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In vitro comparison of four rapid antigen tests for group A streptococcus detection

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Abstract

Aims To examine the analytical sensitivity of four rapid antigen tests (RADT) for detection of group A streptococcus (GAS).

Methods The sensitivities of four RADT kits to detect clinical and reference strains of GAS at different dilutions were compared. Test results were read by two people, and differences in interpretation were settled by a third reader.

Results A total of 697 tests were performed. For all kits, detection increased with increasing colony counts of GAS. One kit [ulti med Products, Deutschland, GmbH (UM)] was found to have the highest sensitivity, although there was no significant difference between it and one other kit (Testpack Plus). All kits were only faintly positive or negative at low colony counts.

Conclusions The sensitivity of RADT for detecting GAS is related to inoculum size and the faint appearance of a positive test at low colony counts contributes to inter-observer variability. Sore throats with low colony counts have been shown to be clinically relevant.

Non-suppurative complications of pharyngitis due to group A streptococcus (GAS), i.e. acute rheumatic fever (ARF) and acute post-streptococcal glomerulonephritis (APSGN), continue to disproportionately afflict socially disadvantaged children in New Zealand (NZ). Rates of ARF and APSGN in Māori and Pacific children in NZ are among the highest in the world.[1,2]

Antibiotic treatment of GAS pharyngitis can prevent the development of ARF,[3] and a school-based programme which identifies and treats children with GAS pharyngitis could result in fewer cases of ARF and perhaps APSGN.[3,4] The NZ Ministry of Health (MOH) has indicated that they wish to reduce the incidence of ARF in Māori and Pacific people to Pakeha (NZ European) rates by 2020.[5] There are several school-based sore throat clinics running in high risk communities in North Island, with the specific aim of reducing the incidence of ARF.[6]

Sore throat is one of the top 10 presenting symptoms in primary care;[7] however, the signs and symptoms of bacterial and viral pharyngitis overlap making differentiation on clinical grounds problematic. A throat swab for culture (48 hours incubation) is the current gold standard for diagnosing GAS throat infection,[8] but throat swab culture does not allow for point of care diagnosis and treatment, and responding to a positive culture result requires subsequent re-contact with the patient.

Disadvantaged children, often Māori or Pacific, in addition to suffering from preventable diseases disproportionately, are well documented as having limited ability to access healthcare.[9]

An attractive alternative to culture is rapid antigen detection tests (RADT). In contrast to culture, RADTs can be performed at the time the patient presents and provide a result in less than 15 minutes. It has been demonstrated in adults that compliance to antibiotic therapy for GAS throat infections is higher when a RADT is used for point of care diagnosis. [10] However, to date, the implementation of RADTs has been hampered by sensitivity concerns necessitating back up culture for all negative throat swab RADT for GAS. [11]

Presently, and in contrast to point of care pregnancy tests, RADTs for GAS are not funded by the NZ government. Pharmac have expressed interest in funding RADTs if a testing strategy with appropriate test performance can be identified. [12] In addition to *ruling in* GAS pharyngitis among high risk (for ARF) children, RADTs may also have a role in *ruling out* GAS pharyngitis in low risk settings; which could have a positive impact by reducing unnecessary empiric antibiotic prescription. [13]

Norris *et al* have recently shown that antibiotic prescription in Te Tairawhiti (an area with a high incidence of ARF) is higher among those less likely to require it (urban, non-Māori living in areas with lower socioeconomic deprivation scores), and lower among those with greatest need. [14]

GAS RADTs are usually considered to be of moderate complexity. None of the kits used in this study are Clinical Laboratory Improvement Amendment (CLIA)-waived (as pregnancy tests for home use are). Thus, some training is recommended if non-laboratory personnel, such as school nurses, whanau workers, GP practise nurses etc, are performing the tests.

As a precursor to a planned clinical study examining the possible role for RADTs in selected schools, and perhaps other sites, in NZ, we performed a laboratory study comparing the in vitro test performance of four RADTs for GAS.

