BACTERIA AND DISEASE

BABS 3081
2017
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1. **Information about the Course**

   NB. Some of this information is available @ myUNSW (https://my.unsw.edu.au)

<table>
<thead>
<tr>
<th>Year of Delivery</th>
<th>2017</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Course Code</strong></td>
<td>BABS3081</td>
</tr>
<tr>
<td><strong>Course Name</strong></td>
<td>Bacteria and Disease</td>
</tr>
<tr>
<td><strong>Academic Unit</strong></td>
<td>Biotechnology &amp; Biomolecular Science</td>
</tr>
<tr>
<td><strong>Level of Course</strong></td>
<td>3rd UG</td>
</tr>
<tr>
<td><strong>Units of Credit</strong></td>
<td>6UOC</td>
</tr>
<tr>
<td><strong>Session(s) Offered</strong></td>
<td>S1</td>
</tr>
</tbody>
</table>

**Assumed Knowledge, Prerequisites or Co-requisites**

Prerequisite: BABS2011  
Highly recommended: BABS3041 or BABS3641

**Hours per Week**  
6

**Number of Weeks**  
12

**Commencement Date**  
27th February, 2017

### Summary of Course Structure (for details see 'Course Schedule')

<table>
<thead>
<tr>
<th>Component</th>
<th>HPW</th>
<th>Time</th>
<th>Day</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>e.g. Lectures</td>
<td>2</td>
<td>1-2pm</td>
<td>Wednesday</td>
<td>Mathews D</td>
</tr>
<tr>
<td>Lecture 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lecture 2</td>
<td>9-10 am</td>
<td>Thursday</td>
<td>Mathews C</td>
<td></td>
</tr>
<tr>
<td>Laboratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lab – Component 1</td>
<td>4 hrs: Weeks 2 - 13</td>
<td>2-6pm</td>
<td>Tuesday</td>
<td>BioScience 110</td>
</tr>
<tr>
<td>Online</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other activities, e.g., field trips</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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2. **Staff Involved in the Course**

<table>
<thead>
<tr>
<th>Staff</th>
<th>Role</th>
<th>Name</th>
<th>Contact Details</th>
<th>Consultation Times</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Course Convenor (please see note below)</strong></td>
<td></td>
<td>Assoc Professor Ruiting Lan</td>
<td>Rm 301B, Ph 9385 2095 <a href="mailto:r.lan@unsw.edu.au">r.lan@unsw.edu.au</a></td>
<td>By appointment</td>
</tr>
<tr>
<td><strong>Additional Teaching Staff</strong></td>
<td>Lecturers &amp; Facilitators</td>
<td>Professor Hazel Mitchell</td>
<td>Rm 301A, Ph 9385 2040 <a href="mailto:h.mitchell@unsw.edu.au">h.mitchell@unsw.edu.au</a></td>
<td>By appointment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dr Jai Tree</td>
<td>Samuels S110, Ph 9385 9142. <a href="mailto:j.tree@unsw.edu.au">j.tree@unsw.edu.au</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tutors &amp; Demonstrators</td>
<td>Dr Jani O’Rourke</td>
<td><a href="mailto:j.orourke@unsw.edu.au">j.orourke@unsw.edu.au</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Technical Staff</td>
<td>Li Zhang</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Course Description

Bacteria and Disease aims to develop a high level understanding of bacterial pathogenesis, disease control and prevention. We examine in depth a select number of pathogens that portray the diverse characteristics seen in different pathogenic bacterial species. In conjunction with the lecture program, contemporary medical laboratory training is given through a simulated diagnostic unit. Development of communication skills constitutes part of this course.

### Course Aims

That at the completion of the subject students:

1. Understand basic mechanisms by which bacteria can cause disease.
2. Recognise common themes in microbial pathogenesis.
3. Appreciate the process of biological research that has resulted in the current state of knowledge of bacterial pathogenesis.
4. Use the literature to research a topic on medical microbiology and present this in a professional manner.
5. Understand the basic principles of diagnostic bacteriology.
6. Acquire an understanding and be able to carry out a number of the basic skills of the medical microbiologist such as the ability to recognise and identify common bacterial pathogens.

### Student Learning Outcomes

Students should understand the pathogenic mechanisms used by common bacteria to cause disease and be able to identify common pathogenic mechanisms used by these bacteria. In addition students should understand what approaches can be used to control the spread of disease as well as strategies that can be taken to prevent disease. From a practical perspective students should understand and be able to undertake the steps required to isolate, identify and report bacterial pathogens in a routine microbiology laboratory. Students will also develop research skills in a mini-research project.

### Graduate Attributes Developed in this Course

<table>
<thead>
<tr>
<th>Science Graduate Attributes</th>
<th>Select the level of FOCUS</th>
<th>Activities / Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research, inquiry and analytical thinking abilities</td>
<td>3</td>
<td>The development of research inquiry and analytical thinking will be an integral part of the Lectures and practical sessions. In addition the major presentation and examinations requires students to demonstrate a competency in these areas.</td>
</tr>
<tr>
<td>Capability and motivation for intellectual development</td>
<td>3</td>
<td>These attributes will be integral to Lectures, practicals, the major presentation and examinations</td>
</tr>
<tr>
<td>Ethical, social and professional understanding</td>
<td>3</td>
<td>These attributes will be covered in Lectures, practicals and the major presentation. In particular the practical sessions will develop a professional understanding of the importance of bacteria in disease and the role of the medical microbiologist in diagnosing infective agents.</td>
</tr>
<tr>
<td>Communication</td>
<td>3</td>
<td>The student’s ability to communicate will be developed in the practical component of the course, in the presentation of their research topic and in the examination where students will be required to write essays that will bring together their understanding of bacterial infections.</td>
</tr>
<tr>
<td>Teamwork, collaborative and management skills</td>
<td>3</td>
<td>In the practical program students will learn to work in collaboration with other students and staff to identify bacterial pathogens present in unknown samples. Time management and organisational skills will also be developed.</td>
</tr>
</tbody>
</table>

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1. myUNSW: Handbook (https://my.unsw.edu.au)
2. Learning and Teaching Unit: http://www.ltu.unsw.edu.au
4. Learning and Teaching Unit – Graduate Capabilities: http://www.ltu.unsw.edu.au/graduate-capabilities
5. Science Faculty – Graduate Attributes: http://www.science.unsw.edu.au/our-faculty/science-graduate-attributes
## Lecture/Tutorial Program

For the organisms listed below students should be able to:

1. List major diseases caused.
2. Explain in detail the mechanisms of pathogenesis in the diseases you have listed above.
3. Understand the limitations of our knowledge and identify unsolved problems in our understanding of how bacteria cause disease.
4. Identify the experiments which you feel have contributed most to our understanding of the pathogenesis of the specific diseases you have listed above.

### Bacteria covered:

<table>
<thead>
<tr>
<th>Staphylococcus aureus</th>
<th>Staphylococcus epidermidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>Streptococcus pyogenes</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Viridans group of streptococci</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>Mycobacterium avian-intracellular complex</td>
</tr>
<tr>
<td>Corynebacterium diphtheriae</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Clostridium botulinum</td>
<td>Clostridium tetani</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>Helicobacter pylori</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>Campylobacter concisus</td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>Neisseria gonorrhoeae</td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td></td>
</tr>
</tbody>
</table>

### Practical Program

1. For the specimens listed below
   - Throat swabs
   - Wound swabs
   - Rectal swabs
   - Urine sample

   Students should be able to:

   a. Name the major pathogens that could be isolated from the specimen
   b. Name normal flora commonly found in that specimen
   c. Describe a protocol for culture and identification that would result in diagnosis of the most common bacterial infections.
   d. Set up and examine
      i. microscopic preparations
      ii. bacterial culture plates
      iii. tests for the identification of microorganisms and interpret the findings.

2. Describe the major distinguishing features of the bacteria demonstrated in kits 1-2.
3. Given appropriate plates and tests, identify organisms in Kits 1-2.
4. Isolation and characterisation of pathogenic *E. coli* from dog faecal samples

## Relationship to Other Courses within the Program

This course constitutes a Stage 3 subject as part of a Bachelor of Science Program (3970) or Bachelor of Advanced Science (3972) with a major in Microbiology or Medical Microbiology and Immunology.
## 4. Rationale and Strategies Underpinning the Course

<table>
<thead>
<tr>
<th>Teaching Strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>The focus of this course is to understand the mechanisms by which bacteria colonise a host and subsequently cause disease. In constructing the themes of this course, the bacteria covered were specifically chosen to provide good examples of the range of pathogenic mechanisms used by bacteria to cause disease. For example, toxin production is an important and common mechanism by which bacteria cause disease, however the structure of the toxins involved, the mechanism by which they act and their site of action varies considerably among bacteria. The bacterial examples chosen for inclusion clearly demonstrate such differences but in addition demonstrate that in relation to toxin related disease, some bacteria have very similar mechanisms despite the fact they cause very different diseases. To encourage interactions between students and staff, questions are often posed in lectures. This approach not only reinforces understanding of the concepts being taught but also encourages discussion of the similarities/differences in the mechanisms used by bacteria discussed in previous lectures. To stimulate debate, discussion and to demonstrate that science is an evolving field areas of controversy in bacterial pathogenesis are introduced.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rationale for learning and teaching in this course⁶</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>The rationale behind the Bacteria and Disease course is to provide students with a learning environment that stimulates and excites their interest in Medical Microbiology, develops their intellectual abilities and clearly demonstrates the relevance of medical microbiology to their life and future careers. While the aspirations of students clearly differ, the major expectations of the majority of students are to be engaged (UG 1) throughout their degrees, gain knowledge and develop skills that will equip them to find interesting and fulfilling jobs (UG 12). In the design of this course our aim as an educator was to help students fulfil these goals by providing a stimulating, safe and supported learning environment that not only provides opportunities to develop their conceptual knowledge but excites and steers them along a path that enables independent and critical thinkers and decision makers (Science GA 1,2), the ability to resource information (Science GA 6), work collaboratively (Science GA 5), communicate in a clear and informative manner (Science GA 4) and meet the challenges of the workplace. To help a student complete a University degree and attain these attributes makes facilitating this process an extremely rewarding experience.</td>
<td></td>
</tr>
</tbody>
</table>

⁶ LTU – Beliefs about Learning: http://teaching.unsw.edu.au/beliefs

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## Course Schedule

Some of this information is available on the [UNSW Timetable](http://www.timetable.unsw.edu.au/).

### LABORATORY PROGRAM

All lab classes will be held in BioScience Lab 110 on Tuesdays from 2 pm to 6 pm

<table>
<thead>
<tr>
<th>Week</th>
<th>Date</th>
<th>Lab activity</th>
<th>Research project</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28/02/2017</td>
<td>No lab</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7/03/2017</td>
<td>Kit 1 (Gram positive pathogens)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14/03/2017</td>
<td>Kit 2 (Gram negative pathogens)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>21/03/2017</td>
<td>Wound infection</td>
<td>Project briefing</td>
</tr>
<tr>
<td>5</td>
<td>28/03/2017</td>
<td>Urinary tract infection</td>
<td>Research planning</td>
</tr>
<tr>
<td>6</td>
<td>4/04/2017</td>
<td>Gastrointestinal tract infection</td>
<td>Sample collection</td>
</tr>
<tr>
<td>7</td>
<td>11/04/2017</td>
<td>Throat infection/Mid-session exam</td>
<td>Isolation</td>
</tr>
<tr>
<td>8</td>
<td>25/04/2017</td>
<td>ANZAC DAY</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2/05/2017</td>
<td>Throat infection/Research project</td>
<td>Identification</td>
</tr>
<tr>
<td>10</td>
<td>9/05/2017</td>
<td>Research project</td>
<td>Characterisation</td>
</tr>
<tr>
<td>11</td>
<td>16/05/2017</td>
<td>Research project</td>
<td>Characterisation</td>
</tr>
<tr>
<td>12</td>
<td>23/05/2017</td>
<td>Research project</td>
<td>Characterisation</td>
</tr>
<tr>
<td>13</td>
<td>30/05/2017</td>
<td>Research project</td>
<td>Report</td>
</tr>
</tbody>
</table>
The assessment is divided into three components as listed below.

- **EXAMINATIONS**
  
  **Mid-session examination (1 hour)**
  
  Four short answer questions covering any part of the lecture program from week 1 up to and including week 5.  
  
  **End of session examination (2 hours)**
  
  This will consist of eight short answer questions covering the material covered in lectures from weeks 6-12.

- **PRACTICAL ASSESSMENT**
  
  The practical assessment is comprised of the following four components:
  
<table>
<thead>
<tr>
<th>Part</th>
<th>Description</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prelab tests (open book 4 questions per test)</td>
<td>7%</td>
</tr>
<tr>
<td>2</td>
<td>Diagnostic reports (4 reports)</td>
<td>8%</td>
</tr>
<tr>
<td>3</td>
<td>Literature review</td>
<td>10%</td>
</tr>
<tr>
<td>4</td>
<td>Project report</td>
<td>15%</td>
</tr>
</tbody>
</table>

  **Total Practical Assessment Weight:** 40%

  *Students who fail the practical assessment will fail the subject outright.*
7. Additional Resources and Support

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Course Manual</td>
<td>Available in print and on-line</td>
</tr>
<tr>
<td>Required Readings</td>
<td>More advanced reading on the topics covered in the lecture series can be obtained through Medline. In addition specific journal articles will be recommended by individual lecturers.</td>
</tr>
<tr>
<td>Additional Readings</td>
<td>Reviews and current papers relevant to the topic covered in the major presentation should be accessed through Medline.</td>
</tr>
<tr>
<td>Societies</td>
<td>Australian Society for Microbiology</td>
</tr>
<tr>
<td>Computer Laboratories or Study Spaces</td>
<td>NA</td>
</tr>
</tbody>
</table>

8. Required Equipment, Training and Enabling Skills

<table>
<thead>
<tr>
<th>Equipment Required</th>
<th>Personal protection equipment (PPE) such as safety glasses, lab coat. Please see following pages for full details of Risk assessments and laboratory rules and procedures.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enabling Skills Training Required to Complete this Course</td>
<td>HS, ELISE, LILT</td>
</tr>
</tbody>
</table>
9. Course Evaluation and Development

Student feedback is gathered periodically by various means. Such feedback is considered carefully with a view to acting on it constructively wherever possible. This course outline conveys how feedback has helped to shape and develop this course.

<table>
<thead>
<tr>
<th>Mechanisms of Review</th>
<th>Last Review Date</th>
<th>Comments or Changes Resulting from Reviews</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major Course Review</td>
<td>November 2009</td>
<td>A major aim of Bacteria and Disease is to encourage students to integrate and recognise recurrent themes in bacterial pathogenesis. Thus in the final exam both short answer questions on specific topics and two essays that require the students to bring together material from across the course are included. While good students liked this approach and performed well in these essays, examination of the student comments from 2005 showed that an increasing proportion of students did not like these essays and had great difficulty in writing an essay. To address this issue, in 2013, the structure of both the mid-session and end of session exam was changed. Instead of setting 10 short answer questions in the mid session exam, the number of short answer questions was reduced to 2 and one essay based on integrating material across a number of lectures was introduced. In 2007 students felt that there was too many assessments and that the assessments were conducted over too short a time frame. As a result the tests associated with the SDU have been removed, replaced with a take home test, and the other assessments spread out over the session.</td>
</tr>
<tr>
<td>CATEI</td>
<td>June 2014</td>
<td>Q1 5.15 Q2 4.92 Q3 5.15 Q4 5.15 Q5 5.23 Q6 5.23 Q7 4.92 Q8 4.92 Q9 5.31 Q10 5.23</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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### Expectations of Students

**MINIMUM REQUIREMENTS TO ACHIEVE A PASS IN BABS3081**

All students are required to achieve a satisfactory performance in all components of the subject.

Students who have achieved an aggregate mark of 50% or more overall, but only obtain a mark of 45% or less in the final theory examination or have an unsatisfactory performance in other components of the subject, may fail outright or be required to undertake further assessment.

Where further assessment is required, unless there are special circumstances that need to be taken into consideration, the student will be awarded either a Pass or a Fail.

Should further assessment be required it is the responsibility of the student to be available at the time and place notified. The supplementary examinations will be held the week after the results are released on *myUNSW*, on Tuesday 12th July. The time and place of the exams will be given via UNSW email by BSB student office.

### Health and Safety

*Information on relevant Health and Safety policies and expectations both at UNSW: [https://safety.unsw.edu.au](https://safety.unsw.edu.au)*

*In addition please see details of risk assessments and laboratory procedures on pages 18-20.*

### Assessment Procedures

**Students have been known to suffer a major crisis such as death or illness in the family or a major personal trauma during session. Please let your subject convenor or tutor know of any such events that may affect your performance in the subject as soon as possible, so that appropriate assistance can be rendered. If you are colour blind or have a specific disability please advise the subject convenor at the beginning of session.**

### Special Consideration and Further Assessment

**Students who believe that their performance, either during the session or in the end of session exams, may have been affected by illness or other circumstances may apply for special consideration. Applications can be made for compulsory class absences such as (laboratories and tutorials), in-session assessments tasks, and final examinations. Students must make a formal application for Special Consideration for the course/s affected as soon as practicable after the problem occurs and within three working days of the assessment to which it refers.**

Students should consult the “Special Consideration” section of Moodle for specific instructions related to each BABS course they are studying. Further general information on special consideration can also be found at [https://student.unsw.edu.au/special-consideration](https://student.unsw.edu.au/special-consideration).

**HOW TO APPLY FOR SPECIAL CONSIDERATION**

Applications must be made via Online Services in myUNSW. *You must obtain and attach Third Party documentation before submitting the application. Failure to do so will result in the application being rejected.* Log into *myUNSW* and go to My Student Profile tab > My Student Services channel > Online Services > Special Consideration. After applying online, students must also verify supporting their documentation by submitting to UNSW Student Central:

- Originals or certified copies of your supporting documentation (Student Central can certify your original documents), and
- A completed Professional Authority form (pdf - download here).
The supporting documentation must be submitted to Student Central for verification **within three working days** of the assessment or the period covered by the supporting documentation. Applications which are not verified will be rejected.

**Students will be contacted via the online special consideration system as to the outcome of their application. Students will be notified via their official university email once an outcome has been recorded.**

**SUPPLEMENTARY EXAMINATIONS:**
The University does not give deferred examinations. However, further assessment exams may be given to those students who were absent from the final exams through illness or misadventure. Special Consideration applications for final examinations and in-session tests will only be considered after the final examination period when lists of students sitting supplementary exams/tests for each course are determined at School Assessment Review Group Meetings. Students will be notified via the online special consideration system as to the outcome of their application. It is the responsibility of all students to regularly consult their official student email accounts and myUNSW in order to ascertain whether or not they have been granted further assessment.

For Semester 1 2017, BABS Supplementary Exams are:
- All BABS coded courses (except BABS1201): Wednesday 12th July

Further assessment exams will be offered on this day ONLY and failure to sit for the appropriate exam may result in an overall failure for the course. Further assessment will NOT be offered on any alternative dates.

**Equity and Diversity**
Those students who have a disability that requires some adjustment in their teaching or learning environment are encouraged to discuss their study needs with the course Convenor prior to, or at the commencement of, their course, or with the Equity Officer (Disability) in the Equity and Diversity Unit (9385 4734 or http://www.studentequity.unsw.edu.au/)

Issues to be discussed may include access to materials, signers or note-takers, the provision of services and additional exam and assessment arrangements. Early notification is essential to enable any necessary adjustments to be made. Information on designing courses and course outlines that take into account the needs of students with disabilities can be found at: http://www.gs.unsw.edu.au/policy/documents/equitystatement.pdf

<table>
<thead>
<tr>
<th><strong>Grievance Policy</strong>[^9]**</th>
<th><strong>School Contact</strong></th>
<th><strong>Faculty Contact</strong></th>
<th><strong>University Contact</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contact BSB Office</td>
<td>Dr Gavin Edwards</td>
<td>University Counselling Services[^10]</td>
</tr>
<tr>
<td></td>
<td>G17, Tel: 9385 8047</td>
<td><a href="mailto:g.edwards@unsw.edu.au">g.edwards@unsw.edu.au</a></td>
<td>Tel: 9385 5418</td>
</tr>
<tr>
<td></td>
<td><a href="mailto:babstudent@unsw.edu.au">babstudent@unsw.edu.au</a></td>
<td>OR Dr Louise Lutze-Mann, <a href="mailto:l.lutze-mann@unsw.edu.au">l.lutze-mann@unsw.edu.au</a></td>
<td>Tel: 9385 2024</td>
</tr>
</tbody>
</table>


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11. **UNSW Academic Honesty and Plagiarism**

**What is Plagiarism?**

Plagiarism is the presentation of the thoughts or work of another as one’s own. *Examples include:

- direct duplication of the thoughts or work of another, including by copying material, ideas or concepts from a book, article, report or other written document (whether published or unpublished), composition, artwork, design, drawing, circuitry, computer program or software, web site, Internet, other electronic resource, or another person’s assignment without appropriate acknowledgement;
- paraphrasing another person’s work with very minor changes keeping the meaning, form and/or progression of ideas of the original;
- piecing together sections of the work of others into a new whole;
- presenting an assessment item as independent work when it has been produced in whole or part in collusion with other people, for example, another student or a tutor; and
- claiming credit for a proportion a work contributed to a group assessment item that is greater than that actually contributed.†

For the purposes of this policy, submitting an assessment item that has already been submitted for academic credit elsewhere may be considered plagiarism.

Knowingly permitting your work to be copied by another student may also be considered to be plagiarism.

Note that an assessment item produced in oral, not written, form, or involving live presentation, may similarly contain plagiarised material.

The inclusion of the thoughts or work of another with attribution appropriate to the academic discipline does not amount to plagiarism.

The Learning Centre website is main repository for resources for staff and students on plagiarism and academic honesty. These resources can be located via:

[www.lc.unsw.edu.au/plagiarism](http://www.lc.unsw.edu.au/plagiarism)

The Learning Centre also provides substantial educational written materials, workshops, and tutorials to aid students, for example, in:

- correct referencing practices;
- paraphrasing, summarising, essay writing, and time management;
- appropriate use of, and attribution for, a range of materials including text, images, formulae and concepts.

Individual assistance is available on request from The Learning Centre.

Students are also reminded that careful time management is an important part of study and one of the identified causes of plagiarism is poor time management. Students should allow sufficient time for research, drafting, and the proper referencing of sources in preparing all assessment items.

* Based on that proposed to the University of Newcastle by the St James Ethics Centre. Used with kind permission from the University of Newcastle

† Adapted with kind permission from the University of Melbourne.
12. Risk Management

Working in a laboratory is inevitably associated with certain risks. Good laboratory practice means working in such a way as to eliminate, or at least minimise, these hazards. In order to perform your work safely and to comply with government legislation, a risk assessment has been conducted on all of work that will be performed in this subject in the laboratory and the following potential risks have been identified:

Biological hazards: All microorganisms are potentially harmful if ingested or exposed to body surfaces. Some organisms used in this class may be opportunistic human pathogens, however none are considered to pose a significant risk if handled appropriately (see procedures below).

Chemical hazards: Most of the chemicals used in this subject (e.g., in solid and liquid media and most buffers) are not hazardous at the concentrations that are being used, however all chemicals should be considered potentially harmful. Some practical classes employ hazardous chemicals. In these cases the hazard is described in the class directions for that specific exercise. The concentrations of antibiotics in media are generally not harmful, however contact with skin should be avoided.

Note: Material Safety Data Sheets (MSDS) are available for all of the hazardous chemicals from your tutor. You should be familiar with the relevant MSDSs prior to commencing your practical work.

Physical hazards: Bunsen burners and heat from other sources such as water baths, breakable glassware, sharp objects such as plastic tips and needles.

Hazards involving work environments: The combination of large numbers of students performing laboratory work (e.g., with bunsen burners alight) and the necessity to wear protective clothing (see below), especially in summer weather, may cause discomfort to some students.

PROCEDURES FOR DEALING WITH RISKS

In addition to the general risks that have been identified with laboratory work for this subject (see above), any additional risks associated with specific practicals are written in this manual at the beginning of each practical description. At the commencement of each new practical your tutor will review the risks with you. At the commencement of each class the procedures may be reviewed. You may be examined on your understanding.

It is imperative that you be present at the beginning of each class to ensure that you are available to review safety procedures. If you are not present you may be excluded from the class.

Following are some simple rules that you must follow which will ensure good laboratory practice and minimise the consequences of risks:

a. Wear adequate protective clothing. This will protect you from contamination by cultures and chemicals as well as protecting the cultures and chemicals from contamination by you. A laboratory coat must always be work while in the lab, and removed on leaving. Where necessary protective clothing also includes footwear. Fully enclosed shoes must be worn and thongs and other open, loose footwear are not permitted. Safety glasses may also be required. You should protect yourself from the possibility of falling equipment (glass) and
spilling chemicals. Also there may be broken glass, spilled liquids, etc. in the floor. Students who do not comply with these stipulations will be asked to leave the laboratory.

b. You must not eat, drink, smoke, apply make-up etc. in the lab. Neither should you bring food, drink etc. into the lab. Habits such as chewing the ends of pens and pencils, nail biting etc. are often difficult to avoid, but you should make a conscious effort not to do them. Do not sit on laboratory benches.

c. Do not invite anyone into the lab. They may not be aware of the hazards and may themselves create additional hazards.

d. Practice good aseptic techniques when handling microorganisms. If you do not know what this involves you must ask. It is essential to wear gloves at all times when handling potentially pathogenic agents.

e. Never pipette by mouth. Use plugged, sterile pipettes and mechanical pipetting devices to transfer cultures and solutions. This will prevent contamination in both directions. Take care to avoid breaking glass pipettes when inserting them into mechanical pipettors.

f. Keep everything covered. Do not leave the plugs off flasks or caps off tubes and bottles. As well as minimising spillages this will prevent contamination of cultures and solutions.

g. If there is an accident with a microbial culture, or hazardous chemical, ask a fellow student to call someone in authority immediately. Do not move and risk the spread of contamination. If there is a fire, remove yourself immediate danger and call someone in authority immediately.

h. Place PIPETTES (ONLY) in the labelled containers of disinfectant. Pasteur pipettes should be disposed of in the pasteur pipette discard container. Place all other glassware in the containers provided as soon as possible after use. Broken glass, and only broken glass, should be placed in the “glass only” bins located at the front of the class. All other waste (infected or not), including used Petri dishes, should be placed in the plastic, autoclaved bags. These will be autoclaved before disposal. Never place any infectious material in the sinks, to

i. Avoid blockages, never drop anything solid into the sinks. Special discard bottles will be provided for particular chemicals.

j. All materials for incubation or refrigeration should be adequately labelled and placed in the relevant containers provided.

k. Equipment such as centrifuges, baths, etc. should be operated carefully and correctly. If any doubt exists regarding the correct method of operation of any equipment, then consult a demonstrator before proceedings.

l. Before leaving the laboratory tidy your bench, clean your bench area using disinfectant provided and wash your hands with Bioprep hand wash and water.

m. If you feel discomfort from your work (e.g. heat exhaustion or back pain), consult your tutor or the subject authority.

n. If you feel you may have allergic reactions or be sensitive to any of the biological or chemical components (e.g., antibiotics) used in the practicals, consult your tutor or the subject authority.
If you get any biological or chemical substance (e.g., sodium hydroxide) in your eye, ensure that you immediately go to a tap and wash your eye.

While washing your eye, alert someone to your situation so that they can assist you and gain the attention of someone in authority. Continue to wash your eye until someone in authority indicates for you to do otherwise. It is imperative that you take this seriously as you may risk permanent eye damage if it is a harmful chemical. Note: you should always wear safety glasses when handling hazardous substances.

Health and Safety Precautions for Electronic devices including laptop computers and mobile phones.

Mobile phones: For your own safety when using your mobile phone in class please ensure it is placed in a plastic zip lock bag. Every student will be provided with a zip lock bag in their first practical class which they should continue to use during the session (keep it with your lab book). If you misplace or lose this bag you will be expected to provide your own zip lock bag.

Computers and tablets: this applies to either your own device or those supplied by the school. Please cut a section of benchcoat (this will be provided in the lab class) and place your device on this on the lab bench to separate it from your other laboratory work. DO NOT wear gloves when using these devices. If the lab is being used as a dedicated DRY LAB (please check signage on the door) you will not be required to do this as the benches will be cleaned and decontaminated before your class.

STUDENTS MUST NOT GO INTO THE PREPARATION ROOM AREA UNLESS DIRECTED TO DO SO BY THEIR TUTOR.
I, ........................................................................................................

Student Number ...............................................................................

Here by certify that I have read the laboratory rules in this manual. I also certify that I am aware that the microorganisms that I will be handling in the laboratory are human pathogens that require due care and attention to aseptic technique in their handling.

