5.5.3 Plating Procedures

The contents of each blood sample tube were used to inoculate three different types of bacterial culture plate. The plates were assembled in sets of three including one plate of each type. The types of plates used were chocolate horse blood agar (Oxoid, Basingstoke, England), Blood/Haemin/Vitamin K (BHV), (Oxoid, Basingstoke, England) and chromogenic agar (CHROMagar® Orientation, Chromagar Microbiology, Paris, France). The plates were prepared on the premises as per the procedures outlined in the Westmead Hospital Microbiology Department Media Preparation Manual, which can be found in Appendix 4. The bases of the plates in each set were labelled with the patient number and the sample number corresponding to the respective tube, using a permanent marker, or a white China Graph pencil for the BHV plates.

The plating procedure involved distributing the contents of each concentrate pipette evenly onto the three plates in each respective set. This was done within the laminar flow cabinet to minimize settling of airborne contaminants on the growth medium. One third of the contents of each concentrate pipette were deposited on each plate type, in a straight line passing through the centre of the plate, taking care not to touch the side of the plate with the inoculum. The inoculum was then streaked using the tip of the pipette. Fifteen to 20 passes were made with the tip of the pipette, streaking the inoculum perpendicular to the original streak, taking care not to touch the edges of the plate.
Following inoculation, the plates were transported to their respective incubators where they were kept for seven days before being read. The chocolate agar plates were incubated at 35°C, 5% CO₂ (Sanyo model MCO-15A carbon dioxide incubator). The chromogenic agar plates were incubated in a hot room in ambient atmosphere at 37°C. The BHV plates were incubated in an anaerobic cabinet (Forma Scientific, Model 1029, Marietta, Ohio) at 37°C, 10% CO₂, 80% N₂, 10 % H₂.
5.6 IDENTIFICATION OF BACTERIA

5.6.1 Interpretation

Following seven days of incubation, the culture plates were examined in order to detect the growth of micro-organisms. The examination of the plates was carried out in the presence of senior staff of the Microbiology Department within the Westmead Hospital Institute of Clinical Pathology and Medical Research (ICPMR) unit. ICPMR staff members with the appropriate expertise were responsible for all the identification procedures carried out. The staff did not know which treatment group each set of plates belonged to as the plates only carried two sequence numbers for identification.

Screening of the isolates to discriminate between species that were likely to have come from plaque and those that might be contaminants was done in two stages. The first stage in the screening process, to decide whether an isolate was from the blood sample or from a contaminant during the laboratory processing procedure, was decided on the following basis:

- If a colony appeared only within the area inoculated, it was initially considered a significant positive culture, pending further identification (second stage of screening).
• If colonies appeared on both the inoculated area and outside the inoculated area, the colony within the inoculated area was considered significant (and was further identified as above). The one outside was considered a contaminant. If both colonies were identical morphologically following a Gram stain, they were both considered contaminants.

• If a colony appeared only outside the inoculated area, it was considered a contaminant.

The first stage of the screening process for contaminants is represented diagrammatically in Appendix 5.

All colonies on the inoculated areas that were not excluded for the reasons above were identified on a morphological basis (second stage of screening). Initial identification involved a Gram stain. These colonies were sub-cultured as required, using an appropriate culture medium. Further identification procedures were as follows:

5.6.2 Colonies recovered from aerobic/facultative anaerobic cultures

Following morphologic identification, *Staphylococcus* spp. were further identified using a Coagulase Plasma Test. The test procedure and the preparation of the constituents for the test are shown in Appendix 6. Colonies were incubated in 10% human plasma in 10% nutrient broth at 37°C. The tubes were checked at two and four hours. The colony
was proven coagulase negative if there was no evidence of coagulase activity after four hours.

The coagulase negative status of these species was confirmed with the aid of an additional test, the latex agglutination test (remel BACTi™ STAPH, TI No. 21143, Lenexa, Kansas, USA). The reagent induces an agglutination reaction in the presence of coagulase negative Staphylococci.

5.6.3 Colonies recovered from culture

Colonies were sub-cultured onto two BHV plates. One was incubated anaerobically at 37°C, 10% CO₂, 80% N₂, 10% H₂ as before. The other was incubated aerobically at 37°C, 10% CO₂. Following incubation for three days, the colonies were Gram stained.

For those that grew better at 37°C, 10% CO₂ (aerobically)

- If the colony was a Gram-positive coccus, coagulase activity was checked as above. If the organism was coagulase negative, it was classed as a coagulase negative spp.
- If the colony was a Gram-positive rod, it was classed as a diphtheroid. Further identification of diphtheroids was done on a morphologic basis.
- *Streptococcus* spp. were identified on a morphologic basis following a Gram stain.
For those that grew better at 37°C, 10 % CO₂, 80 % N₂, 10 % H₂ (anaerobically)

- Gram-positive rods were identified using an anaerobe test kit (Microbact 24AN System, Medvet Science Pty. Ltd., Adelaide, Australia). Those colonies that were catalase and indole positive were classed as Propionibacterium spp.

- Gram-negative rods that morphologically resembled black-pigmented spp. and that showed β-haemolytic activity were classed as Prevotella spp.

- Gram-positive cocci were classed as Peptostreptococcus spp.

5.6.4 Calculating Colony Forming Units

The lysocentrifugation system is a semi-quantitative culture technique, which allows for the calculation of the concentration of isolates in the 10ml blood samples. The number of bacterial colony forming units (CFU) of isolates found on each plate was counted. All CFU on the area inoculated with the blood samples were counted and later tabulated as either a positive culture or a contaminant. The procedure for calculating CFU per ml of blood is as follows:

Each 10 ml blood sample was divided and plated onto three plates. This meant that a count of the isolates on a given plate would have revealed the cultivable bacterial concentration per 3.3 ml of blood. The concentration could then expressed in appropriate units, such as CFU per ml or CFU per 10 ml, depending on the number of isolates recovered.
5.7 STATISTICS

The patient data was summarized using means and standard deviations for continuous variables and percentages in each category for categorical variables. The computer software utilized to create a spreadsheet for data entry and for statistical analysis of the results was SPSS v 8.0.

The homogeneity of the povidone-iodine and saline groups was tested using 2 sample t-tests for continuous variables (age, bleeding on instrumentation, probing depth, recession, plating time), the Mann-Whitney U Test for ordered categorical variables (PII, mPMAI) and Chi Square tests for categorical variables (gender, smoking status). All tests were 2-sided and significance of 5% or less was considered statistically significant throughout.

The primary outcome variable was the presence or absence of a bacteraemia. Logistic regression analysis was used to test for associations between this variable and the treatment (povidone-iodine or saline) and to test whether clinical variables or patient data were predictive for bacteraemia. A stepwise elimination procedure was used to identify the best fitting multivariate logistic regression model. Odds ratios together with their 95% confidence intervals (CI) were used to quantify the levels of association.
6. RESULTS

6.1 COMPARISON OF STUDY GROUPS

The study population consisted of 60 subjects, 30 in each of the povidone-iodine and saline groups.

6.1.1 Age and Gender

The ages and genders of the study groups are shown in Tables 2 and 3. The mean age of the subjects in the saline group was 43.95 years (S.D. ± 20.8) and the mean age of subjects in the povidone-iodine group was 45.9 years (S.D. ± 19.5). The difference between the mean ages of the two groups was not statistically significant (t=0.38, p=0.7, 58df, Independent t-test). The gender distribution between the two groups was similar. Overall, 28% of study participants were male and 72% were female. In the saline group, 23% of subjects were male and 77% were female. In the povidone-iodine group, 33% of participants were male and 67% were female. The difference in male to female ratio between the saline and povidone-iodine groups was not statistically significant ($\chi^2=0.74$, p=0.4, 1df).

6.1.2 Smoking

The smoking status of the experimental groups is shown in Table 4. Of the participants in the saline group, 53% were non-smokers, whereas 57% of the
povidone-iodine group volunteers were non-smokers. The two treatment groups were homogeneous with respect to smoking status. The difference in the proportion of smokers between the saline and povidone-iodine groups was not statistically significant ($\chi^2 = 1.00$, $p = 0.3$, 1df).

6.1.3 Probing Depths

The mean probing depths (± S.D.) for the saline and povidone-iodine groups are shown in Tables 2 and 3. Both groups had the same mean probing depth of 2.16mm and the difference between the two groups was not statistically significant ($t = 0.03$, $p = 0.97$, 58df, Independent t-test).

6.1.4 Recession

The mean values for gingival recession, measured in millimetres, were similar for both groups and are shown in Tables 2 and 3. The mean gingival recession for the saline group was 0.54 mm (S.D. ± 0.53) and for the povidone-iodine group was 0.58 mm (S.D. ± 0.52). The difference in mean gingival recession between the saline and povidone-iodine groups was not statistically significant ($t = -0.31$, $p = 0.8$, 58df, Independent t-test).
6.1.5 Gingival Index

The values for the gingival index used (mPMAI) for the study groups are shown in Tables 5 and 6. The index means for the saline and povidone-iodine groups were 1.28 (S.D. ± 0.51) and 1.22 (S.D. ± 0.48) respectively. The difference between the two groups was not statistically significant (Z=0.22, p=0.8, Mann Whitney U-Test).

6.1.6 Plaque Index

The values for the PII are shown in Tables 5 and 6. The mean values for the saline and povidone-iodine groups were 1.33 (S.D. ± 0.62) and 1.10 (S.D. ± 0.42) respectively. The difference in values for the PII between the two treatment groups was not statistically significant (Z=1.6, p=0.1, Mann Whitney U-Test).

6.1.7 Bleeding on Instrumentation

Bleeding on instrumentation relates to the proportion of sites (six sites per tooth) that bled following scaling. This proportion was expressed as a percentage. The findings for bleeding on instrumentation are shown in Tables 5 and 6. The values for bleeding on instrumentation for the two treatment groups displayed a trend towards a greater amount of bleeding for the povidone-iodine group. The mean percentage of sites that bled following instrumentation for the saline group was 63.7% (S.D. ± 21.2) and the corresponding mean for the povidone-iodine group was 73% (S.D. ± 17.8). However,
a statistically significant difference was not observed (t= -1.84, p=0.07, 58df, Independent t-test).

6.1.8 Plating Time

Plating time refers to the time interval between collection of the blood samples and plating out onto the growth media. This consisted of transit time to the laboratory and time taken to centrifuge and recover the pellet for plating.

Table 7 shows the summary statistics for plating time. The mean plating time for the saline group was 188.2 minutes (S.D. ± 128.6) and for the povidone-iodine group, it was 156.2 minutes (S.D. ± 94.7). The high values for the standard deviations reflect large variations in the time taken to process the blood samples. The difference in mean plating times between the saline group and povidone-iodine groups was not statistically significant (t=1.10, p=0.3, 58df, Independent t-test).

6.2 TREATMENT OUTCOME

6.2.1 The Effect of Povidone-Iodine

The primary outcome variable for the effect of rinsing with each of the mouth rinses was the presence or absence of a bacteraemia. Logistic regression was used to test for
associations between the mouthwash used for rinsing and the subsequent outcome in terms of whether or not a bacteraemia was recovered following scaling.

Logistic regression analysis showed that the odds ratio for developing a bacteraemia after rinsing with povidone-iodine relative to the saline control group was 0.189 (p=0.03, 95% CI, O.R.= 0.043, 0.827). The use of povidone-iodine mouth rinse was therefore a significant negative predictor for the development of a post-scaling bacteraemia, being approximately 80% effective in reducing the incidence of bacteraemias, relative to saline. (O.R.≈ 0.2).

6.2.2 Other Factors Associated with Bacteraemia

Logistic regression was used to identify possible predictors for the likelihood of developing a bacteraemia following scaling. Factors that were analysed included patient characteristics (age, gender and smoking status), clinical parameters (PII, mPMAI, probing depth, recession, bleeding on instrumentation) and plating time. A stepwise elimination procedure was used to identify the best fitting multivariate logistic regression model.

Age was the only other factor predictive for the development of a post-scaling bacteraemia, apart from the mouth rinse used (p=0.05, O.R.= 1.40, 95% C.I. O.R.= 1.00 - 1.97). For every ten-year increase in age of these subjects, the propensity for developing a bacteraemia was shown to increase. The odds ratio for the recovery of a
bacteraemia following scaling increased by 1.4, for every ten-year increase in age of the subjects. The effect of age on bacteraemia recovery was statistically significant.