Methods

The four RADT kits known to be commercially available in NZ in mid-2011 were included in the study [ulti med Products, Deutschland, GmbH (UM), SD-Bio, Standard Diagnostics, Hagal-dong, Korea (SD), Clearview Exact, Inverness Medical, Bedford, UK (CV), and Testpack Plus, Inverness Medical, Bedford, UK (TP)]. Their NZ suppliers were contacted by the senior author, and all sought and obtained agreement from the manufacturers to supply approximately 200 test kits free of charge for the study.

GAS strains used were a combination of a reference strain (ATCC 19615) and strains isolated from patient throat swabs. Clinical isolates were identified as GAS by colonial appearance on blood agar (beta-haemolytic and >0.5 mm colony size) and latex agglutination testing (PathoDX® Strep Grouping, Remel, Lenexa, Kansas).

Following overnight incubation (CO_2, 37°C), on sheep blood agar, fresh GAS colonies were diluted in saline to a concentration of approximately 10×10^6/L (MacFarland 0.5 by turbidimetre). In order to establish more accurate counts of bacteria, 1 mcL of solution was plated onto blood agar and incubated overnight in CO_2 at 37°C. The number of colonies present was used to calculate colony forming units (CFU)/mcL. RADTs were tested using different volumes of the GAS solution (10–100 mcL), starting at 100 mcL and reducing the volume (and corresponding total colony count) to a discriminating volume near the cut off between negative and positive results. A total CFU count was calculated using the colony count and the volume used, e.g. colony count=140 CFU/mcL and volume used=100mcL gives total colony count used in testing of 14×10^3 CFU.

Tests were performed as per the manufacturer's instructions. All four kits were laid out on the bench, and the order of kits was changed in a random fashion each day. A negative control was performed for all kits at the beginning of the study.

Test results were read by a final-year Medical Laboratory Science student (CL), who was blinded to the inoculum size, and a Clinical Microbiologist (AU). When their interpretation of a test was disparate a microbiology scientist also read the result, and a consensus was reached. Test results were reported in a graded fashion (positive, faint/positive and faint/negative). The last category was used when the investigators thought they could detect a line indicating a positive result, but it was so faint that they could not be certain.

Organisms known to colonise the oropharynx were used for specificity testing and included: group one (*Streptococcus anginosus, S. salivarius, S. mitis, S. mutans, S. sanguinis,* and *S. dysgalactiae*), group 2 (*Bacteroides melaninogenicus, Fusobacterium nucleatum,* and *Veillonella parvula*), group 3 (*Neisseria sicca, N. pharyngitis, Moraxella catarrhalis, Haemophilus influenzae,* and *H. parainfluenzae*), and group 4 *(Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Staphylococcus aureus, and S. epidermidis*).

Specificity testing was performed using the two RADTs found to have the highest sensitivity. Groups of commensal organisms were made up to at least 0.5 MacFarland by mixing one colony of each organism in sterile saline.

Binary logistic regression was used to determine whether a difference in the detection rate across kits could be found. Absolute colony count and kit were included as explanatory variables. Initially their interaction was also included. The outcome was whether the test was negative or not.

Results

A total of 697 tests were performed for determination of sensitivity (Table 1) using 10–100 mcL of solution giving CFU/test between 4.875×10^3 and 3.5×10^6 There was weak evidence that the difference in the kits was influenced by the colony count ($p=0.07$) with the greatest difference being at the lower colony counts. When the interaction was removed from the analysis to examine the overall effect of kits, there was strong evidence of an effect of both colony count and kit (both $p<0.0001$), with detection increasing with concentration.

Kit UM had the highest estimated rate, which was significantly higher than SD and CV ($p<0.0001$ and $p=0.008$ respectively) but no difference could be demonstrated between UM and TP ($p=0.15$) (Table 1).

Approximately 30% of the results included as ‘positive’ were barely discernable (faint/negative); if these were not included as positive the overall sensitivity dropped considerably for all RADTs by between 18 and 38.3% (Table 1).

Specificity testing using the UM (three tests on each organism group=12 tests in total) and TP (one test on each organism group=4 tests in total) kits were all negative.

Technical errors where no result was available occurred for all kits but most frequently for TP. All technical errors were due to a reagent not being added in the correct sequence or at all.