- I understand that I must wear a laboratory coat and covered shoes at all time while in the laboratory.
- I understand that I must wear gloves at all times when handling potentially pathogenic agents.
- I understand that I must wash my hands using the appropriate disinfectant provided on each occasion that I leave the laboratory.
- I understand that I am not permitted to smoke, eat or drink in the laboratory.
- In the event of an accident I understand that where appropriate I should remain where I am and request assistance from another student to contact a member of staff.
- I understand that I must obey the safety directions of staff members at all time and recognise that failure to do so may result in my exclusion from the laboratory.

Signed ..........................................................................................

Date ........../......./.........

This page should be signed, removed from the manual and handed to one of the tutors at the start of the first laboratory session.
Demonstration kits of microorganisms of medical and public health importance

<table>
<thead>
<tr>
<th>Week</th>
<th>Laboratory Material</th>
<th>Page</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td><strong>Kit one: Gram positive organisms and yeasts</strong></td>
<td>23</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus, Streptococcus, Enterococcus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Clostridium and Candidia</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal throat flora</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exercises for Kit One</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><strong>Kit two: Gram negative organisms and parasites</strong></td>
<td>33</td>
</tr>
<tr>
<td></td>
<td><em>Bacteriodes, Campylobacter and Pseudomonas</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Escherichia, Enterobacter and Klebsiella</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Salmonella, Shigella and Proteus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parasite Infections</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal faecal flora</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exercises for Kit Two</td>
<td></td>
</tr>
</tbody>
</table>
1. DEMONSTRATION KITS OF MICROORGANISMS OF MEDICAL AND PUBLIC
HEALTH IMPORTANCE

The two demonstration kits include cultures, slides etc. of the medically important genera
that may be encountered in the practical program on diagnosis of bacterial infections.
Learning objectives of demonstration kits and exercise in weeks 2 and 3 are:

1. To familiarise with morphological and phenotypic properties of organisms commonly
encountered in medical microbiology and public health laboratories.

2. To familiarise with the media used for culture and identification of medically important
microorganisms.

3. To refresh and/or develop skills in subculture, laboratory tests and knowledge required
for the diagnosis of bacterial infections practicals followed.

ABBREVIATIONS COMMONLY USED FOR MEDIA

CSA   Campylobacter Selective Agar
CCA   Candida Chromogenic agar
CUTIM Chromogenic Urinary Tract Infection Medium
HBA   Horse Blood Agar
MAC   MacConkey Bile Salt Agar
MSA   Mannitol Salt Agar
NAG   Nagler Medium
NA    Nutrient Agar
SAB   Sabourauds Agar
SCM   Salmonella Chromogenic Medium
SENS  Antibiotic Sensitivity Agar
XLD   Xylose Lysine Desoxycholate Agar
WEEK 2
Kit One: Gram positive bacteria and Yeasts

GENUS STAPHYLOCOCCUS

Material:
Culture of *Staphylococcus aureus* (A) and *Staphylococcus epidermidis* (B) on
- HBA incubated aerobically and anaerobically
- MAC
- MSA

Culture of *Staphylococcus epidermidis* (B) and *Staphylococcus saprophyticus* (C) on
HBA plate with a novobiocin disk.

Gram stain of *Staphylococcus aureus* (A), *Staphylococcus epidermidis* (B).

Description:
Gram-positive spherical cocci, 0.8 - 1.0 µm, occurring in grape-like clusters; in pus
may be in pairs, catalase positive. Facultatively anaerobic. Fermentative action on
glucose. Grow well on basal media (e.g. NA), often pigmented. Non-motile. Usually non-
encapsulated, no spores. Circular colonies 2-4 mm, opaque, may be pigmented, catalase
positive, haemolysis variable. They can grow in the presence of bile salts and in media
containing 6.5% NaCl.

This genus is in the family *Micrococcaceae* and it is most simply differentiated from
the genus *Micrococcus* on the basis of anaerobic fermentation of glucose (Hugh and
Leifson test) and production of acid from glycerol-erythromycin medium. Both tests are
positive for *Staphylococcus*.

Diagnosis:
Pathogenic strains isolated aerobically from pus, sputum etc. on HBA, or from food
and faeces on HBA or MAC (after enrichment in 10% NaCl broth) or MSA. Normal habitat
on skin and nasal cavity of 60-90% of healthy persons. May also be found in foods which
have been contaminated by handling.

There are now more than 45 species of Staphylococci however the 3 main species of
medical importance are *S. aureus* and 2 coagulase negative species (*S. epidermidis* and
*S. saprophyticus*). Many of the other species are of animal origin and may be
encountered in public health microbiology.
Following establishment of isolates as being members of the genus Staphylococcus, the coagulase test may be employed as a rapid test to identify pathogenic species.

### Coagulase Test

- **Positive (Pathogenic)**
  - Mannitol positive
    - Staph. aureus
    - Staph. epidermidis
    - Novobiocin sensitive
    - Staph. saprophyticus*
- **Negative (Usually non-pathogenic)**
  - Mannitol negative
  - Novobiocin resistant

*Involved in urinary tract infections, particularly in females.

Strains of *Staph aureus* can be further differentiated by:

1. Phage typing which is based on the reaction of individual strains to infection with bacteriophages An International Set of 25 typing phages is used.
2. Strains may differ in sensitivity to a range of antibiotics. The drug sensitivity patterns may be used as a basis for typing organisms encountered in epidemiological surveys.

### GENERA STREPTOCOCCUS & ENTEROCOCCUS

**Material:**

Culture of *Streptococcus pneumoniae* (D) and one member of the "viridans" streptococci, *S. viridans* (E) on:
- HBA incubated aerobically with Optochin (OPT)
- HBA incubated anaerobically with Optochin (OPT)

Culture of *Streptococcus pyogenes* (F) and *Streptococcus agalactiae* (G) on:
- HBA incubated aerobically with Bacitracin (BC)
- HBA incubated anaerobically with Bacitracin (BC)

Culture of *Enterococcus faecalis* (H) on:
- HBA incubated both aerobically and anaerobically.
- MAC
- MSA
- CUTI
Gram stain of *Streptococcus pneumoniae* (D), *Streptococcus viridans* (E), *Streptococcus pyogenes* (F), *Enterococcus faecalis* (H).

Demonstration of Streptex streptococcal grouping.

**GENUS STREPTOCOCCUS**

**Description:**
Gram-positive spherical or ovoid cocci, less than 2mm in diameter, arranged in long or short chains or pairs. Catalase negative. Usually require enriched media and may produce characteristic changes on blood agar. Facultatively anaerobic or strictly anaerobic.

**Diagnosis:**
Streptococci are widely distributed in humans and animals with some also found in soil, water and food products. In addition to the well-recognised pathogens such as *Strep pyogenes* and *Strep pneumoniae* many of the commensal species are capable of causing disease under appropriate conditions. Similarly, many Streptococci are responsible for disease in domestic, farm and aquatic animals. In humans they can be isolated from nose, throat and other parts of the respiratory tract, skin lesions, female genital tract, wounds, blood and other body fluids on HBA. The most logical differentiation of species is by antigenic structure but practical differentiation can be made first using haemolytic activity.

**GENUS ENTEROCOCCUS**

**Description:**
In the early 1980's a number of species of *Streptococcus* were reclassified into a new genus, *Enterococcus*. These included *E. faecalis, E. faecium, E. avium* and *E. gallinarum*. *E. faecalis* and *E. faecium* are residents of the intestinal tracts of humans and most animals, with the latter two species occurring in poultry. The majority of Enterococci species fall into Lancefield Group D and are capable of growing on media containing 6.5% NaCl. The development of vancomycin resistant enterococci (VRE) is now a major health concern in clinical situations.
The following is a minimally acceptable scheme for differentiation of enterococci and streptococci (Small opaque colonies 1 mm diameter on HBA)

**Type of haemolysis**

<table>
<thead>
<tr>
<th>ALPHA (green zone)</th>
<th>BETA (clear zone)</th>
<th>NON-HAEMOLYTIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optochin sensitivity</td>
<td>Bacitracin</td>
<td>Fac. AnO₂</td>
</tr>
<tr>
<td>Bile sensitivity</td>
<td>Sensitive</td>
<td>Strict AnO₂*</td>
</tr>
<tr>
<td>Positive</td>
<td>Resistant</td>
<td>Enterococcus faecalis (Group D)</td>
</tr>
<tr>
<td>Strep. pneumoniae</td>
<td>Other streptococci (Groups B (including Strep agalactiae, C or G)</td>
<td></td>
</tr>
<tr>
<td>Viridans streptococci</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strep. pyogenes (Group A)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Some anaerobic cocci are only isolated using special anaerobic techniques.*

**OTHER TESTS**

**Serodiagnosis:** (Antistreptolysin "O" test (ASO). See page 86.

**Lancefield grouping**

The division of haemolytic streptococci into serologic groups depends on the occurrence of group-specific cellular antigens, which are carbohydrate in nature and are designated as C carbohydrates. Streptococci that cause disease in humans usually belong to Lancefield group A. The names Group A streptococci and *Streptococcus pyogenes* are used interchangeably.

**M typing and T typing**

Group A streptococci are subdivided into at least 55 different M types based on a precipitin reaction involving a type specific protein. These are numbered 1-55. A slide agglutination method is also useful for sub-typing strains into T types based on the presence of T protein antigens.
**GENUS CLOSTRIDIUM**

**Material:**
Culture of *Clostridium perfringens* (I) and *Clostridium tetani* (J) on HBA incubated aerobically and anaerobically.

Culture of *Clostridium perfringens* (I) on Nagler plate with antitoxin (demonstration).

Gram stain of CMM cultures of *Clostridium perfringens* (I) and *Clostridium tetani* (J).

**Description:**
Gram-positive motile (except *Cl. perfringens* = *Cl. welchii*) rods with spores usually distending the bacilli. Usually rhizoid colonies (except *Cl. perfringens*). Catalase negative. Grow on basal media. Proteolytic species produce digestion, blackening and foul odour in CMM. Saccharolytic species reddens meat and produce gas. Pathogens produce powerful exotoxins. Strictly anaerobic. Show resistance to a number of antibiotics (e.g. kanamycin, polymyxin, cyclosterine) which may be used in selective media. They are commonly found in soil, aquatic sediments and in human and animal intestines.

**MYONECROTIC CLOSTRIDIA:**

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>MORPHOLOGY</th>
<th>PATHOGENICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cl. perfringens</em></td>
<td>Thick rods, spores rarely formed</td>
<td>Type A: gas gangrene and food poisoning man. Type D: enterotoxaemia in sheep.</td>
</tr>
<tr>
<td><em>Cl. oedematiens</em></td>
<td>Large rods, oval spores</td>
<td>Gas gangrene in man and animals</td>
</tr>
<tr>
<td><em>Cl. septicum</em></td>
<td>Pleomorphic rods, oval spores</td>
<td>Gas gangrene in man</td>
</tr>
<tr>
<td><em>Cl. chauvoei</em></td>
<td>Pleomorphic rods, oval spores</td>
<td>Blackleg in cattle</td>
</tr>
<tr>
<td><em>Cl. histolyticum</em></td>
<td>Rods, large oval spores</td>
<td>Occurs in mixed anaerobic infections</td>
</tr>
<tr>
<td><em>Cl. sporogenes</em></td>
<td>Rods, distended by oval spore</td>
<td>Usually non-pathogenic</td>
</tr>
<tr>
<td><em>Cl. difficile</em></td>
<td>Rods with terminal spores</td>
<td>Antibiotic-associated diarrhoea and colitis</td>
</tr>
</tbody>
</table>
NEUROTOXIC CLOSTRIDIA:

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>MORPHOLOGY</th>
<th>PATHOGENICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl. botulinum</td>
<td>Large rods, spores infrequent</td>
<td>Types A, B, E, F cause botulism in man</td>
</tr>
<tr>
<td>Cl. tetani</td>
<td>Thin rods, &quot; drumstick &quot; form when sporing</td>
<td>Tetanus in man and animals</td>
</tr>
</tbody>
</table>

Additional features:

Cl. tetani - difficult to isolate from natural sources of wounds. Strict anaerobe giving very fine spreading growth on HBA. *Tetanus neonatorum* following umbilical infection very common in developing countries (e.g. in South East Asia). Virulent strains detected by injection into 2 mice, one protected by antitoxin.

Cl. perfringens (syn. welchii) - most common cause of gas gangrene in man following injury or abortion. Some strains of Type A, usually heat-resistant and non-haemolytic, cause food poisoning. One of the few non-motile Clostridia. Main identifying features are morphology, capsulation and positive Nagler reaction Acid and gas from sugars.

Cl. botulinum - extremely heat-resistant spores, survive 5 hr. boiling. Powerful neurotoxins produced in infected foods, e.g. home preserved non-acid (>pH 5.4) foods, preserved meats and liver paste (0.7-0.9µg inhaled or 70µg ingested can kill an average human). Botulism mostly due to Type A strains. Toxin detected in suspected food by inoculating 2 guinea pigs, one protected by polyvalent serum.

Cl. difficile can be responsible for diarrhoea and colitis, most commonly pseudomembranous colitis, in humans as a result of overgrowth of this organism following antibiotic therapy for other infections. It possesses both an entertoxin and a cytotoxin, both implicated in damage to intestinal mucosa.
Yeast - Genus Candida

Culture of Candida albicans (K) on:
- HBA incubated aerobically
- MAC
- SAB

Gram stain of Candida albicans (K)

Demonstrations:
- Germ tube production of for identification of Candida albicans
- Cultures of Candida species on CCA

Description:
Infected mucous membranes show greyish-white patches with underlying inflammation. Candida albicans and related species are often found in flora of respiratory and alimentary tracts without signs of clinical infection. On the other hand, it should be considered that clinical infection with Candida may indicate the existence of serious underlying disease. Skin lesions may resemble ringworm.

Predisposing conditions for candidiasis are: pregnancy and early infancy; endocrine disorders; cancer and tuberculosis; therapy with antibacterial, immunosuppressive or cytotoxic drugs, which cause imbalance of normal flora or suppress host defence mechanisms.

Diagnosis:

Direct microscopy - Gram-stained smear of skin scrapings, sputum, urine, faeces and vaginal swabs examined for Gram-positive oval, budding yeast cells (3-6m) and pseudohyphae (elongated cells or filaments), which may also show budding.

Culture
Specimens plated on CCA, HBA, malt extract agar or Sabouraud's agar and incubated 24-48 hr. at 30°C or 37°C. All species give cream coloured colonies consisting of budding cells only. Grow on media containing cycloheximide. Chlamydospores and germ tubes used in identification.

Note: While the majority (80 - 90%) of Candida infections are due to C. albicans an increasing number of other species are now being identified. These include Candida glabrata and Candida krusei. These latter two species show reduced
susceptibility/resistance to commonly used antibiotics. Thus it is important to accurately identify the Candida species present (via the germ tube test and/or the use of chromogenic agar) and determine its antibiotic sensitivity pattern.

**Cell morphology and germ tube production:**

<table>
<thead>
<tr>
<th>Test</th>
<th>C. albicans Principal pathogen</th>
<th>C. stellatoidea, C. tropicalis and others. Occasionally of pathological significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells incubated in human serum (37°)</td>
<td>Germ tubes produced in two hours or less</td>
<td>Germ tubes produced only after three hours or longer</td>
</tr>
</tbody>
</table>

**NORMAL THROAT FLORA**

The pharynx (throat) is normally colonized by both α and β streptococci as well as a number of anaerobes, staphylococci, Neisseria and diphtheroids. Sometimes pathogens such as *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae* and *Neisseria meningitidis* colonize the pharynx.

For the purposes of the SDU you will be examining blood agar plates for the presence of haemolytic organism sand for any streptococci detected you will be determining their identification at species level.
## Exercises for Kit One

1. Record the appearance and characteristics e.g. colour, haemolysis, etc of the gram-positive organisms examined on the various media.

<table>
<thead>
<tr>
<th>Organism</th>
<th>HBA aerobic</th>
<th>HBA anaerobic</th>
<th>MAC</th>
<th>OTHER MEDIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph aureus (A)</td>
<td></td>
<td></td>
<td>MSA:</td>
<td></td>
</tr>
<tr>
<td>Staph epidermidis (B)</td>
<td></td>
<td></td>
<td>MSA:</td>
<td></td>
</tr>
<tr>
<td>Staph Saprophyticus (C)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>Strep pneumonia (D)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>Strep viridans (E)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>Strep pyogenes (F)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>Strep agalactiae (G)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis (H)</td>
<td></td>
<td></td>
<td>MSA:</td>
<td>CUTI:</td>
</tr>
<tr>
<td>Clostridium perfringens (I)</td>
<td></td>
<td></td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>Clostridium tetani (J)</td>
<td></td>
<td></td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>Candida albicans (K)</td>
<td>na</td>
<td></td>
<td>SAB:</td>
<td></td>
</tr>
</tbody>
</table>
2. Record the diagnostic results of the following organisms:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Diagnostic disks</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph epidermidis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staph saprophyticus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strep pyogenes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strep agalactiae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strep pneumonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strep viridans</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Diagnostic disks: BC = Bacitracin, OP = Optochin, NV = Novobiocin

3. You will be given 2 unknown organisms, perform a Gram stain and then determine what tests you would need to perform to make a presumptive identification. Carry out these tests and include their results in the table below and make a presumptive ID.

<table>
<thead>
<tr>
<th>Unknown</th>
<th>Test</th>
<th>Result</th>
<th>Presumptive ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Subculture the mixed bacterial suspension for single colonies onto MAC and XLD agar – these plates will be examined next week.

Tutor’s signature: ......................................................
IDENTIFICATION KEY

GRAM POSITIVE COCCI
(GPC)

Anaerobes
Peptococcus spp
Peptostreptococcus spp

Facultative Anaerobes
catalase test

negative Streptococcus spp
(usually form chains in broths)

positive Staphylococcus spp
(should form cluster/clumps in broth)

coagulase
effect on blood agar

α-haemolysis (green)
β haemolytic (clear)
non-haemolytic Group D (Enterococcus)

Optochin sensitivity
Bacitracin sensitivity

S. pneumoniae
resistant Viridans strep

Sensitive Group A strep
(Strept pyogenes)

resistant non-group A streps
(eg. Group B)

positive Staph. aureus
negative Staph. epidermidis
Staph. saprophyticus

The School of Biotechnology & Biomolecular Sciences, UNSW 2017
IDENTIFICATION KEY

GRAM POSITIVE RODS (GPR)

Atmosphere requirements

Aerobes

Facultative Anaerobes

Strict anaerobes

Corynebacterium spp

Listeria spp

Proprionibacterium spp

Clostridium spp

large sporing rods, dry colony

Bacillus spp

Chinese letter with swelling, often curved

P. acnes

Indole positive

Straight rods, often large, may spore

Lecithinase specific

Glucose ferm

Cl. perfringens + +

Cl. tetani - -

Cl. difficile - +

glucose ferm

haemolysis

C. diptheria + -

C. hofmani - -

L. monocytogenes + +
GENUS BACTEROIDES

Material:
Culture of *Bacteroides fragilis* (L) on HBA incubated aerobically and anaerobically.
Gram stain of *Bacteroides fragilis* (L).

Description:

*Bacteroides* species belong to a group of anaerobic gram negative rods which now also includes *Prevotella* and *Porphyromonas* species. These organisms are found in humans and other mammals where they are common members of the normal flora of the gastrointestinal tract, the mouth and the female genital tract. They are opportunistic pathogens and are often associated with polymicrobial infections. The most common organism associated with infections in humans is *Bacteroides fragilis*, which is a normal resident of the lower bowel. Infections can arise if the mucosal wall of the intestine is disrupted by factors including gastrointestinal surgery, perforated appendicitis or ulcers, diverticulitis, trauma or inflammatory bowel disease. While they are predominant in intra-abdominal infections they can also result in infections in the central nervous system, bloodstream, neck, head, chest, pelvis and skin and soft tissue. Infections are generally of endogenous origin, from the patient’s own intestinal flora.

*Bacteroides* are not considered to be invasive but they have been shown to contribute to infections via their ability to induce abscesses formation, reduce phagocytosis by polymorphonuclear leukocytes and inactivate antibiotics by β-lactamase production. Abscess formation is a pathological response of the immune system to the *Bacteroides* capsular polysaccharide.

Diagnosis:

Preferred samples are aspirates or swabs of pus from the infected site. Samples should be transported in media suitable for anaerobes however *B. fragilis* is known to be aerotolerant. A smear of the sample should be gram-stained and then cultured anaerobically on enriched agar such as blood agar. Antibiotics can be added to increase the selectivity of the agar plates. Organisms can then be further identified by biochemical or enzymatic tests or nucleic acid probes or by chromatography of metabolites.
GENUS CAMPYLOBACTER

Material:
Culture of *Campylobacter jejuni* (M) on:
- HBA incubated aerobically
- HBA incubated anaerobically
- MAC incubated aerobically
- Campylobacter selective agar (CSA) incubated microaerophilically

Gram stain of *Campylobacter jejuni* (M)

Description:
*C. jejuni* is a member of the genus *Campylobacteraceae*. It is a Gram-negative, small S-shaped bacterium that can transform to a coccoid form with age, or exposure to toxic concentrations of oxygen. It is motile, having a characteristic corkscrew motion. It grows at both 37°C and 42°C and is microaerophilic with a respiratory type of metabolism. In humans, *C. jejuni* is generally regarded as the most common bacterial cause of gastroenteritis; it can also cause septicemia and abortion. Infection with certain strains of *C. jejuni* may be a predisposing factor to the development of the neurological disorder Guillain-Barre syndrome. Strains of *C. jejuni* are also found as normal flora of the intestinal tract of poultry and other bird species, cattle, sheep, pigs, goats, dogs, rabbits and monkeys.

Diagnosis:
*C. jejuni* can be isolated from human faeces on enriched media (blood or charcoal) with a range of different selective antibiotic supplements and it is commonly referred to as *Campylobacter* Selective agar (CSA). Colonies on this agar often appear as a spreading or swarming growth along the direction of the streaks. *C. jejuni* is both catalase and oxidase positive.
GENUS PSEUDOMONAS:

Material:
Culture of *Pseudomonas aeruginosa* (N) on:
- HBA incubated aerobically
- HBA incubated anaerobically
- MAC

Gram stain of *Pseudomonas aeruginosa* (N)

Demonstration:
- Growth on Pseudo P (King's A) incubated aerobically

Description:

Diagnosis:
Isolated from respiratory tract, ears, eyes, wounds, burns, urine, bone or joint infections or moist reservoirs in the hospital environment. Isolated on media that are both selective and promote pigment production (e.g. cetrimide agar).
### IDENTIFICATION OF PSEUDOMONAS SPECIES

<table>
<thead>
<tr>
<th>TEST</th>
<th>Pseudomonas aeruginosa</th>
<th>Other pseudomonads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyocyanin production observed on Pseudomonas P medium or King A.</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Fluoroscein production observed on Pseudomonas F medium or cetrimide agar</td>
<td>Positive</td>
<td>Negative (some positive)</td>
</tr>
<tr>
<td>Growth at 42°C</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Slime in gluconate broth.</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>&quot;Fishy&quot; odour (trimethylamino)</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*Ps. aeruginosa* may be further divided into serological, phage and pyocin types. No typing system is as yet universally accepted for epidemiological purposes although pyocin typing seems to be the most useful.

Pyocins are bacteriocins (bacteriocidal substances active against other strains of the same or closely related species). It is likely that these are defective bacteriophages and their production is controlled by plasmids. Bacteriocin-producing strains are resistant to their own bacteriocins, thus pyocins can be used for typing of *Pseudomonas aeruginosa*. 
ENTERIC GRAM-NEGATIVE MICROORGANISMS

The enteric organisms are a large group of Gram-negative, non-spore forming, oxidase negative rods whose natural habitat is the intestinal tract of man and animals. Some (e.g. *Escherichia coli*) form part of the normal flora of the intestinal tract; others (e.g. *Salmonellae, Shigellae*) are regularly pathogenic for man. Enteric bacteria are aerobes and facultative anaerobes, ferment a wide range of carbohydrates, and possess a complex antigenic structure. In the laboratory the genera are separated by a few simple biochemical tests as indicated in the table below.

**Identification of Gram-negative enteric bacteria:**

<table>
<thead>
<tr>
<th>Lactose fermented rapidly</th>
<th>Lactose not fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli</strong></td>
<td><strong>Shigella species</strong></td>
</tr>
<tr>
<td>motile; flat, non-viscous colonies; indole positive</td>
<td>Non-motile; no gas from glucose</td>
</tr>
<tr>
<td><strong>Enterobacter aerogenes</strong></td>
<td><strong>Salmonella spp.</strong></td>
</tr>
<tr>
<td>Raised colonies; motile; more viscous growth.</td>
<td>Motile; acid and usually gas from glucose</td>
</tr>
<tr>
<td><strong>Klebsiella pneumoniae</strong></td>
<td><strong>Proteus species</strong></td>
</tr>
<tr>
<td>Very viscous, mucoid growth; non-motile.</td>
<td>Some species “swarm” on agar; urea rapidly hydrolysed (smell of ammonia).</td>
</tr>
<tr>
<td><strong>Serratia, Citrobacter, Hafnia, etc.</strong></td>
<td></td>
</tr>
</tbody>
</table>

The reactions of the most important species of enteric Gram-negative microorganisms are shown in the following pages. While identification of these Gram-negative bacteria is often achieved using a few biochemical tests and agglutination reactions, there are a significant number of other bacteria with very similar reactions which may confuse the identification. To identify these bacteria a widely expanded range of tests has to be used.
THE COLIFORM BACTERIA

The coliform bacteria are a large and heterogeneous group of Gram-negative rods resembling, to some extent, *Escherichia coli*. The complexity of the group, the variations in biochemical test results, and the changing ecologic relationships have led to a confusing profusion of names. Besides *E. coli*, derived from the intestinal tract, the following organisms can be included among the "coliforms".

Typical *Klebsiella pneumoniae*, originally known as a respiratory pathogen, is now the most commonly encountered member, especially in hospital infections. It is characterised by mucoid growth, large polysaccharide capsules, and lack of motility. *Enterobacter aerogenes* is motile, exhibits less mucoid growth, has small capsules and may be found free-living as well as in the intestinal tract, in urinary tract infections and in sepsis. (*Enterobacter* was formerly called *Aerobacter*). *Serratia marcescens* is a small usually free-living Gram-negative rod that may produce an intense red pigment in culture. *Serratia* usually ferments lactose very slowly.

**GENERAE ESCHERICHIA, ENTEROBACTER AND KLEBSIELLA**

**Material:**
Cultures of *Escherichia coli* (O), *Enterobacter aerogenes* (P) and *Klebsiella pneumoniae* (Q) on:
- MAC
- XLD
- Chromogenic Urinary Tract Infection agar

Gram stains of *Escherichia coli* (O), *Enterobacter aerogenes* (P) and *Klebsiella pneumoniae* (Q).

**Description:**
Gram-negative rods, motile or non-motile, may be capsulated. Grow well on basal media. Biochemically active, acid and usually gas produced from glucose, lactose and mannitol. Facultatively anaerobic.

**Diagnosis:**
Isolated from faeces, urine, wounds, sputum and water on MAC - pink colonies (i.e. lactose fermenters).
GENERALSALMONELLA, SHIGELLA AND PROTEUS

Material:
Cultures of *Salmonella entericia* serovar Typhimurium (S. Typhimurium) (R), *Salmonella entericia* (S), *Shigella sonnei* (T) and *Proteus mirabilis* (U) on:
- MAC
- XLD
- Salmonella Chromogenic agar
- Chromogenic Urinary Tract Infection agar (*Proteus* only)

Gram stains of *Salmonella* Typhimurium (R), *Shigella sonnei* (T), and *Proteus mirabilis* (U).

GENUS SALMONELLA

Description:
Gram-negative rods, usually motile. Grow well on basal media. No growth in KCN broth. Biochemically active, producing acid and gas in sugars (S. Typhi acid only). *Salmonellae* fail to ferment lactose or sucrose, to produce indole, to hydrolyse urea or to liquefy gelatin, H₂S usually positive. Significant changes in bacterial names have resulted in 2 valid species with a number of subspecies and serovars now recognised. Widely distributed in animals, humans and birds.

Diagnosis:
Isolated from faeces, foods, blood (*Salmonella Typhi*) etc. directly onto MAC or XLD plate. With specimens preliminary enrichment in selenite broth may be useful. Colourless colonies (non-lactose fermenters) on MAC. Species can be identified by agglutination reactions and grouped using the Kauffman-White classification.

Serodiagnosis:
- Widal agglutination for 'H' and 'O' antibodies.
- Vi antibody for typhoid carriers (haemagglutination)
- In doubtful cases repeat serology for evidence of a rising titre.

Phage typing:
*Salmonella Typhi*
Over 90 different types can be identified by lytic phage reactions. The most common types in Australia are EI, then A and DI. *Salmonella Paratyphi* B and *Salmonella Typhimurium* may also be typed by phage reactions.
GENUS SHIGELLA

Description:
Gram-negative, non-motile rods, usually confined to humans. Grows well on basal media. No growth in KCN broth. Biochemically active, producing acid only (no gas) in sugars. H₂S not produced. Do not ferment lactose (except *Sh. sonnei*: late lactose fermenter, 2-8 days). Facultatively anaerobic.