There were no other characteristics or clinical parameters identified that had a significant influence on the occurrence of bacteraemia. Rinsing with povidone-iodine and age were the only factors shown to be predictive for the occurrence of bacteraemia following scaling.

6.3 Bacterial Isolates Recovered

A decision was made as to the most likely origin of the isolates. Those isolates recovered from blood samples that normally reside in the oral cavity were classified as "Oral Species". It is assumed that these isolates entered the bloodstream via the periodontal tissues as a result of the scaling procedure. Those isolates recovered, including those that are not normal residents of the oral cavity or that are normal skin commensals or known to contaminate blood cultures, were classified as "Non-oral Species". It is assumed that these isolates were contaminants that entered the blood sample as a result of the venepuncture procedure or settled on the growth plates during the plating procedure.
6.3.1 Isolates Recovered from Baseline Blood Samples

6.3.1.1 Oral Species

No bacteria considered to be of oral origin were recovered from any of the study participants in the baseline (pre-scaling) blood samples, as shown in Tables 8 and 9.

6.3.1.2 Non-Oral Species

Bacterial isolates of non-oral origin were recovered from the baseline blood samples of four volunteers; three in the saline group and one in the povidone-iodine group. The numbers and identities of the isolates, which were termed contaminants, are shown in Tables 8 and 9. The baseline non-oral isolates included Coagulase-Negative Staphylococci, one mixed colony of *Staphylococcus epidermidis / Corynebacterium* spp. and a yeast.

6.3.2 Post-Scaling Blood Samples

Tables 8 to 11 show the isolates recovered from both groups following scaling.
6.3.2.1 Oral Species

Tables 10 and 11 show the oral isolates recovered from the saline and povidone-iodine group volunteers. There were three isolates considered “oral species” recovered from the povidone-iodine group, two of which were recovered from the two minute blood samples. More isolates were recovered from the saline group than the povidone-iodine group. There were 24 isolates recovered from the saline group, five from the samples taken at 30 seconds and the remainder from the two minute blood samples. In general, the recovery of isolates was greatest from the two minute blood samples. Approximately 70% of the oral species in the saline group were recovered from the final blood samples.

It is evident from Table 10 that patients number 6 and 7 had multiple isolates of oral species in both the 30 second and two minute samples. Volunteers from the povidone-iodine group produced only single isolates of oral species, as shown in Table 11.

Viridans Group Streptococci comprised 11 of the 24 isolates recovered from the saline group volunteers. Of these, seven isolates were classified as *Streptococcus milleri* on a morphologic basis and the remainder were unclassified Viridans Group Streptococci. In contrast, there were no Viridans Group Streptococci isolated from the povidone-iodine group volunteers.
Three isolates considered Oral Species were recovered from the povidone-iodine group in total. These included one Moraxella (possibly M. catarrhalis) isolate and two Enterobacteriaceae spp. isolates. The oral species recovered from the saline group, apart from the Viridans Group Streptococci, included numerous bacterial isolates comprising predominantly species such as P. gingivalis, Prevotella intermedia and Actinomyces spp.

Figures 4 and 5 show (BHV) bacterial culture plates with positive cultures considered “oral” isolates. Figure 4 shows two isolates of Viridans Group Streptococci recovered from patient 6. Both colonies were within the inoculated area of the plate, as shown by the reflection of the flash by the secondary streak lines in association with the isolate at the top edge of the plate. Figure 5 shows two isolates each of P. intermedia (dark pigmented colonies) and Viridans Group Streptococci, recovered from patient 7. One isolate of each species was present on the (horizontal) primary streak line and the other two isolates were present on secondary streak lines, as shown by the flash reflection.
Figure 4  Viridans Group Streptococci isolates recovered from patient 6.
Figure 5 Viridans Group Streptococci and *P. intermedia* isolates recovered from patient 7.
6.3.2.2 Non-Oral Species

Tables 8 and 9 show the non-oral isolates recovered from both groups. There were 14 non-oral isolates recovered from the saline group whilst 12 isolates were recovered from the povidone-iodine group. The predominant species amongst the contaminants were Coagulase-negative staphylococci, diphtheroids (saline group) and *S. epidermidis*. There were two poly-microbial isolates recovered from two different saline group volunteers. Three colonies of yeasts were recovered including two *Oiccha monray* isolates from the povidone-iodine group and one *Candida* spp. (non-albicans) isolate from the saline group. The recovery of non-oral species, in general, was more evenly spread amongst the blood samples at all three time points. This was particularly evident for the povidone-iodine group, as shown in Table 8. In the saline group, non-oral species tended to be more prevalent amongst the isolates recovered from the final blood samples, as shown in Table 9.
<table>
<thead>
<tr>
<th>Pt. ID</th>
<th>Gender</th>
<th>Age (Y)</th>
<th>PD (mm)</th>
<th>REC (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>F</td>
<td>55</td>
<td>2.5</td>
<td>1.7</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>51</td>
<td>2.5</td>
<td>0.3</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>59</td>
<td>2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>62</td>
<td>2.6</td>
<td>0.9</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>69</td>
<td>1.7</td>
<td>0.6</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>62</td>
<td>2.8</td>
<td>1.2</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>63</td>
<td>1.6</td>
<td>0.2</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>23</td>
<td>2.4</td>
<td>0.1</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>37</td>
<td>2.3</td>
<td>1.0</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>47</td>
<td>2.4</td>
<td>1.5</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>58</td>
<td>2.5</td>
<td>0.2</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>52</td>
<td>2.7</td>
<td>0.6</td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>60</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>75</td>
<td>1.9</td>
<td>0.1</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>30</td>
<td>1.8</td>
<td>0.4</td>
</tr>
<tr>
<td>27</td>
<td>F</td>
<td>39</td>
<td>2.4</td>
<td>0.5</td>
</tr>
<tr>
<td>29</td>
<td>F</td>
<td>29</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>31</td>
<td>M</td>
<td>46</td>
<td>2.1</td>
<td>0.6</td>
</tr>
<tr>
<td>33</td>
<td>M</td>
<td>21</td>
<td>1.6</td>
<td>0.1</td>
</tr>
<tr>
<td>34</td>
<td>M</td>
<td>20</td>
<td>1.5</td>
<td>0.9</td>
</tr>
<tr>
<td>39</td>
<td>M</td>
<td>78</td>
<td>2.0</td>
<td>0.6</td>
</tr>
<tr>
<td>41</td>
<td>M</td>
<td>40</td>
<td>2.2</td>
<td>0.3</td>
</tr>
<tr>
<td>42</td>
<td>M</td>
<td>22</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>44</td>
<td>M</td>
<td>28</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>46</td>
<td>F</td>
<td>21</td>
<td>1.7</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>M</td>
<td>29</td>
<td>2.3</td>
<td>0.3</td>
</tr>
<tr>
<td>56</td>
<td>F</td>
<td>24</td>
<td>1.9</td>
<td>0.6</td>
</tr>
<tr>
<td>58</td>
<td>F</td>
<td>79</td>
<td>1.9</td>
<td>0.7</td>
</tr>
<tr>
<td>59</td>
<td>M</td>
<td>63</td>
<td>1.7</td>
<td>1.6</td>
</tr>
</tbody>
</table>

| n(Male) | 10 |
| n(Female) | 20 |

RANGE: 20-79 | 1.5-2.8 | 0-1.7

MEAN: 45.9 | 2.16 | 0.58

S.D. ±: 20.8 | 0.38 | 0.52
### Table 3
Patient gender, age and mean probing depth (PD) and recession (REC)
Saline Group

<table>
<thead>
<tr>
<th>Pt. ID (n=30)</th>
<th>Gender</th>
<th>Age (Y)</th>
<th>PD (mm)</th>
<th>REC (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>27</td>
<td>2.7</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>16</td>
<td>2.5</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>19</td>
<td>2.6</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>18</td>
<td>2.6</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>28</td>
<td>2.7</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>52</td>
<td>2.2</td>
<td>1.2</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>83</td>
<td>2.1</td>
<td>0.8</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>65</td>
<td>1.9</td>
<td>0.9</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>55</td>
<td>1.9</td>
<td>0.2</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>43</td>
<td>2.7</td>
<td>0.8</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>45</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>F</td>
<td>40</td>
<td>2.4</td>
<td>0.2</td>
</tr>
<tr>
<td>30</td>
<td>F</td>
<td>43</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>32</td>
<td>F</td>
<td>80</td>
<td>1.8</td>
<td>0.8</td>
</tr>
<tr>
<td>35</td>
<td>F</td>
<td>18</td>
<td>2.1</td>
<td>0.2</td>
</tr>
<tr>
<td>36</td>
<td>F</td>
<td>68</td>
<td>1.8</td>
<td>0.9</td>
</tr>
<tr>
<td>38</td>
<td>F</td>
<td>37</td>
<td>1.9</td>
<td>0.1</td>
</tr>
<tr>
<td>40</td>
<td>M</td>
<td>21</td>
<td>2.0</td>
<td>0.1</td>
</tr>
<tr>
<td>43</td>
<td>F</td>
<td>21</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>45</td>
<td>F</td>
<td>71</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>47</td>
<td>F</td>
<td>23</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>49</td>
<td>M</td>
<td>37</td>
<td>1.9</td>
<td>0.3</td>
</tr>
<tr>
<td>50</td>
<td>F</td>
<td>35</td>
<td>2.0</td>
<td>0.1</td>
</tr>
<tr>
<td>51</td>
<td>F</td>
<td>66</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>52</td>
<td>F</td>
<td>79</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>53</td>
<td>M</td>
<td>36</td>
<td>2.1</td>
<td>0.6</td>
</tr>
<tr>
<td>54</td>
<td>M</td>
<td>33</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>55</td>
<td>F</td>
<td>75</td>
<td>1.8</td>
<td>0.7</td>
</tr>
<tr>
<td>57</td>
<td>F</td>
<td>38</td>
<td>2.4</td>
<td>0.3</td>
</tr>
<tr>
<td>60</td>
<td>M</td>
<td>43</td>
<td>2.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

- **n(Male)**: 7
- **n(Female)**: 23
- **RANGE**: 16-83  1.8-2.7  0-2.0
- **MEAN**: 43.95  2.16  0.54
- **S.D. ±**: 20.8  0.32  0.53

---

91
**Table 4** Smoking status, summary statistics and significance (Chi Square test).

<table>
<thead>
<tr>
<th>Smoking Status</th>
<th>Saline (n=30)</th>
<th>Povidone-Iodine (n=30)</th>
<th>Significance of Difference (1df)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>23</td>
<td>53</td>
<td>26</td>
</tr>
<tr>
<td>Smoker</td>
<td>7</td>
<td>47</td>
<td>4</td>
</tr>
</tbody>
</table>

