Original Article

Experiences and Factors that Influence Potassium Hydroxide Examination by Microscopists

Sumanas Bunyaratavej¹, Penvadee Pattanaprichakul¹, Sorachai Srisuma²
and Charussri Leeyaphan¹

¹Department of Dermatology, Faculty of Medicine Siriraj Hospital Mahidol University

²Department of Physiology, Faculty of Medicine Siriraj Hospital Mahidol University

ABSTRACT

Background: Potassium hydroxide (KOH) examination is commonly used in dermatological practice. Despite its simplicity, rapidity, and minimal invasiveness, experience in specimen collection, preparation, and interpretation is extremely important. **Aims:** To determine the ability to interpret KOH examination of six microscopists with different levels of experience within the Department of Dermatology. **Methods:** Six volunteer microscopists, who have different experiences in KOH examination in terms of specimens per week (SPW), were assigned to prepare and examine 10 unknown slides of skin scrapings. All participants were then paired into three groups and exchanged the slides set to their partner in each group for a second round of slides interpretation. **Results:** Results of examinations were classified as correct, false negative, false positive, and misinterpretation. The highly experienced microscopists achieved more correct answers than the fairly experienced group in both sessions. There was a significant positive correlation between SPW ($r = 1.0$, Spearman rank, $p = 0.01$) and the correct answers; and a significant negative correlation between SPW and misinterpretation ($r = -1.0$, Spearman rank, $p < 0.01$), exclusively for the second session. **Limitations:** A small number of volunteer microscopists was enrolled in this study. **Conclusions:** Experience in routine slide examination and time spent during examination were significant factors for accurate interpretation of KOH examination. Positive correlation between experience and correct answers, and negative correlation between experience and misinterpretation were particularly observed under limited examination time

Key words : potassium hydroxide, KOH, experience, superficial fungal infection

Introduction

Potassium hydroxide (KOH) examination from the scale of the skin is a simple, rapid, inexpensive, and minimally invasive procedure. Therefore, physicians can use it to promptly prescribe appropriate management that reduces complications or unnecessary costs from misdiagnosis¹⁾.

KOH examination is not only mainly used in dermatological practice, it is also applied in other medical fields, such as obstetrics-gynecology²⁾, ophthalmology^{1,3)} or even for intestinal parasitic

infectious disorders⁴⁾. In dermatology, it is commonly ordered for the confirmatory diagnosis of superficial skin infection at the keratinous layer of epidermis, e.g., dermatophytosis, pityriasis versicolor, and candidiasis^{1,5)}. This technique can also be used to diagnose less common fungal skin infectious diseases, such as tinea nigra, piedra of the hair, etc. Keratin, which is affected by fungal elements, can be easily scraped using scalpel blades. KOH solution is then applied to dissolve keratin from the slides and make hyphae clearly visible under the microscope¹⁾.

Despite its simplicity, false positive and false

Address for correspondence : Charussri Leeyaphan

Department of Dermatology, Faculty of Medicine Siriraj Hospital Mahidol University, 2 Wanglang Road, Bangkoknoi, Bangkok, THAILAND 10700

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E-mail : charussrilee@gmail.com

negative results from KOH examination occur in clinical practice, which may be caused by incorrect sampling at the lesion, or inexperience in interpretation^{1,5)}. Most studies focused on the diagnostic value⁶⁾ and effectiveness of KOH interpretation compared with fungal culture preparation⁷⁻¹⁰⁾. Thus far, no studies have been conducted to clearly determine the effects of technique and experience in KOH preparation and interpretation. This study was designed to examine the ability in KOH preparation and interpretation among microscopists with various levels of experience within the Department of Dermatology, Faculty of Medicine Siriraj Hospital. It was also used as a means to improve routine services of the microscopists in the Department.

Methods

Six microscopists in the Department of Dermatology, as volunteers, were recruited in this study in September 2008. All of them had received sufficient training in the basic technique and interpretation of KOH examination. Each of the microscopists worked under a different division of the Department of Dermatology and was routinely assigned with various job responsibilities in addition to KOH examination. Any of the microscopists is occasionally asked to substitute the regular working staff to perform KOH examination. This resulted in each working microscopist having different levels of experience in KOH examination in terms of average number of specimens per week (SPW).