Diagnosis:
Isolated from faeces, rectal swabs, either by direct plating or after selenite enrichment, on MAC. Shigella species are identified by agglutination reactions and rapid biochemical kits.

GENUS PROTEUS

Description:
Gram-negative, pleomorphic, motile rods. Grow well on NA, often with characteristic swarming (these bacteria do not swarm on bile containing media, examine the blood agar plate to see swarming). Growth in KCN broth. Biochemically active; acid and gas from many carbohydrates; non-lactose fermenters; hydrolyse urea. Facultatively anaerobic.

Diagnosis:
Isolated from urine, pus, faeces, etc. on MAC or XLD; colonies colourless; rapid urea hydrolysis; mannitol negative (except *Pr. rettgeri*).
PARASITIC INFECTIONS

Gastrointestinal infections can be the result of either viral, bacterial or protozoal infections. Viral infections are usually determined by using immunoassays and this will not form a part of this course. You will be primarily detecting bacterial pathogens by cultivation of faeces on a range of selective agars however it is also important to be aware that protozoal infections can also be detected by microscopic examination of fresh faecal samples. A wet preparation of the faecal samples is prepared and then scanned by microscopy (40x magnificent is sufficient due to the size of the protozoa). Identification of the causative organism is based on the characteristic morphologies of the different protozoa.

In addition direct microscopic examination of faecal samples also allows for the detection of blood cells if present. These are usually associated with more severe infections and are indicators of damage to the intestinal epithelium as often seen with cases of dysentery.

NORMAL FAECAL FLORA

The flora of the large intestine (colon) is qualitatively similar to that found in faeces. Populations of bacteria in the colon reach very high levels of $10^{11}$/g faeces. The predominant species are anaerobic *Bacteroides* and anaerobic lactic acid bacteria in the genus *Bifidobacterium* (*Bifidobacterium bifidum*). Coliforms (e.g. *E. coli*), enterococci, clostridia and lactobacilli can be regularly found however they are usually outnumbered (1000:1 to 10000:1) by *Bacteriodes* and *Bifidobacterium* species. *Bacteriodes*, *E. coli* and some enterococci are some of the organisms that can result in serious disease when transferred into other sites of the body. Harmless protozoans can also occur in the intestine (e.g. *Entamoeba coli*) and these can also be considered as part of the normal flora of the intestine.
Exercises for Kit Two

1. Record the appearance and characteristics e.g. colour, haemolysis, etc of the gram-positive organisms examined on the various media.

<table>
<thead>
<tr>
<th>Organism</th>
<th>HBA aerobic</th>
<th>HBA anaerobic</th>
<th>MAC</th>
<th>Selective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteriodes fragilis (L)</td>
<td></td>
<td></td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Campylobacter jejuni (M)</td>
<td></td>
<td></td>
<td></td>
<td>CSA:</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (N)</td>
<td></td>
<td></td>
<td></td>
<td>Pseudo A:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism</th>
<th>MAC</th>
<th>XLD</th>
<th>Chromogenic UTI</th>
<th>Chromogenic Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli (O)</td>
<td></td>
<td></td>
<td></td>
<td>na</td>
</tr>
<tr>
<td>Enterobacter aerogenes (P)</td>
<td></td>
<td></td>
<td></td>
<td>na</td>
</tr>
<tr>
<td>Klebsiella pneumonia (Q)</td>
<td></td>
<td></td>
<td></td>
<td>na</td>
</tr>
<tr>
<td>Salmonella Typhimurium (R)</td>
<td></td>
<td></td>
<td></td>
<td>na</td>
</tr>
<tr>
<td>Salmonella enterica (S)</td>
<td></td>
<td></td>
<td></td>
<td>na</td>
</tr>
<tr>
<td>Shigella sonnei (T)</td>
<td></td>
<td></td>
<td></td>
<td>na</td>
</tr>
<tr>
<td>Proteus mirabilis (U)</td>
<td></td>
<td></td>
<td></td>
<td>na</td>
</tr>
</tbody>
</table>
2. Describe the colony appearance of the mixed culture set up last week and give an interpretation of this appearance. Perform a gram stain on the different colonies on the MAC plate and record the results.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Appearance On MAC</th>
<th>Interpretation</th>
<th>Appearance On XLD</th>
<th>Interpretation</th>
<th>Gram stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Perform oxidase tests on the organisms you isolated above (unknowns 1 and 2) and unknown 3. Make a presumptive identification based on these and the above results.

<table>
<thead>
<tr>
<th>Unknown</th>
<th>Oxidase</th>
<th>Presumptive ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tutor's signature:  ………………………………………………..
IDENTIFICATION KEY

GRAM NEGATIVE COCCI (GNC)

Atmospheric requirements

Aerobes +/- CO₂ oxidase positive
Neisseria spp.

Strict anaerobes
Veillonella

Sugar fermentation

Organism Enriched agar required (eg CBA) glucose maltose sucrose

N. meningitidis + + + -
N. gonorrhoeae + + - -
Branhamella catarrhalis - - - -
N. sicca - + + +
IDENTIFICATION KEY

GRAM NEGATIVE RODS (GNR)

- Strict anaerobes
  - *Bacteroides* spp
- Facultative anaerobes
  - Oxidase negative
- Strict aerobes
  - *Pseudomonas* spp
    - Oxidase positive
    - Non-lactose fermentor

OR

- Microaerobes
  - *Campylobacter* spp
    - Catalase positive
    - Oxidase positive

Fastidious growth requirement

No

Lactose fermentation

- Positive
  - Indole test
  - Positive *E. coli*
  - Negative *K. pneumoniae*

- Negative
  - Motility
    - motile
      - *Shigella* spp
        - (divided into species by reaction with antibody)
    - non-motile
      - *Salmonella* spp
        - (divided into species by reaction with antibody)

Requires CBA / V & X factors

*Haemophilus influenzae*
WEEKS 4 – 9
Diagnosis of Bacterial Infections

For this part of the course, students will work on 1 specimen each from simulated wound, urines, faecal and throat samples to isolate and identify bacterial pathogens. The aim of these practicals is to give you an opportunity to experience the work of a clinical microbiology laboratory, and to consolidate your knowledge in medical microbiology.

This work will be assessed by the following criteria:

1. **Laboratory workup.**
   Observations. Reasons for actions. (Workups on specimens must be available for inspection by your tutor at any time). Evidence of the use of appropriate procedures for isolation, identification and sensitivity testing.
   Correct identification of organisms.

2. **The completed signed laboratory report.**
   Content of the report, choice of amount, relevance and appropriateness of information included, interpretation of significant findings, use of summary comments.
   Format. Accuracy and completeness. Choice of a sensible and clear format for presenting the report.

3. **Student Laboratory Work.**
   Technical skills (e.g., streak plates, inoculation of biochemical tests, slide agglutination etc.). Evidence of familiarity with diagnostic procedures. Careful bench work and follow-ups and evidence of appropriate attitude and application.

**SPECIMENS**

All specimens potentially contain human pathogens. Therefore use strict aseptic precautions at all times and discard specimens into the appropriate containers.

**LABORATORY PROCEDURES**

1. **Receipt of Specimens**
   Record batch number (A, B or C), sample number, nature of the specimen, date, name of the patient and examinations requested into your lab book.
2. Inoculation of Specimens

As soon as possible inoculate specimens onto appropriate media as described below. **Write a description of the macroscopic appearance of the specimen on the back of the request form, together with the results of any direct microscopic examination, e.g. Gram stain, wet preparation.**

On the back of the request form make a note of the media used and the atmosphere in which the cultures were incubated and the results of any other tests performed.

3. Examination of Incubated Plates

Examine the cultures by the naked eye and a hand lens having a focal length of approximately three inches. Note each different type of colony and, working in order of predominance of growth, label with small Roman letters a, b, c etc.

Use the following semi-quantitative criteria for recording the amount of growth observed.

i) confined to 1st set of streaking inoculum +
ii) reaching to 2nd set of streaking ++
iii) reaching to 3rd and 4th set of streaking +++

Note the approximate diameter, measured in millimetres, of an isolated colony of each type, followed by a sufficient description of the colony to enable anyone looking at the culture later to be able easily to distinguish it from the others present. Next, make smears from single colonies of each type from all the plate cultures and Gram stain them. Enter the result of microscopic examination on the form.

N.B., As you become more experienced in colony recognition, it will not be necessary to do so many Gram stains.

4. Preparation of laboratory report

A work sheet is for use in the laboratory and is seldom suitable for final transmission to the clinician. Production of a suitable report with interpretive comments is an issue discussed in all the following sections.
THE LABORATORY REPORT

Once you have completed your investigation and have identified the organisms present and done the appropriate sensitivities, it is time to write the report.

The laboratory report is the major method of communication between the laboratory and the clinician. In special instances doctors will contact the microbiologist by telephone but usually they rely on the report.

Deciding what to write on the report is the most difficult feature of medical microbiology. Guidelines for writing reports include:

1. Where possible microbiologists should indicate on the report their interpretations of the significance of the laboratory results.

2. If the request form gives insufficient data or the specimen was poorly taken, this should be communicated back to the clinician. In certain cases material, e.g. urine and sputum, should not be processed if a poor specimen is provided. However, if the specimen appears important the clinician should be notified by phone that a new specimen is required.

3. Only sensitivities to the most useful and relevant antibiotics should be reported. Sensitivity results should only be reported when there is a likelihood that chemotherapy would be appropriate. In other cases, the clinician should be notified that sensitivities are available on request.

4. The report should give only relevant information and should be free of laboratory jargon: Details of any investigation or cultural methods should be omitted unless they would be useful to the clinician in interpretation of results.

5. The names of unusual organisms should not be reported unless there is comment on its origins and likely significance, e.g. *Enterobacter cloacae*, Group C β-haemolytic Streptococci, *Acinetobacter* sp.

6. When numerical data is reported normal values should be included.

7. Where special techniques have yielded negative results or specific important pathogens have been excluded, this should be stated, e.g. "No growth on anaerobic culture" in correctly taken specimen from an abdominal wound. "*Neisseria gonorrhoeae* not isolated" in urethral discharge from patient with urethritis.
8. Bacteria that are normal flora at the site from which the specimen is taken should not be reported by name. However if "normal flora" are reported, the location of the normal flora should be written down, e.g. skin, intestinal, vaginal etc.

9. Any comment with regard to possible significance of isolates must be qualified in the absence of complete knowledge of the patient's history.

10. When interpretation of significance is difficult and the microbiologist is uncertain, then this should be communicated to the clinician.

11. Precedent from the literature should be quoted where relevant.

12. Semi-quantitative terms of growth should be restricted. The amount of growth should be reported only when it reinforces the likely interpretation, e.g. "Heavy growth" when significant or "scanty growth" when significance doubtful. The terms heavy, moderate and light without any qualification should be avoided.

13. If alternative interpretations are possible they should be written on the report.

14. All data that contribute to possible interpretation of significance should be reported.
**AN EXAMPLE OF A COMPLETED CASE STUDY WORKSHEET & REPORT:**

**Specimen**: Wound swab - right leg

**Workup**: On the back of the request form.

<table>
<thead>
<tr>
<th>Gram stain:</th>
<th>Gram pos cocci (+++). Numerous PMN seen (+++)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media inoculated:</td>
<td>Results</td>
</tr>
<tr>
<td>HBA/O₂</td>
<td>+++ 1-2mm round col. Gram pos cocci</td>
</tr>
<tr>
<td>HBA/AnO₂</td>
<td>+++ as per O₂, smaller col.</td>
</tr>
<tr>
<td>MAC</td>
<td>+++ 1mm round col. Lactose ferm.</td>
</tr>
<tr>
<td>MSA</td>
<td>+++ 1mm round col. Mannitol ferm.</td>
</tr>
<tr>
<td>Further tests:</td>
<td>Catalase pos.</td>
</tr>
<tr>
<td></td>
<td>Coagulase pos.</td>
</tr>
<tr>
<td>Presumptive ID:</td>
<td><em>Staph. aureus</em></td>
</tr>
<tr>
<td>Sens:</td>
<td>Meth, Ery, Gent, Ceph, Vancomycin</td>
</tr>
<tr>
<td>Res:</td>
<td>Pen</td>
</tr>
</tbody>
</table>
DR J.L. HARKNESS
MICROBIOLOGY DEPT.
L1S O'BRIEN BUILDING
ST VINCENT'S HOSPITAL

MRN: (9000)9-05-34-38
Name: TEST, MICROBIOLOGY
Age: 27 YRS DOB: 2008
Sex: F

SWAB CULTURE
SOURCE: WOUND
RIGHT LEG
Accession No: M-96-29265

Collected: 13AUG96 1806
Received: 13AUG96 1810
Started: 13AUG96 1810

-------------GRAM STAIN------------------
13AUG96 1827
3+ Polymorphs
3+ Gram positive cocci resembling Staphylococci

FINAL REPORT----------------------
13AUG96 1827
3+ Staphylococcus aureus

----------ANTIMICROBIAL SENSITIVITY REPORT----------
S aureus

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>R</td>
</tr>
<tr>
<td>Meth/Flucloxac</td>
<td>S</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>S</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>S</td>
</tr>
</tbody>
</table>

Printed: 13AUG96 1830 hrs
Page: 1
End of Report
PERMANENT REPORT
Comment:

A potential pathogen in a wound has been isolated. The request form states that the wound looks infected. Therefore, it is possible the clinician will treat with antibiotics although in many cases local treatment, e.g. drainage of pus, would be sufficient. Thus the sensitivity to a limited range of antibiotics should be reported.

Resistance to Penicillin should be reported.

Methicillin (Flucloxacillin drug of choice)

Cephalothin - alternative to Flucloxacillin if hypersensitive to penicillin.

Erythromycin - alternatives to Flucloxacillin if hypersensitive to penicillin.

Gentamycin and Vancomycin should not be reported as there are less toxic alternatives.

Interpretative comment is not necessary on this report although it could be argued that the senior microbiologist when signing the report could include a comment emphasizing that antibiotic therapy would probably be unnecessary unless the patient is showing signs of systemic involvement, e.g., fever. Drainage of the wound would usually be satisfactory in the absence of complications.
WEEK 4
Wound Swabs

PROCEDURES

Week 4 – Setting up plates
1. Collect your plates and label them with your name, date, specimen number, and incubation conditions (aerobic, $O_2$ or anaerobic, $AnO_2$). Plates required are:
   - MAC ($O_2$)
   - MSA ($O_2$)
   - 2 x HBA (one $O_2$ and one $AnO_2$)
2. Streak the primary inoculum onto all four plates and then swab sample onto a glass slide for a gram strain. Streak the plates for single colonies.
3. Place plates in appropriate incubation boxes (note place $AnO_2$ plates straight into anaerobe jars provided).
4. Perform a gram stain on the primary swab and record the presence of organisms and any inflammatory cells.

Week 5 – Reading and interpreting plates:
5. Examine all plates and record the type and number of any organisms isolated.
6. Gram stain representative colonies from each plate.
7. For any strict anaerobes note the following:
   a. Bacteroides fragilis: - Gram negative rod, test Ampicillin and Kanamycin sensitivities on HBA plates – Bacteroides are Ampicillin Resistant and Kanamycin Resistant.
   b. Cl. perfringens - Gram positive rod, look for double zone of lysis.
8. For Gram positive cocci perform catalase test. If positive (Staph) perform a coagulase test, if negative (Strep) do a Streptococcal grouping test.
9. For Gram negative rods perform an oxidase test and identify the isolate using a commercial kit (RapID SSU system, note that RapID ONE is used in routine laboratory diagnosis. We use RapID SSU as it requires only 2 hr incubation).
10. Set up sensitivity plates if needed – depends on organisms isolated (see below).
11. Write up lab report using the conventions listed below. If appropriate report sensitivities; depending upon isolate identification.

Note: mixed infections may occur. (Read next section "Significance of Isolates").
Significance of isolates

1. Specimen from normally sterile site, e.g. osteomyelitis, pus from cerebral abscess. Report all organisms. Saprophytes are of doubtful significance.

2. Saprophytes, e.g. *Staph epidermidis*, *Bacillus* sp. Report only if from Hickman's catheter or central line sites.

3. Specimen from external site, eg skin wound. *Staph aureus* and *b haemolytic strep* group A, C, G are always reported.

Other organisms:
- Light growth - describe organisms present and report "probable contamination”.
- Moderate/heavy growth - pure culture - identify and report with sensitivity.
- Mixed culture - list organisms from macroscopic appearance. No sensitivities. Mixed faecal flora can be reported as such without investigation.
- In presence of a lot of pus, identify all organisms and report with sensitivities.

Interpretation and reporting conventions:

An important principle when considering the results of a wound swab is that the primary treatment for an infected wound is drainage. This removes the majority of bacteria from the site and allows the host defence mechanisms to resolve the infection. However there are certain pathogens that have invasive properties that must always be considered as significant, e.g. *Strep. pyogenes* in any numbers, *Clostridium perfringens* in other than very light growth. *Staph. aureus* infections will usually respond to drainage but sensitivities should always be reported.

All other organisms other than skin contaminants should be reported unless in very low numbers. However, whether sensitivities should be reported is a contentious topic. If the request form gives no indication of systemic involvement, it can be argued that sensitivities be not reported, but it is noted on the report that sensitivities are "available on request". At present most hospital laboratories will report sensitivities to all isolates from mixed infections. Certainly, there are no clear data in the literature which show that particular organisms in mixed infections contribute more than others. However, *Bacteroides fragilis* and *Escherichia coli* have been shown to be of major importance in animal experiments with infections caused by organisms from the intestinal tract. Therefore no clear guidelines for interpretation can be written here at present. You are encouraged to discuss this important issue with your tutor. One proven consequence of reporting sensitivities in wound specimens is that it encourages additional usage of antibiotics.
**Transport of pus specimens:** In most hospitals, wound swabs are transported in Stuart's Transport Medium. Certain anaerobes will only survive for brief periods in this medium and so other methods should be used. Aspirates of pus are considered the best specimen for preserving anaerobes as long as air is excluded. However, swab specimens are adequate as long as they are maintained in a moist oxygen free environment. Various commercial swab kits or transport media are available that maintain these conditions. Dry swabs or swabs in Stuart's held for longer than 12 hours should not be processed for anaerobes. The clinician should be notified that the specimen was inadequate. Specimens from the following sites should be cultured for anaerobes: brain abscess, oral cavity abscess, sinus swab, aspirate of pus from chest, aspirate or swab from middle ear, heart abscess, necrotising skin and deep tissue infection, surgical wounds, gas forming cellulitis, gas gangrene, symbiotic gangrene, pilonidal abscess, liver or subphrenic abscess, ischiorectal abscess, endometrial, fallopian tube or tubo ovarian abscess (theatre specimen), intra-abdominal (including pelvic abscess), peritonitis, appendix, post-operative bowel surgery wound, bile, amputation stump.

**Likely causative agents:** Staph. aureus, Strep pyogenes; Gram Negative Rods (GNR), anaerobes.
WEEK 5
Urinary Tract Infections

Background
The important signs and symptoms of infection are that the patient feels pain and tenderness in the loins and abdomen, high fever, pain at micturition and a general feeling of being unwell. Sometimes there are no signs and symptoms in the presence of active infection whereas on other occasions, symptoms may be present without infection.

Factors predisposing to urinary tract infection are those in which stasis occurs, for example: pregnancy, renal stone (calculus), congenital abnormality, urethritis, tumour, paraplegia.

Urinary tract infection is commonly observed in pregnant women. In about 5 per cent of women during the first three months of pregnancy there is a significant bacteriuria, i.e. more than 10^8/L (100,000 orgs/ml). In these women severe infection may occur at later stages of pregnancy; this may predispose to premature birth of the infant.

LABORATORY DIAGNOSIS OF URINARY TRACT INFECTIONS
Laboratory diagnosis of urinary tract infection depends on
- counting of viable bacteria
- on microscopic examination of the urine
- on culture of the organisms for identification and antibiotic sensitivity testing.

Collection and transport of specimens is very important.

1.1 Counts of viable bacteria in urine specimens
*Escherichia coli* is the causative agent in the majority of urinary tract infections (about 80%); however species of *Proteus*, *Klebsiella*, *Pseudomonas* and *Staphylococcus* may also cause infection. Each of these organisms may contaminate urine, thus causing a problem as to the significance of their presence in a specimen of urine. It has been pointed out that generally speaking, when the number of organisms present is less than 3x10^6/litre, they probably represent contamination. A viable count of more than 10^8/litre is considered as significant bacteruria and is associated with infection. Figures between 3x10^6 and 10^8 are equivocal and tests should be repeated.

1.2 Aetiology of urinary tract infection

<table>
<thead>
<tr>
<th>Organism</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Commonest cause by far</td>
</tr>
<tr>
<td><em>Proteus</em> spp</td>
<td>Often associated with renal stones</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp</td>
<td>May be hospital - or catheter-associated</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>Usually low grade pathogens</td>
</tr>
<tr>
<td><em>Staphylococcus saprophyticus</em></td>
<td>Confined to young women</td>
</tr>
<tr>
<td><em>S. epidermidis/aureus</em></td>
<td>Hospital acquired, associated with urinary catheters or bacteraemia</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Recurrent UTI or underlying pathology</td>
</tr>
</tbody>
</table>
URINE

DIAGNOSTIC FLOW CHART

Likely causative agents: Enteric Gram negative rods, *Staph saprophyticus*, *Enterococcus faecalis*

Note macroscopic appearance

Biochemical urinalysis  Test for antibacterial activity (not done)

Microscopic examination (counting chamber): WBC, RBC and epithelial cell numbers

Presence of motile *Trichomonas vaginalis*, bacteria, yeasts, casts and crystals.

Culture on MAC  Direct sensitivity (if +++ (>10^8/L) organisms seen)

Flood sensitivity agar plate with 1 drop of urine in 5 ml saline. Remove excess.

*Culture on Chromogenic Urinary Tract Infection agar/Blood agar (1/2 plates).

Note: if yeasts seen by microscopy also culture on Candida Chromogenic agar.

Record: Viable count (> or <10^8/L)

Pure/mixed growth

PMN/epithelial cells

Identification tests:

Coliforms: Gram stain, oxidase test, RapID SS/u system

*Pseudomonas*: Gram stain, oxidase test, RapID SS/u system

*Staph* species: Gram stain, catalase, coagulase/novobiocin sensitivity testing.

*Strep* species: Gram stain, catalase, Streptex grouping test

Sensitivity test: Ampicillin, Cotrimoxazole, Norfloxacin, Gentamycin, Augmentin, Cephalexin.
LABORATORY PROCEDURES TO BE USED FOR URINARY TRACT INFECTIONS

**Week 5 – Setting up plates**
1. Record the macroscopic appearance of the urine
2. Direct microscopic examination of the urine sample using a counting chamber
   a. Record the number of WBC, RBC and epithelial cells
   b. Examine for the presence of motile Trichomonas vaginalis, bacteria, yeasts, casts and crystals (for the latter 2 see descriptions on page 80).
3. Collect your plates and label them with your name, date, specimen number, and incubation conditions (aerobic, O₂ or anaerobic, AnO₂). Plates required are:
   - MAC (O₂)
   - Chromogenic UTI/Blood (1/2 plates) (O₂)
   - SENS (O₂)
   - Note: if yeasts are seen by microscopy also set up a Candida Chromogenic agar plate (O₂)
4. Streak the urine sample, for single colonies, on half the MAC plate and the Chromogenic UTI/Blood half plates. If yeasts were seen in microscopic examination also inoculate a Candida Chromogenic agar plate.
5. On the remaining half of the MAC plate set up 2 x Urostrips (paper strip method) for viable cell count (see page 74).
6. Perform a direct sensitivity of the urine sample if >10⁹/L organisms were seen by microscopy. Add one drop of the urine sample to ~5 mL of saline and flood a SENS plate. Remove excess liquid (NOTE treat as contaminated waste – DO NOT dispose of in the sink) and let plates dry for ~20 mins and then add antibiotic sensitivity disks (Ampicillin, Cotrimoxazole, Norfloxacin, Gentamycin, Augmentin, Cephalexin).
7. Place plates in appropriate incubation boxes.

**Week 6 – Reading and interpreting plates:**
8. Examine all plates and record the type and number of any organisms isolated.
9. Gram stain representative colonies from each plate.
10. Perform identification tests based on Gram stain results (as listed on the flow diagram) and repeat sensitivity testing if original plate was contaminated.
11. Write up the lab report with interpretive comments as discussed in all the following sections. An example of a typical report is shown on page 66.
**1. VIABLE COUNT ON URINE**

**i) Drop method:** Using the calibrated loop, transfer one drop of undiluted urine to one half of a MacConkey agar plate and spread. Prepare a $10^{-3}$ dilution of the urine and, using 0.02 ml calibrated loop, transfer one drop of this dilution to the other half of the MacConkey agar plate and spread.

**ii) Paper strip method:** Dip two blotting paper strips in the undiluted urine and allow excess fluid to drain off. Touch the surface of a MacConkey agar plate with the folded ends (12 x 6 mm of the strips).

The viable count from the paper strip method is reported as follows:

<table>
<thead>
<tr>
<th>COCCI</th>
<th>BACILLI</th>
<th>COUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;6 colonies</td>
<td>&lt; 5 colonies</td>
<td>$&lt;10^7$ orgs/litre</td>
</tr>
<tr>
<td>6-30 colonies</td>
<td>5 - 25 colonies</td>
<td>$10^7$ orgs/litre</td>
</tr>
<tr>
<td>&gt;30 colonies</td>
<td>&gt;25 colonies</td>
<td>$&gt;10^8$ orgs/litre</td>
</tr>
</tbody>
</table>

In general, when the number of organisms present is less than $3 \times 10^6$ they probably represent contamination. A viable count of more than $10^8$ orgs/litre is considered as significant bacteriuria and is associated with infection. Figures between $3 \times 10^6$ and $10^8$ are equivocal and tests should be repeated. Specimens of urine obtained by suprapubic aspiration may have low counts and any organisms found could be significant.
2. MICROSCOPIC EXAMINATION OF URINE

Normal urine contains less than 10 red cells and white cells per cmm; no bacteria are visible. Infected urines may contain numerous red cells, white cells and organisms.

(a) **Enumeration of cells in urine**

- urine is placed in a counting chamber and the cells counted.

Results:

- Less than $10 \times 10^6$ rbc/litre \} = Normal
- Less than $10 \times 10^6$ wbc/litre \} = Normal
- $10 - 100 \times 10^6$ rbc/litre \} = Probably increased
- $10 - 100 \times 10^6$ wbc/litre \} = Probably increased
- More than $100 \times 10^6$ rbc/litre \} = Increased above normal
- More than $100 \times 10^6$ wbc/litre \} = Increased above normal

Increased **wbc** excretion indicates infection and rbc may also be present.

If only **rbc** are increased it may indicate:

1. trauma, e.g. during catheterisation or renal calculus
2. tumours
3. nephritis

Numerous squamous **epithelial** cells suggest contamination, especially with vaginal secretions.

(b) **Gram-stained preparation of centrifuged urinary deposit**

- **Normal urine** - no cells or bacteria
- **Infected urine** - wbc and bacteria (usually Gram negative rods)
- **Contaminated urine** - numerous epithelial cells and Gram positive rods.
KOVA Glasstic slide 10 with Grid Chamber
Chamber volume: 6.6 µl
Chamber Depth: 0.1 mm
Outer Grid Dimension: 3 mm x 3 mm
Volume within Grid: 0.9 µl
Small Grid Size: 0.33 mm x 0.33 mm
Small Grid Volume: 0.01111 µl

GLASSTIC SLIDE GRID
Estimate the average number of each cell type in each square mm and thus the number of each cell type $\times 10^6$ / L of urine as follows:

<table>
<thead>
<tr>
<th>&lt;1</th>
<th>&lt;1</th>
<th>&lt;1</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

An average of <1 cell in each square millimetre (e.g., 1 in some, 0 in others or <9 in total chamber) is equivalent to:

$<10$ cells $\times 10^6$/L of urine

(as $<1 \times 9 \times 10/9 = <10$ L)

<table>
<thead>
<tr>
<th>1-10</th>
<th>1-10</th>
<th>1-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>1-10</td>
<td>1-10</td>
</tr>
<tr>
<td>1-10</td>
<td>1-10</td>
<td>1-10</td>
</tr>
</tbody>
</table>

An average of 1-10 cells in each square millimetre is equivalent to:

$10-100$ cells $\times 10^6$/L of urine

(as $1 \times 9 \times 10/9 = 10$ and $10 \times 9 \times 10/9 = 100$ L)

<table>
<thead>
<tr>
<th>&gt;10</th>
<th>&gt;10</th>
<th>&gt;10</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

An average of >10 cells in each square millimetre is equivalent to:

$>100$ cells $\times 10^6$/L of urine

(as $>10 \times 9 \times 10/9 = >100$ L)
### INTERPRETATION OF RESULTS
(From the Clinical Microbiology Update Programme Monograph No. 27)

**Summary table: Interpretation and reporting of midstream urine results.**

<table>
<thead>
<tr>
<th>Viable count*, (cfu/L)</th>
<th>Number of bacterial species</th>
<th>PMN</th>
<th>Report</th>
<th>Name organism</th>
<th>Report sensitivities</th>
<th>Interpretation</th>
<th>Note #</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 10^8</td>
<td>1</td>
<td>+**</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>Consistent with UTI</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 10^8</td>
<td>1</td>
<td>-</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>Possible UTI</td>
<td></td>
</tr>
<tr>
<td>10^7-8</td>
<td>1</td>
<td>+</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>Possible UTI</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 10^8</td>
<td>2</td>
<td>+/-</td>
<td>yes</td>
<td>yes</td>
<td>yes (both)</td>
<td>Possible UTI</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 10^8</td>
<td>1</td>
<td>squamous epithelials</td>
<td>no</td>
<td>yes</td>
<td>yes (predominant organism)</td>
<td>Likely UTI</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 10^8</td>
<td>2 species present, (one &gt; 10^8, one &lt; 10^7)</td>
<td>+</td>
<td>yes</td>
<td>yes</td>
<td>yes (predominant organism)</td>
<td>Possible UTI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10^7</td>
<td>Any</td>
<td>+/-</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>No significant growth</td>
<td></td>
</tr>
</tbody>
</table>

* In certain situations lower viable counts are significant.