Table 1. Percentage RADT tests positive at different colony forming units

Test	Colony-forming units per test					Total tests (% positive)	% positive with faint/negatives considered negative
	<15×10^3	**15≤20×10^3**	**20≤40×10^3**	**40≤80×10^3**	**≥80×10^3**		
RADT	Number of tests performed (percentage positive)						
Clearview	38 (68.4)	52 (67.3)	44 (75.0)	27 (88.9)	14 (100.0)	175 (75.4)	37.1
SD Bio	38 (21.1)	25 (17.3)	45 (48.9)	28 (82.1)	14 (100.0)	177 (42.9)	24.9
Testpack	35 (48.6)	49 (85.7)	43 (90.7)	27 (92.6)	14 (92.9)	168 (81.0)	54.2
Ulti-med	37 (70.3)	53 (83.0)	45 (91.1)	28 (100.0)	14 (100.0)	177 (86.4)	54.8

Discussion

This study confirms the relationship between the sensitivity of RADTs for detection of GAS and inoculum size (i.e. colony count). Lasseter et al had similar findings in their laboratory study.[15] We found that the two best performing RADTs had in vitro sensitivities >90% when there were at least 20×10^3 CFU per test. This relationship has also been identified in clinical studies; combining two throat swabs improves the sensitivity of both culture and RADT compared with a single swab. [16] An incubation step prior to RADT has been found to improve the sensitivity of TP. [17]

The issue of inoculum size is clinically important. Significant GAS infection (with symptoms, antibody titre rise, and risk for ARF) can occur in children despite only small numbers (fewer than ten colonies on bacterial culture plate) of GAS isolated from the throat swab. Thus, in order for a RADT to replace culture it must be able to detect GAS pharyngitis in those children with low bacterial load. [18]

To our knowledge, there is only one other study comparing the *in vitro* sensitivity of RADTs for GAS detection. [15] In this study, TP had superior sensitivity to three of the four comparator RADTs, and was found to be easiest to use overall. The only comparator (to TP) examined in both this study and ours was CV.

There was a suggestion that the sensitivity of the kits may be influenced by the colony count. At the lowest colony count ($<15\times10^6$ CFU) UM was more sensitive than the other kits, including TP (70.3% vs. 48.6%) despite not being able to demonstrate an overall difference in sensitivity between UM and TP. The authors did note that at the lowest colony counts, some of the TP kits that were negative at 10 minutes (the upper time limit for the test to be read) became positive if left another two or so minutes.

We found TP to be easiest to read but not easiest to use; the majority of our technical errors were with TP; mostly due to forgetting to add the third reagent. As TP was the only kit that requires a third reagent, it is likely that if TP alone was used in a clinical setting the person performing the test would remember to add all three reagents.

None of the RADTs were easy to read at low colony counts. The positive lines were extremely faint precipitating some dispute between investigators (and calling in a third scientist for arbitration). This highlights the importance of education around reading results, especially when RADTs are utilised in the community by non-laboratory staff.

It is possible that our study design (adding from 10 to 100 mcL of GAS solution to the RADT reagents) may have negatively impacted the test performance by diluting the amount of GAS antigen available for absorption and migration through the membrane, as the membrane can only take so much liquid volume before saturation. However, this would have affected all RADTs equally. In addition, it is possible that the different volumes of inoculum used impacted on test results.

We did not focus on specificity testing as clinical studies have consistently demonstrated excellent specificity (>95%). Lasseter et al found 100% specificity in their laboratory evaluation.[15] We conclude that UM and TP were the most sensitive kits; all kits were simple to use although our technical errors were mostly with TP.

The TP kit was thought to be clearest to read. However, our findings support the current recommendation that RADTs are not used as stand-alone point of care tests in the community without culture back-up. The sensitivity and negative predictive value are insufficient to be reassured by a negative test in a symptomatic patient. In addition, at low colony counts the tests are difficult to read and intra-observer variability is common.

On the basis of this study, we have elected to employ the UM kit for a clinical study which will determine whether or not flocked swab technology is able to sufficiently improve the sensitivity of the RADTs such that they can be used for the diagnosis of GAS pharyngitis as a point of care test at high risk schools and in primary care.

Competing interests: Nil.

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