The 10 unknown slides of scale scrapings from glabrous skin of patients were prepared in one set for each microscopist. The slide of each set was taken from the same group of patients (10 patients) with various dermatological disorders. The clinical and laboratory diagnosis for this group of patients, for positive result of KOH examination, were confirmed as either one of the following superficial fungal skin infections: dermatophytosis, candidiasis, or pityriasis versicolor. For negative KOH findings, the slides were prepared from cases of psoriasis and eczema. Specimens taken from nail scrapings were not included in this study due to more time needed for KOH preparation. All of the skin scrapings were cultured for fungal growth to carry out standard diagnosis.

All six microscopists were assigned to examine

KOH preparation on a set of the same 10 unknown slides labeled and coded number 1 to 10 at randomly different orders for each microscopist. All slides were prepared and coded by the investigator. Each microscopist then interpreted the unknown slides independently without consulting with or receiving suggestions from one another.

At the beginning of the study, the microscopists were randomly paired into three groups, and the study was divided into two sessions. The first session included the steps for slide preparation and interpretation; namely, by using 20% KOH solution, putting the cover slip on the slide, warming the prepared slide briefly by the flame, applying a little pressure to blot away the excess KOH, and allowing the scale to be thinned for further viewing, and interpretation under microscopic examination. In the first session, 10 minutes were allowed for each slide; therefore, total examination time was approximately 100 minutes. The microscopist then swapped the set of slides to his/her partner to begin the second session. Each microscopist then interpreted the unknown coded slides numbered 1-10, which had been already prepared by his/her partner during the first session. It took 3-5 minutes in the second session for interpreting each slide. At the end, each microscopist handed in the sheets with his/her own interpretation for each of the two sessions, as positive or negative results, including the description of the microscopic findings. The interpretation from each microscopist was further evaluated by the investigator, who did not participate in the examination.

A correct interpretation must include both true positive or true negative results. The 10 specimens used consisted of positive results from eight specimens (four dermatophyte, two *Candida*, and two *Malassezia* specimens) and negative results from two specimens. Especially for positive results, descriptive microscopic findings must be additionally interpreted in an accurate fashion. As an example of inaccurate microscopic description, one microscopist described the microscopic findings for dermatophytes (long, branching, straight septal hyphae) instead of *Candida* (budding yeasts and pseudohyphae) for specimens taken from a lesion of candidiasis. This response was considered as a misinterpretation.

This study was approved by the Institutional Review Board of the Faculty of Medicine Siriraj

Table 1. Data for all microscopists and results of KOH interpretation

Microscopists	Working experience (years)	Average number of Specimens per week	First session: 10 minutes per specimen				Second session: 3 to 5 minutes per specimen			
			Correct	False positive	Misinterpretation	False negative	Correct	False positive	Misinterpretation	False negative
A	> 20	< 0.05	6	0	3	1	4	0	5	1
B	> 20	3.0	9	0	1	0	5	0	4	1
C	> 20	5.0	8	0	2	0	7	0	3	0
D	1-2	17.0	8	0	0	2	8	0	2	0
E	< 1	60.0	8	0	2	0	9	0	1	0
F	> 20	250.0	10	0	0	0	10	0	0	0
Mean (SD)			8.17 (1.33)	0	1.33 (1.21)	0.50 (0.84)	7.17 (2.32)	0	2.50 (1.87)	0.33 (0.52)

Hospital, Mahidol University.

Statistical analysis

Data were analyzed using statistical software PASW Statistics 17 for Windows (SPSS Inc., Chicago, IL). Mann-Whitney U test was performed to compare the numbers of correct responses between microscopists with "High" and "Fair" experience. Wilcoxon's Signed-Rank test was used to compare the numbers of correct answers from the slide interpretation between the first and second session in each group of microscopists. Spearman rank correlation was used to determine the relationship between the numbers of correct or misinterpreted answers in each session and the work experience, and between working duration and average number of SPW. A P-value of 0.05 or less was considered to be statistically significant.