df: Degrees of Freedom
**Table 5**
Patient Clinical Data: Povidone-Iodine Group

<table>
<thead>
<tr>
<th>Pt. ID</th>
<th>GI(^1)</th>
<th>PLI(^2)</th>
<th>BOI(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>1.0</td>
<td>0.5</td>
<td>30</td>
</tr>
<tr>
<td>13</td>
<td>0.8</td>
<td>1.1</td>
<td>57</td>
</tr>
<tr>
<td>14</td>
<td>0.8</td>
<td>0.6</td>
<td>60</td>
</tr>
<tr>
<td>15</td>
<td>0.7</td>
<td>1.0</td>
<td>30</td>
</tr>
<tr>
<td>16</td>
<td>0.9</td>
<td>1.3</td>
<td>60</td>
</tr>
<tr>
<td>17</td>
<td>1.6</td>
<td>1.9</td>
<td>77</td>
</tr>
<tr>
<td>18</td>
<td>0.3</td>
<td>0.6</td>
<td>63</td>
</tr>
<tr>
<td>19</td>
<td>1.5</td>
<td>1.0</td>
<td>80</td>
</tr>
<tr>
<td>20</td>
<td>1.1</td>
<td>1.0</td>
<td>77</td>
</tr>
<tr>
<td>21</td>
<td>1.0</td>
<td>0.7</td>
<td>83</td>
</tr>
<tr>
<td>22</td>
<td>1.2</td>
<td>1.0</td>
<td>90</td>
</tr>
<tr>
<td>23</td>
<td>2.4</td>
<td>1.3</td>
<td>97</td>
</tr>
<tr>
<td>24</td>
<td>1.7</td>
<td>0.9</td>
<td>77</td>
</tr>
<tr>
<td>25</td>
<td>1.3</td>
<td>1.4</td>
<td>66</td>
</tr>
<tr>
<td>26</td>
<td>1.4</td>
<td>1.4</td>
<td>90</td>
</tr>
<tr>
<td>27</td>
<td>1.4</td>
<td>0.8</td>
<td>77</td>
</tr>
<tr>
<td>29</td>
<td>0.6</td>
<td>0.6</td>
<td>63</td>
</tr>
<tr>
<td>31</td>
<td>1.4</td>
<td>1.2</td>
<td>70</td>
</tr>
<tr>
<td>33</td>
<td>1.0</td>
<td>1.0</td>
<td>67</td>
</tr>
<tr>
<td>34</td>
<td>0.8</td>
<td>0.9</td>
<td>70</td>
</tr>
<tr>
<td>37</td>
<td>0.6</td>
<td>0.7</td>
<td>77</td>
</tr>
<tr>
<td>39</td>
<td>0.6</td>
<td>0.4</td>
<td>47</td>
</tr>
<tr>
<td>41</td>
<td>1.6</td>
<td>1.4</td>
<td>73</td>
</tr>
<tr>
<td>42</td>
<td>1.6</td>
<td>1.9</td>
<td>97</td>
</tr>
<tr>
<td>44</td>
<td>2.1</td>
<td>1.5</td>
<td>100</td>
</tr>
<tr>
<td>46</td>
<td>0.9</td>
<td>0.8</td>
<td>90</td>
</tr>
<tr>
<td>48</td>
<td>1.7</td>
<td>1.9</td>
<td>97</td>
</tr>
<tr>
<td>56</td>
<td>1.4</td>
<td>1.2</td>
<td>67</td>
</tr>
<tr>
<td>58</td>
<td>1.5</td>
<td>1.5</td>
<td>90</td>
</tr>
<tr>
<td>59</td>
<td>1.6</td>
<td>1.6</td>
<td>70</td>
</tr>
</tbody>
</table>

| RANGE  | 0.3-2.4 | 0.4-1.9 | 30-100 |
| MEAN   | 1.22    | 1.10    | 73.04  |
| S.D. ± | 0.48    | 0.42    | 17.75  |

Gingival Index\(^1\) (mPMAI, after Schour & Massler 1947); Plaque Index\(^2\) (Silness & Löe 1964); Bleeding on Instrumentation\(^3\) (% of sites bleeding on scaling).
### Table 6
Patient Clinical Data: Saline Group

<table>
<thead>
<tr>
<th>Pt. ID</th>
<th>GI(^1)</th>
<th>PLI(^2)</th>
<th>BOI(^%)^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=30)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
<td>1.3</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>1.1</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>1.2</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>1.8</td>
<td>66</td>
</tr>
<tr>
<td>5</td>
<td>0.6</td>
<td>2.5</td>
<td>66</td>
</tr>
<tr>
<td>6</td>
<td>2.1</td>
<td>2.4</td>
<td>66</td>
</tr>
<tr>
<td>7</td>
<td>0.8</td>
<td>1.3</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>1.6</td>
<td>1.7</td>
<td>80</td>
</tr>
<tr>
<td>9</td>
<td>0.8</td>
<td>1.2</td>
<td>66</td>
</tr>
<tr>
<td>10</td>
<td>1.3</td>
<td>1.1</td>
<td>70</td>
</tr>
<tr>
<td>11</td>
<td>1.5</td>
<td>0.8</td>
<td>30</td>
</tr>
<tr>
<td>28</td>
<td>0.9</td>
<td>0.9</td>
<td>80</td>
</tr>
<tr>
<td>30</td>
<td>1.2</td>
<td>1.3</td>
<td>83</td>
</tr>
<tr>
<td>32</td>
<td>1.3</td>
<td>1.1</td>
<td>43</td>
</tr>
<tr>
<td>35</td>
<td>0.8</td>
<td>1.1</td>
<td>60</td>
</tr>
<tr>
<td>36</td>
<td>1.2</td>
<td>0.9</td>
<td>73</td>
</tr>
<tr>
<td>38</td>
<td>0.9</td>
<td>1.0</td>
<td>70</td>
</tr>
<tr>
<td>40</td>
<td>1.2</td>
<td>1.4</td>
<td>73</td>
</tr>
<tr>
<td>43</td>
<td>1.0</td>
<td>1.2</td>
<td>70</td>
</tr>
<tr>
<td>45</td>
<td>1.3</td>
<td>1.2</td>
<td>60</td>
</tr>
<tr>
<td>47</td>
<td>0.4</td>
<td>0.2</td>
<td>57</td>
</tr>
<tr>
<td>49</td>
<td>2.0</td>
<td>1.6</td>
<td>53</td>
</tr>
<tr>
<td>50</td>
<td>0.9</td>
<td>0.6</td>
<td>53</td>
</tr>
<tr>
<td>51</td>
<td>2.4</td>
<td>2.8</td>
<td>93</td>
</tr>
<tr>
<td>52</td>
<td>1.9</td>
<td>1.7</td>
<td>90</td>
</tr>
<tr>
<td>53</td>
<td>2.6</td>
<td>2.4</td>
<td>97</td>
</tr>
<tr>
<td>54</td>
<td>1.3</td>
<td>1.4</td>
<td>57</td>
</tr>
<tr>
<td>55</td>
<td>1.5</td>
<td>1.6</td>
<td>67</td>
</tr>
<tr>
<td>57</td>
<td>1.5</td>
<td>0.8</td>
<td>83</td>
</tr>
<tr>
<td>60</td>
<td>1.5</td>
<td>1.4</td>
<td>87</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RANGE</th>
<th>0.4-2.6</th>
<th>0-2.8</th>
<th>20-97</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN</td>
<td>1.28</td>
<td>1.33</td>
<td>63.72</td>
</tr>
<tr>
<td>S.D. ±</td>
<td>0.51</td>
<td>0.62</td>
<td>21.2</td>
</tr>
</tbody>
</table>

Gingival Index\(^1\) (mPMAI, after Schour & Massler 1947); Plaque Index\(^2\) (Silness & Löe 1964); Bleeding on Instrumentation\(^3\) (% of sites bleeding on scaling).
Table 7 Plating time, summary statistics and significance (Independent t-test).

<table>
<thead>
<tr>
<th>Laboratory Parameter</th>
<th>Saline (n=30)</th>
<th>Povidone-Iodine (n=30)</th>
<th>Significance of Difference (58 df)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \bar{x} )</td>
<td>S.D.</td>
<td>( \bar{x} )</td>
</tr>
<tr>
<td>Plating Time (minutes)</td>
<td>188.17</td>
<td>128.6</td>
<td>156.17</td>
</tr>
</tbody>
</table>

df: Degrees of Freedom
Povidone-Iodine Group: Non-Oral Species Recovered

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Baseline</th>
<th>30 secs</th>
<th>2 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>S. epidermidis</td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>CNS (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Bacillus spp.</td>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td></td>
<td></td>
<td>CNS (1)</td>
</tr>
<tr>
<td>37</td>
<td>Oiccha monray</td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td></td>
<td></td>
<td>CNS (1)</td>
</tr>
<tr>
<td>42</td>
<td></td>
<td></td>
<td>Oiccha monray (1)</td>
</tr>
<tr>
<td>58</td>
<td>G + coccus</td>
<td>(1)</td>
<td></td>
</tr>
</tbody>
</table>

CNS: Coagulase Negative *Staphylococcus* spp.

**Table 8** Non-oral species considered contaminants recovered from blood samples taken from povidone-iodine group volunteers at baseline and after 30 seconds and two minutes of scaling. The numbers in brackets refer to the number of isolates recovered per 10 ml blood sample, at each time point.
**Saline Group: Non-Oral Species Recovered**

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Baseline</th>
<th>30 secs</th>
<th>2 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td></td>
<td></td>
<td><em>Corynebacterium spp. (1)</em></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td><em>Corynebacterium spp. (1)</em></td>
</tr>
<tr>
<td>8</td>
<td><em>Corynebacterium spp.</em></td>
<td><em>S. epidermidis (1)</em></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td><em>Corynebacterium spp. (1)</em></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td><em>CNS + P. acnes (1)</em></td>
</tr>
<tr>
<td>36</td>
<td><em>CNS (1)</em></td>
<td><em>CNS (1)</em></td>
<td><em>CNS (1)</em></td>
</tr>
<tr>
<td>47</td>
<td></td>
<td></td>
<td><em>S. epidermidis (1)</em></td>
</tr>
<tr>
<td>51</td>
<td></td>
<td></td>
<td><em>Bipolaris spp. (1)</em></td>
</tr>
<tr>
<td>52</td>
<td></td>
<td></td>
<td><em>CNS (2)</em></td>
</tr>
<tr>
<td>53</td>
<td></td>
<td></td>
<td><em>P. acnes (1)</em></td>
</tr>
<tr>
<td>54</td>
<td><em>Candida spp. (1)</em></td>
<td>(Non-albicans)</td>
<td></td>
</tr>
</tbody>
</table>

(X)* Denotes mixed colony  
CNS: Coagulase Negative *Staphylococcus* spp.

**Table 9** Non-oral species considered contaminants recovered from blood samples taken from povidone-iodine group volunteers at baseline and after 30 seconds and two minutes of scaling. The numbers in brackets refer to the number of isolates recovered per 10 ml blood sample, at each time point.
## Saline Group: Oral Species Recovered

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Baseline</th>
<th>30 secs</th>
<th>2 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>A. naeslundii (1)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>A. odontolyticus (1)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>S. milleri (1)</td>
<td>P. intermedia (1)</td>
<td>S. viridans spp. (1)</td>
</tr>
<tr>
<td>7</td>
<td>S. milleri (1)</td>
<td>P. intermedia (2)</td>
<td>S. viridans spp. (1)</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>P. intermedia (1)</td>
<td>P. intermedia (1)</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>P. intermedia (1)</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td>P. gingivalis (2)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>Streptomyces spp. (1)</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td></td>
<td>S. viridans spp. (1)</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td></td>
<td>G + coccus (1)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 10** Oral species recovered from blood samples taken from the saline group volunteers at baseline and after 30 seconds and two minutes of scaling. The numbers in brackets refer to the number of isolates recovered per 10 ml blood sample, at each time point.
### Povidone-Iodine Group: Oral Species Recovered

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Baseline</th>
<th>30 secs</th>
<th>2 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
<td><em>Enterobacteriaceae spp.</em> (1)</td>
</tr>
<tr>
<td>58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>59</td>
<td></td>
<td></td>
<td><em>Moraxella spp.</em> (1) (catarrhalis?)</td>
</tr>
</tbody>
</table>

**Table 11** Oral species recovered from blood samples taken from the Povidone-Iodine group volunteers at baseline and after 30 seconds and two minutes of scaling. The numbers in brackets refer to the number of isolates recovered per 10 ml blood sample, at each time point.
7. DISCUSSION

The aims of this study were to investigate the effect of a povidone-iodine solution pre-treatment mouth rinse on the incidence of bacteraemia associated with ultrasonic scaling in patients with plaque-induced gingivitis; to identify the species of microorganisms recovered from positive blood cultures; to assess the magnitude of bacteraemia; and to assess patient clinical and social data for any relationship with the occurrence of bacteraemia.

The povidone-iodine solution used in this study (Betadine® sore throat gargle) was selected as it is an over the counter product available in Australia which is approved for use in the oral cavity. The solution was supplied as 7.5% w/v povidone-iodine which was then diluted 1:20 in water according to the manufacturer’s instructions. Since the dilution was performed using the measuring container provided with the Betadine® bottle and not a pipette, the final dilution of the povidone-iodine solution may not have been exactly 0.375% w/v. Thus, a 0.4% w/v concentration was considered to be the most appropriate dilution to be referred to during the study. No pilot studies were conducted to determine the optimal rinsing time prior to scaling. Instead, two minutes was selected on the basis of use in previous studies (Macfarlane et al. 1984).

