β -D-Glucuronidase activity assay for rapid differentiation of species within β -haemolytic group C and G streptococci

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Abstract

Methylumbelliferyl-conjugated enzyme substrates were assessed for their ability to differentiate β haemolytic streptococci in Lancefield groups C and G. Both *Streptococcus equisimilis* (group C) and large colony human biotype group G strains were consistently differentiated from group C and G "*Streptococcus milleri* group" bacteria by their ability to hydrolyse the β -D-glucuronide substrate. The test was completed in less than one hour.

The role of the β -haemolytic non-group A streptococci in community acquired pharyngitis has been studied extensively.¹ Recent clarification of the taxonomy of these bacteria, however, has rekindled interest, and investigators have suggested that few β -haemolytic non-group A streptococcal subgroups (large colony group C—that is, *Streptococcus equisimilis* and *Streptococcus zooepidemicus*, and group G streptococci) are likely aetiological agents.² A series of outbreaks have provided most of the support for these latter bacteria as causative agents.³⁻⁶

Although molecular genetic studies have provided the ultimate foundation for our current appreciation of the taxonomy of the β -haemolytic non-group A streptococci,^{7,8} there are several simple laboratory methods which are useful for differentiating "*Streptococcus milleri* group" organisms from other β -haemolytic streptococci bearing group C and group G Lancefield antigens: colony size, quality of haemolysis, and the rapid Voges-Proskauer test.⁹ The prevalence of various group C and G streptococcal subgroups is such that a simple laboratory scheme for their differentiation is justified.^{2,10} Because colonial morphology and haemolysis may be difficult to appreciate within the context of cultures yielding mixed oropharyngeal flora and because the rapid Voges-Proskauer test requires about four to six hours to perform, we evaluated the ability of rapid enzyme hydrolysis to facilitate rapid differentiation. Our use of the term "*S. milleri* group" is based on the understanding that despite many phenotypic similarities, there may be several distinct species in this group.⁷ This term is synonymous with *Streptococcus anginosus* which would have taxonomic priority if, in fact, the group were only one species.¹¹

Methods

One hundred and thirty three isolates of β -haemolytic group C and group G streptococci were isolated from the throats of children between 1988 and 1989 inclusive. These bacteria included 24 *S. equisimilis*, 54 large colony human biotype group G strains,¹² and 55 "*S. milleri* group" strains (23 group C, 32 group G). These strains were previously characterised by haemolysis on sheep blood agar (Columbia base), colony size, Lancefield grouping (Streptex, Wellcome), rapid Voges-Proskauer test, sugar fermentation (trehalose, sorbitol, lactose, raffinose), and aesculin hydrolysis. Strains were maintained at -70°C before this study.

After one subculture from frozen stocks, all strains were tested for the ability to hydrolyse three 4-methylumbelliferyl-linked substrates previously used by Slifkin and Gil¹³ for identifying certain β -haemolytic non-group A streptococci. The latter included N-acetyl-beta-D-glucosaminide, β -D-glucuronide, and β -galactoside conjugates. All stock solutions were prepared in 0.2 ml of dimethyl sulfoxide: 15 μmol of each β -D-glucuronide and N-acetyl- β -D-glucosaminide, and 5 μmol of β -D-galactoside. Each stock solution was admixed with 10 ml of 0.2 M acetate buffer (pH 5.2) to yield a working solution. Five colonies of each strain were touched with the wooden end of a cotton stick swab and imprinted on to a filter paper (Whatman No 1). One drop of each substrate was applied over the colony imprints and the filter paper was incubated in a plastic Petri dish in a humidified chamber for 30 minutes at 37°C .

The fluorogenic response was visualised with long wave ultraviolet light after 0.1 N NaOH alkalisation (1 drop) as a blue-white fluorescence.

Results

The table shows the results of the fluorogenic enzyme substrate hydrolysis. The substrate for determining β -D-glucuronidase activity clearly served to separate the "*S. milleri* group" from the large colony forms. All of the *S. equisimilis* and large colony group G streptococci yielded a strong positive reaction for fluorescence which was clearly interpretable. Both N-acetyl β -D-glucosaminidase and β -D-galactosidase activities were common in either small or large colony subgroups. Occasional N-acetyl β -D-glucosaminidase reactions were

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Fluorescence conjugate hydrolysis profiles for β -haemolytic group C and G streptococci

Lancefield group	Bacteria	(n =)	Enzyme activities (No positive):		
			Glucosaminidase	Glucuronidase	Galactosidase
C	<i>S. equisimilis</i>	(24)	24	24	24
C	" <i>S. milleri</i> group"	(23)	18	0	23
G	Large colony human biotype	(54)	53	54	54
G	" <i>S. milleri</i> group"	(32)	20	0	30

weakly positive, especially in the "*S. milleri* group". Two strains of *S. zooepidemicus* were also previously found to be positive for β -D-glucuronidase activity. After filter sterilisation the β -D-glucuronide conjugate could be maintained in the working solution state for a minimum of four months.

Discussion

Although large colony β -haemolytic group C and G streptococci can often be readily differentiated from the "*S. milleri* group" by the colonial morphology and quality of haemolysis alone after subculture, the distinction is often not as apparent on primary culture plates. Length of incubation, incubation atmosphere, overgrowth by other bacteria, and synergistic haemolysis are among the several reasons for this difficulty. Both Lancefield grouping and the rapid hydrolysis of the fluorescent β -D-glucuronide conjugate can be performed on material available from primary plates and can therefore facilitate rapid differentiation of these bacteria from important specimens. β -D-glucuronidase activity is very strong in *S. equisimilis* and large colony group G streptococcal strains and allows for a simplified interpretation.

We also examined the ability of three group C streptococcus agglutinating lectins (from *Dolichos biflorus*, *Helix pomatia*, and *Wisteria floribunda*) to differentiate *S. equisimilis* and the "*S. milleri* group".¹³⁻¹⁵ All group C strains, however, are agglutinated by the three lectins, and we propose that the N-acetyl-D-galactosamine bearing group C carbohydrate is considerably conserved to preclude differentiation, albeit among different species.

Although the rapid Voges-Proskauer test when used for these bacteria, will reduce the identification time compared with the use of the conventional Voges-Proskauer test, four to six hours are still required and is much more inoculum dependent than rapid enzyme hydrolysis. Any method, however, will require that contamination by other bacterial colonies be avoided. The combination of first

Lancefield grouping and then rapid enzyme hydrolysis using the β -D-glucuronide conjugate will not only facilitate a more rapid processing time for technologists, but will also more rapidly identify patients who may be recruited for clinical studies related to disease associated with these large colony streptococci.

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