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### EVALUATING THE USE OF DEDICATED SWAB FOR RAPID ANTIGEN DETECTION TESTING IN GROUP A STREPTOCOCCAL PHARYNGITIS IN CHILDREN

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#### RUNNING TITLE: RAPID ANTIGEN DETECTION TESTING IN GAS PHARYNGITIS IN CHILDREN

#### ABSTRACT

**Background:** Group A streptococcus (GAS) is the most common and fearful bacterial cause in pediatric acute pharyngitis due to its serious complications. Several generations of rapid antigen detection tests (RADTs) have been developed to facilitate rapid detection of GAS pharyngitis. We assessed the value of using a dedicated swab for RADT rather than using the same swab for throat culture and RADT.

**Methods:** We conducted a prospective, single-center study that included children with suspected GAS pharyngitis. Paired throat swabs were taken simultaneously from each child. We dedicated one swab for RADT (RADT#1) and used the other swab to inoculate blood agar plate surface, and then immediately to process the RADT (RADT#2).

**Results:** The prevalence of GAS pharyngitis among the participants was 28% based on throat culture positive results. The RADT#1 and RADT#2 had sensitivity results of 92.9% and 84.3% respectively. Both RADT#1 and RADT#2 had 96.1% specificity.

**Conclusion:** We found that RADT resulted in a better sensitivity when one swab was dedicated for the test. Therefore, physicians are encouraged to use separate swabs for each diagnostic test when both RADT and throat culture are performed.

**Key words:** GAS, pharyngitis, rapid antigen detection test

### ÉVALUATION DE L'UTILISATION D'UN SWAB DEDICÉ POUR DES ESSAIS RAPIDES DE DÉTECTION D'ANTIGÈNE DANS LE GROUPE A PHARYNGITE STREPTOCOCALE EN ENFANTS

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#### TITRE FONCTIONNEL: TEST DE DETECTION ANTIGENNE RAPIDE DANS LA PHARYNGITE DE GAZ DANS LES ENFANTS

#### ABSTRAIT

**Contexte:** Le streptocoque du groupe A (GAS) est la cause bactérienne la plus fréquente et la plus effrayante de la pharyngite aiguë pédiatrique due à ses graves complications. Plusieurs générations de tests de détection d'antigène rapide (RADT) ont été développés pour faciliter la détection rapide de la pharyngite GAS. Nous avons évalué la valeur de l'utilisation d'un tampon dédié pour RADT plutôt que d'utiliser le même écouvillon pour la culture de la gorge et la RAD

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**Méthodes:** Nous avons mené une étude prospective et à centre unique comprenant des enfants atteints de pharyngite soupçonnée de GAS. Des écouvillons de gorge appariés ont été pris simultanément par chaque enfant. Nous avons dédié un écouvillon pour RADT (RADT # 1) et utilisé l'autre écouvillon pour inoculer la surface de la plaque d'agar de sang, puis immédiatement pour traiter le RADT (RADT # 2).

**Résultats:** La prévalence de la pharyngite GAS chez les participants était de 28% selon les résultats positifs de la culture de la gorge. Le RADT # 1 et le RADT # 2 ont des résultats de sensibilité de 92,9% et 84,3% respectivement. Les deux RADT # 1 et RADT # 2 avaient une spécificité de 96,1%.

**Conclusion:** Nous avons constaté que RADT a permis une meilleure sensibilité lorsqu'un tampon a été dédié au test. Par conséquent, les médecins sont encouragés à utiliser des écouvillons séparés pour chaque test de diagnostic lorsque la RADT et la culture de la gorge sont effectuées.

**Mots clés:** GAS, pharyngite, test rapide de détection d'antigène

## INTRODUCTION

Group A streptococcus [GAS] is considered the most prevalent and important bacteria that causes pediatric acute pharyngitis due to its serious complications (1, 2). It causes a considerable economic burden to the community due to the cost of medical care. Moreover, delay in school progress of children due to the disease is also a matter of concern. In the United States of America, GAS in children costs millions of US dollars per year (3).

The rapid and accurate diagnosis of GAS pharyngitis in children enables an early treatment with proper antibiotics reducing organism transmission in the community along with its complications (4). Furthermore, an accurate diagnosis of GAS pharyngitis allows proper use of antibacterial drugs with subsequent reduction in the potential risk of drug resistance (5,6). The clinical diagnosis of GAS pharyngitis is a challenging task as the symptoms are non-specific, and similar manifestations are also observed in other types of pharyngitis (7). Therefore, clinical scoring systems such as Centor and McIsaac scores were developed to identify the patients with GAS pharyngitis prior to prescribing antibiotics (8). Unfortunately, these clinical diagnostic tools have yielded inaccurate results, therefore performing laboratory tests is a necessity in these cases (9).