A randomised controlled clinical trial was undertaken to test the effect of 0.4% povidone-iodine on the incidence of bacteraemia following routine ultrasonic scaling in patients with plaque-induced gingivitis. A double-blind placebo-controlled trial was not undertaken for the principal reason that it was not possible to obtain a suitable
placebo solution. Following rinsing, povidone-iodine leaves a yellowish stain, which is obvious to the clinician. Thus, a suitable placebo mouth rinse would also have to leave a yellowish residue whilst not having any antimicrobial activity (or containing iodine). The placebo solution would also have to have a similar taste and colour. No such mouth rinse was identified and therefore a sterile 0.85% saline solution was selected. The problem with a non-coloured negative control was that those who had rinsed with povidone-iodine were readily identifiable, which raises the possibility of operator bias. For example, operator bias may have resulted in reduced scaling force and subsequently less scaling trauma in the povidone-iodine group, which may have influenced the bacteraemia incidence in this group. However, the results showed a tendency for more bleeding on instrumentation in the povidone-iodine group, which was not statistically significant. Thus, both treatment groups were likely to be homogeneous with respect to the amount of trauma due to the scaling procedure.

Secondly, povidone-iodine has a distinctive taste that many of the volunteers commented on, but generally described as palatable. A small proportion described the taste as pleasant and several found the taste very unpleasant. Removing the active ingredient, iodine, from povidone-iodine solution would remove the odour that is characteristic of iodine (British Pharmacopoeia 1993) and the strong taste of the solution would also be affected. Thus, the patients may react differently to the taste, again making operator blinding difficult. Placebo solutions have been used in previous povidone-iodine studies (Randall & Brenman 1974; Brenman & Randall 1974; Scopp & Orvieto 1971). However, the formulae for these placebo solutions have not been described.
The inclusion of a solution to use as a positive control, such as chlorhexidine, was considered but rejected. Chlorhexidine, although extensively researched in terms of its anti-plaque effect, has shown conflicting results with respect to the capacity to reduce bacteraemia related to dental treatment (Greenstein 1999). A recent double-blind placebo controlled study investigating the effect of chlorhexidine used as a pre-treatment mouth rinse showed no effect on the incidence of bacteraemia related to tooth extraction (Lockhart 1996). Therefore, chlorhexidine was not considered a suitable positive control. In the absence of a suitable positive control or placebo solution, it was decided that the control group would rinse with sterile normal saline, which would act as a negative control and ensure repeatability of the study. Normal saline has been used previously as a negative control in dentally induced bacteraemia studies (Keosian et al. 1956; Macfarlane et al. 1984).

Patients with plaque-induced gingivitis only were included in the present study in order to select patients who did not have periodontal pockets, which are not reliably penetrated by mouth rinse solutions even when delivered as a subgingival irrigant (Pitcher et al. 1980). Plaque-induced gingivitis is a common form of periodontal disease, with 44% of all subjects in one study found to have gingivitis at an average of 2.7 sites per subject (Brown et al. 1990). In order to exclude patients with chronic periodontitis without having first to perform a detailed periodontal examination, it was decided to screen potential subjects by use of their OPG radiographs and CPITN scores, which were available. It has been shown that information from OPG radiographs is useful when determining the presence of alveolar bone loss and correlates well with probing depths in screening for the presence of periodontitis (Jenkins & Mason 1984; Walsh et al. 1994). Two patients (39, 45) were diagnosed as
having plaque-induced gingivitis on a reduced periodontium (Mariotti et al. 1999). Patient 39 was in the povidone-iodine group and 45 in the saline group.

Since periodontal probing can cause bacteraemia (Daly et al. 1997), this procedure could not be performed at the visit when blood samples were to be taken. Although patients could have been examined perhaps one to two weeks earlier, this requirement may have reduced the chance of the patients volunteering for the study. It may have also led to an improvement in oral hygiene due to the Hawthorne effect, such that the clinical parameters recorded may not have been valid a few weeks later. Previous studies where treatment had been scheduled over a series of appointments have permitted up to 24 days to elapse between the measurement of clinical parameters and performance of the study treatment (Witzenberger et al. 1982). The patients in the present study were screened initially using the OPG radiographs and CPITN scores and were interviewed on the telephone before arranging an appointment to attend for the study. This permitted a single clinical visit for the blood sampling and scaling and ensured that the values for the clinical parameters were relevant when evaluating possible predictive factors for bacteraemia.

The male to female ratio amongst the selected patients was 1:2.7 and although it was anticipated that the male to female ratio should have been 1:1, this was not possible to achieve. The male to female ratio reflected the gender proportion of the waiting list from which the patients were selected. During the period of the study, the male to female ratios of patients on the dental hospital waiting list were generally of the order of 1:2.
The random allocation of volunteers to the two treatment groups was accomplished by the toss of a coin, following determination of each participant’s age, gender, smoking history, PII and mPMAI. The patients were randomly allocated to the treatment groups after recording of the indices that may arguably be subjective: mPMAI and PII. Bleeding on instrumentation, recession and probing depths were recorded after allocation to the treatment groups and after the actual scaling. The most important reason for blinding a study is to prevent errors due to lack of randomisation and to minimize operator bias, therefore ensuring homogeneity within and between study groups. Despite the lack of operator blinding in this study, it appears that the two study groups were homogeneous.

The patient inclusion criteria were designed to ensure homogeneity of the study sample population so that the effect of povidone-iodine could be tested without undue confounding variables. The homogeneity of the two mouth rinse groups was verified by performing statistical analysis on the social and clinical data obtained from the patients. No statistically significant differences were found between the two mouth rinse groups, in terms of the clinical parameters measured (mPMAI, PII, bleeding on instrumentation, recession, probing depths and the plating time), or the social data recorded (age, gender, smoking status). Since both groups were homogeneous in terms of the above parameters, it was considered that there was a sound basis for comparing the incidence of bacteraemia in the povidone-iodine group, relative to the saline group.

An ultrasonic scaler was used for the scaling procedure for two reasons. Firstly, ultrasonic scalers are commonly used in dental practices for scaling teeth. Secondly,
it was considered that it would be easier to control the parameters of the scaling instrument if an ultrasonic were used. For example, if hand instruments were used it would be difficult to control for sharpness and width of the blade and for wear during use, which may have had an influence on the degree of resulting scaling trauma. In addition, a decision whether to use site specific or universal hand instruments would have been necessary.

A piezo-electric scaler was used rather than a magnetostrictive type ultrasonic scaler. This ensured that variations in the efficiency of the stacks of different magnetostrictive inserts would not result in variations in the power output of the instrument, a potential source of method error affecting scaling trauma. It is likely that any variation in scaling force between patients was due to variations in the force applied to the tissues via the ultrasonic, by the hand of the operator. The scaling force applied by the operator is a difficult parameter to quantify and control for and was not measured in this study.

Bleeding on instrumentation was used as an indirect measure of tissue trauma, which was assumed to be related to scaling force. However, bleeding on instrumentation is likely to be as much a reflection of the degree of tissue inflammation (akin to bleeding on probing; Lang et al. 1991) as it is for operator-induced scaling trauma. Thus, it is possible that the gingival bleeding would have been a result of tissue injury from operator-induced scaling trauma and the extent of gingival inflammation present in the tissues. The saline and povidone-iodine groups were considered homogeneous for gingival inflammation, as a statistically significant difference between the mean scores for mPMAI for the saline and povidone-iodine groups was not shown. There
was a greater tendency for bleeding on instrumentation in the povidone-iodine group, which was not statistically significant. As bleeding on instrumentation tended to be greater for the povidone-iodine group whilst the gingival inflammation levels were similar for both mouth rinse groups, it is possible that the scaling force was slightly greater for the povidone-iodine group patients. However, this could not be verified.

It has been shown that ultrasonic scalers have no inherent antimicrobial effect by virtue of their sonication and cavitation effects, as shown by an *in vitro* study (Schenk et al. 2000). In this regard therefore, ultrasonic scalers appear the same as hand instruments, in that the only apparent effect these instruments have on plaque is to physically remove it from the tooth surface to which it has adhered. However, the flushing effect of the coolant in ultrasonic scalers may wash away plaque bacteria, allowing fewer bacteria to encounter gingiva denuded of epithelium, resulting in a possible decreased propensity for bacteraemia to occur.

For reasons of patient comfort and to minimise the risk of patients withdrawing from the study during the scaling procedure, it was decided to limit the time spent scaling to two minutes. Two minutes was considered a reasonable treatment time for scaling the five treatment teeth and none of the study teeth had visible remaining deposits of plaque or calculus at the end of this time interval. Two minutes was a relatively short treatment time, although it should compare to treatment times for procedures such as single tooth extraction studies. The duration of extraction procedures have rarely been defined in bacteraemia studies. However, Yamalik and co-workers (1992) reported that the single tooth extraction procedures in their study were completed within three minutes. As the scaling time and technique were kept uniform for each
patient, the scaling protocol permitted direct comparison of the incidence of bacteraemia in both mouth rinse groups.

The timing of blood sampling procedures has been shown to have an important bearing on the recovery of micro-organisms involved in bacteraemia. Animal and human studies have shown that the recovery rate of micro-organisms from transient bacteraemia peaks when samples are taken 30 seconds after the commencement of treatment (Silver et al. 1975; Roberts et al. 1992). Thus, in order to optimise the detection rate for bacteraemia in the present study, the second blood samples were collected 30 seconds after the commencement of scaling. The finding that more isolates were recovered in the third blood samples (two minutes) than in the second blood samples (30 seconds) suggested that the bacteraemia had peaked towards the end of the scaling procedure rather than at 30 seconds following the commencement of scaling. It is possible that the bacteraemia may have peaked after the final sample was taken, but this cannot be verified. The peak bacteraemia may occur at different time points in different patients but only a continuous sampling technique could verify this. It is possible that the peak bacteraemia in scaling occurs at different times to that of a tooth extraction, as determined by Roberts and co-workers (1992). Extractions result in an explosive rupturing of tissue whereas scaling possibly causes tissue damage at a slower, more progressive rate. In order to determine the peak bacteraemia induced by scaling procedures, a similar study to that of Roberts and co-workers (1992) needs to be performed, using scaling as the treatment modality in the place of extractions.
As a bacteraemia may be produced at any time during manipulation of the gingival tissues (Carranza 1979), it may be preferable to measure transient bacteraemia by sampling continuously from the commencement of scaling and for up to two minutes after the completion of scaling. The lysocentrifugation technique does not lend itself well to situations where multiple blood samples are to be processed. The most rapid sample collection technique using a cannula in a peripheral vein yields a 10 mL sample, enough to fill one lysocentrifugation tube, approximately every 15 seconds. If a continuous technique were used for the present study where blood was sampled during the scaling procedure and for two minutes after at a rate of one sample per 15 seconds, each volunteer would have generated 16 blood samples yielding a total of 160 ml of blood. This would then have required the processing of nearly 50 bacterial culture plates per patient, the time involved and expense of which would have been prohibitive. It is unlikely that a protocol requiring the drawing of 160 ml of blood for testing from each patient would gain ethical approval. Further research is required to determine the peak recovery period for bacteraemia in human subjects related to a variety of dental procedures, in order to refine the timing of the blood sampling for studies related to the reduction of bacteraemia.

The blood samples were obtained using a venepuncture technique in which aseptic conditions were employed whereby isopropyl alcohol wipes were used to disinfect the skin before insertion of the cannulae. Seventy per cent alcohol was used as it has been shown to be more effective than povidone-iodine when used as a skin preparation, with respect to the reduction of cannula related bacteraemia (Maki et al. 1991). Heparinised saline was used to flush the intravenous cannulae between blood samples in order to minimise the risk of cannula contamination, since heparin acts by
reducing thrombus formation in the lumen of cannulae, which is a predisposing factor to bacterial colonization (Randolph et al. 1998).

Had a cannula been contaminated by a blood sample containing micro-organisms, the subsequent samples may have contained micro-organisms that were not circulating in the bloodstream, but were dislodged from the lumen of the cannula as the sample was drawn. This effect would create false positives for bacteraemia. The incidence of cannula-related bacteraemia, which is defined as cannula colonization by bacteria already in the bloodstream, may be reduced by Heparin flushes (Randolph et al. 1998). This study was based on a meta-analysis of randomised controlled trials involving central venous and arterial cannulae where they remained in situ for much longer than those used in the present study. Therefore, it is possible that the incidence of cannula-related bacteraemia may have been lower in the present study.