Results

Having various assignment responsibilities and working durations within the Department of Dermatology, each working microscopist had different levels of experience in KOH examination, ranging from several specimens every day to only one specimen for a 3-4-month period (Table 1). This study specifically defined the experience level in KOH examination as average number of specimens per week (SPW). Among all the six microscopists, microscopist F had the highest SPW (250). Microscopist F was the only one who was able to provide completely accurate re-

sponses for the entire test in both sessions. It is noteworthy that even though microscopist E had working duration in the Department that is less than that of microscopist C (< 1 vs. > 20 years, respectively), the number of SPW for microscopist E was higher than microscopist C (60.0 vs. 5.0). Their correct answers for slide interpretation, however, were not different in the first session. Microscopist E provided higher scores of correct answers and less misinterpretation than microscopist C during the second session. This study showed that SPW had no correlation with working duration. There was a poor negative correlation between working duration and SPW (Spearman's rank correlation, $r = -0.44$, $p = 0.383$).

This study enabled further classifying the microscopists into two groups according to experience, namely, Fair experience and High experience groups, based on SPW numbers (Table 2). Microscopists A, B, and C (average SPW < 10) were classified as Fair experience group; and Microscopists D, E, and F (average SPW > 10) were classified as High experience group. The average number of correct specimen identification for both sessions and level of experience are shown in Table 2. The average number of correct specimens in both sessions of the High experience group is more than the Fair experience group, but the difference is not statistically significant.

This study compared the average number of correct specimen identification between the first session (KOH preparation and examination) and second session (KOH examination from slides prepared by one's partner) within the same group.

Table 2. Numbers of correct answers in Fair- and High-experience groups

Level of experience	Numbers of correct answers: Mean (SD)		Exact p-value
	First session: 10 minutes	Second session: 3-5 minutes	
Fair experience: Microscopist A, B, C	7.67 (1.53)	5.33 (1.53)	0.250 [@]
High experience: Microscopist D, E, F	8.67 (1.16)	9.00 (1.00)	1.000 [@]
Exact p-value	0.700 [#]	0.100 [#]	

Mann-Whitney U test

@ Wilcoxon's Signed-Rank test

Table 3. Correlation between specimens per week (SPW), correct answers, and misinterpretation

	Spearman rank correlation coefficient, r
SPW, Correct answers in first session (10 minutes)	0.577 (p = 0.231)
SPW, Correct answers in second session (3-5 minutes)	1.000 (p = 0.01)
SPW, misinterpretation in first session	-0.618 (p = 0.191)
SPW, misinterpretation in second session	-1.000 (p < 0.001)

The group with fair experience provided lower number of correct responses in the second session than that in the first session; whereas a high number of correct responses in slide interpretation was observed in the individuals in the High experience group, both in the first and second sessions (Table 2).

This study sought to determine the potential correlation between experience in terms of SPW and the correct/incorrect responses in the individual's tasks assigned in the first and second session (Table 3). This study was able to demonstrate the positive correlation between the correct answers and SPW in the first and second sessions. Notably, this study detected a perfectly positive correlation (correlation coefficient [r] = 1.0) between SPW and correct answers obtained in the second session, in which tasks were assigned to be performed within a shorter period than the first session. This study was also able to demonstrate the negative correlation between SPW and misinterpreted responses, particularly with a perfect negative correlation (r = -1.0) for the second session.

Discussion

This study focused on the interpretation skill of each examiner regarding KOH preparation from cutaneous lesions. As an examiner, each microscopist from the Department of Dermatology had

various levels of experience in KOH examination. Each of them was assigned to interpret findings from scales obtained from patients with various skin disorders. The official reports for the results must include the positive or negative KOH results and the descriptive findings. The analysis in this study showed that the level of working experience (in terms of SPW) was perfectly correlated with correct interpretation and inversely correlated with misinterpretation under limited time allowed for examination.

Laboratory diagnosis of fungal infection is composed of several techniques including fungal culture, Periodic acid-Schiff (PAS) staining, and KOH preparation. KOH preparation is known to be a simple investigation procedure, nevertheless, it requires a significant level of examiner's experience^{1, 11-12}. In order to obtain an accurate result, the procedure must begin with accurate specimen sampling by properly choosing the area of skin lesion, and collecting the appropriate amount of the scale^{1, 5}. Collecting samples from inappropriate areas could lead to negative results. Too much scale in the sample from scraping causes excessive amount of keratin to be dissolved, thus leads to difficulty in interpretation. On the other hand, a small amount of scale provides inadequate specimen for evaluating the fungal elements. The sample collection steps were not tested because they are not the main focus in this study. All specimen samples were already avail-

able on the slides used in the study.

