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Simplification of a Locus-Specific DNA Typing Method (Vir Typing) for *Streptococcus pyogenes*

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We describe a simplification of a highly discriminatory molecular typing method, called Vir typing, for *Streptococcus pyogenes* (D. Gardiner, J. Hartas, B. Currie, J. D. Mathews, D. J. Kemp, and K. S. Sriprakash, PCR Methods Appl. 4:288–293, 1995). The procedure can be completed within a day, is reproducible, and can be applied directly to colonies growing on primary culture plates, allowing rapid establishment of strain identity in an outbreak.

Streptococcus pyogenes (group A streptococcus [GAS]) is historically typed based on the antiphagocytic M protein. There are nearly 74 recognized M types (11). However, a great majority of isolates collected from regions of GAS endemicity, such as northern Australia, Malaysia, and Thailand, could not be typed by the available sera (10, 12, 14) despite the presence of M protein in these nontypeable strains (17). Several molecular typing methods for GAS have been reported (3, 4, 7, 8, 13, 15). One of these, Vir typing (8), is based on the restriction fragment length polymorphism (RFLP) of a PCR-amplified region representing the *mga* regulon of GAS. This locus encodes M and M-related proteins, their positive regulator (the *mga* product), and C5a peptidase (5, 6). This method is applicable to all isolates from diverse geographic origins. Vir typing has been found to be highly discriminatory and very useful in epidemiological studies (9).

The size of the PCR product is in the range of 4 to 7 kb. To consistently obtain PCR products corresponding to the *mga* regulon, template DNA was routinely prepared in gel blocks (8). On average this procedure added a delay of at least 2 days to the time until typing results could be obtained. For a quick epidemiological or hospital surveillance, this delay is not acceptable. In our hands, a DNA template from boiled colony materials provided variable results. Furthermore, the PCR using such templates often gave a background smear upon gel electrophoresis. We report here the use of alkali lysis, which has been successfully used for PCR before (2), to obtain good templates for long PCR.

Template DNA was obtained either by the procedure described by Gardiner et al. (8) or by alkali lysis as described below. A single colony from a plate containing Todd-Hewitt broth plus 0.2% yeast extract in agar, a horse blood agar (HBA) plate, or a plate containing HBA and colistin sulfate plus nalidixic acid (a primary plate used for streptococcus/staphylococcus isolation) was picked up and suspended in 100 μl of 50 mM sodium hydroxide. The suspension was incubated at 95°C for 1 min, cooled to 4°C, and then neutralized with 16 μl of 1 M Tris-HCl (pH 8.0). After centrifugation for 2 min at high speed in a microcentrifuge, the template was ready for the amplification reaction. The PCR conditions were essentially as described before (8). Briefly, the 50-μl reaction mixture contained 5 μl of template DNA, 0.4 μM primers (VUF and SBR), 5 μl of the template preparation, 200 μM deoxynucleotide triphosphates, and 0.2 μl of an 8:1 mixture of *Taq* and *Pfu* thermostable polymerases, in PC2 buffer (1). The reaction included 1 cycle at 95°C for 1 min and 30 cycles at 95°C for 15 s, 60°C for 2 min, and 68°C for 6 min. The reaction product was digested at 37°C for 1 h by simply adding 2 U of *Hae*III. RFLP was determined by size fractionation by electrophoresis.

Fifty-two isolates corresponding to different Vir types (VTs) were analyzed by the old and modified methods. In all cases, the two methods gave identical results. A further 30 isolates were successfully typed by the modified method only, yielding RFLP patterns identical to those for members of the same VT typed by the old method. Thus, the modification gave reproducible results. Comparison of the two methods for a subset of 18 VTs is shown in Fig. 1. Colony material from all three media could be used directly for Vir typing using this simplified protocol (results not shown). Figure 1 presents results for colonies grown on HBA plates. The presence of contaminants, such as hemoglobin (from the HBA plate), other medium components, and other microbiological contaminants, on the primary plate did not interfere with the PCR. Minor *Staphylococcus* contamination, often found in impetiginous specimens, did not interfere. Prior to digestion with *Hae*III, the PCR product of GAS (3.5 to 7.5 kb) can be easily distinguished from the products of other hemolytic streptococci, such as those of groups G and C, which yield a much smaller product (1.5 to 1.8 kb) (16). Thus, this procedure is highly specific, robust, and applicable to all GASs.

Alkali lysis is commonly used in plasmid DNA purification, wherein the large chromosomal DNA is removed with cell debris. The consistently successful amplification reported here suggests the presence in the supernatant of nicked chromosomal DNA possibly due to autolysis of some cells in a colony or due to large amounts of DNases characteristically present in streptococci.

With this simplification, Vir typing results could be obtained within 8 h. The template preparation could be stored frozen for at least 2 months without deterioration, as judged by the quality of the Vir typing profiles. With this proposed simplification, Vir typing is the fastest typing method for *S. pyogenes*. Moreover, there is a considerable cost savings in the preparation of the template. The universality of Vir typing, the limited number of reagents and their long shelf life, and

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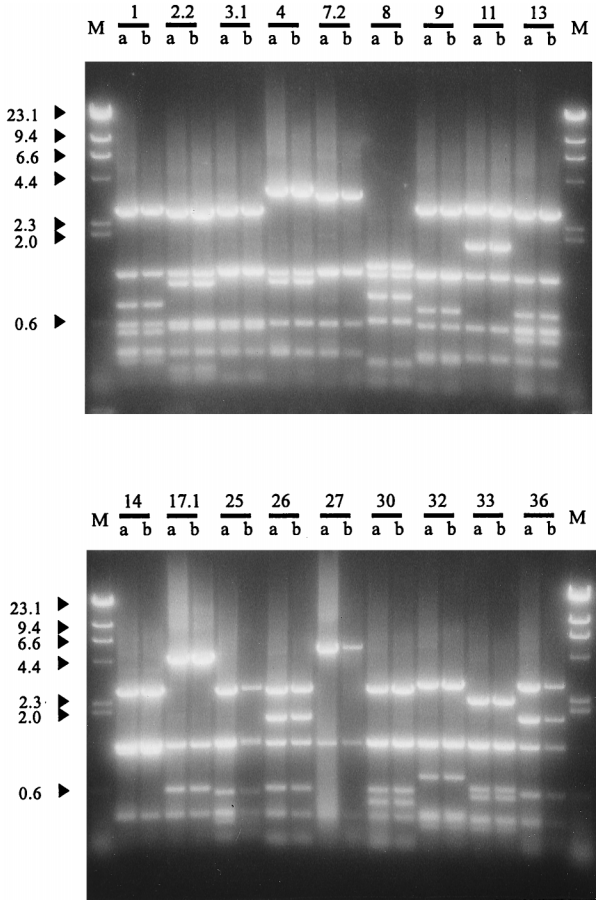

FIG. 1. Ethidium bromide-stained agarose gels containing *Hae*III-digested PCR products corresponding to the *mga* regulon of GAS isolates. Lanes M contain lambda *Hin*dIII markers. Band sizes, in kilobases, are indicated in the left margin. The remaining lanes contain paired preparations of the same local isolate representing a particular VT. The VT is indicated by the number above the bar. The patterns in lanes a are from template DNA obtained by the gel block method (8), and those in lanes b are from template DNA obtained by the alkali lysis method.

the ease of interpretation of the RFLP patterns makes this a method of choice in routine epidemiological surveillance. This is particularly so in places such as Thailand, Malaysia, northern Australia, and India, where traditional M typing is not applicable or informative across the spectrum of endemic isolates.

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