The incidence of bacteraemia was 33% in the saline group and 10% in the povidone-iodine group. This means that the povidone-iodine group experienced a bacteraemia incidence that was 70% less than that experienced by the saline group. Regression analysis showed that the odds ratio for a bacteraemia following rinsing with povidone-iodine was 0.189 (95% C.I. O.R.= 0.043 - 0.827), which means that povidone-iodine was approximately 80% effective in reducing bacteraemia under the conditions of this study. The Null Hypothesis (which stated that rinsing with povidone-iodine mouthwash for two minutes before scaling had the same effect with regards to reducing the incidence of bacteraemia as rinsing with saline) was therefore rejected. It is difficult to claim that these findings are clinically significant in isolation. However, if in addition to the effect povidone-iodine had on the incidence
of bacteraemia, the magnitude of bacteraemia recovered from the individual patients had also decreased, povidone-iodine would then have had a more significant effect on iatrogenic bacteraemia. If povidone-iodine was also shown to be effective against micro-organisms that are commonly found in association with infective endocarditis, then it would suggest that povidone-iodine may be useful in preventing infective endocarditis.

There are no studies dealing specifically with bacteraemia due to scaling with an ultrasonic scaler in patients with gingivitis, with which to compare the level of bacteraemia in the saline group. Roberts and co-workers (1997) reported a bacteraemia incidence of 24.5% in paediatric patients following tooth polishing and a 40% bacteraemia incidence following scaling with a sonic scaler. However, it is not known whether these patients had periodontitis or gingivitis. Two minutes of scaling were found to produce a bacteraemia in 9.5% of those with gingivitis and in up to 42% of periodontitis patients (Winslow & Kobernick 1960), although many of the isolates may have been skin contaminants, as large numbers of *S. aureus* isolates were recovered. It is not possible to make a reliable comparison between the present study and that of Witzenberger and co-workers (1982), as the latter study did not identify the bacterial isolates recovered. The studies in this area show considerable variation in bacteraemia incidence, which may be due in part to factors such as the use of differing blood culturing techniques and failure to consider the presence of skin contaminants amongst the isolates recovered. Furthermore, some studies fail to take into consideration the effect of varying periodontal pocket depths on exposure of the plaque to the mouth rinse solution, which has been shown to be variable where periodontal pockets exist (Pitcher et al. 1980).
The saline group bacteraemia incidence of the present study was 33%, which contrasts with the findings of Reinhardt and co-workers (1982), who reported that the incidence of bacteraemia in patients undergoing ultrasonic scaling was 51.7%. However, there was no information regarding the periodontal status of the patients in that study. There are no studies reporting on bacteraemia resulting from the hand scaling of gingivitis patients who have used saline as a control, which would allow a direct comparison with the bacteraemia rate from ultrasonic scaling in patients with plaque-induced gingivitis. However, in a study consisting of patients with moderate to advanced periodontitis with pockets 4-6 mm deep, scaling with an ultrasonic scaler resulted in a bacteraemia rate of 61% from blood cultures obtained five minutes after completion of scaling (Baltch et al. 1982). However, no mouth rinse was given prior to scaling and the duration of the cleaning procedure and the number of teeth cleaned was not stated.

It may not be possible to extrapolate the results of this study to infer what is likely to happen with hand scalers and curettes. The incidence of bacteraemia in the control group (no rinsing) of the Witzenberger and co-workers (1982) study, which involved the hand scaling of patients with periodontitis, was 55%. This is the better of the two hand scaling studies involving a povidone-iodine solution with which to compare the present study, the other study being that of Randall and Brenman (1974). The incidence of bacteraemia in the Witzenberger and co-workers (1982) study appears significantly higher than that of the present study. However, Witzenberger and co-workers (1982) did not identify the bacterial isolates to species level, to enable exclusion of bacteraemia due to skin contaminants from their incidence calculations.
Therefore, it is possible that the apparent difference between this study and the present study is not significant.

All micro-organisms recovered from positive blood cultures were identified to species level. They were classified as oral isolates if they were considered to have originated from the oral cavity or were otherwise classified as contaminants.

Viridans Group Streptococci accounted for 13 of the 24 oral isolates (54.2% of isolates) recovered from the saline group patients. This result correlates well with bacteraemia studies relating to other dental procedures. Viridans Group Streptococci were found to be the most commonly recovered species (45% of isolates) following probing in patients with periodontitis (Daly et al. 1997). A study investigating dental extractions and intra-ligamentary injections showed that the incidence of bacteraemia involving Viridans Group Streptococci amongst the control group was 32.5% (Rahn et al. 1995). Of the remaining 11 isolates recovered from the saline group patients in the present study, ten were anaerobes. Given that a large proportion of the isolates recovered from the saline group were anaerobic species (41.6% of isolates) and that the lysocentrifugation technique is less sensitive at detecting anaerobic species, it is possible that this technique may have under-represented the incidence of bacteraemia. However, the total proportion of isolates appears to be consistent with other oral bacteraemia studies using broth culture techniques (Daly et al. 1997), which have been shown to be the most sensitive for recovering Streptococci spp.

There were no Viridans Group Streptococci recovered from those who rinsed with povidone-iodine. The results of the present study are in agreement with those of Rahn
and co-workers (1995), who also found that povidone-iodine rinses reduced the incidence of bacteraemia involving Viridans Group Streptococci. In that study, there were 13 isolates of Viridans Group Streptococci recovered from the control group whereas in the group that underwent sulcus irrigation with povidone-iodine, there were only four Viridans Group Streptococci isolates recovered. Taken together, the findings of the present study and those of Rahn and co-workers (1995) are significant since Viridans Group Streptococci are the most common isolates from patients with native valve endocarditis and have been shown to be commonly associated with cases of late prosthetic valve endocarditis (Otaki 1994). Viridans Group Streptococci have also been isolated from 29% of cases of infective endocarditis between 1979 and 1992 at Westmead Hospital, Sydney, Australia (Dwyer et al. 1994). If povidone-iodine is truly effective against Viridans Group Streptococci, then it may be of value in the prevention of infective endocarditis.

The lysis centrifugation (lysocentrifugation) technique was used for processing of the blood samples because this is a semi-quantitative culture technique, which enabled the magnitude of bacteraemia to be measured. Lysocentrifugation is a technique that aims to preserve the viability of the micro-organisms recovered by protecting them from the effects of microbicidal serum factors and any systemically administered antibiotics (Henry et al. 1983). Sodium polyanetholesulfonate was added by the manufacturers to the Oxoid Wampole lysocentrifugation tubes which were used in the present study, to increase the survival of Streptococci spp. (Shanson et al. 1995). The lysocentrifugation technique is less sensitive for the recovery of anaerobic microorganisms (Henry et al. 1983). However, lysocentrifugation is considered overall the
most sensitive semi-quantitative culture technique for the recovery of bacteria in the bloodstream (Lockhart 2000).

Apart from Viridans Group Streptococci, most of the isolates identified are constituents of dental plaque in patients with gingivitis (Moore & Moore 1994). The three bacterial isolates recovered from the povidone-iodine group patients included *Enterobacteriaceae* spp. and *Moraxella* spp. (possibly *M. catarrhalis*). *Moraxella* spp. can cause sinusitis and otitis media but rarely cause infective endocarditis (Douer et al. 1977). *Moraxella* spp. are known constituents of dental plaque, in particular *M. catarrhalis* (Moore & Moore 1994). *Enterobacteriaceae* spp. are Gram-negative bacilli commonly found in the genitourinary system. *Enterobacteriaceae* spp. are recognised as an increasingly important cause of nosocomial infection. However, these micro-organisms have only been responsible for a few cases of infective endocarditis (Weinstein & Brusch 1996b).

The most likely source of those micro-organisms considered as contaminants would have been from the venepuncture site or from the laboratory processing procedure (Henry et al. 1983). An aseptic venepuncture technique was used to minimise contaminants from the cannula site and the blood cultures were processed in a laminar flow cabinet, which is likely to have significantly reduced the contamination rate (Thomson et al. 1984). Some of the species that were classed as contaminants are also found in dental plaque, such as *P. acnes*, *Corynebacterium* spp. and *S. epidermidis*. In order to make the results for bacteraemia incidence less equivocal, these species were classed as ‘non-oral’ contaminants. Therefore, it is possible that the incidence of bacteraemia may have been understated in both mouth rinse groups,
in particular the bacteraemia incidence in the saline group. The isolates classed as contaminants in the povidone-iodine group (Coagulase Negative Staphylococci spp., *Oiccha monray, S. epidermidis*) in general are more easily regarded as skin or laboratory contaminants and are less likely to be found in plaque than those classed as contaminants in the saline group (*P. acnes, Corynebacterium* spp., *S. epidermidis, Candida* spp.).

Coagulase Negative Staphylococci spp. was the most prevalent group of micro-organisms judged to be contaminants, with six isolates in the povidone-iodine group and five isolates and one mixed colony in the saline group. They were the most frequently isolated micro-organisms from baseline blood samples. These micro-organisms are rarely found in dental plaque (Moore & Moore 1994) but are commonly found on the skin.

Isolates of *P. acnes* were recovered (one isolate and one mixed colony in the saline group), which are frequent blood culture contaminants and usually inhabit the skin and rarely cause infective endocarditis (Hernandez et al. 1988). *P. acnes* was therefore regarded as a contaminant. However, *P. acnes* has been found to be a constituent of dental plaque in health and disease (Moore et al. 1987) and therefore the incidence of bacteraemia in the saline group may have been underestimated by not including *P. acnes* as a positive culture. Similarly, both *Corynebacterium* spp. and *S. epidermidis* were classified as contaminants. Although both micro-organisms are commonly found on the skin, they have both been recovered from dental plaque (Moore & Moore 1994). *S. epidermidis* is known to be a frequent contaminant recovered using the lysocentrifugation technique (Henry et al. 1983).
There were 12 isolates recovered from eight patients in the povidone-iodine group that were classed as ‘non-oral’ contaminants and 14 isolates from 11 patients in the saline group classed as ‘non-oral’ contaminants. Since the numbers of contaminants were similar for both mouth rinse groups, this would support the argument that the species classed as “non-oral” were not from the oral cavity, as the “background count” of skin and laboratory contaminants would be expected to be similar in both groups. Kiehn and co-workers (1983) found that in their laboratory, the contamination rate for the lysocentrifugation procedure ranged from 1.6 to 12.8% from month to month. However, these authors did not use a laminar flow cabinet for processing of the blood samples.

In addition to the incidence of bacteraemia and identification of the recovered species, another outcome that was measured was the magnitude of the bacteraemia, in terms of the total number of “oral” bacterial isolates recovered from the blood samples. The magnitude of bacteraemia was calculated for each patient, which was facilitated by the use of the semi-quantitative lysocentrifugation technique. The numbers of bacterial isolates recovered represent those in a 10 mL blood sample, the volume of a lysocentrifugation tube. Using a semi-quantitative culture technique such as lysocentrifugation enables the calculation of the approximate concentration of micro-organisms in the blood. However, the numbers of oral micro-organisms recovered from patients positive for bacteraemia were too few to permit whole numbers for CFU/mL to be presented. Therefore, only the number of isolates recovered per patient was reported and this was used for the purposes of determining the magnitude of bacteraemia.
If it is assumed that the blood samples were a representative sample of the circulating peripheral blood at a given point in time, then one isolate in a 10 mL sample tube would represent a bacteraemia concentration of 100 micro-organisms per litre of blood. Given that a person’s blood volume in litres is about 10% of their body weight, a 70 kg person would have a blood volume of approximately seven litres. Therefore, one isolate recovered in a 10 ml lysocentrifugation tube could represent a bacteraemia involving approximately 700 micro-organisms in the circulation at that point in time. It would be increasingly difficult to detect a bacteraemia once the total number of micro-organisms in the bloodstream falls below 700, or one organism per 10 ml of blood if 10 ml samples were taken. This would decrease the chance of obtaining a representative sample of blood. Roberts and co-workers (1992) attempted to address the problem of false negatives for bacteraemia that can result from relatively small blood sample volumes, by doubling the volume of blood sampled. However, Roberts and co-workers (1992) found that doubling the sample volume to 16 ml only increased the detection rate of bacteraemia by 1.9%, from a baseline of 39% for the 8 ml samples. This may indicate that false negatives are less of a problem and that blood samples are highly representative of the bacterial concentration in the peripheral circulation. Alternatively, it may indicate that a much larger volume of blood needs to be sampled in order to detect and determine reliably the magnitude of bacteraemia.