** See page 75 - generally counts of WBC > 10^8/L indicate a level increased above normal and indicate an infection

# See Notes on following page for details of the basis for the comment.
Details of the basis for the comments in the table.

**Note a.** Pure culture of $\geq 10^8$ cfu/L.

**Note b.** Reduced viable count, pure growth. Children's Hospital reports the organism with sensitivities and the comment "Possible infection". St. Vincent's and Douglass do the same if PMN are present and if not, the organism is named without sensitivities together with the comment "Doubtful significance. Suggest repeat if clinically indicated."

**Note c.** Mixed growth of two organisms, both $\geq 10^8$/L. This usually represents contamination especially if squamous epithelial cells are present. In this case the report reads: "Mixed growth of A + B. Indications are that external genital flora have been introduced during specimen collection" or "Probable contamination. Repeat if necessary."

However, certain conditions predispose to infection with multiple organisms, and if repeated cultures from a patient show mixed organisms the case should be investigated to find a cause. Both organisms are reported + sensitivities and the presence of PMN could be influential in commenting "Possible UTI".

**Note d.** Predominant growth by one organism of $\geq 10^8$/L. The predominant organism is named with its sensitivities and the comment "Likely UTI". The likelihood falls as the number of PMN decreases.

**Note e.** Mixed growth with no predominance and reduced viable count. "Mixed growth of A + B. Indications are that external genital flora have been introduced during specimen collection" or "Probable contamination. Repeat if necessary".

**Note f.** Mixed growth of 3 organisms showing no predominance. Report as in Note e.
4. APPEARANCE OF CRYSTALS UNDER THE MICROSCOPE

**CRYSTALS OF NORMAL URINE**

- Uric acid
- Ammonium Urates
- Calcium Oxalate
- Amorphous Phosphates
- Calcium Phosphate
- Triple Phosphate
- Calcium Carbonate
- Amorphous Urates

**CRYSTALS OF ABNORMAL URINE**

- Cystine
- Cholesterol
- Bilirubin
- Leucine
- Tyrosine
- Sulfa
- Hippuric Acid
Specimen: Surname: Smith
Cather Specimen Urine (CSU) Other names: John
(State site of collection of all swabs and method of collection of all urine samples) Address: 28 The Boulevarde Kensington

Time collected...10am... Date:...10.04.09
Copies of report to Dr Mitchell
Provisional diagnosis Urinary tract infection
Clinical notes Fractured femur – indwelling cather, blood in urine
Epidemiological features (if viral or other contagions suspected)
Onset/Duration 09.04.09 Occupation Retired Recent travel
Current antibiotic therapy Amoxycillin

Dose and time of last dose (for assay request)

The Health Insurance Act requires the following section to be hand written by the doctor
Tests requested Requesting MO Employee No. Signature
Microscopy, Culture & Sensitivity (MC&S)

Report:

<table>
<thead>
<tr>
<th>Chemistry:</th>
<th>pH 6</th>
<th>Ketones -</th>
<th>Urobilinogen -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose +</td>
<td></td>
<td>Leucocytes -</td>
<td>Nitrite +</td>
</tr>
<tr>
<td>Protein –</td>
<td></td>
<td>Bilirubin -</td>
<td>Acetoacetic acid -</td>
</tr>
<tr>
<td>Blood –</td>
<td></td>
<td>Specific gravity 1.2</td>
<td></td>
</tr>
<tr>
<td>Cell Count:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC: &gt; $10^5$/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC: &gt; $10^6$/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial cells &lt; $10^6$/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria: &gt; $10^6$/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pure culture of E. coli ($10^9$/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Antibiotic sensitivity test:

| Sensitive: Amoxycillin/Clavulanate, Gentamycin, Trimethoprim, Norfloxacin, Cephalexin | Resistant: Ampicillin |
WEEK 6
Faecal samples

PROCEDURES

Week 6 – Setting up plates
1. Collect your plates and label them with your name, date, specimen number, and incubation conditions (aerobic, O2, microaerobic (MO2) or anaerobic, AnO2). Plates required are:
   - MAC (O2)
   - XLD (O2)
   - Campylobacter Selective Agar (CSA) (MO2 for CSA plate)
   - chromogenic Salmonella (O2)
2. Streak the primary inoculum onto all four plates and then streak the plates for single colonies.
3. Place plates in appropriate incubation boxes (note place MO2 plates straight into anaerobe jars provided).

Follow up: Week 7 – Reading and interpreting plates:
4. Examine all plates and record the type and number of any organisms isolated. (Please note: if no non-lactose fermenters (NLF) were seen on agar after 24 hr incubation usually the lab would sub culture a selenite broth onto MAC (O2) to look again for NLF (Salmonella, Shigella and others) however this is not being done as part of this practical program.)
5. Gram stain representative colonies of suspect pathogens from each plate.
6. For suspect Salmonella perform the following:
   a. Salmonella antisera slide agglutination test
7. For suspect Shigella perform the following:
   a. Shigella antisera slide agglutination test
8. For suspect Campylobacter perform the following:
   a. oxidase test
   b. catalase test
   c. wet preparation
9. If a pathogen is detected, report it. Sensitivities are not done as antibiotic therapy is not indicated in the majority of gastroenteritis cases. If no pathogen is detected, report "No Campylobacter, Salmonella or Shigella detected".
Other less frequent pathogens not examined in this practical program include *Vibrio cholerae, Vibrio parahaemolyticus, Clostridium difficile* and *Yersinia* species.

1. *Vibrio* spp - *Vibrio cholerae* in patients with diarrhoea who have returned from cholera endemic area *Vibrio parahaemolyticus* in patients with gastroenteritis after ingesting seafood.

2. *Clostridium difficile* - *C. difficile* colitis is associated with antibiotic therapy. It is present as normal flora in a small percentage of adults and many infants, but no toxin is present in these cases; in patients with the disease the organism is present in large numbers and toxin is present. Culture for organism and detection of toxin production needs to be determined.

3. *Yersinia enterocolitica* - Causes mild and occasionally severe gastroenteritis in children and adults. Can be cultured from faeces on Blood agar or MacConkey's agar.
WEEK 7
Throat swabs
THROAT SWAB DIAGNOSTIC FLOW CHART

Likely bacterial pathogens for pharyngitis: \( \beta \)-haemolytic strep group A (strep group C or G also possible)

- Suspect pharyngitis
  - No Gram stain
  - HBA (O\(_2\)) without diagnostic discs
  - HBA (AnO\(_2\)) plus two Bacitracin diagnostic discs

- Suspect Gonorrhea
  - No Gram stain
  - Gonococcal agar

- Suspect oral candidiasis
  - Gram stain: look for yeast cells and psuedomycelium
  - SAB + HBA
  - Germ tube test if significant numbers of yeasts
    - \textit{Candida albicans}

\( \beta \) haemolytic colonies, Gram positive cocci, catalase negative

- Bacitracin sensitive
  - \( \beta \)-haemolytic strep A (\textit{Strep pyogenes})
    - Confirm Identity using Strep Grouping kit
- Bacitracin resistant
  - Identify using Strep Grouping kit
    - \( \beta \)-haemolytic strep group B, C, G

\textbf{NOT DONE IN THIS PRACTICAL PROGRAM}

The School of Biotechnology & Biomolecular Sciences, UNSW 2017
PROCEDURES

Week 7 – Setting up plates

1. Collect 2 x HBA plates and label them with your name, date, specimen number, and incubation conditions (one plate is incubated aerobically, O₂ and one anaerobically, AnO₂).

2. Streak out for single colonies and aseptically place 2 bacitracin discs on the first and second intersections of streak lines (see diagram in flow diagram).

3. Place plates in appropriate incubation boxes (note place AnO₂ plates straight into anaerobe jars provided).

Follow up Week 9 – Reading and interpreting plates:

4. Examine plates for any β-haemolytic organisms and note whether they are sensitive or resistant to bacitracin.

5. For any β-haemolytic organisms perform a gram strain and catalase test to confirm they are Streptococci and then a Streptococcal grouping test.

6. If Strep pyogenes is isolated set up a sensitivity plate (HBA sens) with penicillin, erythromycin, tetracycline disks.

7. Write up lab report using the conventions listed on the following page.
Interpretation and reporting conventions for throat wabs:

A. Growth of bacitracin sensitive β haemolytic colonies.
   **Report:** β haemolytic streptococcus Group A (Strep pyogenes)
   **Note:** Test and report sensitivity profile for Pen, Tet & Ery only.

B. i. Growth of bacitracin resistant β-haemolytic colonies.
   Group C and G
   **Report:** β haemolytic Strep group C/G.
   Unusual but established cause of pharyngitis

ii. Growth of bacitracin resistant β-haemolytic colonies.
    Group B and D
   **Report:** No Group A streptococcus isolated

C. No β haemolytic colonies grown.
   **Report:** No Group A streptococcus isolated

D. Growth of *Haemophilus influenzae*, *Strep. pneumoniae* and *Staph. aureus*.
   While these organisms can cause pneumonia, meningitis or ear infections, presence in the throat is not significant. Normal carriage rates may be quite high. Therefore these organisms are NOT reported.
   **Report:** No Group A streptococcus isolated.

E. Growth of coliform bacilli.
   Present when patient on antibiotics or very ill. Represents colonisation and should not be reported unless notes indicate a new-born baby with suspected acute generalised bacterial infection (neonatal sepsis).

F. Neisseria gonorrhoea is a not uncommon cause of pharyngitis.
   **Report:** Neisseria gonorrhoea isolated.
   Test and report sensitivity to antibiotics

G. Yeasts.
   Presence of pseudomycelia in Gram or significant growth of yeast suggest infection. Usually occurs in neonates, immunosuppressed patients and with antibiotic therapy.
   **Report:** Germ tube positive reported as *Candida albicans*.
   Germ tube negative reported as Yeast, not *Candida albicans*.

H. Throat swab from neonate with suspected acute generalised bacterial infection.
   Look for and report any of:
   - Enterobacteriaceae
   - Groups A and B streptococci
   - *Listeria monocytogenes*
   - *Staphylococcus aureus*
**Research project: isolation and characterisation of *Escherichia coli* from pet animals**

**Learning objectives**

1) Develop students' research skills.
2) Develop students' scientific report writing skills.
3) Develop students' understanding of human pathogens in the context of our environment.
4) Develop students' skills in data analysis.

**Assessments**

1. Literature review on molecular diagnosis of *E. coli* infections: 10%
2. Report of research results in a scientific paper format: 16%

1. Literature review on molecular diagnosis of *E. coli* infections

**Specifications:**

Total mark: 10%
Number of words: 1500-2000
Number of figures/tables: 1 to 5 in total.

**Content of literature review:**

The literature review aims to provide an overview of the diversity of *E. coli* including the types of pathogenic *E. coli* that may cause human infections, both intestinal and extraintestinal infections including uropathogenic *E. coli* (UPEC), enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), and enteroaggregative *E. coli* (EAEC) and build the knowledge base for the research project.

Diagnosis of diarrheagenic *E. coli* infections is often difficult or time consuming using traditional culture based diagnostic methods. Pathogenic *E. coli* strains may be classified into pathovars by the presence of specific virulence genes. The classification of EPEC, EHEC, STEC or ETEC is relatively more straightforward. However, there are significant difficulties for others such as EAEC, EIEC and UPEC. Many genes have been reported to be promiscuously associated with UPEC. Multiplex PCR is often used to allow detection of different pathogenic *E. coli* simultaneously.

The assignment will review current methods available for molecular diagnosis of *E. coli* infections using PCR or other molecular methods and the challenges in the diagnosis of *E. coli* infections. The pathogens should be covered are UPEC, EPEC, STEC, ETEC and EAEC. You may include additional *E. coli* pathogens such as enteroinvasive *E. coli* (EIEC) and any new pathogenic forms. The review should include the genetic basis of the molecular methods. This can include brief information of the functions of the genes, but
detailed mechanisms of pathogenesis are not required and should not be included. The review must also include specificity and sensitivity of the methods.

The references cited for this lab manual are a starting point. You will need to perform a PubMed search using Keywords such as *Escherichia coli*, multiplex PCR detection, Diarrhoea, urinary tract infection. You may use key words of specific pathogens to narrow down your searches such as diarrheagenic *E. coli*, enteroaggregative *E. coli*, enterotoxigenic *E. coli*, enteropathogenic *E. coli*, Shiga toxin-producing *E. coli*.

**Due date:** Tuesday 2 pm, Week 9.

**Penalty if late submission:** 10% per day including weekends.

2. **Report of research results in a scientific paper format**

**Specifications:**

Total mark: 16%
Number of words: 2500
Number of figures/tables: 1 to 6 in total

**Content of the project report**

The report must be in a research paper format including introduction, materials and methods, results and discussion. Materials and methods should be presented in brevity and shall not exceed 600 words. This restriction is to prevent reports disproportionately describing the materials and methods. An abstract of no more than 100 words should be included (not in the 2500 word count).

Supplementary material (appendix) is allowed but will not be used for marking.

**Due date:** 5 pm Friday, Week 13.

**Penalty if late submission:** 10% per day including weekends.

**Project aims**

The overall aim of this project is to determine the diversity of *E. coli* in pet animals (dogs in particular) including the carriage of pathogenic *E. coli* as potential zoonotic human pathogens and to determine the antibiotic resistance profiles of *E. coli* carried by pet animals as a potential pool of antibiotic resistant pathogens and antibiotic resistance genes.

Specific aims:

1) Isolate *E. coli* from pet faecal samples, with 2 samples per students.

2) Determine their antibiotic resistance profiles to antibiotics commonly used for treating Gram negative infections.
3) Determine the phylogroup of one isolates obtained using Clemont’s multiplex PCR method.

4) Determine whether the isolate is a potential human UPEC pathogen by multiplex PCR.

5) Determine whether the isolate is a potential human intestinal (diarrheagenic) pathogen by multiplex PCR.

6) Determine the prevalence of \( E. \ coli \) phylogroups, pathogenic \( E. \ coli \) and antibiotic resistance in the pet animal \( E. \ coli \) population using class data.

**Project overview and planning**

1. Overview

\( E. \ coli \) is a diverse species. It is generally present in the large intestine and lower end of the small intestine of many mammals as a commensal. \( E. \ coli \) is also a major pathogen to humans and other animals causing intra-intestinal and extra-intestinal infections [1]. \( E. \ coli \) has been involved in infections of humans of virtually all body systems and tissues. Pathogenic \( E. \ coli \) can be distinguished into several pathogenic classes or pathovars based on mode of pathogenesis including enteropathogenic, enterohaemorrhagic, enterotoxigenic, enteroaggregative, enteroinvasive and extraintestinal pathogenic \( E. \ coli \) (EPEC, EHEC, ETEC, EAEC, EIEC and ExPEC respectively). EHEC is a subset of Shiga toxin \( E. \ coli \) (STEC). A new pathogenic form emerged recently during an outbreak in Germany of an O104:H4 strain that is a Shiga toxin producing enteroaggregative \( E. \ coli \) [2]. \( E. \ coli \) is present in the environment as a faecal contaminant and may also be a long-term inhabitant as environmental \( E. \ coli \) was found to be distinctive from other strains [3]. The intestinal pathogens are collectively referred to as intestinal pathogenic \( E. \ coli \) (InPEC) or diarrheagenic \( E. \ coli \) (DEC).

Domestic and other animals are natural reservoirs for some human pathogenic \( E. \ coli \) strains. A typical example is STEC which does not normally cause disease in these animals. On the other hand, the human ETEC and domestic animal ETEC strains are differentiated by their adhesion factors and as a result, human ETEC strains normally do not cause disease in domestic animals and vice versa [4]. UPEC strains can cause urinary tract infection in both humans and other animals and may be shared among human and pet animals with long term persistence and cross transmission.

\( E. \ coli \) has been divided into four main phylogenetic groups (phylogroups) (A, B1, B2, and D) [5]. Three additional minor phylogroups, F, G and H, have been defined. The four main phylogroups: A, B1, B2 and D have been well studied and a multiplex PCR based on presence/absence of three genes \( chuA \), \( yjaA \) and TSP.E4.C2 has been devised to type \( E. \ coli \) strains into one of the four groups. The typing method is often referred to as the Clemont method as it was developed by Clemont *et al.* [6]. The distribution of \( E. \ coli \) phylogroups has been extensively studied with a differential prevalence of the phylogroups in different hosts or human populations from different regions [5]. Some phylogroups have
been shown to be more likely to contain pathogens. Group A is more likely to be commensal while group B2 and to a lesser extent group D contain a larger proportion of ExPEC pathogens.

Antibiotic resistance is a major concern of public health. *E. coli*, both pathogenic and commensal forms, face the selection pressure from antibiotics used for treatment of *E. coli* infections or other infections. Therefore, antibiotic resistance in *E. coli* is rapidly rising, especially resistance to fluoroquinolones and cephalosporins [7]. Multidrug resistance (resistance to three or more drugs) is also common [8]. *E. coli* is capable of acquiring new resistance mechanisms. For example, NDM-1 mediated broad spectrum β-lactam resistance was first discovered in a patient who suffered an infection caused by NDM-1 resistant *Klebsiella pneumoniae*. From that patient’s faecal sample an NDM-1 resistant *E. coli* was also isolated, suggesting acquisition of NDM-1 resistance *in vivo* by faecal *E. coli* [9]. The recently discovered MCR-1 mediated colistin resistance was first reported in an animal *E. coli* [10]. As such companion animals may be an important reservoir of resistant *E. coli*.

This project will examine *E. coli* from pet animals to determine the prevalence of *E. coli* phylogroups, types of pathogenic *E. coli* and patterns of antibiotic resistance.

2. Project planning

The project manual has set out the tasks for the duration of the project. The minimum required time is 6 weeks and will run over weeks 6 – 12 (no class in week 8 as public holiday). Students are required to draw up a weekly plan for the project to map the project tasks to be allocated to each week’s lab class so that there is a good understanding of the amount of work, the nature of the work and the tasks to be completed each week. Some tasks overlap with other laboratory activities.

It is imperative that students understand the overall timeline so their projects run smoothly as no catch up of laboratory work is possible. Any student who cannot attend a practical class for medical or other legitimate reasons shall inform their tutors and seek assistance from other students to help complete that week’s task so that the project is not disrupted.
Week 6
Sample collection and culture on selective media

Task 1: Collection of dog faecal samples

You will need to collect a faecal sample from two different pets (dogs) using the equipment provided. If you do not have a pet, you may want to use a friend’s pet or could go to a local park. Note that if the sample is fresher it will be easier to work with in the lab.

Safety Warnings (Refer to pages 97-102 for Risk Assessments)

- If you are pregnant or immunocompromised, please contact your tutor on additional safety requirements.
- You must only attempt to collect the samples once the animal has left the area to avoid bites or scratches. You may also ask the dog owner to give you a sample if they collected their own dog droppings while in the park.
- You must take all equipment back to the lab to dispose of correctly.
- Try to collect the samples the day before the lab.
- Please make sure you have completed the fieldwork form. Tutor must sign off the form. You must carry the tutor’s contact or course convenor’s contact number in case of emergency.

Materials

- Two screw capped faecal sample collection tubes
- Two zip lock bags for samples
- Gloves for sample collection if required
- One large zip lock bag for used gloves

Procedure

1. Label your two screw capped tubes with your name, the date of collection and whether it is sample 1 or 2.
2. Locate faecal samples for your experiment. Wait a safe distance away; then approach the sample once the animal has left the area.
3. Use the spatula provided to collect a small amount of the sample.
4. Place the sample inside the screw-capped tube and screw the lid shut.
5. Place the tube inside the zip lock bag provided and put the loop into a different zip lock bag.
6. Repeat the procedure for steps 2-5 for your other sample. Make sure that the second sample is not from the same animal.
7. Keep the samples inside the zip lock bags overnight and store away from any food.
8. Take all equipment back to the lab (do not dispose of any material outside of the lab because it may contain pathogens).
9. In the lab, dispose of the bag containing the gloves into the bin provided.
10. Do a quick spin of your sample then place your labelled sample in the location provided by the tutors.
11. Write down all information into the class table including your name, sample number, type of pet, date of sample collection and location sample was collected from (postcode and/or latitude/longitude from Google maps).

**Task 2: Subculturing the Samples on selective plates**

**Subculturing the Sample onto EMB agar**

The next step is to dilute the sample and then streak it onto Eosin Methylene Blue agar (EMB). EMB agar is both selective and differential. It is selective for gram-negative bacteria, so will inhibit gram-positive growth and is differential for lactose and non-lactose fermenters. Lactose fermenters produce coloured colonies and *E. coli*, in particular, should have a metallic green sheen (refer to image in the appendix).

**Safety Warnings (Refer to pages 97-102 for Risk Assessments)**

- Never open centrifuge when it is still spinning
- Be aware of the open flame on the Bunsen Burner, so make sure hair is tied back
- Avoid infection by always handling samples carefully and wearing gloves

**Materials**

- P1000 pipette and tips
- Saline
- Loop and Bunsen burner
- 2 EMB plates
- Your two diluted and spun down samples

**Procedure:** Do this for both samples

1. Label the EMB plate with your name, sample number, date and ‘Possible *E. coli*’.
2. Add 500 μL saline to your sample and mix gently with the pipette.
3. Vortex the sample to allow the saline to mix well.
4. Spin down using a centrifuge so that faecal matter settles to the bottom.
5. Take a loop of the liquid at the top of the tube and streak onto EMB agar, following the directions in your lab manual (Make sure that you sterilise the loop in between each streak).
6. Place your streaked plate into the incubation box provided.

The samples will be stored in the fridge for you for the following week, then incubated for 24 hours prior to your next class.


**Week 7**

**Subculturing Colonies onto MacConkey Agar**

To further confirm that *E. coli* was isolated on the EMB agar, suspected *E. coli* colonies will be subcultured onto MacConkey agar. MacConkey agar is also selective and differential, selecting for gram negative bacteria. It is differential based on lactose fermentation and lactose fermenting colonies can be recognised by their pink appearance (non-lactose fermenters are colourless), so *E. coli* should appear pink.

Today you will choose one suspected *E. coli* colony from each EMB plate and streak them onto MacConkey agar to further confirm their identity.

Note: If one of your plates did not grow any suspected *E. coli*, you can choose 2 colonies from the sample plate however you must let your tutor know and write down that you are working with 2 colonies from the same animal.

If you did not get any suspected *E. coli* growth on either EMB plate you should talk to your tutor- they will find you a sample that you can use for the following stages of the project.

**Safety Warnings (Refer to pages 97-102 for Risk Assessments)**

- Be aware of the open flame on the Bunsen Burner, so make sure hair is tied back.
- Avoid infection by always handling samples carefully and wearing gloves.

**Materials**

- 2 MacConkey Plates
- Loop and Bunsen burner
- Your EMB plates containing suspected *E. coli*

**Procedure**

1. Label the two plate with your name, date and sample number and then label one ‘Sample 1’ and the other ‘Sample 2’
2. Observe your EMB plates and identify one metallic green colony on each plate (larger ones will be easier to work with) and circle them, labelling the colonies as ‘1’ and ‘2’
3. Streak each colony onto a separate MacConkey plate (make sure that you sterilise the loop in between each streak as single colonies are required).
4. Place the plate into the incubation box. Once again, it will be refrigerated for you and incubated in the 24 hours prior to your next class.
**Week 9**

**Confirmation via biochemical test, Antibiotic Sensitivity Testing, DNA Preparation and Preservation of bacterial isolate**

This week you have four tasks to complete:

1. Confirm that your suspected colonies are *E. coli* by using RAPID SS/U.
2. Set up your antibiotic sensitivity tests of your isolated *E. coli*.
3. Prepare your samples for PCR by extracting DNA from the samples.
4. Set up glycerol stocks to store your samples for later use.

**Task 1: Biochemical test to confirm *E. coli* using RAPID SS/U**

If you have identified possible *E. coli* colonies on the MacConkey agar (pink colonies) you need to confirm that it is *E. coli* and not other species such as *Aerobacter aerogenes* that appears quite similar on the agar plates used. The RAPID SS/U test uses a series of 11 biochemical tests that relies on colour change to indicate which bacteria is present. Results are entered into a sheet which generates a code that can be entered into the computer to tell you the likely identification of your bacterium.

**Safety Warnings (Refer to pages 97-102 for Risk Assessments)**

- Be aware of the open flame on the Bunsen Burner, so make sure hair is tied back
- Avoid infection by always handling samples carefully and wearing gloves

**Materials**

- 2 x RAPID SS/U kit
- 2 x inoculation broths
- Loop and Bunsen burner
- Your MacConkey plate containing suspected *E. coli*

**Procedure:** **Do this for both samples.**

1. Observe your MacConkey plate to see if suspected *E. coli* is present (pink colonies). If it is, then select one colony and circle it. Consult your tutor if necessary.
2. Label both your RAPID SS/U kit and inoculation broth with your name, date and sample number.
3. Dip your loop in the flame to sterilise it, then after cooling pick up your colony and mix it into the inoculation broth tube. Ensure the colony has come off into the broth and isn’t stuck to the tube- you may need to move it around a bit.
4. Vortex the tube until the mixture becomes cloudy.
5. Take the RAPID SS/U kit and peel back the top to expose a hole in the side. Pour the entire tube of your broth into the hole.
6. Mix the RAPID SS/U kit so that the liquid is dispensed equally along the kit, then tip the entire kit forward, allowing the liquid to settle into the holes at the front.
7. Place both your RAPID SS/U kits into the incubation box.
8. They will be incubated for you for the following 2 hours. During this time you should move onto Step 2 (setting up your antibiotic sensitivity testing).
9. After incubation, follow the RAPID SS/U protocol to identify colour change; and write down your results on the supplied RAPID SS/U paper.
10. Finally enter the code into the computer to discover if it is *E. coli* or not.

**Task 2: Antibiotic Sensitivity Testing**

Normally you would wait until the samples are confirmed to contain *E. coli* before starting this stage. However, due to time constraints you will begin this next step during the RAPID SS/U Incubation. You will identify which antibiotics your isolated *E. coli* is sensitive to by testing it against commonly used gram-negative antibiotics. The disc diffusion method will be used to see whether or not it is sensitive to Augmentin (AMC), Cephalothin (KF), Ampicillin (AMP), Co-trimoxazole (SXT), Gentamicin (CN) and Ciprofloxacin (CIP). To complete the disc diffusion method, a sensitivity plate is flooded with a diluted colony, and then antibiotic discs are added. After incubation, the zone of clearing around the disc is measured. If the zone is greater than or equal to 6 mm the bacterium is sensitive to the antibiotic, if it is smaller it is resistant. Refer to CDS method on page 106 as has been used previously.

Safety Warnings (Refer to pages 97-102 for Risk Assessments)

- Be aware of the open flame on the Bunsen burner, so make sure hair is tied back
- Avoid infection by always handling samples carefully and wearing gloves

**Materials**

- 2 x glass tubes filled with 2.5mL saline
- 2x sensitivity plates
- Loop and Bunsen burner
- P1000 pipette and tips
- Antibiotic disc dispenser for gram-negative bacteria.
- Your MacConkey plate containing suspected *E. coli*

**Procedure: Do this for both samples**

1. Label the glass tube containing saline with your name, date and sample number.
2. Label the sensitivity plate with the same details.
3. Sterilise your loop in the Bunsen burner and after cooling, pick up one of the MacConkey colonies and mix into the saline. (If the colonies are too close together you can use 3 small colonies instead).
Pour the entire contents onto the sensitivity plate, swirling the plate gently so the liquid covers the entire plate.

Using your pipette, gently suck up all excess liquid and discard.

Allow the plate to dry.

