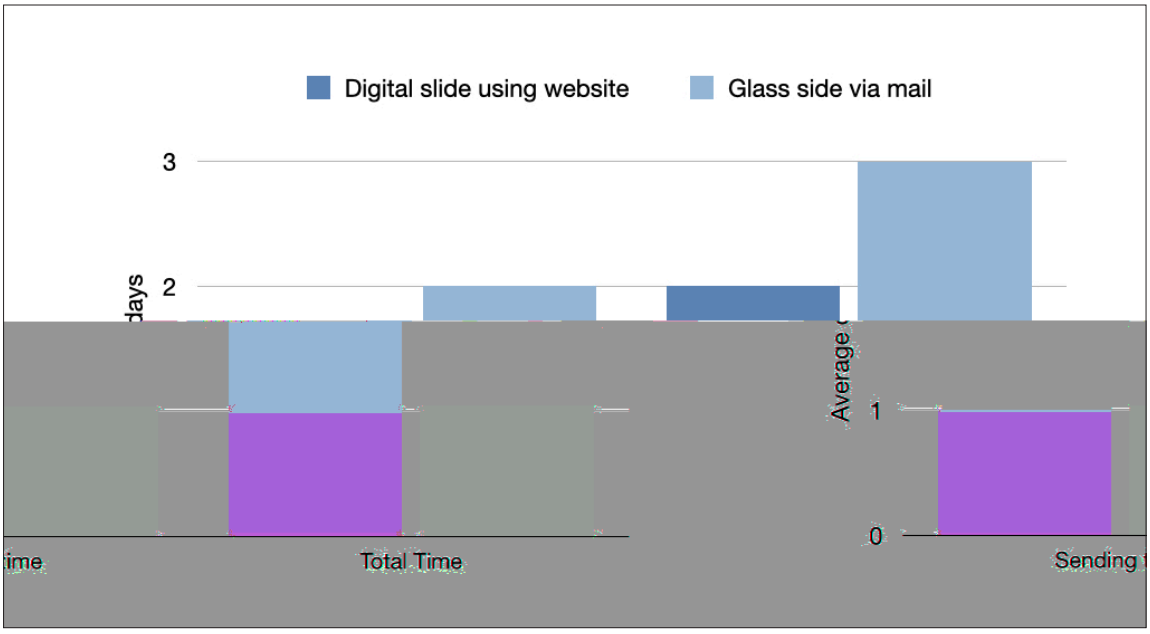

Figure 1. Comparison of the average time from sample sending to completion of submission (days), n=30.

Table 3. Comparison between the glass and digital slide diagnoses.

		Digital slides			*P* value
		True	False	Total	
Glass slides	True	205 (97.6)	0 (0)	205 (97.6)	.317
	False	1 (0.5)	4 (1.9)	5 (2.4)	
	Total	206 (98.1)	4 (1.9)	210 (100)	

Numbers in parentheses are percentages. Digital slides: True if participant's results concordance assigned value. Glass slides: True if participant's results concordance assigned value. Digital slides: False if participant's results discordance assigned value. Glass slides: False if participant's results discordance assigned value.

ple, 100% of the results of participants were consistent with the assigned values in **Table 2**. The sample P*1-03 (one participant) presented discordant results between glass and digital slides (**Table 2**).

Similarity of glass and digital slide-reading results

A comparison of the reading results of glass and digital slides revealed that 97.6% (205) of the readings were correct for both methods, whereas 1.9% (4) were incorrect. No participant correctly read glass slides and incorrectly read digital slides. However, one participant (0.5%) incorrectly read glass slides and correctly read digital slides. There were no significant differences in the diagnostic results between glass and digital slides (**Table 3**).

The convenience because of the digital slides

The median sending time for the participants to receive glass slide samples was 2 (1–3) days (via mail), whereas for digital slide samples, it was <1 day (sent by the website). The use of digital slides contributed to a reduction in sample delivery time (**Figure 1**).The overall median time, from sending the sample to receiving the report, was 3 (2–4) days for glass slides and 2 days for digital slides. The shorter duration of digital slide testing primarily resulted from decreased shipping time. The Mann–Whitney test showed statistically significant differences in the sending and overall time between the glass and digital slides (*P*<.001) (**Figure 1**).

The survey results of the implementation of external testing via website interface were good and stable (66.7%) and considered easy to operate (63.3%). The

Table 4. The ratio levels of ease of use, stability, and quality characteristics during the use of digital slides by participants.

Measuring ease of use in EQA website	
Interfaces very easy to operate	2 (6.7)
Interfaces easy to operate	19 (63.3)
Average	9 (30)
Difficult to operate	0 (0)
Very difficult to operate	0 (0)
Evaluation of "stability" during digital slides zooming	
Excellent	2 (6.7)
Good	20 (67.3)
Neutral	20 (67.3)
Poor/Very poor	0 (0)
Evaluation of digital slides image quality	
Excellent	10 (33.3)
Good	18 (60)
Neutral	2 (6.7)
Poor/Very poor	0 (0)
Total time taken in a slide	
Digital slides have a shorter implementation time	30 (100)
Appropriate time to read one (01) digital specimen	
Less than 10 minutes	17 (56.7)
15 minutes	13 (43.3)
20 minutes	1 (6.7)
≥25 minutes	0 (0)

Numbers in parentheses are percentages. EQA: external quality assessment

image quality of the digital slides was high (60%). The method of using digital slides was convenient, and the execution time was shorter than that by transferring glass slides via mail (**Table 4**). Over 60% of the participants responded that using digital slides on the EQA website was easy to operate, stable when zoomed, and had good image quality.