The magnitude of bacteraemia is difficult to measure accurately when only small numbers of micro-organisms are involved. The number of isolates per millilitre of blood therefore would be best used as a relative measure of the magnitude of
bacteraemia. In the present study, the weight of the patients was not recorded. It is possible that a bacteraemia of given magnitude may be detected more easily in a person of lower body mass (smaller blood volume, therefore greater concentration of bacteria in the blood). However, this effect has not been verified and has never been taken into account in oral bacteraemia studies.

In the saline group, 22 isolates were recovered from 10 subjects. In the povidone-iodine group, three isolates were recovered from the three povidone-iodine group patients. A semi-quantitative culture technique was used in order to calculate the concentration of bacteria (or the magnitude of the bacteraemia) isolated in the blood samples. It was not possible to compare the two mouth rinse groups based on the magnitude of the resulting bacteraemia, as the numbers of patients experiencing a bacteraemia in both groups were too small to permit statistical analysis. However, there appears to be a trend towards a reduced magnitude of bacteraemia in the povidone-iodine group. In the saline group, four of the ten patients positive for bacteraemia produced more than one ‘oral’ bacterial isolate, with patient 7 producing nine isolates and patient 6 producing five isolates. However, in the povidone-iodine group, the three patients positive for bacteraemia produced only one ‘oral’ bacterial isolate each.

The magnitude of bacteraemia may be as important as the incidence of bacteraemia with respect to the potential for precipitating infective endocarditis in humans, as has been observed in animal studies, where a large inoculum of micro-organisms is required to produce infective endocarditis (Glauser & Francioli 1987). The present study is the first that attempts to quantify the magnitude of bacteraemia with respect
to quantifying bacteraemia reduction from pre-treatment rinsing with an antimicrobial solution. However, greater numbers of patients would need to be tested to permit statistical analysis of the effect on bacteraemia magnitude.

Two patients in the saline group (patients 6 and 7) experienced poly-microbial bacteraemia from “oral” isolates recovered. However, bacteraemia from single species only was recovered from the povidone-iodine group patients. It has been shown that poly-microbial cultures are recovered more frequently when lysocentrifugation is used to process blood samples (50% increase in detection rate) compared to non-vented tryptic soy broth bottles (Henry et al. 1983). Poly-microbial infection has been shown to increase significantly the morbidity associated with infective endocarditis (Sandre & Shafran 1996). If povidone-iodine is effective in reducing the incidence of poly-microbial bacteraemia then the incidence of poly-microbial infective endocarditis may be reduced.

The average time taken to process the blood samples (plating time) was 188 minutes for the saline group and 156 minutes for the povidone-iodine group. The difference in plating times was not statistically significant and hence both treatment groups were homogeneous with respect to the processing times of the blood samples. The manufacturers of the Isolator™ lysocentrifugation system used, recommend that specimens are stored in the sample tubes for no longer than 16 hours before processing. This ensures that the reagents in the tubes, which are designed to lyse blood cells, do not start to inactivate any micro-organisms recovered in the blood sample. Since the average plating times were well within the manufacturer’s
recommendations, the plating time was unlikely to have affected recovery of the bacteraemia isolates.

In addition to investigating the incidence and microbiology of bacteraemia in the study groups, clinical and social data were investigated for any association with a positive bacteraemia. The study protocol was designed so that non-invasive clinical indices were used where possible, to assess periodontal parameters (PII, mPMAI). Regression analysis was then used to determine whether any clinical parameters could be used to assess the likelihood of inducing a bacteraemia by scaling teeth. If it were possible to predict the likely occurrence of a bacteraemia by assessing these clinical parameters, such information would be useful to the clinician when deciding on prophylactic measures for patients at risk of infective endocarditis. The clinical measurements that such a decision would be based on should not in themselves induce a bacteraemia. It was therefore decided to use a visual index of inflammation (mPMAI, after Schour & Massler 1947) rather than to use the Gingival Index (Löe & Silness 1963), which involves probing the marginal gingiva to assess the propensity for gingival bleeding. Care was taken during PII measurements not to contact the gingiva with the probe and not to unduly disturb plaque that was adhering to the surfaces of the teeth. Since periodontal probing has been shown to cause bacteraemia (Daly et al. 1997), probing depths could not be used as indicators for potential bacteraemia in patients requiring antibiotic prophylaxis. Procedures related to the recording of clinical parameters that may have produced a bacteraemia (measurement of recession and probing depths), were performed after collection of the final blood samples.
Patient means were used for the clinical parameters. Baelum and co-workers (1986) pointed out the drawbacks of using patient means to summarise data for clinical measurements in patients with periodontitis, as important data relating to the deeper pockets is likely to be lost, leading to under-estimation of disease severity. In the present study, patient means could have "hidden" periodontal pockets that may harbour plaque that is not likely to be exposed to the mouth rinse solutions. However, patients with plaque-induced gingivitis exhibit less variation in probing depths than patients with periodontitis with probing depths usually being three millimetres or less.

Regression analysis was used to test variables, in terms of clinical and patient data, that may have been predictive for a bacteraemia. Of the social and clinical variables entered into the regression model (age, gender, smoking, PII, mPMAI, probing depths, bleeding on instrumentation, recession, plating time, mouth rinse group), the strongest factor to correlate with the bacteraemia outcome was the mouth rinse group. Rinsing with povidone-iodine had a statistically significant negative correlation with the incidence of bacteraemia.

Of the variables correlated with bacteraemia, the only other variable that had a statistically significant correlation with bacteraemia was the age of the patients. For every ten-year increase in the age of the patients, the odds ratio for the recovery of an iatrogenic bacteraemia increased by 1.4. The odds ratio was statistically significant (95% C.I. O.R. 1.00 - 1.97). This effect may be of concern for dentists treating elderly patients, as it implies that for a given dental procedure, elderly patients are more likely to be exposed to a bacteraemia. In addition, epidemiological studies of infective endocarditis have shown that it is a disease with higher incidence amongst
the elderly. The median age for infective endocarditis patients in a large study was 60-70 years (Watanakunakorn & Burkert 1993). However, age as a predictive factor for bacteraemia does not appear to have a high level of specificity.

Patient 7, who was 83 years of age and the oldest patient in the saline group, produced nine bacterial isolates, the highest number of isolates from a single patient in the study. However, of the four next oldest patients in the saline group, (patient 32 was 80 years old; patient 52 was 79 years old; patient 55 was 75 years old and patient 45 was 71 years old), three were negative for bacteraemia and one, patient 52, produced one bacterial isolate. Therefore, only two of the five oldest patients in the saline group were positive for bacteraemia. Of the bacteraemia positive patients in the povidone-iodine group (patients 27, 58 and 59), the more elderly patients were better represented. Patient 27 was 39 years of age, less than the average age of 45.9 years for this group. The other two bacteraemia positive patients in the povidone-iodine group were patient 58 who was 79 years of age and patient 59 who was 63 years of age.

The elderly have been shown to experience an increased incidence of bacteraemia in several dental bacteraemia studies. An early study investigating the factors influencing the occurrence of bacteraemia resulting from oral surgical procedures found that younger and older patients were more likely to experience bacteraemia (Lazansky et al. 1949). Patients less than 30 years of age were grouped with those 60 years of age or above. This composite group was compared to those patients 30-59 years of age. A greater incidence of bacteraemia was found in the younger and older age groups, which was statistically significant. Chi square values only were quoted.
and these ranged from 4.63 to 9.41 depending on the oral surgical procedure performed. Okabe and co-workers (1995) identified factors associated with bacteraemia resulting from dental extractions. Bacteraemia was detected in 42.9% of patients less than 20 years of age, whereas 86.8% of patients over 60 years of age experienced a bacteraemia. When the study patients were grouped according to whether they experienced a bacteraemia or not, there was a statistically significant difference in the mean age of the patients in both groups. The mean age of the bacteraemia positive group was 44.3 years and the mean for the bacteraemia negative group was 32.9 years.

If age were shown to be a sensitive and specific indicator for the occurrence of bacteraemia resulting from dental treatment, then a threshold age may be identifiable above which it would be necessary to give antibiotic cover. Since this is not the case, it is not feasible to restrict antibiotic prophylaxis to elderly patients.

Bleeding on instrumentation, which has been defined in this study as bleeding resulting from ultrasonic scaling, was the only parameter to show a tendency to be dissimilar between the two treatment groups. However, the difference was not statistically significant. Bleeding on instrumentation, in addition to bleeding on probing, fits the criteria for “Bleeding on Provocation”, as defined by the American Academy of Periodontology (Mariotti 1999). Bleeding on provocation is recognised as a clinical characteristic of plaque-induced gingivitis (Mariotti 1999).

Bleeding has been considered an indicator that a bacteraemia may have occurred since it shows that the epithelial barrier has been breached, allowing micro-organisms
access to the underlying tissues and subsequently to the bloodstream. The likelihood of bleeding occurring during a dental procedure is currently used by the American Heart Association as the basis for deciding whether antibiotic prophylaxis should be given (Dajani et al. 1997). However, the importance of gingival bleeding in the development of bacteraemia has not been documented scientifically, in terms of a positive or negative correlation between gingival bleeding and bacteraemia (Lockhart 2000). If the degree of bleeding that results from a dental procedure is a determining factor in the occurrence of bacteraemia, then the effect of povidone-iodine on the resulting bacteraemia incidence has been shown by this study to have a more important over-riding effect on the incidence of bacteraemia than bleeding on instrumentation. There was a tendency for a greater degree of bleeding on instrumentation in the povidone-iodine group, but the incidence of bacteraemia was significantly less in the povidone-iodine group. Logistic regression did not show that bleeding on instrumentation was predictive for bacteraemia in this study.

It has been shown that the gingival tissues of smokers have a reduced tendency to bleed following dental manipulations (Preber & Bergstrom 1985). It is commonly thought that the occurrence of bacteraemia is related to the degree of bleeding induced by a dental procedure (Dajani et al. 1997), which is the principal factor taken into consideration when deciding on the need for antibiotic prophylaxis for a given dental procedure. If the occurrence of bacteraemia was dependent on the degree of bleeding only, smokers may be expected to show a decreased incidence of bacteraemia. However, this effect was not reflected in this study by a negative correlation between smoking status and the incidence of bacteraemia. It is possible that the occurrence of bacteraemia is related primarily to the degree of epithelial denudation resulting from
trauma, which results in less bleeding in smokers but still allows micro-organisms a similar degree of access to the bloodstream. It was not possible to verify the effect of smoking on the incidence of bacteraemia by comparing the bacteraemia incidence between smokers and non-smokers in the saline group, given the low numbers of study participants in these categories.

Any possible effect of povidone-iodine pre-treatment mouth rinses on the incidence of infective endocarditis cannot be determined without a population-based study. A bacteraemia is required to precipitate infective endocarditis in a patient predisposed to development of infective endocarditis (Bansal 1995). Therefore, the observation that povidone-iodine mouth rinses were 80% effective in reducing bacteraemia and that the recovery of Viridans Group Streptococci was diminished, may indicate that pre-scaling povidone-iodine mouth rinses could be useful in helping to prevent infective endocarditis related to dental procedures in patients with plaque-induced gingivitis. However, povidone-iodine did not eliminate transient bacteraemia from scaling completely and for this reason, it is unlikely that povidone-iodine mouth rinses would replace antibiotic prophylaxis for infective endocarditis. Since the administration of prophylactic antibiotics does not prevent viable micro-organisms from gaining access to the blood stream during invasive dental procedures (Hall et al. 1993), povidone-iodine, which has been shown to reduce the incidence of transient bacteraemia from dental scaling, may be a valuable adjunct to existing antibiotic prophylaxis regimes. It is possible that povidone-iodine mouth rinses will complement the action of prophylactic antibiotic cover, reducing the load of micro-organisms that enter the blood stream, thereby improving the efficacy of antibiotic cover. Povidone-iodine rinses may reduce the number of failures related to antibiotic cover (Durack et al.
1983), as 75% of the failure cases in that study were due to Viridans Group Streptococci and alpha-haemolytic Streptococci spp., which povidone-iodine was found to effectively eliminate in the present study.