The bacterial culture of collected throat swabs using blood agar plates continues to be the gold standard laboratory test for GAS pharyngitis (10). Although throat swab culture allows further investigations such as subtyping and antimicrobial susceptibility testing, it has many limitations for example laboratory infrastructure, costs, and the lag period to obtain the result (24–48 hours) which can delay an effective management (10,11). These limitations could be a problem with low resources, as most of the patients cannot come back for another visit and management (12).

RADTs have been used for detection of GAS pharyngitis since the 1980s (13). Their quick turnaround time enables the diagnosis of GAS

pharyngitis within few minutes and thus helps clinicians decide appropriate management at their healthcare facility. In addition, it is easy to perform in both outpatient clinics and professional laboratory settings (14).

Rapid antigen detection tests have a specificity ranging from 90- 99% which is considered high, however, the sensitivity is variable, ranging from 75% to 95%, compared to the throat culture technique (15). Although RADTs have been used in many societies in America and Europe as clinical practice guidelines, (16,17). their widespread use is limited by their variable sensitivities. Owing to the reported variable sensitivity of RADTs, the international protocols emphasize utilizing throat culture as a supporting method in the case of negative RADTs to avoid missing any positive instance of GAS pharyngitis (10,17,18).

Regardless of the test method, careful sampling from the posterior pharynx and tonsils is essential for the accurate results as per the recommendations of Infectious Diseases Society of America (11). Most of the studies comparing the performance of RADT with throat culture used either a single swab to perform culture and RADT or separate swabs for each diagnostic test. However, none of the previous studies, up to the best of our knowledge, presented a parallel comparison of these two approaches from a single patient.

In the current study, we evaluated the performance of using a dedicated swab for RADTs rather than using the same swab for a throat culture and RADTs.

## MATERIALS AND METHODS

### Study Participants

We included eligible patients from the outpatient clinics of Mansoura University Children Hospital during the period from October 2014 till June 2015. The inclusion criteria were based on the Modified Centor score as clinical manifestations of GAS pharyngitis, including the absence of cough, temperature > 38°C, anterior cervical lymphadenitis and the presence of pharyngeal or tonsillar exudates

(19). We included the participants with Modified Centor score of  $\geq 2$  in our study. The patients who had a tonsillectomy or received antibiotics during the preceding week were excluded. An informed consent was obtained from at least one of the parents or legal guardians before enrollment in the study. Demographic and clinical data were also collected from the participants.

### Study Design

We conducted a prospective, single-center study. Paired swabs, using the sterile swabs provided in RADT kit, were collected simultaneously from each child by rubbing the two swabs together against the back of the throat and tonsillar area (especially the areas of inflammation, ulceration or exudation), while avoiding contact with teeth, tongue, gums, and cheek surfaces. The swabs were placed in dry test tubes and immediately transported to the microbiology laboratory for further processing. We dedicated one swab for RADT (RADT#1) and used the other swab to inoculate blood agar plate surface, and then immediately to process the RADT (RADT#2).

### BinaxNOW® Strep A Card RADT

We used a BinaxNOW® Strep A Card immunochromatographic test (Alere Scarborough, Inc. USA). The sample line in the card is a strip of antibody (anti-strep A), which is coated on a nitrocellulose membrane. The internal control line is formed by the anti-species antibody, which is coated on the same membrane forming the second stripe. The test was executed according to the manufacturer guidelines. The results were read within five minutes and interpreted by the presence or absence of pink to purple colored lines. A positive result indicated the detection of both sample and control lines, while a negative result showed only the control line. BinaxNOW® Strep A Card RADT is readily available in Egypt and it costs as low as 3 US dollars per patient that may be suitable for low and middle-income countries.

### Culture and Identification

Incubation of blood agar plates (Oxoid, UK) was done at 37° C for 24 hrs. If there was no growth visible, re-incubation of the plates for further 24 hrs was done. We identified potential GAS by beta-hemolytic colonial morphology, Gram staining, catalase test and bacitracin test. The SLIDEX® Strepto Plus A latex

agglutination test (bioMérieux SA, France) was used for grouping to confirm GAS identification.

### Statistical Analysis

Both sensitivity and specificity together with predictive values were calculated based on Greenhalgh's formulas (20). The data were presented as numbers and percentages. Categorical variables were compared using the Chi-squared test and are presented as percentages (%). Statistical values were considered significant at a P-value is less than 0.05. All statistical data were analyzed by using version 15.0 of SPSS software package (Chicago, IL, USA).

### RESULTS

We included 250 patients into our study. All the demographic data of the participating subjects are illustrated in Table-1. The prevalence of GAS among participating subjects with pharyngitis was 28% (70/250) based on throat culture which was considered as the gold standard in our study. Cervical lymphadenitis and the presence of pharyngeal or tonsillar exudates were significantly associated with GAS pharyngitis as shown in Table 2.

