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Effect of swab type on the analytical sensitivity of five point-of-care tests for group A streptococci

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The majority of point-of-care rapid antigen detection tests (RADTs) for group A β-haemolytic streptococci (GABHS) are sold by manufacturers with kit swabs provided. This is convenient for the purchaser but may have unexpected effects on kit performance.

Most clinical validation studies compare the performance of RADTs against culture and in these studies the clinical throat samples are often collected using various swab types; usually swabs provided with bacteriology transport media. It is widely assumed that swab type has no impact on RADT performance, and despite the fact that manufacturers often provide swabs with their kit that have been specifically validated for use with a RADT,[1] many clinical validation studies routinely disregard these recommendations by using a variety of swab types.[2–7]

This study presents a brief report on the impact of swab type on the analytical sensitivity of five point-of-care RADTs used for GABHS throat infection. The full study protocol is

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outlined in previous work by Lasseter *et al*.[8] and is outside the remit of this paper, which reviews the impact of swab types on test sensitivity. This assessment is part of a larger clinical evaluation of RADTs for the diagnosis of GABHS (PRImary Streptococcal Management [PRISM] study), in which RADTS were compared to clinical scoring systems.

The aim of this study is to identify the most sensitive RADT via *in vitro* evaluation (parallel *in vitro* studies using different clinically achievable GABHS concentrations and control strains) and clinical validation (two double swabs per adult, using the same swab for both RADT and culture). Thus, in the *in vitro* study we also evaluate the effect of using a selection of swab types, usually those used for culture, on RADT sensitivity.

Five commercially available kits were evaluated: Clearview Exact Test (Inverness Medical Professional Diagnostics, Bedford, UK), IMI Test Pack Plus Strep A (Inverness Medical, Bedford, UK), OSOM Ultra Strep A (Bio-Stat, Stockport, UK), Quickvue Dipstick Strep A test (TK Diagnostic, Oxford, UK) and Streptatest (Dectrapharm, Strasbourg, France).

Manufacturer's instructions in each evaluated RADT specifically recommended the use of either polyester or rayon swabs (Table 1). Consequently, all kits were tested *in vitro* with single-tipped polyester (170C, Copan Diagnostic, Barloworld Scientific, Staffordshire, UK), rayon (141C, Copan Diagnostic) and the kit swabs provided by the manufacturer. All swabs used were fibre-tipped and had solid shafts.

For RADT sensitivity testing, four strains of *Streptococcus pyogenes* were used because of an association with clinical sore throat infection (NCTC, Heath Protection Agency [HPA], Colindale, UK). Serial dilutions of each GABHS strain were prepared (2.5×10^6, 5×10^6, 7.5×10^6 and 10×10^6 colony-forming units [cfu]/mL) and a 100 µL aliquot was administered on a swab for testing.

Previous studies have shown that some GABHS RADTs have a sensitivity of 80–90%.[4] Assuming that the best RADT in this study would achieve a sensitivity of 85–95% and to estimate with 95% confidence that the sensitivity of an RADT was within ±5%, between 1460 and 3920 samples were required for all five RADTs. Consequently, each RADT was tested 20 times (following the manufacturer's instructions) at each dilution with four GABHS strains and three different swab types (960 tests per RADT). Subsequently, analytical sensitivity was calculated on the number of positive tests versus the number of tests performed per kit.

Combining the results from all RADTs demonstrated the impact of swab type. Kit swabs were associated with the highest overall sensitivity at 55% (95% confidence interval [CI]: 53–57%), compared to polyester at 52% (95% CI: 50–54%) or rayon at 37% (95% CI: 35–39%).

Figure 1 presents the compiled sensitivity results for all GABHS strain types and GABHS concentrations. The kit swabs provided produced the highest sensitivity results with the OSOM, Quickvue dipstick, IMI Test Pack Plus and Streptatest kits. However, Clearview sensitivity was notably better with polyester swabs at 77% (95% CI: 67–87%) compared to the kit swabs provided, which had a sensitivity of only 38% (95% CI: 28–48%), and rayon swabs at 23% (95% CI: 13–33%).

Figure 2 shows the sensitivity results for Clearview with

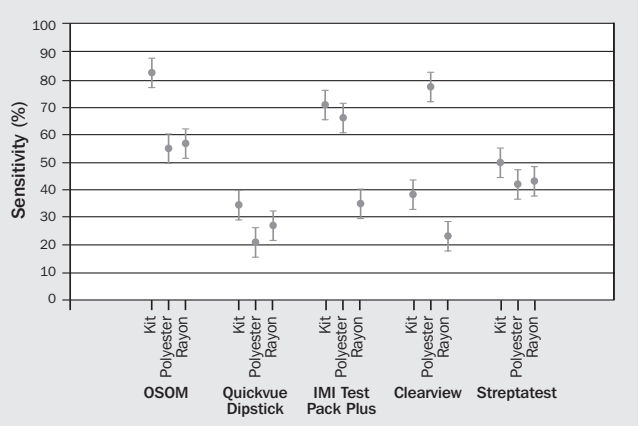

Fig. 1. Sensitivity (with 95% confidence intervals [CI]) for five RADTs, in relation to swab type used; all GABHS strains and concentrations.