Random bacteraemia and bacteraemia due to home care procedures are possibly more important in the development of infective endocarditis than bacteraemia resulting from dental treatment (Guntheroth 1984). However, the bacteraemia studies that Guntheroth's (1984) assertions are based upon are equivocal and should be repeated using modern blood sampling and culturing techniques. It is possible that povidone-iodine may have more use in the home, to be used as a pre-rinse before carrying out home-care procedures that have also been shown to produce transient bacteraemia (Romans & App 1971; Sconyers et al. 1973). However, these home-care bacteraemia studies need to be repeated using modern culture techniques in order to quantify the resulting bacteraemia and to verify that the micro-organisms recovered originated from the oral cavity. Updating these studies could validate the assumptions underlying the calculations of exposure times to 'random' and dentally induced bacteraemia (Guntheroth 1984). In addition, updated studies could verify that home care procedures are capable of inducing bacteraemia that may contribute to infective endocarditis.

It has been shown that solutions used for oral rinsing are unlikely to penetrate into the gingival sulcus effectively. Pitcher and co-workers (1980) were able to demonstrate that following rinsing with a dye solution, only the plaque in the coronal 0.2 mm of the gingival sulcus had been stained by the dye. This suggests that antimicrobial solutions are unlikely to be effective against subgingival plaque, since the flow of
gingival crevicular fluid is likely to flush any povidone-iodine out of the pocket. Studies investigating the use of povidone-iodine solutions in patients with periodontitis have produced variable results with respect to the reduction of bacteraemia (Winslow & Millstone 1965; Witzenberger et al. 1982). It is possible that plaque within periodontal pockets provides the micro-organisms that become the source of bacteraemia in patients with periodontitis. However, supragingival plaque may provide this source in plaque-induced gingivitis. This may explain the significant reduction in incidence of bacteraemia in the present study, as the plaque that could have provided a source of micro-organisms for bacteraemia may have been exposed to the povidone-iodine mouth rinse.

There are no studies that document accurately the pharmacokinetic properties of povidone-iodine in the oral cavity and relate the clearance time of povidone-iodine to the time it takes for povidone-iodine to neutralise supragingival plaque microorganisms. Povidone-Iodine has not been recommended for use as an anti-plaque antimicrobial on the basis that it does not have substantivity (Addy & Wright 1978). Lack of substantivity may not be a disadvantage if an adequate concentration of povidone-iodine exists in the oral cavity to neutralize plaque bacteria before it is cleared from the oral cavity. Substantivity is important in terms of prolonging the plaque inhibiting effect of the antimicrobial for long term benefit. If it is necessary only to suppress plaque for the length of a dental appointment, then substantivity may not be a relevant property for a possible adjunctive topical prophylactic antimicrobial such as povidone-iodine.
There are no published studies of *in vivo* experiments that test the effect of rinsing with povidone-iodine on natural plaque biofilm. It would be possible to conduct such a study using confocal scanning laser microscopy with vital staining techniques, that enable visualisation of the micro-organisms that survive and those which have been killed (Pan et al. 2000). A study such as this would provide valuable evidence supporting the effect of povidone-iodine on plaque biofilm in the presence of saliva and would help to corroborate the results of this study.

At present, blanket antibiotic cover of all persons considered at high risk of contracting infective endocarditis from dental procedures resulting in bleeding is advocated (Dajani et al. 1997). This approach is necessary as it is not possible to identify the patients who are likely to develop bacteraemia from dental treatment, based on general factors such as age and gender or clinical observations relating to the health of the oral tissues. Thirty three per cent of the patients in the saline group developed a bacteraemia resulting from scaling, but no clinical or social parameters were identified that could be used reliably to indicate which patients would be likely to develop bacteraemia. Thus, it is necessary to administer prophylactic antibiotics to a large number of patients.

In some patient groups, such as those with mitral valve prolapse, it is not likely to be cost-effective to administer antibiotic cover for the prevention of infective endocarditis (Pallasch & Slots 1996). However, it may be cost effective at the population level to administer povidone-iodine as a prophylactic measure aimed at reducing the incidence of infective endocarditis in this group of patients. For patients such as those with mitral valve prolapse, considered at relatively low risk of
contracting infective endocarditis, povidone-iodine rinses alone may provide a sufficient level of prophylactic cover. At present, a bottle of povidone-iodine gargle costs approximately $10 and is sufficient to treat 20 patients. In contrast, antibiotic cover costs the patient approximately $20, the cost of a prescription, to provide antibiotic cover for one visit to the dentist. Although povidone-iodine has not been shown to eliminate bacteraemia, it may reduce bacteraemia and in particular bacteraemia due to Viridans Group Streptococci to a degree that might give a significant degree of protection against infective endocarditis in this group of patients. The cost of povidone-iodine would allow patients to be treated on a large scale.

It has been shown that even subgingival irrigation does not reliably deliver irrigant solutions to the apical extension of subgingival plaque in periodontal pockets (Pitcher et al. 1980) and therefore an antimicrobial mouth rinse is not likely to be effective for patients with chronic periodontitis. Since patients with periodontitis account for only 15% of the population (Brown et al. 1990), povidone-iodine mouth rinses may be potentially useful for the majority (85%) of the population who do not have periodontitis, as an adjunct to antibiotic prophylaxis against infective endocarditis. However, the proportion of patients requiring antibiotic cover who also have periodontitis is not known.
8. CONCLUSION

It has been shown that two one-minute pre-treatment 0.375% povidone-iodine mouth rinses were effective in reducing the incidence of bacteraemia resulting from dental scaling by approximately 80%, in patients with gingivitis. Furthermore, Povidone-Iodine mouth rinses were shown to eliminate the recovery of Viridans Group Streptococci, which are commonly associated with infective endocarditis lesions. A trend towards a decreased magnitude of bacteraemia in the povidone-iodine group was also evident.

On the basis of these results, it is therefore recommended that two one-minute pre-treatment rinses with 0.4% povidone-iodine may be used as an adjunct to prophylactic antibiotic cover in patients with plaque-induced gingivitis who are considered at risk of developing infective endocarditis and who require ultrasonic scaling. However, the use of povidone-iodine mouth rinse would not be recommended for patients with thyroid disease, known allergy to iodine or for pregnant females.
9. APPENDIX

Appendix 1

The Duke criteria for the clinical diagnosis of Infective Endocarditis (Durack et al. 1994).

I. Definite Infective Endocarditis. Two major criteria or one major and three minor criteria or five minor criteria.

A. Major Criteria.

1. Isolation of Viridans streptococci, S. bovis, HACEK group organisms, or (in the absence of a primary focus) community acquired S. aureus or Enterococcus spp. from two separate blood cultures or isolation of a microorganism consistent with endocarditis in (1) blood cultures ≥12 hours apart or (2) all of three or most of four or more blood cultures, with first and last at least one hour apart.

2. Evidence of endocardial involvement on echocardiography: oscillating intracardiac mass or abscess or new partial dehiscence of prosthetic valve or new valvular regurgitation.

B. Minor Criteria.

1. Predisposing lesion or intravenous drug use.
2. Fever of ≥38.0°C
3. Major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial haemorrhage, conjunctival haemorrhages or Janeway lesions.
4. Glomerulonephritis, Osler’s nodes, Roth’s spots, rheumatoid factor.
5. Positive blood cultures not meeting the major criterion (excluding single cultures positive for organisms that do not typically cause endocarditis) or serologic evidence of active infection with an organism that causes endocarditis.
6. Echocardiogram consistent with endocarditis but not meeting the major criterion.
II. Possible Infective Endocarditis. Findings that fall short of "definite" but do not fall into the "rejected" category.

III. Rejected. Alternative diagnosis or resolution of the syndrome or no evidence of Infective Endocarditis at surgery or autopsy with ≤ four days of antibiotic therapy.
CONSENT TO PARTICIPATE IN RESEARCH

Title of Research Project:

Name of Researcher:

1. I understand that the researcher will conduct this study in a manner conforming with ethical and scientific principles set out by the National Health and Medical Research Council of Australia and the Good Clinical Research Practice Guidelines of the Therapeutic Goods Administration.

2. I acknowledge that I have read, or have had read to me the Participant Information Sheet relating to this study. I acknowledge that I understand the Participant Information Sheet. I acknowledge that the general purposes, methods, demands and possible risks and inconveniences which may occur to me during the study have been explained to me by _______________ (the researcher) and I, being over the age of 16 years or over the age of 14 years but under the age of 16 years (delete as applicable), acknowledge that I understand the general purposes, methods, demands and possible risks and inconveniences which may occur during the study.

3. I acknowledge that I have been given time to consider the information and to seek other advice.

4. I acknowledge that refusal to take part in this study will not affect the usual treatment of my condition.

5. I acknowledge that I am volunteering to take part in this study and I may withdraw at any time.

6. I acknowledge that this research has been approved by the Western Sydney Area Health Service Human Research Ethics Committee.

7. I acknowledge that I have received a copy of this form and the Participant Information Sheet, which I have signed.

8. I acknowledge that sponsoring pharmaceutical companies and any regulatory authorities may have access to my medical records to monitor the research in which I am agreeing to participate. However, my identity will not be disclosed to anyone else. (Please delete this paragraph if not applicable)

Name of Participant _____________________________________________________________

Address _______________________________________________________________________

Signature of participant (refer below for definition) ___________________________________

Signature of Researcher _________________________________________________________

Signature of Witness ____________________________________________________________

Definition of Participant
This may only be signed by:
1. Participants over 16 years of age; or
2. Participants between the age of 14 and 16 years together with the signature of their parent or guardian
3. Parent or guardian of participants under 14 years
4. Where patient or participant has a medical or legal disability then signature must be that of
   (a) The legal guardian; or
   (b) Spouse or de facto spouse; or
   (c) Caregiver [refer (a) below]; or
   (d) The Guardianship Board (Telephone 02 9555 8500)
   (e) Family member or friend but not a professional caregiver (eg medical superintendent, director of nursing, nursing home director).
CONSENT TO PARTICIPATE IN RESEARCH

INDEPENDENT WITNESS/INTERPRETER:

Independent Witness:

I, __________________________ of __________________________

hereby certify as follows:

I was present when __________________________ (“the participant”) appeared to read or had read to him/her a document entitled Participant Information Sheet; or

I was told by __________________________ (“the participant”) that he/she had read a document entitled Participant Information Sheet

("Delete as applicable"

1. I was present when __________________________ (“the researcher”) explained the general purposes, methods, demands and the possible risks and inconveniences of participating in the study to the participant. I asked the participant whether he/she had understood the Participant Information Sheet and understood what he/she had been told and he/she told me that he/she did understand.

1. I observed the participant sign the consent to participate in research and he/she appeared to me to be signing the document freely and without duress.

1. The participant showed me a form of identification which satisfied me as to his/her identity.

1. I am not involved in any way as a researcher in this project.

1. (Delete this clause if not applicable) I was present when __________________________ (“the interpreter”) read the Participant Information sheet to the participant in the __________________________ (here insert appropriate language) language. I certify that when the researcher explained the general purposes, methods, demands and possible risks and inconveniences of participating in the study that what was said by both the researcher and the participant was translated by the interpreter from the English language into the __________________________ language and vice versa. When I spoke to the participant what I said and what the participant said was translated by the interpreter from the English language into the __________________________ language and vice versa.

Name of independent witness __________________________

Address ______________________________________

Signature of independent witness __________________________

Relationship to participant of independent witness __________________________

Page 2 of 3
CONSENT TO PARTICIPATE IN RESEARCH

Interpreter:

If an interpreter is used, the following addition is necessary –

I __________________________ (name of interpreter) of __________________________
certify as follows:

1. I am qualified to translate speech and writing from the English language into the
   __________________________ language and vice versa.

1. I read the Participant Information Sheet to the participant in the
   __________________________ language and he/she appeared to understand it.

1. I was present when the researcher explained the general purposes, methods, demands
   and possible risks and inconveniences of participating in the study to the participant and
   I translated all that was said by the researcher and by the participant from the English
   language into the __________________________ language and vice versa.

1. I was present when the independent witness spoke to the participant and I translated all
   that was said by the independent witness and by the participant from the English
   language into the __________________________ language and vice versa.