DISCUSSION

The tropical climate of Viet Nam facilitates the growth and transmission of intestinal helminths. Stool screening tests are widely used in medical facilities because of their simplicity and affordability. However, the accuracy and reliability of testing results are based on the evaluation of the testing participants, including EQA. With an increasing number of participants in the EQA program, the preparation of a large number of samples can be difficult in the future, as sending real samples poses the risks of loss, breakage, or delay. Studies on digital slides for rare samples of intestinal parasites or insufficient EQA sample volume can be a solution to help eliminate these limitations.

Herein, using the characteristics of digital slides, the digital sample set was deployed on the website for concurrent transfer to all participants for evaluation; this method helped in ensuring uniformity. The digital slide testing process was conducted on a website; therefore, all participants evaluated one set of samples to ensure consistency. The use of digital slides presented time-saving advantages for both suppliers and participants regarding the average sample transport time and overall duration of the EQA program. The feedback regarding the website interface, stability, and image quality was consistently positive. Therefore, it is clear that high-resolution digital slides provide better image quality in more detail. Digital slides were easier to access for comparative analysis. The use of digital slides helped the laboratory participating in the study obtain more accurate diagnostic results.

Xue et al compared digital and glass slides to determine human epidermal growth factor receptor 2 (HER2)-low and negative categories in breast cancer. The findings indicated that digital slides might shift HER2-0 to HER2-low, potentially improving access to anti-HER2 antibody-drug conjugates for patients, but further studies are needed on the clinical benefits and biological characteristics.[13]

Accuracy defines the degree of correctness or true values of a given laboratory result compared with that of the gold standard method, which implies freedom of error. Precision is the degree to which a test provides the same measurements over time.[14] The accuracy of our research is recognized by the concordance diagnosis rates of participants between the digital slides and original diagnoses by parasitologists, which were higher than those of the glass slides (99% and 96%, respectively). Applying digital slides and digital image analysis offers enhanced consistency; however, confirmation by a pathologist is essential to ensure accuracy in specific aspects.[15]

The production and application of digital slides in the EQA of intestinal parasites may help control, improve the quality, and ensure the reliability of the laboratory diagnosis. Additionally, the digital slide library on intestinal parasites can be used in education, as a reference, for image analysis. Eliminating transportation-induced incidents of loss and damage, transportation costs, and reducing fees for participating in external quality assessments may encourage more laboratories to participate in external testing, bringing benefits to laboratories, hospitals, and patients.

Farris et al emphasized that digital slides can be shared with collaborators without the risk of loss or breakage. They further emphasized that the most sig-

nificant practical impact of digital slides is facilitating long-term storage and retrieval of images while obviating glass slides on site. Overcoming current barriers in cost, training, software design, and storage will make these digital slides fundamental to precision medicine.[16]

Involving multiple observers in the review process ensures slide image uniformity, thereby eliminating variations that may arise from recuts with different tissue features. With the digital microscopy infrastructure, sharing images or slides with international institutions has become faster, easier, and potentially more cost-effective than shipping fragile glass slides worldwide.[17]

The small sample size in this experimental study limited the statistical significance of the comparisons. With limited resources and experimental research on the type of equipment scan digital specimens, only a Nikon E200 microscope and Nikon DS-Fi3 microscope camera, with control software Nikon's Photodocumentation/ Clinical NIS-Elements Package were used.

The results obtained during the study and production of the digital intestinal parasite test set showed that the process used to produce the test is applicable to the production of samples at a laboratory scale for use in stool examination quality control programs for intestinal parasites.

The findings of this study may provide insights into the better use of digital slides for an EQA program for intestinal parasite testing as a new tool to help optimize time and staffing. Additionally, this study opens up new research directions, allowing assessment approaches in other aspects, such as cost-effectiveness, or directions for other EQA programs that apply digital slides.

In conclusion, the application of digital slides in the EQA program for intestinal parasites revealed results comparable to those of traditional glass slides. Altogether, the use of digital slides not only offers convenience for users through online platforms but also saves operational time in the EQA process.

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Author contributions

Conceptualization: Nguyen Tien Vien and Ngo Quoc Dat; Data curation: Nguyen Tien Vien and Huynh Thi Diem Phuc; Formal analysis: Nguyen Tien Vien, Huynh Thi Diem Phuc, Nguyen Thi Be Phuong and Nguyen Truong Vien; Investigation: Nguyen Tien Vien, Huynh Thi Diem Phuc, Le Van Chuong, Nguyen Thi Be Phuong, Ngo Quoc Dat, Nguyen Truong Vien and Tran Ngoc Dang; Methodology: Nguyen Tien Vien and Le Van Chuong; Validation: Nguyen Tien Vien and Le Van Chuong; Writing – original draft: Nguyen Tien Vien, Huynh Thi Diem Phuc, Nguyen Thi Be Phuong and Nguyen Truong Vien; Writing – review and editing: Tran Ngoc Dang; Supervision: Ngo Quoc Dat.

Ethics approval

This study was approved by the Medical Ethics Committee of the University of Medicine and Pharmacy, Ho Chi Minh City (No. 672/HĐĐĐ).

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