Using the antibiotic disk dispenser, place the machine over the plate and stamp the discs down. (If they don’t all come out, use sterilised forceps to take the individual discs out).

Place the plate in the incubator box with the lid facing up, so that the discs don’t fall off.

This will be incubated for you so you can analyse results next week.

DO NOT DISCARD YOUR MACCONKEY PLATE. THIS WILL BE USED FOR THE FOLLOWING SECTIONS

Task 3: Extracting DNA from Colonies using the Boiling Method

Before you can run the various PCRs in the second half of the project, you need to extract the DNA from the colonies you selected. Boiling is used to lyse the bacterial cells, and then the tubes are spun down to precipitate proteins present. The supernatant can then be used for analysis for the rest of this project.

Safety Warnings (Refer to pages 97-102 for Risk Assessments)

- Be aware of the open flame on the Bunsen Burner, so make sure hair is tied back
- Avoid infection by always handling samples carefully and wearing gloves
- Do not touch the heat block directly until it has cooled
- Do not open the centrifuge until it has stopped spinning

Materials

- 4 x 1.5 mL tubes
- P200 pipette and tips
- TE Buffer
- Loop and Bunsen burner
- Heating block
- Ice box
- Your MacConkey plate containing suspected *E. coli*

Procedure: Do this for both samples

1. Label two 1.5mL tubes with your name, the date, and sample number.
2. Pipette 200 μL of TE buffer into the first tube.
3. After sterilising your loop under the flame, cool the loop and pick up a single colony from the MacConkey plate.
4. Mix the colony in the TE buffer ensuring the colony comes off the loop.
5. Heat the tube for 5 minutes at 98 degrees Celsius on the heating block provided.
6. Sit the tube on ice for 2 minutes.
7. Spin the tube down at maximum speed on the centrifuge for 3 minutes.
8. Carefully pipette as much supernatant as possible out of the first tube without disturbing the pellet and dispense into the second tube. Discard the first tube.
9. Place the second tube in the area provided. It will be stored in the fridge until you do the PCRs.

Task 4: Prepare the Glycerol Stocks

The final task for today is to store the colonies you isolated on MacConkey agar in glycerol, similarly to how you stored your original sample in glycerol. This will enable the pure culture to be used in the future.

Safety Warnings (Refer to pages 97-102 for Risk Assessments)

- Be aware of the open flame on the Bunsen Burner, so make sure hair is tied back
- Avoid infection by always handling samples carefully and wearing gloves

Materials

- 2 x cryovials (one for each sample)
- Loop and Bunsen burner
- P1000 pipette and tips
- Glycerol- peptone storage media
- Your MacConkey plate containing suspected *E. coli*

Procedure: Do this for both samples

1. Label the cryovial with your name, the date and the sample number.
2. Fill the cryovial with 500 μL of glycerol-peptone storage media.
3. Sterilise the loop with the flame, then after cooling, scrape the entire half of the plate containing the sample, picking up as many colonies as possible.
4. Place the loop inside the glycerol and move it around, allowing the colonies to fall off.
5. Sterilise the loop
6. Mix the cryovial by rocking upside down and back and then place in the designated storage area. Samples will be stored at -20°C and available if required.
Task 1: Analysis of the Sensitivity Plates

The incubated sensitivity plates will be returned so you can analyse them. You will need to measure the zones of inhibition to identify what antibiotics your *E. coli* is sensitive or resistant to. To measure the zone of inhibition, you use a ruler to measure from the edge of the disc to the edge of the zone of inhibition (annular radius). Fill in the following tables with your results.

**Sample 1**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Zone of Inhibition (mm)</th>
<th>Sensitive or Resistant (S or R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Augmentin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalothin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Sample 2**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Zone of Inhibition (mm)</th>
<th>Sensitive or Resistant (S or R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Augmentin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalothin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Task 2: Multiplex PCR for phylogrouping and UPEC typing

Note: For all of these PCR reactions you will only use one of your samples. (The other will no longer be needed; it functioned as a back up in case one of your samples did not contain *E. coli.*)

PCR1: The Phylogrouping PCR

*E. coli* can be divided into 4 phylogroups: A, B1, B2 and D. Groups A and B1 are usually commensal while most virulent strains are in B2 and D. Groups are determined based on the combination of the gene fragments of *chuA*, *yjaA* and TSP.E4.C2.

PCR 2: UPEC PCR

Testing for UPEC (which causes urinary tract infections) is quite difficult because identification requires detection of more than one virulence gene. In this PCR, you will test for *PapC*, *fyuA*, *hlyA* and *traT*. If more than one of these virulence genes are present, the strain is likely to be UPEC.

One negative control should be set up per bench for each PCR.

To set up the negative control, follow the exact same directions as you will for your samples, but instead of adding your DNA, add water.

(Refer to pages 97-102 for Risk Assessments)

PCR #1: Phylogrouping PCR

Phylogrouping multiplex PCR detects following three gene fragments:

- *chuA*: 279bp
- *yjaA*: 211bp
- TSP.E4.C2: 152bp

Safety Warnings (Refer to pages 97-102 for Risk Assessments)

- Avoid infection by always handling samples carefully and wearing gloves

Materials

- 2 x PCR tubes
- Your extracted DNA
- MyTaq
- Buffer
- Primer Mix (containing *chuA*, *yjaA* and TSP.E4.C2)
- Water
- P2 and P20 pipettes and tips
- Ice bucket
**Procedure:**

1. Label a mini PCR tube with your initials and ‘P’.
2. To the tube add the following reagents, making sure that the tube has been thawed and mixed before adding it
   - 1 μL DNA
   - 4 μL Buffer
   - 1.2 μL Primer Mix
   - 0.1 μL Taq (added by demonstrator)
   - 13.7 μL Water
3. Mix the tube and then do a quick spin to ensure all the liquid is at the bottom of the tube.
4. Place the tube in the designated location.
5. The PCR will be run for you at the following conditions
   i. 96°C for 1’
   ii. 94°C for 15”
   iii. 55°C for 30”
   iv. 72°C for 1’
   v. Repeat Steps ii to iv 34 times
   vi. 72°C for 5’

**PCR #2: UPEC PCR**

Identifying UPEC is quite complicated because Urinary Tract Infections can be caused by commensal bacteria invading the urinary tract. UPEC infections cannot be determined by one gene alone, so you will test for 4 genes in a multiplex PCR.

- **papC** (148 bp)
- **fyuA** (745 bp)
- **hlyA** (1020 bp)
- **traT** (244 bp)

These genes are fairly common in UPEC and Kudinha *et al* recognised the following prevalence in their 2011 paper.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Role</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>papC</td>
<td>Fimbriae assembly</td>
<td>80</td>
</tr>
<tr>
<td>fyuA</td>
<td>Iron acquisition</td>
<td>78</td>
</tr>
<tr>
<td>hlyA</td>
<td>Haemolysin toxin</td>
<td>68</td>
</tr>
<tr>
<td>traT</td>
<td>Cell surface protein</td>
<td>77</td>
</tr>
</tbody>
</table>

- Avoid infection by always handling samples carefully and wearing gloves
Materials

- Your extracted DNA
- MyTaq
- Buffer
- Primer Mix (containing chuA, yjaA and TSP.E4.C2)
- Water
- P2 and P20 pipettes and tips
- Ice bucket

Procedure

1. Label a mini PCR tube with your initials and ‘U’.
2. Add the following reagents, making sure that each tube has been thawed and mixed before adding it
   - 1 μL DNA
   - 4 μL Buffer
   - 0.1 μL Taq (added by demonstrator)
   - 2 μL primer mix
   - 13.7 μL Water
3. Mix the tube and then do a quick spin to ensure all the liquid is at the bottom of the tube.
4. Place the tube in the designated location.
5. The PCR will be run for you at the following conditions
   i. 96°C for 1’
   ii. 94°C for 15”
   iii. 55°C for 30”
   iv. 72°C for 1’
   v. Repeat Steps ii to iv 34 times
   vi. 72°C for 5’
**Week 11**

**Agarose Gel Electrophoresis/ Analysis of Previous Results and Set up InPEC PCR**

This week you will begin by loading your PCR products in the agarose gel set up by the prep staff. You will then analyse the results and record which virulence factors are present. While the gel is running you will set up your final InPEC typing PCR.

**Task 1: Agarose Gel Electrophoresis of Phylogroup and UPEC PCRs**

**Safety Warnings (Refer to pages 97-102 for Risk Assessments)**

- Agarose gel electrophoresis uses a high voltage so never open chamber when in use
- Do not run the GelDoc without assistance from your demonstrator
- Although “GelRed” is non-toxic, handle with gloves and avoid skin contact. Gloves must be inspected prior to use. Use proper glove removal to avoid contact with skin and dispose contaminated gloves appropriately.

**Materials (per bench)**

- 1 electrophoresis chamber
- Gel loading buffer
- HyperLadder 2
- The negative control

**Loading the PCR Products and Imaging the gel**

1. Do a quick spin of your product.
2. Mix 4 μL of product with 1 μL of gel loading buffer and inject into the well using a yellow tip on a P20 pipette.
3. Once the whole table has loaded their products, load the negative control and then load ‘HyperLadder2 50 bp’ into the final well.
4. Place cover on electrode post and run gel at 150V until the Bromophenol Blue dye has migrated approximately half-way down the length of the gel.
5. After electrophoresis, turn power off, remove dam and electrodes and pour off liquid into liquid waste container. Add GelRed post-staining solution to the gel and leave to stain for 15 minutes.
6. An image of the gel will be captured using the Gel Doc system. You should keep and secure this image in your manual.
7. **DISPOSE OF THE GEL IN THE SCIENTIFIC WASTE CONTAINER PROVIDED.**
Analysis of Phylogroup PCR results

1. Using the ladder as a guide, estimate the sizes of bands present in your sample's lane.

Refer to Figure 2 as an example gel for this experiment. *chuA* is the top band at 279bp, *yjaA* is the middle band at 211 bp and TSP.E4.C2 is the bottom band at 152bp. The band sizes are calculated by comparing with HyperLadder2.

*Example results*

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figures 1 (left) and 1 (right). Figure 1 shows a sample gel where lanes 1 and 3 are positive for all three bands. Lanes 2 and 4 are only positive for TSP.E4.C2. Figure 2 shows HyperLadder2 which is used to calculate the sizes of bands present.

2. Use the following flow chart to identify which phylogroup your sample belongs in.

![Flow chart image]

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Bacteria & Disease 91

Fill in the following table in your book, and in the class records

<table>
<thead>
<tr>
<th>Sample</th>
<th>Colony Number</th>
<th>chuA (+/-)</th>
<th>yjaA (+/-)</th>
<th>TspE4.C2 (+/-)</th>
<th>Suspected Phylogroup</th>
<th>Suspected Pathogen? (Yes or No)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of UPEC PCR results

Use the band sizes present to determine which genes are present and fill results into the following table and into the class data sheet

<table>
<thead>
<tr>
<th>Sample</th>
<th>papC (+/-)</th>
<th>fyuA (+/-)</th>
<th>hlyA (+/-)</th>
<th>traT (+/-)</th>
<th>Suspected UPEC?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Example Results

Figure 3: Example of UPEC bands. Lane 1 is positive for fyuA, traT, and papC. The top band is fyuA, middle band is traT and bottom band is papC. hlyA is not present in this gel. Lane 2 is the negative control.
Task 2: InPEC PCR

The InPEC PCR is used to identify pathogenic *E. coli* types based on virulence factors. Pathovars that are common between dogs and humans include EPEC, EHEC and EAEC so these are the types being tested for. This PCR should identify EHEC, EPEC and EAEC based on the combination of the genes *aggR, astA, pic, escV, bfpB* and *stx2*.

There are 2 multiplex PCR to detect these genes:
- Mix 1: *astA* (102bp), *aggR* (400bp), *pic* (1111bp) and *uidA* (1487bp)
- Mix 2: *escV* (544bp), *bfp* (910bp) and *stx2* (324bp)

Note that *uidA* is present in the majority of *E. coli* strains so serves as a positive control. *uidA* presence does not indicate pathogenic *E. coli*.

**One negative control should be set up per bench for each mix.**

Safety Warnings (Refer to pages 97-102 for Risk Assessments)

- Avoid infection by always handling samples carefully and wearing gloves

Materials

- Your extracted DNA
- MyTaq
- Buffer
- Mix 1 of primers
- Mix 2 of primers
- Water
- P2 and P20 pipettes and tips
- Ice bucket

Procedure

1. Label 2 mini PCR tube with your initials and numbers 1 and 2.
2. Use the following table as a guide to add the various components to each tube. (Note that the volume of primers and water are variable depending on the mix being added).

<table>
<thead>
<tr>
<th>Tube Number and Mix</th>
<th>DNA (μL)</th>
<th>Buffer (μL)</th>
<th>Primer Mix (μL)</th>
<th>Taq (μL) (added by demonstrator)</th>
<th>Water (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1.6 (Mix 1)</td>
<td>0.1</td>
<td>13.3</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1.2 (Mix 2)</td>
<td>0.1</td>
<td>13.7</td>
</tr>
</tbody>
</table>
3. Mix each tube and then do a quick spin.
4. Place the tubes in the designated location.
5. The PCR will be run for you at the following conditions
   i. 96°C for 1’
   ii. 94°C for 30”
   iii. 63°C for 30”
   iv. 72°C for 1’30”
   v. Repeat Steps ii to iv 29 times
   vi. 72°C for 5’
Week 12
Agarose Gel Electrophoresis/ Analysis of InPEC PCR results

Task 1: Setting up Agarose Gel Electrophoresis/ Loading the PCR Products/ Imaging the Gels

Refer to the sections on running the agarose gel electrophoresis from last week and determine sizes of bands present using HyperLadder 2.

Analysis of Results

Use the table below summarising virulence factors associated with pathovars to determine which pathovar your sample belongs to. (Muller et al, 2007)

<table>
<thead>
<tr>
<th></th>
<th>aggR</th>
<th>astA</th>
<th>pic</th>
<th>escV</th>
<th>bfpB</th>
<th>stx2</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPEC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>EHEC/STEC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>EAEC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fill in the following table with your results (+ or – for each virulence factor) and add it to the class list.

<table>
<thead>
<tr>
<th>Colony Number</th>
<th>uidA</th>
<th>aggR</th>
<th>astA</th>
<th>pic</th>
<th>escV</th>
<th>bfpB</th>
<th>stx2</th>
<th>Suspected Pathovar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4: Example results using Primer set Mix 1 which shows positive bands for *uid* (top band), *pic* (middle band) and *astA* (bottom band). MW-molecular weight markers.

Figure 5: Example results positive for *escV* and *bfp*. For this gel, primers were not in a mix, they were added individually with the sample. Lane 1 contains *escV* and Lane 2 contains *bfp*. MW-molecular weight markers.
Summary of Results

Fill in the following table to summarise your results for this entire project. Fill in the information in the class data set also.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Phylogroup</th>
<th>Possible UPEC?</th>
<th>Pathovar</th>
<th>Antibiotics it is sensitive to</th>
<th>Antibiotics it is resistant to</th>
</tr>
</thead>
</table>

Acknowledgement
The course convenor gratefully acknowledges the assistance of Violet Warner for piloting this project and developing the lab manual for this research project.

References

For further reading on distribution of *E. coli* across species


Phylogrouping PCR adapted from:


UPEC PCR and gene selection adapted from:


InPEC PCR adapted from:

### Risk Assessments

#### Risk Assessment 1: Collection of Samples

<table>
<thead>
<tr>
<th>Task</th>
<th>Hazard</th>
<th>Associated Harm</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collecting faecal samples</td>
<td>The animal</td>
<td>Bites or scratches could lead to infection</td>
<td>Only attempt to collect the sample once the animal has left the area.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Never approach or play with dogs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wear gloves to protect hands.</td>
</tr>
<tr>
<td>Transport of samples to the</td>
<td>Spilled samples and</td>
<td>Pathogenic bacteria can lead to infection</td>
<td>Use screw capped tubes provided and keep them inside secondary container of sealed plastic bag. Dispose of waste correctly so that people are aware of the hazard associated with each disposal area.</td>
</tr>
<tr>
<td>laboratory</td>
<td>contaminated equipment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handling faecal samples</td>
<td>Pathogens in faecal sample</td>
<td>Faecal oral transmission leading to infection</td>
<td>Wear gloves to collect samples, washing hands after handling samples. Appropriate aseptic techniques. Correct PPE when work in the lab</td>
</tr>
<tr>
<td></td>
<td>(see table below for possible pathogens)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Human pathogens potentially carried by companion dogs and routes of transmission

1. Pathogens carried by dogs

Zoonotic pathogens potentially carried by dogs include bacteria, fungi, parasites, protozoa and viruses. Pathogens shown to be transmittable to humans from domestic dogs in Australia are summarised in Table 1.

Table 1: Zoonotic pathogens potentially carried by dogs

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Microbiological and epidemiological characteristics</th>
<th>Comments and likely mode of transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>Campylobacter species are commonly found in the intestines of food animals, birds, dogs and cats.</td>
<td>Known to be foodborne; young animals are more likely to excrete the pathogen.</td>
</tr>
<tr>
<td>Shiga toxin-producing <em>Escherichia coli</em> (STEC)</td>
<td>STEC bacteria have been isolated from animals such as cattle, pigs, sheep, dogs, cats, horses, and birds including seagulls and geese.</td>
<td>Known to be foodborne; children and immune-compromised individuals are at higher risk of STEC caused illnesses.</td>
</tr>
<tr>
<td><em>Leptospira interrogens</em></td>
<td><em>Leptospira</em> species, notably <em>L. interrogens</em> are pathogenic, causing leptospirosis in humans and animals.</td>
<td>Leptospirosis is a rare disease of dogs in Australia; can be foodborne but mostly an occupational disease associated with cattle or through exposure to contaminated (by animal urine) waterways.</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td><em>Salmonella</em> spp. are found in a wide range of animals including ruminants, poultry and dogs, and in various environmental sources, such as water, soil and animal faeces.</td>
<td>Known to be foodborne; children and immune-compromised individuals are at higher risk of <em>salmonella</em> cause illnesses.</td>
</tr>
<tr>
<td>Yersinia enterocolitica and <em>Y. pseudotuberculosis</em></td>
<td><em>Y. enterocolitica</em> and <em>Y. pseudotuberculosis</em> infects humans and a wide range of animals including dogs. <em>Y. enterocolitica</em> is usually transmitted to humans through ingestion of insufficiently cooked pork or contaminated water.</td>
<td>Known to be foodborne; transmission to humans is achieved through ingestion of contaminated food.</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptosporidium spp.</td>
<td>Humans and animals such as horses, pigs, sheep, goats, cattle, dogs and cats can be infected by Cryptosporidium spp.</td>
<td>Can be foodborne but person to person transmission is more common; children and immune-compromised individuals are at higher risk of disease.</td>
</tr>
<tr>
<td>Giardia spp.</td>
<td><em>Giardia</em> spp. can infect humans and many animals. <em>Giardia</em> is transmitted from host to host by ingesting cysts through contaminated feed or water.</td>
<td>Can be foodborne but person to person and contact with waterways are more common forms of transmission.</td>
</tr>
</tbody>
</table>

---

2 Zoonotic pathogens refer to pathogens that can be transmitted (sometimes via a vector) to humans through non-human animals, both domestic and wild.
### Parasites

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Description</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dipylidium</em> (dog tapeworm)</td>
<td><em>Dipylidium</em> and <em>Echinococcus</em> are tapeworms of cats and dogs. People become infected when they accidentally swallow tapeworm (<em>Dipylidium</em>) larvae excreted by flea or eggs in (<em>Echinococcus</em>) infected faeces. Infection with <em>Echinococcus</em> results in hydatid disease.</td>
<td>Hydatids are rare in domestic dogs in Australia and infection of dogs requires an intermediate (sheep) host. Not known to be foodborne.</td>
</tr>
<tr>
<td><em>Echinococcus</em> (hydatids)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ancylostoma caninum</em> (dog hookworm)</td>
<td><em>Ancylostoma caninum</em> is a parasite nematode. It lives in the small intestine of its host, such as dogs. <em>A. caninum</em> can infect humans.</td>
<td>Not known to be foodborne; contact with environment and skin penetration is the most common form of transmission to humans.</td>
</tr>
<tr>
<td><em>Toxocara canis</em> (dog roundworm)</td>
<td>Adult worms of the <em>Toxocara canis</em> live in the small intestine of dogs and puppies. Almost all puppies are infected at or soon after birth. <em>Toxocara</em> eggs can survive for years in the environment, and humans typically ingest the eggs via oral contact with contaminated hands.</td>
<td>Not known to be foodborne; direct contact with animals is the most common form of transmission to humans.</td>
</tr>
</tbody>
</table>

Source: Food standards Australia and New Zealand

### Risk Assessment 2: Streaking Colonies onto EMB Agar

<table>
<thead>
<tr>
<th>Task</th>
<th>Hazard</th>
<th>Associated Harm</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using the Bunsen burner</td>
<td>Flame</td>
<td>Burns to hands or hard</td>
<td>Hair should be tied back, never leave flame unattended and never use gloves when using the Bunsen burner.</td>
</tr>
<tr>
<td>Using the Centrifuge</td>
<td>Moving parts in centrifuge</td>
<td>Getting fingers caught in the centrifuge or centrifuge falling off surface</td>
<td>Make sure centrifuge is balanced before spinning and ensure it is turned off before opening the lid.</td>
</tr>
<tr>
<td>Diluting the sample</td>
<td>Sample coming out of tube</td>
<td>Infection</td>
<td>Correct PPE. Make sure the tube has been spun down before opening so that nothing is on the lid.</td>
</tr>
</tbody>
</table>
### Risk Assessment 3: Streaking Colonies onto MacConkey Agar

<table>
<thead>
<tr>
<th>Task</th>
<th>Hazard</th>
<th>Associated Harm</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using the Bunsen burner</td>
<td>Flame</td>
<td>Burns to hands or hard</td>
<td>Make sure hair is tied back, never leave flame unattended and never use gloves when using the Bunsen burner.</td>
</tr>
<tr>
<td>Subculturing</td>
<td>Sample contaminating your hands/clothes</td>
<td>Infection</td>
<td>Correct PPE, keep lid on samples whenever you are not using them, keep your lab books separate from lab work.</td>
</tr>
</tbody>
</table>

### Risk Assessment 4: Confirmation via RAPID SS/U kit

<table>
<thead>
<tr>
<th>Task</th>
<th>Hazard</th>
<th>Associated Harm</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using the Bunsen burner</td>
<td>Flame</td>
<td>Burns to hands or hard</td>
<td>Make sure hair is tied back, never leave flame unattended and never use gloves when using the Bunsen burner.</td>
</tr>
<tr>
<td>Using RAPID SS/U Reagents</td>
<td>Chemical</td>
<td>Irritation</td>
<td>Always wear gloves and appropriate PPE when doing RAPID SS/U testing and if chemicals spill clean up immediately.</td>
</tr>
</tbody>
</table>
### Risk Assessment 5: Antibiotic Sensitivity Testing

<table>
<thead>
<tr>
<th>Task</th>
<th>Hazard</th>
<th>Associated Harm</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using the Bunsen burner</td>
<td>Flame</td>
<td>Burns to hands or hard</td>
<td>Make sure hair is tied back, never leave flame unattended and never use gloves when using the Bunsen burner.</td>
</tr>
<tr>
<td>Selecting colony for testing</td>
<td>Sample</td>
<td>Infection</td>
<td>Correct PPE, keep lid on samples whenever you are not using them, keep your lab books separate from lab work.</td>
</tr>
</tbody>
</table>

### Risk Assessment 6: Extracting DNA from Colony using the Boiling Method

<table>
<thead>
<tr>
<th>Task</th>
<th>Hazard</th>
<th>Associated Harm</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using the Bunsen burner</td>
<td>Flame</td>
<td>Burns to hands or hard</td>
<td>Make sure hair is tied back, never leave flame unattended and never use gloves when using the Bunsen burner.</td>
</tr>
<tr>
<td>Using the Centrifuge</td>
<td>Moving parts in centrifuge</td>
<td>Getting fingers caught in the centrifuge or centrifuge falling off surface</td>
<td>Make sure centrifuge is balanced before spinning and ensure it is turned off before opening the lid.</td>
</tr>
<tr>
<td>Using the Heating Block</td>
<td>It is set to 98 degrees Celsius so is very hot</td>
<td>Burns</td>
<td>Never touch heat block directly, always use tongs to retrieve your sample.</td>
</tr>
<tr>
<td>Selecting colony for testing</td>
<td>Sample contaminating your hands/clothes</td>
<td>Infection</td>
<td>Correct PPE, keep lid on samples whenever you are not using them, keep your lab books separate from lab work.</td>
</tr>
</tbody>
</table>

### Risk Assessment 7: PCRs

<table>
<thead>
<tr>
<th>Task</th>
<th>Hazard</th>
<th>Associated Harm</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipetting the sample</td>
<td>Spilling the sample or disposing of</td>
<td>Infection</td>
<td>Correct PPE. Take care when pipetting</td>
</tr>
</tbody>
</table>
pipette incorrectly and dispose of tips in sharps bin. Clean up any spills immediately.

Using the Centrifuge

Moving parts in centrifuge

Getting fingers caught in the centrifuge or centrifuge falling off surface

Make sure centrifuge is balanced before spinning and ensure it is turned off before opening the lid.

**Risk Assessment 8: Agarose Gel Electrophoresis**

<table>
<thead>
<tr>
<th>Task</th>
<th>Hazard</th>
<th>Associated Harm</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Running the gel</td>
<td>High voltage</td>
<td>Electrocution</td>
<td>Correct PPE. Never turn power on until everything is set up and do not touch the chamber when in use. If there is a problem, turn off at power source immediately.</td>
</tr>
<tr>
<td>Imaging the gel</td>
<td>UV light and Ethidium bromide exposure</td>
<td>Risk of cancer</td>
<td>Do not operate the GelDoc without your tutor and never open the door when the gel is being imaged.</td>
</tr>
</tbody>
</table>
HS018a

Fieldwork Approval Form

Refer to HS917 Fieldwork Procedure for further information. This form is intended for use in documenting approval of a fieldwork activity. For low risk work in a low risk environment it may also serve as notification of the occasion. Fieldwork with a medium or high risk must also provide the information found in HS018b Fieldwork Notification. Approval modes other than this form are acceptable e.g. SafeSys – Activity.

<table>
<thead>
<tr>
<th>*denotes mandatory field</th>
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<tbody>
<tr>
<td>Purpose of fieldwork: collecting dog faecal samples in local parks</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date submitted:</th>
<th>Date Approved:</th>
<th>Approval withdrawn by:</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Date: Reason:</td>
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</table>

<table>
<thead>
<tr>
<th>Approving Person name and Role: A/Prof Ruiting Lan, course convenor</th>
<th>Signature:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fieldwork Leader:</td>
<td>Signature:</td>
</tr>
<tr>
<td>Specialist approver and Role:</td>
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<td>Specialist approver and Role:</td>
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<th>DFAT risk level:</th>
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<td>Travel Approval (&gt;100 km) obtained?</td>
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*Describe what tasks you will be doing:

*Fieldwork Locations:

*Proposed Period of Fieldwork:

*Risk Management documents ref: refer to course manual

<table>
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<th>Indigenous community</th>
<th>Other</th>
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<tr>
<td>Collecting</td>
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<tr>
<td>Indigenous community</td>
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<td>None</td>
<td>None</td>
<td>None</td>
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<tr>
<td>Other</td>
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<th>Students</th>
<th>Volunteers</th>
<th>Collaborators</th>
<th>Contractors</th>
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### Fieldwork communication & Emergency

<table>
<thead>
<tr>
<th>Callback Person: A/Prof Ruiting Lan</th>
<th>Secondary Contact: Group tutor, please fill in tutor contact</th>
</tr>
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<tbody>
<tr>
<td>Phone: 9385 2095 or 0431 577 553</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nearest 24hr hospital:</th>
<th>Telephone:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local Emergency Services contact:</td>
<td>Number of qualified First aiders:</td>
</tr>
<tr>
<td>Emergency Plan Ref:</td>
<td></td>
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<tr>
<td>Means of Communication:</td>
<td></td>
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</tbody>
</table>
TECHNIQUES IN MEDICAL MICROBIOLOGY

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1. THE CDS ANTIBIOTIC SENSITIVITY TEST

1. Sample colony
   use one large colony
   or three small colonies

2. Prepare suspension

3. Inoculate plate
   (pre-dried @ 37°C)

4. Distribute inoculum
   by rocking

5. Remove excess
   inoculum

6. Dry at room temp.
   (apprx 3/4 hr)

7. Load plate with antibiotic
   discs and incubate @ 37°C
   for 18 hr.

8. Measure the
   annular radii
Standard Sensitive Organisms

The sensitivity test on the Oxford *Staphylococcus aureus* (NCTC 6571) for Gram-positive organisms and a sensitive *Escherichia coli* (NCTC 10418) for Gram-negative organisms is generally carried out with each series of tests on unknown strains. *Pseudomonas aeruginosa* (NCTC 10490) is used as a control strain for sensitivity tests of this species.