all three swab types. The full sensitivity results for all kits are discussed in a previous study.[8] At 7.5×10^6 cfu/mL the OSOM/kit swab combination achieved a sensitivity of 95% (95% CI: 87–100%), which was match only by Clearview using polyester swabs. However, Clearview and polyester swabs achieved 100% (95% CI: 96–100%) sensitivity at 10×10^6 cfu/mL, while the sensitivity of OSOM and kit swabs remained at 95%. However, it is worth noting that the sensitivity of Clearview when using the kit swabs provided was considerably lower, reaching a maximum sensitivity of only 62% (95% CI: 51–72%) at 10×10^6 cfu/mL, which was lower than any other RADT. The poorest combination was Clearview with a rayon swab, which achieved a maximum sensitivity of 41% (95% CI: 31–52%) at 10×10^6 cfu/mL.

This study evaluated single-tipped polyester and rayon swabs from one manufacturer (Copan Diagnostic) and compared them to the kit swabs provided. The evaluation, therefore, is not broad enough to allow the authors to recommend the best swab type overall; however, the results do show that swab type can have a detrimental effect on RADT sensitivity and confirms that these tests should be performed using the kit swabs provided, as these often achieve the highest analytical sensitivity results.

Fundamentally, swab transport devices have been developed to maximise sample collection, maintain organism viability and augment specimen release for analysis. Various

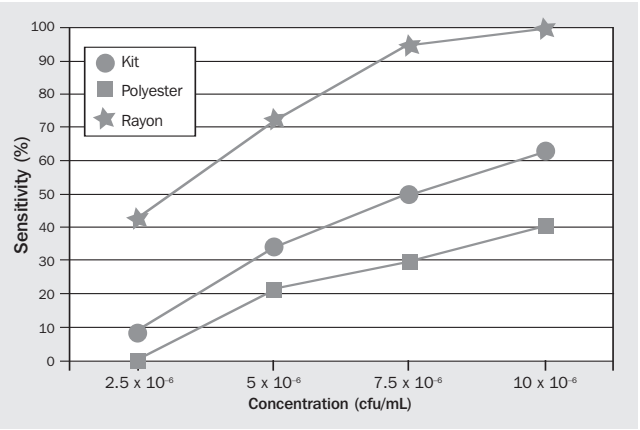

Fig. 2. Clearview RADT sensitivity at each GABHS concentration (combined results for all GABHS strains).

Table 1. Manufacturers' swab recommendations for five rapid antigen detection tests.

Kit	Swab type	Additional suggestions
Clearview Exact Test*	Polyester	Use swabs such as those provided. Plastic shafts only, such as those provided. Do not use calcium alginate, cotton-tipped or wooden-shaft swabs.
OSOM Ultra Strep A†	Rayon	Only use swabs provided. Do not use swabs from other suppliers, not validated.
Quickvue Dipstick Strep A Test‡	Rayon	Any rayon-tipped swab on solid plastic shaft. Do not use calcium alginate, cotton-tipped, hollow or wooden-shaft swabs.
Streptatest§	Polyester	Only use swabs provided.
IMI Test Pack Plus Strep A#	Polyester	Polyester-tipped swabs only. Do not use calcium alginate, cotton-tipped, hollow or wooden-shaft swabs.

*Inverness Medical Professional Diagnostics, Bedford, UK; †Bio-Stat, Stockport, UK; ‡TK Diagnostic, Oxford, UK; §Dectrapharm, Strasbourg, France; #Inverness Medical, Bedford, UK.

studies have investigated swab type and the impact on the organism or specimen,[9–12] yet few have reported on the effect of swab type on RADT sensitivity. The results of a previous study by Scansen *et al.* found that swab type reduced the sensitivity of RADTs for influenza virus, and, although this study investigated two very different swab types (foam swabs versus flocked swabs), these results support the current findings that swab type can significantly affect RADT performance.[13] To our knowledge, the current work is the first study to compare directly the performance of RADTs when using the kit swabs provided versus other recommended swab types (Table 1). The results provide a clear warning to RADT end-users and manufacturers about the importance of swab type for maximising RADT sensitivity, and as a consequence of our work the manufacturer of Clearview has changed the kit swabs provided.

A previous report by Bourbeau noted that swab fibre composition, swab tip preparation, swab tip characterisation (fibre, foam, flocked), shaft type (hollow, solid) and transport medium can have an impact on swab performance.[1] In light of this information, and because the clinical validation part of the PRISM study used rayon swabs with all kits, it was felt that investigating the impact of swab type on RADT performance was warranted.