Each of the laboratory microscopists had different working experiences and durations, ranging from less than a year to longer than 20 years. Each microscopist was assigned to rotate work in the fungal laboratory and substitute for other microscopists. Occasionally, microscopists with minimal working experience (microscopist D and E) were assigned to work at the fungal laboratory to practice their laboratory skill on this aspect. Therefore, the experience in KOH examination or SPW depends upon the time availability in the fungal laboratory as well as on the other tasks assigned to the microscopist.

The findings indicated that SPW, not working duration, reflected the level of experience in KOH examination, and SPW had no correlation with working duration. Additionally, this study was able to determine that two factors are involved in the accuracy of examination: SPW and duration of examination (time spent during the first or second session). This study revealed a perfect positive correlation between numbers of SPW and correct answers exclusively in the second session, suggesting that a higher SPW is correlated with a higher number of correct responses in slide interpretation when individuals perform the examination within a limited period. No significant correlation, however, was observed in the first session. Length of time spent for slide examination is also important. The reasons for the different observations in the first and second session could be the difference in time spent for each slide examination (10 vs. 3-5 minutes, respectively). In the highly experienced group, the scores in both sessions were very similar (p -value = 1.00). With limited duration spent for slide examination in the second session, the fairly experienced group was not able to obtain a high number of correct score as the highly experienced group did. These findings can be interpreted to mean that the highly experienced group can accurately perform KOH interpretation even within a brief or limited time period. It can be concluded from this study that slide examination by individuals with low SPW under limited time could lead to false reports, especially by misinterpretation and false negative reporting.

This study did not observe false positive interpretation of results by any of the microscopists in both sessions. This was despite the fact that false positive interpretation of KOH examina-

tion frequently occurs, such as for cloth fibers¹³⁾, mosaic fungi¹⁾ or other artifacts⁵⁾. Only two specimens for negative KOH findings from other skin disorders were included in the set of scale scrapings used. This could have reduced or even prevented the occurrence of false positive interpretation in this study.

This study demonstrated the perfect inverse correlation between SPW and misinterpretation in the second session. One example was the description of short fragmented hyphae with oval yeasts (descriptive findings for pityriasis versicolor, positive KOH finding), instead of branching septate hyphae with or without arthroconidia (descriptive findings for dermatophytes, positive KOH finding). Misinterpretations affect clinical decisions and could lead to misdiagnosis and improper treatment. Therefore, interpretation of KOH findings should not only include determination of positive or negative results, but also the descriptive findings of structures seen under the microscope. With complete descriptive findings from KOH examination, clinicians will be able to correlate results with clinical features for appropriate therapy.

Most of the statistical tests in this study did not reveal significant differences between High- and Fair-experience groups. A main limitation in this study is the low number of the microscopists enrolled. In fact, there are only six working laboratory microscopists in the Department of Dermatology. With this limitation, it is extremely difficult to increase the sample size. One possibility to rectify this issue would be to recruit more microscopists in the Dermatology laboratory from other medical schools or institutes having similar settings as a university or training hospital. This would allow us to perform the study with a larger group of microscopists. It may, however, introduce more confounding factors, such as the different working schedules or rotation of laboratory microscopists among all the institutes.

This study demonstrated the potential factors, such as working experience and optimal time spent on slide examination, and the significance of proper diagnosis through laboratory slide examination. This principle could be further developed to improve the standards of best practice procedures in laboratory examinations, such as the establishment of proper criteria for SPW and optimal time spent on examination, to reduce errors in laboratory practice as well as in

clinical practice. Improvement of basic laboratory skills, especially in performing KOH examination and interpretation of their results, in both physicians and microscopists, can lead to better patient care. Also, this simple diagnostic technique should continue to be passed on to the future generations of health-care personnel¹⁴⁾.

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Conflict of Interest

If there is COI, please append to that effect.

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