Variation in zone sizes with the control strains are of value in indicating gross deterioration in the potency of batches of antibiotic discs, marked differences in media or generally poor technique of the operator.

**Expected results:**

Users of this method have access to tables showing expected values of zone size for each antibiotic and for each group of organisms. Examples of zones sizes are as following.

*Standard interpretation:* annular radius (AR) \( \geq 6 \text{ mm} = \text{susceptible} \)

\(< 6 \text{ mm} = \text{resistant} \)

*Exceptions:*

*Pseudomonas aeruginosa* v/s aminoglycosides and polymyxin, *Campylobacter* v/s erythromycin, *Neisseria meningitidis* v/s penicillin and enterococci v/s ampicillin, chloramphenicol, gentamicin (200µg) and nitrofurantoin: AR \( \geq 4 \text{ mm} = \text{susceptible} \)

\(< 4 \text{ mm} = \text{resistant} \)

Gram-positive cocci v/s vancomycin or teichoplanin: AR \( \geq 2 \text{ mm} = \text{susceptible} \)

\(< 2 \text{ mm} = \text{resistant} \)

**Antibiotics used in the practical program:**

**Gram-positive organisms** (except for throat swabs):

- Ciprofloxin (CIP 5)
- Erythromycin (E 15)
- Cefoxitin (FOX 30)*
- Penicillin (P 2.0)
- Tetracycline (TE 10)
- Vancomycin (VA 30)

*Note this is used for the detection of methicillin resistance and reported as methocillin resistance or sensitivity (not cefoxitin).

**Throat swabs:**

- Erythromycin (E 15)
- Penicillin (P 2.0)
- Tetracycline (TE 10)

**Gram negative organisms:**

- Ampicillin (AMP 10)
- Augmentin (AMC 30)
- Cephalothin (KF 30)
- Ciprofloxin (CIP 5)
- Cotrimoxazole (SXT 25)
- Gentamicin (CN 10)

**Urines:**

- Ampicillin (AMP 25)
- Augmentin (AMC 30)
- Cephalexin (CL 100)
- Gentamicin (CN 10)
- Norfloxacin (NOR 10)
- Trimethoprim (W 5.0)
# ANTIBIOTIC CODES

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Code</th>
</tr>
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<tr>
<td>Ampicillin</td>
<td>AMP</td>
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<tr>
<td>Augmentin</td>
<td>AMC</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>FOX</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>KF</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>CL</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CIP</td>
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<tr>
<td>Cotrimoxazole</td>
<td>SXT</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>E</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>CN</td>
</tr>
<tr>
<td>Imipenem</td>
<td>IPM</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>NOR</td>
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<tr>
<td>Penicillin</td>
<td>P</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>TE</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>TIC</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>W</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>VA</td>
</tr>
</tbody>
</table>

**Please note:** The number on the disc (e.g., CN10) relates to the amount of antibiotic impregnated into the disc. Certain antibiotics may be available in a number of different concentrations.
2. ANTIBACTERIAL ACTIVITY OF URINE

To test for any antibacterial action (ABA) of urine, plates are prepared by flooding a sensitivity agar plate with a suspension of standard sensitive *E. coli* (touch 5 colonies with a straight wire and emulsify in 5 ml saline; transfer one drop of this into a second 5 ml saline). Remove excess. To perform the ABA test, dip a sterile plain filter paper disc into the urine, drain off excess fluid and place the disc on the prepared plate. Seven tests can be done on one plate.

3. ANTISTREPTOLYSIN "O" TEST

The antistreptolysin 'O' (A.S.O.) test is the most satisfactory serological index of haemolytic streptococcal infection because (1) streptolysin is produced by all Group A β-haemolytic streptococci (*Streptococcus pyogenes*), (2) it is an excellent antigen (3) its antibody, A.S.O., can be accurately determined.

The test depends on the neutralization of a standard amount of streptolysin O by A.S.O., measured by inhibition of haemolysis, and the titre is expressed in units/ml of serum.

A.S.O. titre rises slowly two weeks after infection to a maximum in 4-5 weeks and persists for 6 or more months. The test is most useful in diagnosis of chronic streptococcal infection and doubtful cases of rheumatic fever. The titre has no relation to the severity of the rheumatic process nor is it of any value in predicting a rheumatic complication following acute streptococcal infection.

**Interpretation of Results**

- **<50 - 50 units/ml** No positive evidence of recent β-haemolytic streptococcal infection.
- **50 - 100 units/ml** May be due to recent infections with Group B β-haemolytic streptococci.
- **100 - 150 - 200 ml** Moderate titre. Possible evidence of recent Group A β-haemolytic streptococcal infection. Could repeat in 2 weeks.
- **250 - 400/ml** Titre raised. Evidence of recent Group A β-haemolytic streptococcal infection.
4. BACITRACIN SENSITIVITY

A presumptive identification of group A β-haemolytic streptococci can be made by bacitracin sensitivity testing.

Procedure:

Streak the culture on to a blood plate and place one disc on the first set of streak lines and one on the second set. Incubate at 37°C overnight.

Results:

- Inhibition of β haemolytic streptococci around disc: Group A (Strep. pyogenes)
- No inhibition of β-haemolytic streptococci: Another group

5. CATALASE TEST

Catalase is an enzyme capable of decomposing hydrogen peroxide, liberating gaseous oxygen. It is widely distributed in nature, being present in most aerobic cells. The function of catalase is to remove the toxic H2O2 as it is formed during oxidation-reduction processes involving O2.

\[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

To perform the test place one drop of H2O2 on a glass slide and emulsify a colony from an agar plate in it, OR place one drop of H2O2 on a colony on agar medium.

Results:

- Production of gas bubbles (O2) - positive
- No gas production - negative

6. COAGULASE TEST (STAPHYLOCOAGULASE)

The ability to coagulate plasma is the important criterion of pathogenicity in Staphylococci. The coagulase test identifies whether an organism produces the coagulase exoenzyme, which causes the fibrin of blood plasma to clot. Catalase producing organisms can form protective barriers of fibrin around themselves, making themselves highly resistant to a number of immune responses including phagocytosis and possibly some antimicrobial agents.
At least two substances make up staphylocoagulase, bound and free coagulase. The tube test does not distinguish them. The slide test detects bound coagulase only. Coagulase positive strains of *Staphylococcus aureus* are regarded as pathogenic.

(i) **Slide coagulase test**

Divide a slide into two compartments with grease pencil. In each compartment place a large drop of tap water and in these emulsiy enough growth from solid medium to produce an extremely thick and smooth suspension. Place a loopful of plasma near one of these drops and using the loop, run the drop of plasma into the drop of bacterial suspension.

**Results**

- Firm clumping of suspension immediately - positive
- A uniform suspension or easily dispersed clumps in both drops - negative

**NOTE:** Negative slide test should be checked by tube test for free coagulase.

(ii) **Commercial (Staphylase kit)**

Commercially prepared kits have now replaced the traditional slide coagulation test (using plasma). The staphylase test is a rapid slide identification test for *S. aureus*. The identifying characteristic for this test is still the ability of *S. aureus* to produce coagulase (the clumping factor). The Oxoid Staphylase Test detects the presence of coagulase through clumping of fibrinogen-sensitized sheep red blood cells.

**Instructions for the use of this kit are as an appendix at the back of this manual.**

8. **ENTEROBACTERIAEAE IDENTIFICATION: "SHORT SUGAR' TECHNIQUE**

This technique is commonly used in hospital laboratories. It is cheaper than the commercial kits and is adequate in majority of cases. It involves a set of biochemical tests viz, hydrogen sulphide, indole, motility, urease, citrate.

(a) **HYDROGEN SULPHIDE TEST**

In the routine bacteriology laboratory, the commonest use for tests for hydrogen sulphide production is in the identification of numbers of the Enterobacteriaceae.
Medium

For use in distinguishing members of the Enterobacteriaceae, the most common medium is Kligler's iron agar. This is a single tube differential medium serving as an indicator of glucose and lactose fermentation and of H2S and CO2 production in the identification of the Enterobacteriaceae. The main constituents are peptone, glucose (0.1%), lactose (1%), ferrous sulphate (to show H2S production) and phenol red indicator.

Interpretation

There are four simultaneously occurring reactions:

(i) Organisms which ferment glucose but not lactose will produce a yellow butt and the slope remains pink. Due to the low concentration of glucose, and fermentation taking place anaerobically, only a small amount of acid is produced. This accumulates in the butt of the medium, while at the surface the reaction proceeds to CO2 and H2O.

(ii) Organisms which ferment glucose and lactose will produce a yellow butt and slope.

(iii) Organisms which produce gas from either sugar will occasionally disrupt the medium. Gas production should be confirmed in peptone sugar media.

(b) INDOLE PRODUCTION

Please note appropriate protective clothing including gloves and safety glasses must be worn when handling Kovac's reagent.

Amino acids may arise as a result of the hydrolysis of proteins, peptones and peptides. One such amino acid is tryptophan which can serve as a substrate for biochemical differentiation of certain genera of bacteria within the Enterobacteriaceae. Such bacteria can hydrolyse tryptophan, by means of an enzyme tryptophanase one of the end products of this reaction being indole which can be detected colourometrically by a simple test.

Test

(i) Inoculate tubes of a liquid medium containing tryptophane-peptone water usually with the organism.

(ii) Incubate for at least two days. 7 days incubation may be necessary to obtain a positive result.

(iii) Approx 1 ml Kovac reagent (para-dimethylaminobenzaldehyde in ethyl alcohol)
(c) **MOTILITY TEST**

A motility medium is used for this test. The bottle of medium is inoculated by inserting a straight wire to about one third of the depth of the medium and incubated at 37°C overnight.

**Results**

- Growth away from the line of inoculation: motile
- Growth only along inoculation line: non-motile

(d) **UREA HYDROLYSIS**

This test is important for the identification of *Proteus* which rapidly hydrolyses urea. Other organisms break down urea, e.g., some coliforms, paracolon, *Pseudomonas* and staphylococcal strains, but at a slower rate (1 day or longer). The reaction is based on the action of urease on the substrate urea. Urea is hydrolysed to form ammonia and carbon dioxide.

The rise in pH subsequent upon the formation of ammonia is normally indicated by the change in colour of phenol red indicator incorporated in the medium. At pH 6.8 phenol red is pale orange in colour and at pH 8.1 the indicator is dark pink.

It should be borne in mind that false positives may arise when strongly proteolytic organisms produce ammonia from deamination of amino acids in the medium.

**Test**

- (a) Inoculate urea agar slope.
- (b) Examine after 4 hours or overnight incubation.

**Interpretation**

- Positive - pink colour
- Negative - no change

(e) **CITRATE UTILISATION**

Organisms differ in their ability to use different compounds as sole energy source for growth. In routine bacteriology, ability to use citrate is used to differentiate between members of the coli-aerogenes in the Enterobacteriaceae.
Test

The surface of the slope is lightly inoculated and the butt is inoculated by stabbing. The medium is incubated for 48 hours at 37°C.

Interpretation

**Positive** growth (i.e. citrate utilisation) produces an alkaline reaction - colour changes from green to deep blue.

**Negative** growth results in no change - medium remains green.

Interpretation of “Short sugar” tests based on lactose fermentation reactions:

**Table 1: Frank lactose fermenters on MacConkey Agar**

<table>
<thead>
<tr>
<th>SIM media</th>
<th>Oxidase</th>
<th>H₂S</th>
<th>Indole</th>
<th>Motility</th>
<th>Citrate</th>
<th>Urease</th>
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</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Klebsiella pneumoniae</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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</table>

**Table 2: Variable lactose fermenters on MacConkey Agar**

<table>
<thead>
<tr>
<th>SIM media</th>
<th>Oxidase</th>
<th>H₂S</th>
<th>Indole</th>
<th>Motility</th>
<th>Citrate</th>
<th>Urease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serratia spp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Citrobacter diversus</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
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</table>
Table 3: Non-lactose fermenters on MacConkey Agar

<table>
<thead>
<tr>
<th>SIM media</th>
<th>Oxidase</th>
<th>H₂S</th>
<th>Indole</th>
<th>Motility</th>
<th>Citrate</th>
<th>Urease</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus</em> spp.</td>
<td>-</td>
<td>+</td>
<td>+ (vulgaris)</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>(mirabilis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td><em>Shigella</em> spp.</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
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<tr>
<td><em>Morganella morganii</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

9. GERM TUBE TEST FOR CANDIDA ALBICANS

This test is one of the most valuable tests for rapid, presumptive identification of *Candida albicans*.

**Method:**

A dilute suspension of a yeast colony in 0.5 to 1.0 ml of serum is made. The mixture is then incubated at 37°C for 2 to 4 hours, after which a drop of the mixture is examined microscopically for germ tubes. Germ tubes are short non-septate germinating hyphae.

1. 2. 3. 4.

*C. albicans* produces short lateral hyphal filaments called germ tubes after 2-3 hours' incubation. Other *Candida* species do not produce germ tubes in this time period.
10. **GRAM STAIN**

2. Stain with crystal violet for 1 min.
3. Wash with tap water and drain slide.
4. Stain with fresh concentrated iodine for 1 min.
5. Wash well with water and drain off excess water.
6. De-colourise with alcohol-iodine reagent until violet colour ceases to run from the smear. Wash well with water.
7. Counter stain with carbol fuschin for 20-30 sec.
8. Wash, blot dry and examine with oil immersion objective.

11. **NOVOBIOCIN SENSITIVITY**

Two coagulase negative staphylococci *Staph. epidermidis* and *Staph. saprophyticus*, can be differentiated by their sensitivity to novobiocin.

**Procedure**

Streak the culture on a blood agar plate and place a novobiocin disc on the second set of streak lines. Incubate at 37°C overnight.

**Results**

- Large zone of inhibition of growth around disc: \(\text{Staph. epidermidis}\)
- No zone (or very small zone) of inhibition: \(\text{Staph. saprophyticus}\)

**NB:** The novobiocin disc is used for the purpose of identification and not as any sort of antibiotic sensitivity test.

12. **OPTOCHIN SENSITIVITY**

*Strep. pneumoniae* can be identified by its characteristic growth on blood agar. It is a haemolytic and sensitive to optochin.

**Procedure**

Streak the culture on a blood agar plate and place an optochin disc on the second set of streak lines. Incubate at 37°C in CO₂ overnight.

**Results**

- \(\alpha\) haemolytic, sensitive to optochin- *Strep. pneumoniae*
- \(\alpha\) haemolytic, resistant to optochin- *viridans streptococci*
13. **OXIDASE TEST**

This test detects oxidase enzymes involved in oxidative phosphorylation. Growth is removed from the medium with a platinum loop and placed on to a filter paper soaked with oxidase reagent (tetramethyl-p-phenylene diamine). Alternatively oxidase reagent can be poured over the growth on the plate.

**Results**
- Development of purple colour - positive in growth in 30 secs.
- No colour change of growth - negative

15. **SLIDE AGGLUTINATION TEST TO IDENTIFY AN ORGANISM**

Suspensions of an unknown bacterium are mixed on a slide with antisera prepared against known organisms. If the suspension is mixed with its specific antibody, visible clumps of agglutinated bacteria appear; the identity of the unknown organism can thus be established.

**Procedure**

(a) Divide a clean glass slide into two sections with a grease pencil. Place a large drop of saline on each section.

(b) Using a wire loop emulsify organisms from the culture in the two drops of saline to produce a very thick smooth suspension

(c) Add one drop of antiserum to one drop of suspension (the second drop serves as a control)

(d) Rock the slide gently for up to 2 minutes, and record the reactions

**NOTE:** ALL SLIDES MUST BE DISCARDED IN THE DISINFECTANT DISH AS SOON AS A RESULT IS OBTAINED.
(i) Kauffman-White Classification of Common Salmonella Species

Over 800 serotypes have been identified; those listed below illustrate the principles of separation into serotypes.

<table>
<thead>
<tr>
<th>SEROVAR</th>
<th>GROUP</th>
<th>O ANTIGEN</th>
<th>H ANTIGENS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phase 1</td>
</tr>
<tr>
<td><em>S. paratyphi A</em></td>
<td>A</td>
<td>(1), 2, 12</td>
<td>A</td>
</tr>
<tr>
<td><em>S. paratyphi B</em></td>
<td>B</td>
<td>(1), 4, (5), 12</td>
<td>B</td>
</tr>
<tr>
<td><em>S. Typhimurium</em></td>
<td>B</td>
<td>(1), 4, (5), 12</td>
<td>I</td>
</tr>
<tr>
<td><em>S. hirschfeldii</em></td>
<td>C</td>
<td>6, 7, vi</td>
<td>C</td>
</tr>
<tr>
<td><em>S. choleraesuis</em></td>
<td>Cl</td>
<td>6, 7</td>
<td>C</td>
</tr>
<tr>
<td><em>S. oranienburg</em></td>
<td>Cl</td>
<td>6, 7</td>
<td>m, t</td>
</tr>
<tr>
<td><em>S. montevideo</em></td>
<td>C1</td>
<td>6, 7</td>
<td>g, m, s</td>
</tr>
<tr>
<td><em>S. newport</em></td>
<td>C2</td>
<td>6, 8</td>
<td>e, h</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>D</td>
<td>9, 12, vi</td>
<td>D</td>
</tr>
<tr>
<td><em>S. enteritidis</em></td>
<td>D</td>
<td>(l), 9, 12</td>
<td>g, m</td>
</tr>
<tr>
<td><em>S. gallinarum pullorum</em></td>
<td>D</td>
<td>1, 9, 12</td>
<td>-</td>
</tr>
</tbody>
</table>

Parentheses indicate that antigenic determinant may be difficult to detect.

(ii) Serological Classification of Shigella Species

As with the genus Salmonella, the species of the genus Shigella have similar biochemical reactions and must be identified by slide agglutination with specific antisera. The Genera identification is confirmed with a polyvalent antiserum, then the species can be identified and finally the serotype.

i.e. Group A. *Shigella dysenteriae* Types 1 - 10
Group B. *Shigella flexneri* Types 1 - 6
Group C. *Shigella boydii* Types 1 - 15
Group D. *Shigella sonnei* Type 1
16. STREPTOCOCCAL GROUPING

Streptococci can be identified serologically by Lancefield grouping.

In the classical Lancefield methods C carbohydrate (polysaccharide) is extracted from the cell wall of β haemolytic streptococci and combined with various group specific antisera. The appearance of a precipitate at an extract-antiserum interface allows grouping into Lancefield group A, B, C etc. Some laboratories, due to the time required for these tests, have relied on bacitracin sensitivity as a criterion for identification of Group A streptococci. There is a need for better methods as (a) the bacitracin test is not 100% reliable and (b) the increasing importance of beta-haemolytic streptococci other than Group A (e.g. Group B). Commercially prepared kits are now available for the grouping of Streptococci.

The PathoDXtra Streptococcal Grouping Kit

This Streptococcal grouping kit consists of latex particles previously coated with streptococcal group-specific antibodies. These latex particles will agglutinate in the presence of homologous antigen, but will remain in smooth suspension in the absence of such antigen. Reagents are provided for the identification of the Streptococcal groups A, B, C, D, F and G.

Instructions for the use of this kit are as an appendix at the back of this manual.

17. URO-STRIPS

To obtain a viable count on a urine specimen, mix well then immerse sticks up to crease mark (no further). Drain off excess and then place evenly and entirely on media. Avoid bubbles under paper. Leave the stick in place for one minute. Do duplicate sticks for each specimen. Seven specimens can be tested on one plate.

Interpretation an average of 25 Gram negative rods and 30 Gram positive rods is equivalent to $10^8$ org/litre.
18. WIDAL AGGLUTINATION TEST

This test is used to detect the presence of *Salmonella* "O" and "H" antibodies of patients with suspected enteric fever or of typhoid carriers. Serial dilutions of serum are prepared in sets of tubes. Suspensions of *Salmonella* antigens are then added and the tests are incubated at 52°. The antigens suggested for use in Australia are

- *S. typhi* "O"
- *S. paratyphi* B "O"
- *S. paratyphi* A "H"
- *S. typhi* "H"
- *S. paratyphi* B "H"
- Non specific *Salmonella* "H"

The agglutinability of the bacterial suspensions should be standardised against standard sera and suspension provided by the Standards Laboratory, Colindale, England. It must be stressed that experience with a particular set of agglutinating suspensions is necessary before interpretation of results is possible. Many factors, (e.g. the stage of the disease, previous illness of the patient, previous immunisation courses, normal antibody levels in the community) may affect the results and as a general rule, final evaluation and diagnosis must rest with the clinician in charge of the particular case.

As a general guide:

1. In the unaffected Australian population antibody levels are low and not detectable
2. In endemic areas, antibodies are present against the common organism/s in that community.
3. People who have been immunised have antibodies against the antigens in the vaccine.
4. People with enteric infections caused by *Salmonella* show rising titres of antibodies against the organism involved

In all cases when enteric infection is suspected attempts should be made to isolate the causative organism from faeces, urine and blood cultures.
### MEDIA IN MEDICAL MICROBIOLOGY

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<td>3.</td>
<td>Chromogenic Candida agar</td>
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<td>4.</td>
<td>Chromogenic Urinary Tract Infection agar</td>
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<td>Eosin Methylene Blue Agar</td>
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<td>Sheep blood agar</td>
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<td>SIM medium</td>
<td>126</td>
</tr>
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<td>17.</td>
<td>Stuart's Transport Medium</td>
<td>127</td>
</tr>
<tr>
<td>18.</td>
<td>Xylose lysine desoxycholate agar</td>
<td>128</td>
</tr>
</tbody>
</table>
1. **ANAEROBIC SELECTIVE AGAR**

This is a peptone base agar plus a supplement of haemin and Vitamin K (to produce reducing conditions), and also naladixic acid and vancomycin as selective agents for non sporing anaerobes, particularly *Bacteroides*.

2. **CAMPYLOBACTER SELECTIVE AGAR**

This is essentially horse blood agar or charcoal based agar made selective for *Campylobacter* spp. by the addition of antibiotics bacitracin, cyclohexamide, colistin, cephazolin, novobiocin. In addition ferrous sulphate and sodium pyruvate are added to the charcoal media to enhance growth and aerotolerance. The majority of *Campylobacter* species are microaerophilic requiring an oxygen concentration of between 3-15% (which can be obtained using gas generating systems in anaerobic jars). If only candle jars are available as a means of reducing O₂ tension, then improved results are obtained by adding sodium pyruvate, sodium metabisulphite and ferrous sulphate to the medium.

Typically motile organisms are observed swarming on plates with 1.5% agar incubated at 42°C. Isolated colonies are obtained with 2.5% agar and with the addition of the above mixture of five antibiotics, satisfactory isolation can also be obtained at 37°C.

3. **CHROMOGENIC CANDIDA AGAR**

This is a differential medium which allows for the presumptive identification of clinically important *Candida* species based on the colour of their colony growth on this agar. The medium contains two chromogens that indicate the presence of target enzymes that allow for the differentiation of *Candida* species. These enzymes are X-NAG (5-bromo-4-chloro-3-indolyl N acetyl β-D-glucosaminide) which detects the activity of hexosaminidase and BCIP (5-bromo-6-chloro-3-indoly phosphate p-toluidine salt) which detects alkaline phosphatase activity. Chloramphenicol is also added to inhibit bacterial growth.

4. **CHROMOGENIC URINARY TRACT INFECTION (UTI) MEDIUM**

This is a differential agar which provides presumptive identification of the main pathogens associated with urinary tract infections. The medium contains two specific chromogenic substrates which are cleaved by enzymes produced by enteric bacteria. One chromogen, X-Gluc, is targeted towards β-glucosidase and allows the specific detection of enterococci through the formation of blue colonies. The other chromogen,
Red-Gal, is cleaved by the enzyme \( \beta \)-galactosidase which is produced by \( E. \) \( coli \), resulting in pink colonies. The cleavage of both chromogens occurs in the presence of coliforms, resulting in purple colonies. The medium also contains tryptophan which acts as an indicator of tryptophan deaminase activity, resulting in colonies of \( Proteus, \) \( Morganella \) and \( Providencia \) spp. appearing brown.

### 5. EOSIN METHYLENE BLUE AGAR (MODIFIED) LEVINE

<table>
<thead>
<tr>
<th>Typical Formula</th>
<th>gm/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10</td>
</tr>
<tr>
<td>Lactose</td>
<td>10</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>2</td>
</tr>
<tr>
<td>Eosin Y</td>
<td>0.4</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>0.065</td>
</tr>
<tr>
<td>Agar</td>
<td>15</td>
</tr>
<tr>
<td>pH 6.8 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

EMB is an isolation medium for the differentiation of the \( Enterobacteriaceae \). Colonial characteristics:

**Escherichia coli** - isolated colonies, 2-3mm diameter, with little tendency to confluent growth, exhibiting a greenish metallic sheen by reflected light and dark purple centres by transmitted light.

**Enterobacter aerogenes** - 4-6mm diameter, raised and mucoid colonies, tending to become confluent, metallic sheen usually absent, grey-brown centres by transmitted light.

Non-lactose fermenting intestinal pathogens - translucent and colourless

### 6. HORSE BLOOD AGAR

Blood agar contains whole red blood cells (horse or sheep). It is an enriched medium commonly used in medical microbiology and supports the growth of most pathogenic bacteria and yeasts.

Organisms showing characteristic differentiating reactions around single colonies are:

- \( \beta \) haemolysis (complete clearing): \( Strep. pyogenes \)
- \( \beta \) with one or more zones: some species of \( Clost. perfringens \).
  - haemolysis (partial greenish): \( Strep. pneumoniae \) and the many species known as "viridans" Streptococci.
Most other organisms grow well but any haemolytic effect shown is irrelevant in
differentiation, e.g. some strains of *Staph. aureus* show β-haemolysis while others
show no lysis at all. Almost all organisms produce an effect of partial haemolysis in the
heavy part of the inoculum.

7. **MacCONKEY’S BILE SALT AGAR**

This medium contains bile salts which inhibit many non-enteric organisms making it
useful for the selection of enteric organisms. It also contains lactose and neutral red
and acts as a differential medium for lactose fermenters and non-fermenters.

Important organisms showing characteristic reactions are:

i. Lactose fermentation positive (pink/red colonies): *E. coli*, *Klebsiella* sp.

ii. No lactose fermentation (cream/straw colonies): *Proteus* sp, *Shigella* sp,
    *Salmonella* sp, *Pseudomonas aeruginosa* (note blue green pigment alters the
colour of these colonies and agar).

iii. Staphylococci, if present, also grow producing pale pink colonies.

iv. Important organisms which do not grow include *Streptococci* (except for *Strep.
    faecalis* and other enterococci which grow well as minute brick-red colonies).

8. **MANNITOL SALT AGAR**

Contains beef extract, peptone, mannitol, sodium chloride (75 g/litre), phenol red.
A selective and differential medium used to select members of the micrococcaceae
(high salt concentration) and to distinguish presumptive coagulase positive
*Staphylococcus aureus* (surrounded by yellow zone due to production of acid from
mannitol).

9. **NAGLER MEDIUM**

The Nagler reaction is used to demonstrate the decomposition of lecithin to insoluble
diglycerides and phosphorylcholine by the enzyme lecithinase. Egg-yolk or human
serum can be used as a source of lecithin and the test can be carried out either in
liquid or solid medium. When lecithinase-producing organisms are grown on Nagler
medium the enzyme diffuses from the colonies into the agar and causes the production
of a halo of opalescence which can be specifically inhibited by antilecithinase serum.
Note - growth is not inhibited. The test can also be used for rapid identification of
*Clostridium perfringens* since lecithinase is the major component of the α-toxin
produced by this organism and can be inhibited by antitoxin. Note - growth is not
inhibited.
Method:
On one half of a dried Nagler plate spread 2 or 3 drops (50 - 100 units) of Cl. perfringens antitoxin with a sterile glass spreader. Dry in the 37°C incubator. Inoculate each half of the plate separately with the culture or swab under test, spreading the inoculum in the same pattern on both halves of the plate. Incubate for 24 hours anaerobically.

10. PSEUDOMONAS AGAR P (KING A)

Pseudomonas Agar Media are recommended for the detection and differentiation of Pseudomonas aeruginosa on the basis of pigment production.

History / Principles
Pseudomonas Agar P is patterned after the formulations described by King, Ward and Raney. Pseudomonas Agar P enhances the elaboration of pyocyanin by these cultures and inhibits the formation of fluorescent. The pigments diffuse from the colonies of Pseudomonas into the agar.

11. SABOURAUDS AGAR

Contains a high concentration of glucose at a low pH which inhibits bacteria. In medical microbiology used for the isolation of pathogenic yeasts (e.g. Candida albicans) and fungi including dermatophytes. Widely used in general microbiology.