Signature of Interpreter __________________________ Date __________________________
Patient Information Sheet

**Title of research project:** The effect of Povidone-Iodine mouthwash on bacteraemia after the routine cleaning of teeth.

**Persons conducting the research:** The following staff members of the Dept. of Periodontics at Westmead Hospital Dental Clinical School, The University of Sydney and Westmead Hospital.

Dr M. Cherry: Registrar in Periodontics, Westmead Hospital Centre for Oral Health.
Postgraduate student in Periodontics, The University of Sydney.

A. Prof. C. Daly: Associate Professor, Discipline of Periodontics.
Faculty of Dentistry, The University of Sydney.

Dr D. Mitchell: Staff Specialist.
Dept. of Clinical Microbiology and Infectious Disease
Westmead Hospital.

A. Prof. B. Pearlman: Head of Department, Department of Periodontics.
Westmead Hospital Dental Clinical School.

Dr J. Highfield: Senior Lecturer in Periodontology, Faculty of Dentistry.
The University of Sydney.

**The purpose of the study:** Bacteria and other germs may enter the blood stream (bacteraemia) very frequently when teeth are cleaned or extracted. We are looking at whether the mouthwash that you will use is effective in reducing the number of bacteria that may enter the blood stream whilst teeth are being cleaned. This is harmless in normal healthy people, as we believe you to be. Only patients with heart valve defects or a few other cardiac conditions may be at risk of a heart valve infection (endocarditis). If the mouthwash is effective in this way, then it may help to prevent certain susceptible patients such as those with heart valve defects becoming ill from this exposure to bacteria during dental treatment.

**Who will be asked to participate in the study?** Volunteers will be sought from patients attending Westmead Hospital Dental Clinical School. Those volunteers invited to take part in the study should not have any underlying medical conditions that would affect the outcome of the study. They also should not have periodontal disease (advanced gum disease) or be sensitive to preparations containing iodine.
What will your involvement entail? Your involvement will entail attending for a single appointment, during which time your teeth will be cleaned and the blood samples will be obtained. **We would ask that you do not brush your teeth, chew gum or eat for about thirty minutes before this appointment.**

During the appointment, we will take blood samples from a vein in your arm using a very small needle. This procedure is very similar to that for a standard blood test, as your medical doctor may have arranged for you in the past. We will need to take a total of three blood samples at various stages during the appointment. By the end of the appointment, you will have given 30ml of blood, that is less than 10% of the blood volume that blood donors give each time they donate to the Red Cross. All blood samples will be taken from the same needle so that we are able to avoid inserting the needle again. The only adverse effects from taking the blood sample are a slight prick as the needle is inserted and occasionally there may be slight discomfort or even a little bruising.

The sequence of events is as follows:
- We will ask you to sign a consent form once you are fully aware of the study procedures and after any questions you may have are answered
- We will ask questions to determine your status of general health and well-being. We will examine and measure your gums and record our observations.
- We will insert a needle into your vein
- We will ask you to rinse for one minute with the given mouthwash
- We will take a blood sample
- We will then clean five of your teeth in the usual manner for 30 seconds
- Another blood sample will be taken
- We will clean your teeth for a further 90 seconds
- A final blood sample will be taken after 2 minutes of cleaning is completed
- If you decide not to volunteer for this study, this also will not affect your dental treatment in any way. You are free to stop the study and leave at any time.

We will look at the blood and count the numbers of bacteria in the samples. We will also determine the types of bacteria if we find any. We will only be looking for the types of bacteria we would expect to find. We will **NOT** be performing any unrelated tests such as blood cell counts, HIV or Hepatitis tests.

Page TWO of THREE: Patient Information.
The appointment should last approximately thirty minutes. Should you decide that you no longer wish to participate in the study at any time during the appointment, we shall of course respect your decision and stop the study. The blood results obtained will not be recorded in your Westmead Hospital patient file. Particulars relating to the study will be held by the above named researchers at Westmead Hospital and used for the purposes of research only. **Your patient confidentiality will be preserved at all times.**

We will ensure that you experience the minimum amount of discomfort during the appointment. The procedures performed within your mouth will be very similar to those encountered during the course of a routine dental check-up and clean. Should the mouthwash you will use stain your teeth, your teeth will be thoroughly cleaned before you leave our clinic. The mouthwashes we will be using are not new or experimental and are available in pharmacies without prescription.

Should you have any further questions before continuing with the study or after completion of your involvement, please phone the numbers given below and ask to speak to Dr Cherry.

Westmead Hospital Dept. of Periodontics: **02 9845 7837**  
Dr Cherry (After hours): **0417 960 893**

We would be very grateful for your assistance should you decide to participate in our study. Your participation in this study will not affect your ongoing or future dental treatment. Should you decide not to participate in our study, this will not affect your dental treatment in any way either.

**Page THREE of THREE: Patient Information.**
Appendix 3

ISOLATOR™ Tube Reagents

Each Oxoid Wampole ISOLATOR™ 10 tube contains the following reagents in 0.7 ml aqueous solution (content prior to sterilization).

- Purified Saponin: 28 mg/ml.
  Action: cell lysing agent, non-toxic to microorganisms.

- Polypropylene Glycol: 8 ml/L.
  Action: blocks the foaming action of saponin.

- Sodium Polyanetholesulphonate (SPS): 15.3 g/L.
  Actions: anticoagulant, neutralizes the antibacterial effects of blood, inhibits phagocytosis.
Appendix 4

Media Preparation.

Media were prepared as per the Westmead Hospital Institute of Clinical Pathology and Medical Research (ICPMR) Media Preparation Manual.

BHV PLATES

This medium is used as the basic non-selective medium for anaerobic organisms.

Formulation of Oxoid Columbia Agar Base (CM 331)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Special Peptone</td>
<td>23g/L</td>
</tr>
<tr>
<td>Starch</td>
<td>1g/L</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5g/L</td>
</tr>
<tr>
<td>Agar No 1</td>
<td>10g/L</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td><strong>7.3 ± 0.2</strong></td>
</tr>
</tbody>
</table>

Weigh out 234 g of Oxoid Columbia agar base and 30gm of Oxoid Yeast Extract and suspend in 6 litres of purified water in the vessel of a media bench sterilizer.

Add 6 mL of Vitamin K solution and 60mL of Haemin/Cysteine solution. (See below for the recipe for Vit K and Haemin/Cysteine).

The pH of the media should be 7.5 after the addition of the above, however this can only be checked when making 3 L batches in a flask as the sterilizer cannot be opened once started.

Autoclave at 121°C for 15 minutes with the pouring temperature set at 40°C. When the pouring temperature has been reached, aseptically add 300 ml of fresh horse blood to the vessel.
Dispense the medium into 90 mm Petri dishes with approx 20 ml per plate. Label each plate with the date of manufacture and “BHV”.

**Haemin/ Cysteine Solution.**

Weigh out 50 mg of haemin and 4 gm of cysteine; dissolve in 40 ml of IN NaOH. Make up to 100 ml with sterile distilled water.

**Haemin:** Final concentration required in the medium is 5 mg/l.
We have 50 mg in 100 mL = 0.5 mg/ml.
For 6 litres of BHV we need 30 mg, i.e.: 60 ml of the stock solution.

**Cysteine:** Final concentration required in the medium is 400 mg/l.
We have 4 g in 100 ml = 40 mg/ml.
For 6 litres of BHV we need 2400 mg, i.e.: 60 ml of the stock solution.

The stock solution should be made only as required as it is highly light sensitive and produces a better quality medium when prepared fresh. However, should there be a need to make up in advance, two lots of the stock solution can be prepared in a dark bottle and stored in the fridge for short periods (not exceeding one week).
Chocolate Agar Plate Preparation

The following describes the protocol for preparation of chocolate agar plates.

Weigh out 400gm of Oxoid Columbia Base Agar powder (CM331, as used in the preparation of BHV plates). Note the batch number and brand in the media room daybook.

Measure out 10 litres of distilled water.

Combine the powder and water in the pressure vessel of the bench sterilizer.

Set the sterilizer to autoclave the medium at 121°C for 15 minutes.

Set the pouring temperature to approximately 73°C.

When the medium has been sterilized and the temperature has dropped to 73°C, open the pressure release valve of the sterilizer and add 500ml of defibrinated horse blood. Hold the medium at 73°C for 30-60 seconds and then reset the pouring temperature to 41°C.

When the cycle is complete and the medium has reached 41°C, pour the plates using the plate pourer and stacker.

Label the batch with a batch number.

The quality control officer must check the performance and sterility of the batch prior to its use.
Chromogenic Agar Plate Preparation

Prepared according to the protocol supplied by the manufacturer CHROMagar™ Orientation, CHROMagar Microbiology, Paris, France.

Composition

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>15.0 g/l</td>
</tr>
<tr>
<td>Peptone, meat and yeast extract</td>
<td>16.1 g/l</td>
</tr>
<tr>
<td>Chromogenic mix</td>
<td>1.3 g/l</td>
</tr>
<tr>
<td>PH</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Preparation

According to the quantities required, weigh out powder and use in the proportion of 32.4 g per litre of purified water, or use the full pre-weighed dose with the corresponding volume of purified water.

Disperse the powder slowly in the water by stirring gently while the agar swells.

Bring to the boil (100°C) by repeated heating, stirring regularly. The mixture may also be brought to the boil using a microwave oven. If this is the case, after initial boiling remove the mixture from the oven and stir gently. Return to the oven for short repeat heating. Continue until complete fusion of the agar grains has occurred (large bubbles replacing foam, about 2 minutes). Autoclave at 121°C.

Cool in a water bath to 45°C, stirring gently to homogenize before pouring into sterile Petri dishes.

Let dry.
Label the batch with a batch number and date.

The plates are stored in a dark drawer for immediate use.

The quality control officer must check the performance and sterility of the batch prior to its use.

**Interpretation of colour development**

<table>
<thead>
<tr>
<th>Colony colour and size</th>
<th>Identity to be confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>Turquoise</td>
<td><em>Streptococcus</em> spp.</td>
</tr>
<tr>
<td>Blue grey</td>
<td><em>Klebsiella</em> spp.</td>
</tr>
<tr>
<td>Brown halo</td>
<td><em>Proteus</em> spp.</td>
</tr>
<tr>
<td>Cream, translucent</td>
<td><em>Pseudomonas</em> spp.</td>
</tr>
<tr>
<td>Golden, opaque, small</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Pink, opaque, small</td>
<td><em>Staphylococcus saprophyticus</em></td>
</tr>
</tbody>
</table>
Appendix 5
Screening of Contaminants, First Stage

Example 1.

Example 2.

Example 3.

- Example 1 shows a single isolate on the area of the inoculum.
- Example 2 shows two isolates, one on the inoculated area and one outside the inoculated area.
- Example 3 shows a single isolate outside the area of the inoculum.
Appendix 6

Preparation and Use of Coagulase Plasma Test

10% human plasma in 10% nutrient broth is used in the ICPMR clinical laboratories for the tube coagulase test.

Procedure and Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>160 ml</td>
</tr>
<tr>
<td>Nutrient broth</td>
<td>20 ml</td>
</tr>
<tr>
<td>Sterile human plasma</td>
<td>20 ml</td>
</tr>
</tbody>
</table>

Mix, dispense accurately in 0.5 ml amounts into sterile tubes. Cap tubes.

Label tube and date rack. Store in a freezer.

Quality control

The quality control officer will check the performance of each batch of 10% plasma prepared.

Directions to users

Inoculate 0.5 ml of 10% plasma with a single colony of *Staphylococcus* and incubate in the 37°C water bath. Examine for clotting after 2 and 4 hours’ incubation.

Known positive and negative strains should always be tested in parallel, in addition, an un-inoculated control should also be set up.

A positive coagulase test is represented by any degree of clotting; a loose clot suspended in plasma to a solid clot

False positive tests may occur with mixed cultures or with pure cultures of some gram rods, e.g. *Pseudomonas* spp. but the mechanism of clotting is different. Such organisms, utilising the citrate anticoagulant in the plasma, will produce a clot. The organism to be tested therefore, must first be shown to possess characteristics consistent with the genus *Staphylococcus*.

Plasma is obtained by telephone request from the Parramatta Blood Transfusion Service.
10. REFERENCES


