

## RAPID IDENTIFICATION OF GROUP A STREPTOCOCCI BY PYRROLIDONYL- $\beta$ - NAPHTHYLAMIDE HYDROLYSIS

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### ABSTRACT

In a clinical trial a new pyrrolidonyl- $\beta$ -naphthylamide (PYR) hydrolysis test was compared with the bacitracin disk susceptibility test for accuracy in the presumptive identification of group A streptococci (GAS). Among 128 isolates of beta-hemolytic streptococci 93 group A isolates were found. The sensitivity of the PYR and bacitracin tests were similar (98.9%), but the bacitracin test had a lower specificity (80%) than the PYR test (100%). The efficiency of the PYR and bacitracin test were 99.2% and 93.7%, respectively. All bacitracin tests were performed on subcultures of the isolates from the primary plate, whereas PYR testing was performed on colonies from the primary plate. This shortened the turnaround time for the PYR test compared to the bacitracin test by at least 24 hours.

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### INTRODUCTION

The bacitracin disk susceptibility test is commonly used to presumptively identify group A streptococci (GAS). The test is highly sensitive but not specific. Although nearly all GAS are susceptible to bacitracin, 5 to 20% of beta-hemolytic non-group A streptococci may also be susceptible, resulting in misidentification of them as GAS.<sup>1-3</sup> A further drawback of this test is the length of time required to obtain a final result. Many laboratories place a bacitracin disk on the primary blood agar plate and make a final report after 24h of incubation, although this is not the recommended procedure.<sup>4,5</sup>

Godsey et al. have demonstrated that L-pyrrolidonyl- $\beta$ -naphthylamide (PYR) hydrolysis is a reliable test for the identification of GAS.<sup>6</sup> Earlier PYR tests required 4 hours of overnight incubation to obtain results. Recent modifications however allow detection

of PYR hydrolysis in 10 to 15 minutes.<sup>7,8</sup> All of these methods require specific kits which are commercially made for the PYR test. These are however expensive and not always available in some countries.

In this study the PYR test is compared with the bacitracin test for sensitivity and specificity in the presumptive identification of GAS. Furthermore, the required substrate and indicators were purchased and prepared by the authors, making it more feasible and practical for areas where kits are not always available.

### MATERIALS AND METHODS

All of the streptococcal isolates were from human specimens. Body sites from which they were obtained included the throat, wounds, the genitourinary tract, and cerebrospinal fluid. Over 90% of the strains however were either from the upper respiratory tract or from

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wounds. All strains were finally identified by co-agglutination (phadebact streptococcus, Pharmacia Diagnostic).

Trypticase soy agar (BBL) supplemented with 5% defibrinated sheep blood was used as media.

### Bacitracin

Colonies of beta-hemolytic streptococci from the primary plate were tested for susceptibility to a 0.04 unit bacitracin disk (Bio-Merieux). When isolated colonies were not present subcultures were made and the test was repeated after 24 hours of incubation.

### PYR test

Four to six colonies of each isolate were removed by a sterile swab or rubbed on sterile filter paper (1 × 3 cm) and moistened with two drops of PYR solution, prepared by dissolving 25mg of L-pyrrolidonyl-β-naphthylamide (Fluka Chemie AG, Switzerland) in 1mL of methanol and adding distilled water up to 100 mL. After incubation for 15 minutes at room temperature, 1 drop of dimethylaminocinnamaldehyde solution, prepared daily by dissolving 30 mg of 4-dimethylaminocinnamaldehyde (Fluka Chemie AG, Switzerland) in 10mL of N/10 HCl, was added to the filter paper or the swab. Final reactions were read after 2 minutes. Positive reactions were indicated by the formation of any color ranging from light pink to cherry red, and all other colors including orange were interpreted as a negative reaction.

### Test accuracy parameters

Sensitivity, specificity and efficiency define a laboratory test's diagnostic accuracy.<sup>9</sup> A true-positive (TP) result is the number of bacterial strains (i.e., group A streptococci) correctly classified by the test; a false-positive (FP) result is the number of bacterial strains (i.e., non-group A streptococci) misclassified by the test; a false-negative (FN) result is the number of bacterial strains (i.e., group A streptococci) misclassified by the test, and a true-negative (TN) result is the number of bacterial strains (i.e., non-group A streptococci) correctly classified by the test.

Sensitivity is defined as the percentage of true-positives among all positive situations.

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} \times 100$$

Specificity is defined as the percentage of true-negatives among all negative situations.

$$\text{Specificity} = \frac{\text{TN}}{\text{FP} + \text{TN}} \times 100$$

Efficiency is the percentage of correct results, both positive and negative.

$$\text{Efficiency} = \frac{\text{TP}}{\text{TP} + \text{FP} + \text{FN} + \text{TN}} \times 100$$

## RESULTS

A total of 128 isolates of beta-hemolytic streptococci were found during this study. These included 93 GAS, 23 group B, 8 group C and 4 group G streptococci.

Table I. Comparison of PYR and bacitracin disk susceptibility tests in the presumptive identification of group A and non-group A streptococci.

Test results	PYR	bacitracin
True-positive (group A) (n=93)	92	92
True-negative (non-group A) (n=35)	35	28
False-positive	0	7
False-negative	1	1

The number of true-positive and -negative results given by PYR and bacitracin on initial testing of GAS is given in Table I. The 7 non-group A isolates which gave false-positive results by the bacitracin test were group B, group C and group G. None of these strains yielded a false-positive reaction by the PYR test. Thus the bacitracin test had a lower specificity (80%) than the PYR test (100%). The corresponding values for the sensitivity and specificity of the test are given in Table II. The efficiency of the PYR and bacitracin tests were 99.2 and 93.7%, respectively.

Table II. Sensitivity, specificity and efficiency of PYR and bacitracin disk susceptibility tests in the presumptive identification of GAS

Parameter	PYR	bacitracin
Sensitivity	98.9	98.9
Specificity	100	80
Efficiency	99.2	93.7

## DISCUSSION

The high sensitivity and relatively low specificity of the bacitracin disk susceptibility test reported by many

others<sup>2,10-12</sup> was again proven in this study. The sensitivity of the bacitracin and PYR test were similar, but the PYR test is more specific than the bacitracin test; this is shown by the fact that no group B, C or G beta-hemolytic streptococci were PYR-positive, whereas 19.6% of the same strains were positive by the bacitracin test.

Contaminants may also cause false-positive results. It is therefore important to follow aseptic procedures while performing the test.<sup>13</sup> The false-negative results obtained with the PYR and bacitracin test are probably due to an atypical GAS strain which is poor in M protein.<sup>5</sup> Since more false-positive bacitracin reactions occurred in those groups which were commonly found in wounds and in the respiratory tract,<sup>2</sup> and in our study nearly 90% of the isolated strains were from these areas, this high rate of false-positive reactions with bacitracin is acceptable.

The PYR test therefore provides a rapid, reliable, simple and cost-effective method for the identification of GAS. The specificity of the test is higher than the bacitracin disk method. It can be used as an alternative to the bacitracin test or as a confirmatory test whenever bacitracin results are not satisfactory.

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