12. SALMONELLA CHROMOGENIC MEDIUM

This is a selective medium for the presumptive identification of Salmonella species. The medium combines two different chromogens, 5-Bromo-6-Chloro-3-Indolyl caprylate (Magenta-caprylate) and 5-Bromo-4-Chloro-3-Indolyl β-D-galactopyranoside (X-gal). Hydrolysis of Magenta-caprylate by lactose negative Salmonella species results in purple colonies. Most other members of the family Enterobacteriaceae can utilise X-gal resulting in blue colonies. The medium also contains bile salts which inhibits Gram positive organisms.

13. SELENITE (BRILLIANT GREEN SULPHONAMIDE) BROTH

This medium is available in different forms. The buffered basal medium is used for selection and enrichment of Salmonella species (and some Shigella) and inhibition of many coliforms. However Proteus and Pseudomonas are not inhibited by selenite.
Selenite with brilliant green alone or brilliant green and sulphonamide helps overcome this problem.

Highly selective for *Salmonella*, including *Sal. typhi* from faeces, urine, water and foodstuffs. *Shigella* species may also grow. Selenite inhibits coliforms, brilliant green *Proteus* and *Pseudomonas* species. The sulphapyridine has a similar and additive effect.

NOTE: This medium must be handled carefully as it is potentially toxic to humans.

14. **SENSITIVITY TEST AGAR**

'Sensitest' and other formulations of agar for antibiotic sensitivity testing contain sufficient nutrients for the growth of most major pathogens (and are free of metabolic inhibitors of sulphonamides and trimethaprim). Contain casein, peptones, starch, dextrose, nucleoside bases and thiamine.

15. **SHEEP BLOOD AGAR** See horse blood agar.

16. **SIM**

A medium for differentiation of enteric bacteria on the basis of sulphide production, indole production and motility.

**Description:**
A motility-indole medium has been found to be helpful in the identification of the Enterobacteriaceae; eg. in the differentiation of *Klebsiella* from *Enterobacter* and *Serratia* species. For convenience, these two important tests have been combined with sulphide-production in one tube.

**Technique:**
The medium should be dispensed in tubes or bottles and when cool, inoculated once with a pure culture, by inserting a straight wire to about one third of the depth of the medium. If papers are used for the detection of indole, then these are wedged between the cotton wool plug or cap, and slide of the container. The inoculated medium is incubated at 35°C for 18 hours or longer, if necessary, and examined for motility, hydrogen sulphide production and finally indole production from tryptophan.
To test for indole production:
Add 0.2 ml of Kovac's Reagent to the tube and allow to stand for 10 minutes. A dark red colour in the reagent constitutes a positive indole test. No constitutes a negative test.

Colonial Appearances:
Non-motile organisms grow only along the line of inoculation, whereas motile species show either a diffuse even growth spreading from the inoculum, turbidity of the whole medium, or more rarely, localised outgrowths which are usually fan-shaped or occasionally nodular.
Hydrogen sulphide production is shown by blackening of the line of inoculation.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Motility</th>
<th>H₂S</th>
<th>Indole</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>V</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Shigella sonnei</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Precautions: To avoid delay in initiating growth always subculture from solid media. The reactions given by SIM medium are not sufficient to speciate organisms. Additional biochemical and serological tests are required for confirmation.

17. STUART’S TRANSPORT MEDIUM

The pathogenic *Neisseria* species are extremely fastidious organisms and are very susceptible to environmental changes. The organism will die in transit from patient to laboratory unless the specimen is properly collected. Use of the Stuart swab enables *Neisseria gonorrhoeae* to remain viable for 48-72 hours. Other less fastidious organisms may survive for periods of many months. Best survival is obtained if the medium is kept at room temperature.

A wet preparation from a Stuart swab will also show the protozoan flagellate *Trichomonas vaginalis* if it is present. This organism may cause infection in male and female which can be confused clinically with gonorrhoea.

STUART SWAB

A charcoal impregnated plain swab is prepared by dipping the swab in a 1% suspension of activated charcoal in Ringers solution and sterilised by dry heat for one hour at 160°C; alternatively, an alginate swab may be used. With the swab is supplied a 1/4 oz. screw-capped bottle completely filled with 0.3% semi-solid agar containing
0.1% thioglycollic acid adjusted to pH 7.2 with NaOH and buffered with 0.5% sodium glycerophosphate and 0.02% calcium chloride containing a trace of methylene blue. The agar gel is sterilised by autoclaving 15 lb. for 15 minutes. This anaerobic agar gel should only be used when colourless (i.e. Eh < 0.01 volts). After the specimen has been collected the swab is thrust into the Stuart's medium and the shaft broken off. The cap is screwed down and the swab is later plated onto an appropriate medium.

18. **XYLOSE LYSINE DESOXYCHOLATE AGAR**

This medium is designed to support the growth of all enteric pathogens, especially Shigellae, and to differentiate the genera on the basis of xylose fermentation, lysine decarboxylation, and H₂S production. Sodium desoxycholate is added to inhibit the growth of other organisms. It is claimed that the medium affords a greater degree of differentiation than is found in eosin methylene blue or MacConkey agars and that it provides greater sensitivity for fastidious organisms than is found with SS, brilliant green or bismuth sulphite agars.

Xylose is included since it is fermented by most of the Enterobacteriaceae except *Shigella*. *Shigella* is also unable to ferment lactose or sucrose to acid end products and thus remains neutral-to-alkaline red on this medium. Fermentative coliforms show the acid reaction (yellow) together with opacity due to precipitated bile. In order to "encourage" *Salmonella* sp. to produce a reaction similar to *Shigella* (and thus group pathogens together) a high concentration of lysine has been added. Salmonellae are amongst the few organisms which decarboxylate lysine, producing a sufficiently alkaline reaction to reverse the otherwise acid reaction. The thiosulphate/ferric ammonium citrate indicator system allows detection of organisms producing H₂S.

**Growth characteristics on X.L.D.**

- **Opaque yellow:** *Escherichia, Enterobacter, Klebsiella, Citrobacter, Proteus, Serratia*
- **Red colonies:** *Shigella, H₂S negative Salmonella, Providencia*
- **Red colonies with black centre:** H₂S positive *Salmonella, Edwardsiella.*

**Note:** False positive, red colonies may occur with some *Proteus* and *Pseudomonas* species.
Appendix 1.

COLLECTION AND TRANSPORT OF SPECIMENS

Diagnosis of microbial infection is often delayed due to improper specimen collection and inadequate transport methods. As future clinicians you have a responsibility to ensure that you know the methods described below and understand the reasons for the use of these methods.

GENERAL

All specimens must be labelled with the patient's name, date and time of sampling and be accompanied by a signed request form.

Specimens for culture must never be in contact with antiseptics or disinfectants. If the site must be cleaned, a dry sterile swab moistened with sterile saline should be used; the site should be dried before sampling.

All specimens must be sent to the laboratory on the day of collection and with as little delay as possible.

Stuart's transport medium (STM) is widely used to transport specimens from the patient to the laboratory. It is a semi-solid, non-nutritional medium which preserves even the most delicate pathogens (e.g. *Neisseria*) in a viable condition. It contains a reducing agent and the indicator methylene blue. This medium is not suitable for use unless reduced (i.e. when it appears colourless; blue colour indicates absence of reducing conditions). (Why?). Specially prepared non-toxic swabs must be used. After collection of the specimen the swab is placed in the middle of the bottle of medium, the shaft of the swab broken and the lid screwed tightly on the bottle.

THROAT SWABS

Transport medium kits or a fresh swab are suitable for throat swabs. The area to be swabbed is the tonsillar surface, pillars of the fauces and posterior pharyngeal wall. Areas of exudation, membrane formation or inflammation are choice sites. All other areas should be avoided, including the tongue and teeth when withdrawing the swab. (Why?)

The patient facing a good light, is asked to open his mouth and say 'ahh'. Some people give a good exposure without the use of a tongue depressor; in others it is necessary to press down the tongue gently but firmly with the depressor. Swab rapidly and with slight pressure the two tonsillar areas, pillars of the fauces and the posterior pharyngeal wall behind the uvula.

In children it is an advantage for the child to sit on the mother's knee. The patient's hands are held with one hand by the mother and her other hand holds the child's forehead firmly against her chest, thus preventing the child from retracting its head. In swabbing a child it is important to be successful the first time, and if he/she refuses to open his/her mouth this can usually be effected by pushing the spatula gently into the mouth; when it touches the back of the tongue there is a reflex opening of the mouth giving a short opportunity which must not be missed!

Similarly with a baby, the moment it opens its mouth to cry the opportunity to swab should be seized.
Check that the patient has not recently had an antiseptic mouth gargle or lozenge. Mouth washing with water may be necessary before swabbing.

Two swabs should be taken if Vincent's angina or moniliasis (Candida infection) is suspected. One can be used for microscopy.

PUS

Unfortunately the inflammatory exudate commonly called pus is frequently sampled with a swab. If possible it is better to obtain a liquid sample with a syringe (replacing the cover over the needle immediately), or an excised piece of tissue. If a swab is used, it must be transported in whatever transport media is used in the hospital, e.g., Prince of Wales use Stuart’s for all routine specimens and recommend a special medium if anaerobes are suspected. St Vincent’s Hospital uses a special "transport deep" for all specimens. They also issue two swabs with their specimen collection kits and a clean slide and encourage clinicians to make a smear at the time of specimen collection and send the slide down with the pus swab. If this is not done a swab in transport and a plain swab would be adequate.

From a sinus, clean the sinus opening. Pass a fine wire swab (such as per nasal swab) into the sinus tract as far as possible. Sample before treatment with antiseptics. Take care to avoid contamination by skin flora.

Pus from an undrained abscess should contain the aetiological agent in large numbers. When the abscess is incised and drained the pus should be aspirated directly into a sterile syringe or vacuum tube and sent to the laboratory with minimum delay. Advise the laboratory in advance of the expected arrival of the specimen.

Fluids. It is an advantage to receive the fluid in collection bottles (200 ml) which contain 3.8% citrate, or in 10 ml heparinised tubes. The bottles can be prepared as for anaerobic blood culture bottles with a sterile injection site on the caps.

URINE FOR ROUTINE BACTERIOLOGY & MICROSCOPY

MID STREAM SPECIMEN

Contamination of the urine specimen by bacteria commonly present in the urethra and on the external genitalia must be avoided. Disinfectants should not be used in the patient preparation unless they can be adequately washed off.

Preparation:
Male: Retract foreskin (if necessary). Cleanse the glans penis carefully with swabs dipped in saline.

Female:
Ambulant: Patient showers, uses bidet, or is given swabs and instructions for cleaning perineum.
Bedridden: Place patient on bedpan in bed or on a low chair. Don gloves. Clean perineum using 4 swabs. Grasp one swab at a time with forceps, dip into saline and swab external genitalia from front to back, left side, right side and centre. Use the final swab to cleanse over the urethral orifice. Dry with sterile cloth or swabs. If there is any vaginal discharge or if previous urine contaminated gently insert a dry swab, or tampon, into the vagina.
Collection
The patient commences voiding into the bedpan whilst the nurse or patient maintains separation of the labia or retraction of prepuce. After 20 - 30 ml have been passed, and without stopping the stream, the next 20 ml is collected into a sterile container. The initial 20 - 30 ml washes out most of the bacteria resident in the urethra which are likely to contaminate the MSU.

Infants and young children who are not toilet trained can be coaxed to produce urine at the desired time by the following manoeuvres:

(a) While the infant is held face down over a sterile container, pressure is applied over the suprapubic area.
(b) The paraspinal muscles are stroked to elicit the Perez reflex. This results in crying, extension of the back, reflexion of the legs and arm and urination.
(c) Cold stimulus.

BAG COLLECTION:

Used if a clean catch is not possible but is a much less desirable method. The genitalia are disinfected and cleaned, a plastic urine collection bag is attached. The bag should be removed as soon as the infant voids to prevent multiplication of organisms. If no urine appears in 30 min., cleaning must be repeated and a new bag applied.

The specimen should be removed to a sterile container as soon as possible by cutting the corner of the bag with sterile scissors or using a pipette or syringe.

CATHETER SPECIMEN OF URINE

Urethral CSU - DIAGNOSTIC (contrast indwelling)
After disinfection and washing of perineum, a catheter is inserted via the urethra. This method of specimen collection should be discouraged because of the risk of introducing microorganisms into the bladder. Suprapubic puncture is preferred if a reliable MSU is not possible.

(SUPRAPUBIC PUNCTURE URINE) Useful when an uncontaminated specimen of urine cannot be reliably produced, e.g. for infants; only performed by experienced doctors;

i) Make sure bladder is full by postponing attempt if urine has been passed during preceding 30 minutes.
ii) Percuss bladder prior to attempt.
iii) Immobilise; clean skin with alcoholic chlorhexidine (60 sec.).
iv) Insert 22 gauge needle on syringe exactly in the midline above the symphysis pubis (5 cm for adults) at an angle 10-20° to the perpendicular towards perineum.
v) With gentle suction on syringe, penetrate until bladder is entered (2 - 3 cm for neonate). Aspirate urine (2 - 3 ml for neonate). Withdraw needle.

Transport

Always refrigerate a urine specimen. Even a carefully collected specimen often contains contaminants and these can multiply if the sample is left standing at room temperature before the viable count is performed. At optimal temperatures E. coli may
divide every 20 minutes, which in a short 3 hours is $x \times 500$ and therefore $2 \times 10^6/L$ becomes $10^8/L$.

**FAECES**

Faeces should be passed directly into a clean container, e.g., a clean bedpan or ice cream container. The sample is then transferred to a special sterile container with a spatula and it should include any pus, blood, mucus or worms that have been passed.

For parasitology:

The specimen must be at least walnut sized. Three specimens passed more than one day apart are desired. If threadworm is suspected, an anal swab (with sellotape) is desirable. Trophozoites of *Entamoeba histolytica* will probably be seen only if the specimen is examined within 30 min. of passage, and kept at near 37°C.

**Storage and transport:** Up to 3 hours - 4°C (Except for amoeba).

Rectal Swab for enteric pathogens:

When faeces are not available, insert swab into rectum, gently rotate so that faeces cover the swab. Use transport medium if examination is delayed.

Perianal sellotape preparation for thread worms

Collect before morning wash!
Press sticky surface firmly 3 times to areas of skin around anal region. Stick it down lengthways onto glass slide provided. Place in small plastic bag for transport to laboratory.

**BLOOD**

For bacterial culture

Blood samples should be taken prior to antibiotic therapy. Dilate the median cubital vein by application of tourniquet to upper arm and instructing patient to clench his fist. Swab the skin with 70% alcohol or other suitable antiseptic. Locate vein. Do not touch the skin again, before venipuncture. Pointing the needle in the direction of the vein and obliquely through the skin with the bevel up, withdraw 10 - 15 ml of blood. Unclench fist and cuff.

Detach needle aseptically from the syringe by means of artery forceps, replace with a new needle before injecting blood through diaphragm in bottle caps. Approximately 1 to 10 dilution of blood in medium is optimal.

Repeated blood cultures are taken to detect intermittent showers of organisms. 2-3 blood cultures during a 24 - 48 hour period are sufficient. If a patient has occasional rigors but is afebrile at other times then sample when the patient feels a rigor coming on.

It is better to use adhesive tape to cover the inoculation site on the bottle top than swab with disinfectant on exposed site.
### Appendix 2. Commercial Kits used in this practical program

<table>
<thead>
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<th>Kit Name</th>
<th>Page</th>
</tr>
</thead>
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<td>Streptococcal Grouping kit</td>
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<td>Salmonella Serogrouping</td>
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<td>Shigella immune sera</td>
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<td>Staphaureus Plus Coagulase test</td>
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<td>Chromogenic agar:</td>
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<tr>
<td>Candida Agar</td>
<td>152</td>
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<td>Salmonella agar</td>
<td>154</td>
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<tr>
<td>Urinary Tract Infection agar</td>
<td>156</td>
</tr>
<tr>
<td>XLD agar</td>
<td>158</td>
</tr>
</tbody>
</table>
STREPTOCOCCAL GROUPING KIT

**INTENDED USE**

PathoDxtra™ Strep Grouping Kit is a latex agglutination test which provides a rapid method for the serological identification of Lancefield A, B, C, D, and G streptococci from primary culture plates. The materials supplied are intended for in vitro diagnostic use, as an aid in the rapid grouping of streptococci.

**SUMMARY**

The streptococcal group carbohydrates of streptococcal groups A, B, C, D, and G are complex antigens usually comprised of mannose oligosaccharides and differing side chains, consisting primarily of glucosamine, either acetylated or non-acetylated. The antigen for group D streptococci is lipoteichoic acid.

The PathoDxtra procedure utilizes a latex agglutination method in conjunction with a nitrous acid extraction procedure. The IgG coupled to the latex is highly specific for a given streptococcal group antigen. This method offers significant advantages over other streptococcal grouping procedures in terms of rapidity, simplicity, and convenience.

**PROCEDURE**

8.1 All components (except latex reagents and control) must be at room temperature (15 to 30°C) before use. If the latex reagents and the control are stored at 2 to 8°C, it is not necessary to wait for these reagents to come to room temperature. Use disposable pipet, capillary or Pasteur pipette to transfer the extract.

A Colonies On Solid Media:

1. Label one 12 x 75 mm test tube for each specimen.
2. Add 1 mL fresh growth of Reagent 1 to each specimen tube by squeezing the bottle gently in a vertical position.
3. Pick 1 to 4 isolated B-haemolytic colonies with a disposable applicator stick or with an inoculating loop and resuspend them in Reagent 1. (If cultures are minute sufficient, they may be resuspended in Reagent 1 to ensure it is turbid.) Do not use a swab, since it will absorb too much of the liquid volume. Remove the inoculum by rubbing the stick or loop against the bottom or side of the tube and mix thoroughly. Discard the stick or loop appropriately.
4. Add 1 mL fresh growth of Reagent 2 to each specimen tube by squeezing the bottle gently in a vertical position. Mix the reagents by tapping the tube with a finger for five to ten seconds. (Incubation of the tubes is not necessary, though they may be left for up to 60 minutes at room temperature (15 to 30°C) as long as precautions are taken against drying. Longer incubation periods have not been tested.)
5. Add 5 mL fresh growth of Reagent 3 to each specimen tube by holding the bottle vertically and squeezing gently. Mix the reagents by tapping the tube with a finger for five to ten seconds. If not tested immediately, store the tube tightly capped at 2 to 8°C and test within 24 hours.
6. Designate a row of test circles on the PathoDxtra slide for each specimen or control to be tested.
7. Add 40-50 µl of extract to each of six test circles.
8. Resuspend the latex reagents by gentle inversion or swirling. Add 1 mL fresh growth of Strips A Latex by holding the bottle vertically and squeezing gently to the first circle, then add 1 mL fresh growth of Strips B Latex to the second circle by holding the bottle vertically and squeezing gently. Continue in the same manner, adding Strips C, D, F, and G Latex to the remaining 4 circles.
9. Mix the latex and extract with a mixing stick, using a clean end for each circle.
10. Hold the slide under suitable lighting and gently rock the slide back and forth. A positive agglutination reaction with one of the latex reagents usually occurs within 30 seconds. Stop rocking the slides when a clearly discernible positive reaction is observed and record the result. Do not rock the slides for more than 60 seconds.

**RESULTS**

**INTERPRETATION**

10.1 POSITIVE RESULT: A positive reaction occurs when there is visible agglutination of the latex microparticles with a clearing of the background within 60 seconds. The PathoDxtra Strep Grouping Kit is designed to give a rapid agglutination reaction with the extract of one to four colonies of an 18 to 24 hour culture of streptococci of Lancefield groups A, B, C, D, and G (large colony variety) in 60 seconds for most streptococcal isolates. Minute Group F colonies and small colony strains of other groups require more colony sizes (heavy sweep) to give a positive agglutination reaction.

10.2 NEGATIVE RESULT: A uniform pale blue appearance with no agglutination after 60 seconds.

10.3 INCONCLUSIVE RESULT: If agglutination should occur with more than one latex reagent, the problem may be resolved as follows:

1. Weak agglutination with multiple latex reagents and distinctly stronger agglutination with one reagent: Interpretation: The weak reactions generally are due to a non-specific reaction (e.g., Staph. aureus) and the stronger reaction is specific for the streptococcal group indicated.
2. Approximately equal agglutination with more than one latex reagent (rarely more than two): Interpretation: Two streptococcal groups with similar colony morphology and B-haemolysis were present on the culture plate. Retest, using pure colony extracts after re-isolation.

10.4 NON-SPECIFIC AGGLUTINATION: At least two types of non-specific agglutination may be observed with latex tests.

1. Some mucoid strains of bacteria may cause non-specific clumping of the latex, probably due to physical entrapment of the particles in the extracted capsule material. This is more prevalent when the direct colony procedure is used.
2. Protein A-bearing strains of Streptococcus aureus may cause false-positive agglutination of latex reagents by binding the Fc portion of the IgG on the latex. The PathoDxtra reagents have been designed not to react with moderate levels of protein A, but high levels may overwhelm the system.

**NOTE:** When performing the test, it is advisable to rock the slides only long enough to obtain clearly readable agglutination. Adherence to this procedure will minimize cross-reactions.
**Bacteria & Disease 135**  

**Interpretation of results**

The test should be considered positive when agglutination occurs with one grouping reagent or when grouping reagent gives a substantially stronger reaction than the other five. The test should be considered negative when no agglutination occurs. Faint traces of granular material may be observed in negative reactions and should be ignored.

**Limitations of the test**

Nearly all the β-haemolytic streptococci isolated from the human infections possess specific carbohydrate antigens which can be recognised by serological reactions. Attempts to extend these procedures to non β-haemolytic streptococci have been unsuccessful except for groups B, D and N. Group N streptococci are not found in human infections.

It should be noted that the Group D reagent may fail to react with some *S. bovis* strains and these strains would require further tests for identification.

### Positive reaction on slide

<table>
<thead>
<tr>
<th>β-haemolytic</th>
<th>Non β-haemolytic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reacts solely in A, B, C, F or G</td>
<td>Reacts in Group D</td>
</tr>
<tr>
<td>Test in 6.5% NaCl broth</td>
<td>Positive in more than</td>
</tr>
<tr>
<td>Subculture and re-test</td>
<td>Reacts in Group B</td>
</tr>
<tr>
<td>Report non-haemolytic group B</td>
<td>Reacts in Group D</td>
</tr>
<tr>
<td>Biochemical identification required</td>
<td>Reacts in A, C, F or G</td>
</tr>
</tbody>
</table>

Growth
- Report Group D enterococcus

No growth
- Report Group D non-enterococcus

Biochemical identification if problem not resolved

| +/- | +/- | +/- |
| Group D enterococcus | Group D non-enterococcus | Viridans streptococci |

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Negative reaction on slide

- **β-haemolytic**
  - Repeat extraction with heavier suspension
  - Repeat test
  - If negative, report ‘Not group A,B,C,D, F or G’

- **α or non-haemolytic**
  - Bile-aesculin / 6.5% NaCl broth
  - Group D enterococcus
  - +/- Group D non-enterococcus
  - +/- Viridans streptococci
The WELLCOLEX Colour Salmonella test provides a simple, rapid latex test procedure for detection and presumptive serogroup identification of *Salmonella* present in broth cultures or on solid culture media.

**Summary and Explanation of the Test:**

The genus *Salmonella* is responsible for a wide spectrum of human diseases ranging from mild forms of gastroenteritis to severe, life-threatening enteric fever and, in addition, asymptomatic carriage can occur. Early, reliable identification is important to the provision of appropriate therapy and to control outbreaks. Minimal identification of the organisms involves both biochemical and serological procedures. Definitive serological testing requires a large battery of antisera to both cell-associated ‘O’ antigens and flagellar ‘H’ antigens, and ideally this process is performed in reference centres. It is useful for the clinical laboratory to identify the isolate to the ‘O’ serogroup level prior to submitting it to a reference laboratory.

The WELLCOLEX Colour Salmonella test provides a rapid, simple procedure for serogroup identification of the majority of *Salmonella* encountered in clinical bacteriology. Some strains of *Salmonella*, notably of *S. typhi* and *S. paratyphi C*, possess surface Vi antigen which obscures the ‘O’ antigens until removed by heating; Vi antigen can also be detected in the test.

**Principle of the Procedure:**

In the WELLCOLEX Colour Salmonella test, a suspension of bacteria is reacted with two grey-brown test reagents, each consisting of a mixture of suspensions of red, blue and green latex particles, each of which is coated with antibody specific for different *Salmonella* serogroups. In the presence of homologous antigen one of the colours in a mixture will agglutinate, and the identity of the antigen is indicated by the colour of the aggregated particles with a contrasting change in the colour of the background. Each combination is easily distinguished from the others and from a negative result in which the particles remain in smooth grey-brown suspension, and the occasional non-specific result in which all the particles agglutinate into grey-brown aggregates against a cleared background.
Colony identification

STEP 1 Dispense approximately 200 µl of saline into a suspension tube. The disposable sample dispenser which is graduated at approximately 200 µl may be used.

STEP 2 From an overnight culture pick one or two average-sized (1 to 2mm) suspected Salmonella colonies from the culture plate using the flat end of a sampling stick and carefully emulsify the bacteria in the saline. With small colonies more will need to be picked; the end of the sampling stick should be covered. Discard the sampling stick for safe disposal.

STEP 3 Resuspend latex reagents 1 and 2 by shaking vigorously for a few seconds. Hold the bottle vertically and dispense one drop of each latex reagent into a separate circle on a flat reaction card. Burst any air bubbles with the end of a sampling stick.

STEP 4 Using a disposable sample dispenser held vertically, transfer 1 free-falling drop (40 µl) of bacterial suspension to each of the two circles, and discard the dispenser for safe disposal. Take care not to dispense air bubbles.

STEP 5 Using a sampling stick, mix the contents of each circle and spread to cover the area of the circle. The same stick may be used for both circles and should then be discarded for safe disposal.

STEP 6 Place the card on a suitable flat-bed rotator and run at 150rpm for 2 minutes. Switch off and observe for agglutination without removing the card from the rotator. The card should be viewed from directly above at a normal reading distance. Do not use a magnifying lens. The patterns obtained are clear cut and can be recognised easily under any normal lighting conditions. If there is any doubt about whether agglutination has occurred the test should be repeated using a 40 µl drop of saline. There should be no visible agglutination. This result should be used as a basis for comparison.

STEP 7 Discard the used reaction circles for safe disposal. Ensure that the latex reagents are returned to the refrigerator.
Interpretation of results

A positive result (coloured agglutination) indicates the presence and at the same time identifies the serogroup of *Salmonella* in the sample (or the presence of Vi antigen), as follows:

<table>
<thead>
<tr>
<th>REACTION</th>
<th>BACKGROUND</th>
<th>REAGENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green or olive agglutination</td>
<td>Purple/pink</td>
<td>D</td>
</tr>
<tr>
<td>Blue agglutination in one reagent</td>
<td>Orange/pink</td>
<td>C</td>
</tr>
<tr>
<td>Red agglutination in one reagent</td>
<td>Blue/turquoise</td>
<td>B</td>
</tr>
<tr>
<td>Irregular dark red/brown clumps or specks in both Reagents 1 and 2</td>
<td>Smooth Grey/brown</td>
<td>Negative</td>
</tr>
<tr>
<td>No agglutination</td>
<td>Smooth Grey/brown</td>
<td>Negative</td>
</tr>
<tr>
<td>Fine grainy, dark red/brown agglutination usually occurring equally in both reagents</td>
<td>Clear or light grey/brown</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Turquoise agglutination in one reagent</td>
<td>Pink</td>
<td>C and D</td>
</tr>
<tr>
<td>Orange agglutination in one reagent</td>
<td>Blue</td>
<td>B and D</td>
</tr>
<tr>
<td>Purple agglutination in one reagent</td>
<td>Green</td>
<td>B and C</td>
</tr>
</tbody>
</table>

The performance of the WELLCOLEX Colour Salmonella test determined by comparison with results of traditional bacteriological methods.

**Sensitivity** and **Specificity** of the WELLCOLEX Colour Salmonella test

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary plate cultures</td>
<td>100%</td>
<td>99.2%</td>
</tr>
<tr>
<td>Enrichment broth subcultures</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Pure plates</td>
<td>99.5%</td>
<td>98.0%</td>
</tr>
<tr>
<td>Selenite broths</td>
<td>94.2%</td>
<td>99.7%</td>
</tr>
</tbody>
</table>
**SHIGELLA IMMUNE SERA**

*Shigella* is a gram-negative, non-spore forming bacillus belonging to the *Enterobacteriaceae* family. The organism has no flagella. These products are typing (grouping) sera containing agglutinins specific to each type (or group) and polyvalent sera used for serological testing of shigella.

These are used for the typing (or grouping) of *Shigella* by slide agglutination. Each immune serum is prepared, according to the report by the International *Enterobacteriaceae* Grouping Subcommittee (1958), by hyperimmunizing rabbits with organisms inactivated by heating, and inactivating the serum at 56°C for 30 minutes. After removing cross agglutinins by absorption, the sera are sterilized by filtration and sodium azide is added at 0.1 w/v%.

**PRINCIPLE**

When this reagent is mixed with homologous *Shigella* organisms, an antigen-antibody reaction occurs to produce agglutination of cells visible to the naked eye and thus serotyping can be carried out.