**TABLE 1: DEMOGRAPHIC FACTORS OF THE STUDY PARTICIPANTS**

Demographic factors	Total patients n = 250 (%)
<b>Gender</b>	
Male	130 (52)
Female	120 (48)
<b>Age</b>	
Age <5 years	109 (43.6)
Age > 5 years	141 (56.4)

TABLE 2: ASSOCIATION OF CLINICAL FINDINGS WITH CONFIRMED GAS PHARYNGITIS

Clinical finding	Children with GAS pharyngitis n = 70 (%)	Children without GAS pharyngitis n = 180 (%)	P value*
Absence of cough	55 (78.6)	150 (83.3)	0.81
Fever (temperature > 38°C)	60 (85.7)	151 (83.9)	0.87
Cervical lymphadenitis	47 (67.1)	34 (18.9)	0.02
Pharyngeal or tonsillar exudates	50 (71.4)	40 (22.2)	0.01

\*P value of the association of clinical findings with confirmed GAS pharyngitis

TABLE 3: PERFORMANCE CHARACTERISTICS OF RADT#1 AND RADT#2 COMPARED WITH THROAT CULTURE

	Culture- positive, assay- positive (n)	Culture- negative, assay- positive (n)	Culture- positive, assay-negative (n)	Culture- negative, assay- negative (n)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RADT#1	65	7	5	173	92.9	96.1	90.3	97.2
RADT#2	59	7	11	173	84.3	96.1	89.4	94

PPV: positive predictive value; NPV: negative predictive value

The performance of RADT#1 and RADT#2, compared with the throat culture, are shown in Table-3. Out of the 70 patients with culture confirmed GAS pharyngitis, RADT#1 and RADT#2 were truly positive in 65 and 59 patients, respectively. The RADT#1 and RADT#2 resulted in 5 and 11 false negative results, respectively. The RADT#1 sensitivity (92.9%) was considerably higher than that of RADT#2 (84.3%) however the difference was found to be statistically not significant. Both RADT#1 and RADT#2 assays had 96.1% specificity. The RADT#1 and RADT#2 had positive predictive values (PPV) of 90.3% and 89.4%, respectively, and negative predictive values (NPV) of 97.2% and 94%, respectively.

## DISCUSSION

Streptococcal pharyngitis has drawn medical attention over the years, particularly because of its potential serious problems such as post-streptococcal autoimmune sequelae. The prevalence of GAS among the study participants with pharyngitis was found to be 28%, which is almost similar to an earlier reported prevalence in Egypt (12). Earlier studies reported that the prevalence of GAS pharyngitis varies from one region to another, reaching up to 41% in some regions. This may be due to the influence of several

regional factors such as the school crowdedness level, basic sanitation, and the efficiency of healthcare systems (12,21-23). Among the clinical manifestations of the disease, tender anterior cervical lymphadenitis and the presence of pharyngeal or tonsillar exudates were significantly associated with GAS pharyngitis, which is consistent with other reports (23,24). Although the sensitivity difference between the RADT#1 and RADT#2 did not reach a statistical significance, the RADT#1 had a considerable better sensitivity than RADT#2 (92.9% versus 84.3%). The false negative results in the case of RADT#2 were more than two-fold higher than those obtained in RADT#1. This lower sensitivity of RADT#2 could be accounted for by an insufficient antigen extraction from the swab after plate inoculation, particularly if the collected swab has a low bacterial load. Such findings were also supported by low colony counts of GAS noticed with the samples that gave false negative results by RADT#2, although truly identified by RADT#1. Previous studies have also reported that the performance of RADTs is directly proportional to the bacterial load present on the collected swab (25,26). Furthermore, a low number of GAS colonies was previously noticed in the patients with false negative RADT results reflecting low bacterial load in the collected swabs (15). The sensitivity difference

between the RADT#1 and RADT#2 could also be attributed to a faulty technique and the interpretation of the RADT results. However, in this study, the processing of swabs from each patient was done by the same trained person to eliminate any user bias in the method. Both RADT#1 and RADT#2 assays had the same specificity of 96.1% that was close to previously reported data (23,27,28).

In some cases, a GAS asymptomatic carrier can be mistakenly identified for the illness that is caused by other organisms; this might be considered a study limitation. However, the discrimination between acute GAS pharyngitis and GAS carriers with acute viral pharyngitis cannot be achieved by either conventional throat culture or RADTs. Therefore, it is acceptable to treat GAS infection based on positive result of either throat culture or RADTs (15).

**CONCLUSION:** BinaxNOW Strep-A Card is a simple and quick test that can be used clinically to reduce the unnecessary use of antibiotics in children with pharyngitis. We found that RADT resulted in a better sensitivity when one swab was dedicated for the test. Therefore, physicians are encouraged to use separate swabs for each diagnostic test when both RADT and throat culture are performed.

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