V. RESEARCH DESIGN

Research design for analyzig GAS M-protein genes

PCR targeting at the *emm* genes was performed to amplify the DNA from twenty-two standard M-typable GAS and twenty-one M-nontypable GAS which showed various degree of homology at N-terminal sequence of *emm* genes. The PCR product from each strain was then digested with several restriction enzymes to search for the enzyme that yielded the best pattern by which one strain could be differentiated from the other. The correlation of the digestion patterns with the homology of N-terminal sequence of *emm* genes and the percentage of homology of their chromosomal DNA were then determined. The study design is shown in diagrammatically below.

GAS were cultured in 50 ml THB + 1% neopeptone

↓ 24-48hr, 37°C

Chromosomal DNA extraction

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Amplification of full length *emm* gene by PCR

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Restriction enzyme digestion and

agarose gel electrophoresis with ethidium bromide staining

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Comparison of the digestion patterns with percent homology of

N- terminal sequences of *emm* genes

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Confirm the homology with percent homology by RFLP of chromosomal DNA