During the *in vitro* evaluation, initial visual assessment found the composition of the five swabs (tip and shaft) to appear identical; however, Figure 1 shows that the difference in RADT sensitivity was not solely attributable to swab material (rayon or polyester), as sensitivity was often lower when using swabs not provided by the manufacturer but made of the same recommended material. This would indicate that other factors cause some swabs to impact on RADT sensitivity and that more work is needed to identify these factors.

Clinical throat swabs are notoriously imprecise samples, with two simultaneous swabs often showing variation in the number of GABHS collected.[14] With no standard method recommended for throat swab collection, swab material, transport medium and environmental factors all can affect GABHS collection and survival, and ultimately RADT performance. This *in vitro* study determined the impact of two of these factors by using three different swab types and precise GABHS concentrations. The results showed that RADT analytical sensitivity altered considerably depending on the swab type used.

Users of RADTs should choose the swab type carefully, and manufacturers should demonstrate that their swab type provides optimal results, as the sensitivity of RADTs can be significantly impaired. In clinical practice, the data presented here have important implications for hospital laboratory services and commissioners when determining what swab type should be used in general practice. □

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Comparison of the identification of *Acinetobacter* spp. with API20NE and 16S rRNA gene sequencing techniques

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Acinetobacter species are aerobic, encapsulated, oxidase-negative, non-motile, non-fermentative Gram-negative coccobacilli. Until recently, the genus *Acinetobacter* contained the single species *A. calcoaceticus*, which was subdivided into two subspecies or biovars (*A. calcoaceticus* subspecies *anitratus* and *A. calcoaceticus* subspecies *Iwoffii*). In 1986, the taxonomy of the genus *Acinetobacter* was altered extensively by Bouvet and Grimont,[1] who outlined 12 different species by DNA-DNA hybridisation, including the named species *A. baumannii, A. calcoaceticus, A. haemolyticus, A. johnsonii, A. junii,* and *A. Iwoffii*, and six unnamed genomic species. More recently, 16S rRNA gene sequence analysis has also revealed that *Acinetobacter* spp. represent a well-defined genus;[2] however, species delineation has been more problematic and although a total of 24 genomic species have so far been recognised, only nine have been provided with valid species names.[3] At present, there are 23 formally described species with standing in the literature, which include *A. baumannii, A. baylyi, A. beijerinckii, A. bereziniae, A. bouvetii, A. calcoaceticus, A. gerneri, A. guillouiae, A. grimontii, A. gyllenbergii, A. haemolyticus, A. johnsonii, A. junii, A. Iwoffii, A. parvus, A. radioresistens, A. schindleri, A. soli, A. tandoii, A. tjernbergiae, A. towneri, A. ursingii* and *A. venetianus* (www.bacterio.cict.fr/a/acinetobacter.html).

Acinetobacter species are opportunistic pathogens of low virulence. They are widely prevalent in nature, being found on both animate and inanimate objects.[4] Although generally regarded as commensals of the skin and the respiratory and genitourinary tracts,[5] they have been implicated as the cause of serious infectious diseases such as meningitis, pneumonia, tracheobronchitis, endocarditis, wound infection and septicaemia, mostly involving the immunocompromised host.[6] The contribution of *Acinetobacter* to nosocomial infection has been increasing over the past 30 years.[6,7] Several outbreaks of hospital infection have been described, some being due to contamination of hospital equipment and the hands of personnel. Treatment of serious *Acinetobacter* spp. infection is further complicated by the widespread multidrug resistance of the organism.[7]

There has been considerable difficulty in the identification of species within this genus.[8] Molecular methods may be able to assist with the correct identification over phenotypic methods, particularly for the correct naming of species causing clinically significant disease in this patient population. Hence, it is the aim of this study to identify retrospectively *Acinetobacter* organisms originating from blood culture from patients with haematological malignancy.

A total of 55 isolates belonging to the genus *Acinetobacter* were revived from storage at –80°C from the culture repository of the Northern Ireland Public Health Laboratory, Belfast City Hospital. These isolates were all originally from blood culture material from haematology/oncology patients at Belfast City Hospital during the period January 2005 to May 2008. All *Acinetobacter* isolates were cultured on Columbia blood agar (Oxoid, Basingstoke, UK) containing 5% (v/v) defibrinated horse blood (CBA+DHB).

All *Acinetobacter* isolates were examined using API20NE (bioMérieux, France). Identification of these isolates was performed in accordance with the manufacturer's instructions. Substrate assimilations were read after 24 and 48 h. Interpretation of the results was carried out after 48 h using the identification software version 6.0. Isolates were classified into one of the following three groups: (i) identification at species level, (ii) identification at genus level, (iii) no identification (i.e., low discrimination). According to the manufacturer's instructions, identification at the species level was divided into four subgroups: (i) excellent species identification (≥99.9% identification,

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