**PROCEDURES**

After isolating organisms using conventional procedures, carry out sero-group and serotype screening on an organism whose biochemical characteristics are identical to those of *Shigella*.

i. Using a glass pencil, divide a glass slide into several parts, and place one drop of each polyvalent serum and physiological saline (control) onto each section of the slide.

ii. Using a platinum loop, place one drop of the test bacterium which has been densely suspended in physiological saline, in the vicinity of the drops of solutions previously put on the slides. Using the platinum loop, mix the antigen and serum drops well and also mix the antigen solution and physiological saline in each section.

iii. Tilt the glass slide back and forth and observe the glass slide for agglutination. Only strong agglutinations occurring within 1 minute should be taken as positive, whereas agglutination occurring later than 1 minute or weak agglutination should be taken as negative. The same of the subgroup of the test organism corresponds to the name of the Polyvalent serum which agglutinated the organism. Spontaneous agglutination of the antigen should be checked using physiological saline as a control.

When a positive reaction is observed with one of the polyvalent sera, carry out the slide agglutination test in the same way as described above, using monovalent sera (not available in this practical program).
**FOR THE BIOCHEMICAL IDENTIFICATION OF COMMONLY OCCURRING URINARY MICROORGANISMS**

INTENDED USE

The RapID SS/u System is a qualitative micromethod employing conventional and chromogenic substrates for the identification of selected medically important microorganisms commonly isolated from urine specimens. The RapID SS/u System will provide the laboratory with identification of microbes associated with a positive urine culture in 2 hours. A complete listing of the organisms addressed by the RapID SS/u System is given in the RapID SS/u Differential Chart (Table 3).

**SUMMARY AND EXPLANATION**

The RapID SS/u System is comprised of (i) RapID SS/u PANELS, and (ii) RapID SS/u REAGENT. Each RapID SS/u Panel has several reaction cavities molded into the periphery of a plastic disposable tray. Reaction cavities contain dehydrated reagents and the tray allows the simultaneous inoculation of each cavity with a predetermined amount of inoculum. A suspension of test organism in RapID INOCULATION FLUID is used as the test inoculum which rehydrates and initiates test reactions. After incubation of the panel, each test cavity is examined for reactivity by noting the development of a color. In some cases, reagents must be added to the test cavities to provide a color change. The resulting pattern of positive and negative test scores is used as the basis for identification of the test isolate by comparison of test results to reactivity patterns stored in a database or through the use of a computer-generated Code Compendium.

**PRINCIPLE**

The tests used in the RapID SS/u System are based upon the microbial degradation of specific substrates detected by various indicator systems. The reactions employed are a combination of conventional tests and single-substrate chromogenic tests and are described in Table 1.

**TABLE 1. PRINCIPLES AND COMPONENTS OF THE RapID SS/u TESTS**

<table>
<thead>
<tr>
<th>Cavity</th>
<th>Test Code</th>
<th>Reactive Ingredient</th>
<th>Quantity</th>
<th>Principle</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEFORE REAGENT ADDITION:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>GMD</td>
<td>Amino-acid arylamide</td>
<td>0.5%</td>
<td>Gram-negative organisms hydrolyze the peptide substrate releasing free yellow p-nitrophenol.</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>ONPG</td>
<td>α-Naphthyl-β-D-galactoside</td>
<td>0.25%</td>
<td>Hydrolysis of the colorless nitrophenylated glycoside releases yellow p- or α-nitrophenol.</td>
<td>1, 2, 5</td>
</tr>
<tr>
<td>3</td>
<td>G1</td>
<td>p-Nitrophenyl-β-D-galactoside</td>
<td>0.25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>G2</td>
<td>p-Nitrophenyl-β-D-glucozide</td>
<td>0.25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>G3</td>
<td>p-Nitrophenyl-β-D-glycoside</td>
<td>0.25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>PHO</td>
<td>p-Nitrophenyl phosphohydrolase</td>
<td>0.5%</td>
<td>Hydrolysis of the colorless phosphoester releases yellow p-nitrophenol.</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>URE</td>
<td>Urease</td>
<td>0.9%</td>
<td>Hydrolysis of urea produces basic products which cause a pH indicator change.</td>
<td>1</td>
</tr>
<tr>
<td>AFTER REAGENT ADDITION:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>IND</td>
<td>Tyrosine</td>
<td>0.5%</td>
<td>The production of mobile and mobile derivatives from tyrosine is detected usingput indole Reagent.</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>A1</td>
<td>Amino acid 3-naphthylamide</td>
<td>0.05%</td>
<td>Hydrolysis of the aryl substituted amide releases</td>
<td>4, 9</td>
</tr>
<tr>
<td>9</td>
<td>A2</td>
<td>Amino acid 3-naphthylamide</td>
<td>0.05%</td>
<td>beta-naphthylamine which is detected by</td>
<td>16, 20</td>
</tr>
<tr>
<td>10</td>
<td>A3</td>
<td>Amino acid 3-naphthylamide</td>
<td>0.05%</td>
<td></td>
<td>21, 22, 23</td>
</tr>
</tbody>
</table>
REAGENTS
The RapID SS/u System requires the following reagents:

Supplied with the RapID SS/u System:

1. **RapID SS/u Reagent**
   - One (1) plastic dropper bottle containing reagent sufficient for 20 panels.
   - Reactive ingredients:
     - 3-Pheny1-4-methylaminosalen 0.01%
     - Hydrochloric acid 0.1%
     - Acetic acid 1.0%
     - Detergent 0.1%
   - Store at 2-8°C in the original container. Avoid skin, eye, or clothing contact. The reagent is for in vitro diagnostic use in conjunction with RapID SS/u Panels.

Not supplied with RapID SS/u System but available from REMEL:

1. **RapID INOCULATION FLUID** (Cat. No. 83-25102)
   - 20 screw-cap tubes containing approximately 1 ml each.
   - Reactive Ingredients:
     - KCl 6.0 gr
     - CaCl2 0.5 gr
     - 0.1N NaOH 1.6 ml
     - Deionized Water to 1000 ml

2. **IDS SPOT INDOLE REAGENT** (Cat. No. 83-09002)
   - Store at 15-30°C in the original container. RapID Inoculation Fluid must be used in conjunction with RapID Identification Panels.

PRECAUTIONS
1. Directions should be read and followed carefully.
2. This product is for IN VITRO DIAGNOSTIC USE and should be used by properly trained individuals.
3. Do not use panels or reagents after kit expiration date.
4. Do not intermingle reagents from different kits.
5. Do not pipet specimens by mouth.
6. All clinical specimens should be considered infectious, and should be handled and disposed of according to acceptable practices.

STORAGE
The expiration date and storage conditions for RapID SS/u System components are stated on the outer package. All materials will retain their reactivity for the period indicated if stored as directed.

RapID SS/u System: Store at 2-8°C in the original packaging.

SPECIMEN COLLECTION AND PROCESSING
The subject of specimen collection and processing is of the utmost importance when culturing clinical samples for the organisms addressed by the RapID SS/u System. Improper specimen collection and processing will adversely affect both the recovery and significance of bacteria from specimens. The collection, transport, and processing of a wide variety of clinical specimens is described in the Manual of Clinical Microbiology (16). It is suggested that the user consult these references for valuable information when using the RapID SS/u System.

MATERIALS NEEDED:
Provided with the RapID SS/u System:
- 20 RapID SS/u Panels
- 20 Report Forms
- 1 Dropper-bottle of RapID SS/u Reagent
- 1 Package Insert
- 2 Chipboard Incubation Trays

Not provided with the RapID SS/u System but available from REMEL:
- **RapID Inoculation Fluid** (Cat. No. 83-25102)
- **IDS Spot Indole Reagent** (Cat. No. 83-09002)
- **RapID SS/u Code Compendium V1.54** (Cat. No. 83-24004)
- **Gram stain reagents** (Cat. No. 40-030)
- **McFarland #1 standard** (Cat. No. 20-411)

Not available from REMEL:
- Cotton swabs
- Pasteur pipettes
- Microscope slides
- Incubator (35-37°C)
PROCEDURE

1. PREPARATION OF RapID SS/u PANELS
1.1 Remove required panels and chipboard incubation trays from storage and reseal the plastic pouch. Do not use if the plastic panel is broken or the lid is compromised. Panels must be used the same day they are removed from storage.

2. PREPARATION OF INOCULA
2.1 Only urine isolates should be selected for testing. The use of isolates from other body sites or fluids is not recommended. Test isolate colonial morphology should be closely examined, since mixed populations cannot be used as inoculum. Where there is an indication of polymicrobial isolation plates, each colony type should be processed independently in a RapID SS/u panel.

2.2 Test organisms may be removed from non-selective agar growth media. The following agar plate media are recommended:

- Trypticase soy blood agar
- Eosin methylene blue agar
- Phenylethyl alcohol agar

- Nutrient agar
- MacConkey agar
- Trypticase soy agar

NOTE: beta-Hemolytic streptococci should not be tested using the RapID SS/u System. The IDS RapID STR System is recommended for these isolates.

NOTE: Media containing or supplemented with mono- or disaccharides (i.e. Sabouraud-dextrose agar or Mannitol salt agar) are not recommended since they may suppress glycolytic activity and reduce test selectivity.

NOTE: Plates used for inocula preparation should be less than 50 hours old.

2.3 Using a cotton swab or inoculating loop, suspend sufficient growth from the agar plate culture in RapID INOCULATION FLUID (Cat. No. 83-25102)

EQUIVALENT TO A #1 McFARLAND TURBIDITY STANDARD.

NOTE: Suspensions significantly less turbid than a #1 McFarland standard will result in aberrant reactions. Suspensions SLIGHTLY more turbid than a #1 McFarland standard will not compromise test performance and are recommended for stock cultures and quality control strains.

Suspensions should be mixed thoroughly and vortexed if required. Suspensions should be used within 15 minutes of preparation.

2.4 An agar plate may be inoculated for purity and any additional testing that may be required using a loopful of the last suspension from the inoculation fluid tube. Incubate the plate for 18-24 hours at 35-37°C.

3. INOCULATION OF RapID SS/u PANELS
3.1 Peel back the panel lid over the inoculation port by pulling the tab marked "Peel to Inoculate" up and to the left.

3.2 Using a Pasteur pipette, gently transfer the entire contents of the Inoculation Fluid tube into the upper right-hand corner of the panel. Reseal the inoculation port by pressing the peel tab back in place.

NOTE: It is important that the panel receive the entire test suspension. Spent Inoculation Fluid tubes, swabs, and other contaminated materials should be autoclaved prior to disposal.

3.3 After adding the test suspension, and while keeping the panel on a level surface, tilt the panel back away from the biochemical wells at approximately a 45° angle. See below:

3.4 While tilted back, gently rock the panel from side to side to evenly distribute the inoculum along the rear baffles as illustrated below:
3.5 While maintaining a level horizontal position (best achieved by using the bench top against the biochemical well bottoms), SLOWLY tilt the panel forward toward the biochemical wells until the inoculum flows along the baffles into the biochemical wells (see below). This should evacuate all of the inoculum from the rear portion of the panel.

NOTE: If the panel is tilted too quickly, air may be trapped at the biochemical well junction restricting fluid movement.

3.6 Return the panel to a level position. If necessary, gently tap the panel on the bench top to remove any air trapped in the cavities.

NOTE: Examine the biochemical wells. Wells should appear bubble-free and uniformly filled. Slight irregularities in test cavity fill are acceptable and will not affect test performance. If the panel is grossly misaligned, the new panel should be inoculated and the misaligned panel discarded.

NOTE: Complete the inoculation of each panel receiving inoculation fluid before inoculating additional panels. Do not let the inoculum rest in the back portion of the panel without completing the procedure.

4. INCUBATION OF RapID SS/u PANELS
4.1 Place inoculated panels into the chipboard incubation trays provided in the package.

   Incubate inoculated panels at 35-37°C
   in a non-CO₂ incubator for 2 HOURS

5. SCORING RapID SS/u PANELS
5.1 Refer to the instructions presented in the INTERPRETATION section that follows for complete information on reading and scoring RapID SS/u Panels.

6. DISPOSAL
6.1 Spent Inoculation Fluid tubes, RapID SS/u Panels, cavity lids, and other contaminated materials should be autoclaved prior to disposal.

INTERPRETATION

<table>
<thead>
<tr>
<th>Cavity No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<tbody>
<tr>
<td>Test Code</td>
<td>GMS</td>
<td>ONPG</td>
<td>G1</td>
<td>G2</td>
<td>G3</td>
<td>PHS</td>
<td>URE</td>
<td>A1</td>
<td>A2</td>
<td>A3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IND</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RapID SS/u Panels contain 10 reaction cavities that, in addition to OXIDASE, provide 12 test scores. Test cavity 7 is BIFUNCTIONAL, containing two separate tests in the same cavity. Bifunctional tests are first scored BEFORE the addition of reagent providing the first test result. The same cavity is scored again AFTER the addition of reagent to provide the second test result. Bifunctional tests are distinct and not necessarily related. The bifunctional test cavities that require RapID SS/u Reagent are indicated with the first test above the bar and the second test below the bar. For example:

- URE:
  - First bifunctional test

- IND:
  - Second bifunctional test

REFER TO THE COMMENTS PRESENTED IN TABLE 2 FOR COMPLETE INFORMATION ON TEST INTERPRETATION AND SCORING

5.1 While firmly holding the RapID SS/u Panel on the benchtop, peel back the label lid over the reaction cavities by pulling the lower right hand tab up and to the left.

5.2 Without the addition of any reagents, read and score cavities 1 (GMS) through 7 (URE) reading from left to right using the Interpretation guide presented in Table 2. Record test scores in the appropriate boxes on the report form using the test code above the bar for bifunctional tests.

5.3 Add the following reagents to the cavities indicated:

   5.3.1: Add 2 drops of IDS Spot Indole Reagent to cavity 7 (URE/IND)
   5.3.2: Add 2 drops of RapID SS/u REAGENT to cavities 8 (A1) through 10 (A3).
LIMITATIONS
The use of the RapID SS/u System and the interpretation of results requires a competent laboratorian who is trained in general microbiological methods, and who should judiciously make use of knowledge, specimen information, and other pertinent procedures before reporting the identity of isolates using the RapID SS/u System. Characteristics such as Gram-stain reaction, oxidase, and cellular and colonial morphology must be considered when using the RapID SS/u System.

The RapID SS/u System must be used with pure cultures of test organisms. The use of mixed microbial populations or direct testing of clinical material without culture will result in aberrant results. The RapID SS/u System is designed to address urine isolates. The use of isolates from other body sites may lead to misidentification. The RapID SS/u System is designed for use with the taxa listed in the RapID SS/u Differential Chart.

Expected values listed for RapID SS/u System tests may differ from conventional test results or previously reported information. The accuracy of the RapID SS/u System is based upon the statistical use of a multiplicity of specially designed tests and an exclusive, proprietary database. The use of any single test found in the RapID SS/u System to establish the identification of a test isolate is subject to the error inherent in that test alone.

Results obtained using the RapID SS/u System are dependent upon the procedures indicated. Any change or modification in the procedure may affect results. In which case REMEL disclaims all warranties, expressed or implied, including merchantability and fitness for use, REMEL, in such an event, shall not be liable for damages direct or consequential.

RANGE OF EXPECTED VALUES
The RapID SS/u Differential Chart (Table 3) illustrates the expected results for the RapID SS/u System. DIFFERENTIAL CHART results are expressed as a series of positive percentages for each system test. This information statistically supports the use of each test and provides the basis, through numerical coding of digital test results, for a probabilistic approach to the identification of the test isolate.

Identifications are made using individual test scores from ITS panels in conjunction with other laboratory information (e.g. Gram-stain, oxidase, microscopic and colonial morphology, growth on different media or selective media) to produce a pattern that statistically resembles known reactivity for taxa recorded in the ITS RapID System database. These patterns are compared through the use of the RapID SS/u CODE COMPENDIUM.

<table>
<thead>
<tr>
<th>TAXON</th>
<th>GMS</th>
<th>ONPG</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>PHG</th>
<th>URE</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
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<th>OXI</th>
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<tbody>
<tr>
<td>Escherichia coli</td>
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<td>0</td>
<td>31</td>
<td>0</td>
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<td>96</td>
<td>92</td>
<td>2</td>
<td>71</td>
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<td>99</td>
<td>95</td>
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<td>2</td>
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<td>96</td>
<td>10</td>
<td>0</td>
<td>5</td>
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<tr>
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<td>98</td>
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<td>71</td>
<td>2</td>
<td>89</td>
<td>94</td>
<td>71</td>
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<td>0</td>
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<tr>
<td>Citrobacter spp.</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>67</td>
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<td>Morganella morganii</td>
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<td>0</td>
<td>98</td>
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<tr>
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PERFORMANCE CHARACTERISTICS
The RapID SS/u System performance characteristics have been established by laboratory testing of reference and stock cultures at REMEL and by clinical evaluations using fresh clinical and stock isolates (3.12.17).
BIBLIOGRAPHY


REMEL, Norcross, GA 1-800-447-3641 Issue 87/98 A4
### RapID™ SS/u Color Guide

<table>
<thead>
<tr>
<th>Test</th>
<th>Cavity</th>
<th>Positive Reactions</th>
<th>Negative Reactions</th>
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<td>o</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>o</td>
<td>o</td>
</tr>
<tr>
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<td>o</td>
<td>o</td>
</tr>
<tr>
<td>URE</td>
<td>7</td>
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</tr>
<tr>
<td>A3</td>
<td>10</td>
<td>o</td>
<td>o</td>
</tr>
</tbody>
</table>

Note: RapID™ Color Guides are intended as an educational aid to be used in conjunction with the instructions for use for the product. The reaction colors shown in the charts represent the typical shades of positive and negative colors.

Remarque: Les guides de coloration RapID™ sont conçus pour être utilisés comme support de formation en association avec le Mode d’emploi du produit. Les couleurs de réaction indiquées dans les tableaux représentent les nuances typiques des colorations positives (+) et négatives (−).

Anmerkung: Die RapID™ Farbskalen sind als Lehrinterleung bestimmt und zusammen mit der Gebrauchsanweisung für das Produkt zu verwenden. Die in den Tabellen enthaltenen Reaktionsfarben stellen die typischen Farbgestaltungen für positive (+) und negative (−) Reaktionen dar.

Nota: Le guida ai colori RapID™ sono un supporto formativo da utilizzare in abbinamento alle istruzioni per l’uso del prodotto. I colori delle reazioni presenti nelle tavole rappresentano le tonalità tipiche dei colori positivi (+) e negativi (−).

Nota: Las Guías de colores RapID™ han sido concebidas como una ayuda de formación para su utilización con las instrucciones de uso del producto. Las colores de reacción mostrados en los diagramas representan los tonos típicos de colores de positivo (+) y negativo (−).

Rev. Date: 6/9/2010
Date de Rev. 04/09/2010
Übersetzung am: 04.30.2010
Data rev.: 6/4/2010
Fecha de revolución: 6-4-2010
Alternative Rapid Identification Systems for the Enterobacteriaceae.

API 20E
The API 20E identification system has become the reference method against which the accuracy of other systems is compared. The 21 characteristics that can be determined by the API 20E is among the largest test set of the packaged kits. The system identifies a high percentage of bacterial species within 24 hours without the need to determine additional physiological characteristics. Currently, this system is the most frequently used in clinical laboratories that has a large-scale database that includes common and atypical strains. The API Profile Index, which can be used manually or with computer assistance, provides the frequency of probability of several strains that must be considered for each biotype number. Thus, the accuracy of identification of members of the Enterobacteriaceae is maximised. Castillo and Bruckner found that the API 20E System correctly identified 97.7% of 339 clinical and stock isolates.

The system is somewhat cumbersome to inoculate – a problem, however, that is overcome quickly with practice. After inoculation, the strips must be handled carefully so that the bacterial suspensions do not spill and contaminate the surrounding environment. Practice is required to interpret occasional borderline reactions, which can affect the biotype number and final identification. Occasionally, biotype numbers may not appear in the profile register; however, the manufacturer maintains a telephone number for consultation.

Enterotube II
Of all the systems, Enterotube II is the easiest to inoculate. The system takes up little space, and the risk of contamination is minimal. The colour reactions are generally easy to interpret; a minor problem exits in differentiating the elevation of the waxy overlaying the glucose chamber (an indicator of gas production) from artifactual shrinkage of the media during storage. A false negative interpretation may also result if a tiny leak in the plastic allows the gas to escape as it forms. Indole and Voges-Proskauer (VP) reagents must be added with a needle and syringe through the thin plastic backing. If this is not done carefully, the added reagents can leak into other compartments, altering reactions. Thus, it is recommended that the reactions in other compartments be interrupted before adding these reagents. One additional disadvantage of the Enterotube, compared with other systems that use dry substrates, is that the incorporation of the conventional agar media shortens the shelf life.

The manufacturer provides a convenient computer coding and identification system ICCIS) that lists the possible bacterial identifications for the five-digit biotype numbers that are derived from the interpretation of colour changes.
remel
Staphaurex Plus®
INTENDED USE
Staphaurex Plus® is a rapid latex agglutination test for the identification of staphylococci which possess clumping factor, protein A and/or surface antigen characteristic of Staphylococcus aureus.

SUMMARY AND EXPLANATION OF THE TEST
S. aureus possess a number of properties which are used to confirm identification. These include free coagulase, clumping factor (bound coagulase), thermonuclease and protein A. The tube coagulase test detects free coagulase and is a reference test for S. aureus. This test, however, takes 3 to 24 hours and slums may show a to-and-fro variation. Over the past decade particle agglutination assays have been developed which give a much more rapid identification. These first generation assays are based on latex particles or red cells coated with either fibrinogen alone, to detect clumping factor, or fibrinogen and immunoglobulin G (IgG), to detect both clumping factor and staphylococcal protein A.

Recently, it has been shown that these tests can fail to detect certain strains of S. aureus, particularly a proportion of methicillin/nosacillin resistant strains (MRSA)4,5. Some of these strains may express undetectable levels of clumping factor and protein A. Two antigens, somatic type 16 and capsular type 5,6,7 have been associated with the methicillin-resistant phenotype. The incorporation of antisera to these antigens may improve the sensitivity of agglutination assays for MRSA strains. Investigations on strains that are negative in rapid assays have shown that antibodies to a single somatic or capsular antigen are insufficient to detect all strains that are negative with the first generation of particle agglutination tests. Staphaurex Plus® uses latex beads coated with fibrinogen to detect the majority of clinical strains and IgG specific for a carefully selected group of strains that are negative in the first generation tests.

PRINCIPLE OF THE PROCEDURE
The Staphaurex Plus® Test Latex consists of yellow latex particles which have been coated with fibrinogen and immunoglobulin G (IgG) specific for S. aureus. When a drop of the reagent is mixed on a card with S. aureus organisms, rapid agglutination occurs through the interaction of (i) fibrinogen and clumping factor, (ii) the Fc portion of IgG and protein A or (iii) specific IgG and cell surface antigens.

Some strains of Staphylococcus spp., particularly S. epidermidis, may cause non-specific agglutination of latex particles. Therefore a Control Latex is provided to assist with the identification of non-specific reactivity.

REAGENTS
KIT CONTENTS
<table>
<thead>
<tr>
<th>Staphaurex Plus®</th>
<th>ZL3/300900102</th>
<th>ZL3/400900001</th>
</tr>
</thead>
<tbody>
<tr>
<td>160 Tests</td>
<td>450 Tests</td>
<td></td>
</tr>
</tbody>
</table>

1. Test Latex (Yellow cap) 1 dropper bottle 3 dropper bottles
2. Control Latex (Grey cap) 1 dropper bottle 3 dropper bottles
3. Disposable Reconstitution Cards (RT64/300900001) 2 packs 6 packs
4. Disposable Mixing Sticks 3 bundles 9 bundles
5. Instructions for Use 1 1

DESCRIPTION OF REAGENTS, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS
See also Warnings and Precautions.

The latex suspensions are prepared ready to use and should be stored in an upright position at 2 to 8°C, where they will retain activity at least until the date shown on the bottle label. Do not freeze. Avoid storage at room temperature (15 to 30°C). Do not stand the reagent in bright light on the bench.

Test Latex
A buffered suspension of yellow polystyrene latex particles coated with human fibrinogen and rabbit IgG. Contains 0.05% Bronidox® preservative. Materials of human origin have been tested for the presence of hepatitis B surface antigen, anti-HIV1 and anti-HIV2 and found to be negative.

Control Latex
A buffered suspension of yellow polystyrene latex particles coated with a bovine serum protein unrelated to S. aureus. Contains 0.05% Bronidox® preservative.

Reagent Cards and Mixing sticks should be stored at room temperature (15 to 30°C). Staphaurex Plus® (ZL3/300900102 and ZL3/400900001) was developed using RT64/300900001 Disposable Reaction Cards. Do not substitute another disposable slide for the RT64/300900001 Disposable Reaction Cards when samples are tested using Staphaurex Plus®.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use only.

For professional use only.

Caution: This product contains dry natural rubber.

Please refer to the manufacturer's safety data sheet and the product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION
1. CAUTION: This kit contains human sourced components. No known test can offer complete assurance that products derived from human sources will not transmit infection. Therefore, all human sourced material should be considered potentially infectious. It is recommended that these reagents and test specimens be handled using established good laboratory practice guidelines.
2. Non-disposable apparatus should be sterilised by any appropriate procedure after use, although the preferred method is to autoclave for 15 minutes at 121°C. Disposables should be autoclaved or incinerated. Spillage of potentially infectious materials should be removed immediately with absorbent paper towel and the contaminated areas washed with a standard hospital disinfectant. Materials used to clean spills, including gloves, should be disposed of as biohazardous waste.
3. Wear laboratory coat, disposable gloves and eye protection while handling specimens and preparing the assay. Wash hands thoroughly when finished.
4. When used in accordance with the principles of Good Laboratory Practice, good standards of occupational hygiene and the instructions in these Instructions for Use, the reagents supplied are not considered to present a hazard to health.

ANALYTICAL PRECAUTIONS
1. Do not use the reagents beyond the stated expiry date.
2. Latex reagents should be brought to room temperature (15 to 30°C) before use. Latex reagents which show signs of aggregation or lumpiness before use may have been frozen and should not be used.
3. If it is important when using dropper bottles that they are held vertically and that the drop forms at the tip of the nozzle. If the nozzle becomes wet an incorrect volume will form around the end and not at the tip. If this occurs dry the nozzle before proceeding.
4. Do not touch the reaction areas on the cards.
5. Do not interpret agglutination that appears after 90 seconds as a positive result. Prolonged rocking can result in false-positive reactions with some coagulase-negative staphylococci.
6. Microbiological contamination of reagents must be avoided as this may reduce the life of the product and cause erroneous results.
Bacteria and Disease 150

SPECIMEN COLLECTION AND STORAGE

For details of specimen collection and treatment, a standard text book should be consulted. Cultures may be tested from any of the following media:

- Blood agar
- Columbia CHA agar
- Nutrient agar
- Mannitol-Shafted agar with 5% blood
- Tryptone soya agar
- Baird-Parker agar
- Tryptone soya agar with 5% blood
- Mannitol-salt agar
- Columbia blood agar

THICKER specimens grown on media containing antibiotics or a high-salt-supplemented medium such as Mannitol-salt agar may give an agglutination containing stringy aggregates.

THE USE OF FRESH CULTURES GROWN OVERNIGHT IS RECOMMENDED.

PROCEDURE

MATERIALS PROVIDED

- Sufficient material is provided for 150 (25/L) or 450 (25/L) tests. See Kit Contents.

TEST PROCEDURE

Please read Analytical Precautions carefully before performing the test.

1. Shake vigorously and examine the latex reagents for aggregation before use. Refer to Quality Control Section and Visual Inspection for additional instructions.

2. For each test sample place one drop of Test Latex and 1 drop of Control Latex in a separate circle. Ensure that the dropper bulbs are held vertically to dispense an accurate drop.

3. Using a mixing stick, remove sufficient growth from a pure culture or well-isolated colonies to cover the bottom end of the stick. As a guide, an amount of growth roughly equivalent to six standard-sized colonies should be used.

4. Emulsify the sample of culture in the drops of Test Latex by rubbing with the flat end of the stick. Rub thoroughly, but not too vigorously as the surface of the latex may be damaged. Some strains, particularly of species other than S. aureus, remain difficult to emulsify and this is the rule. Some types of unresolved culture can make the latex appear too thick or stringy when used. Spoon the latex over approximately half the area of the circle. Discard the mixing stick for safe disposal.

5. Using a separate stick, emulsify a similar culture sample in the Control Latex, as stated in Step 4, sample. Discard the mixing stick for safe disposal.

6. Rock the card slowly for up to 20 seconds while observing for agglutination. The card should be held at normal reading distance (25 to 30 cm) from the eyes. Do not use a magnifying lens.

7. Discard the used Reaction Card for safe disposal.

RESULTS

Positive Result

Agglutination of the Test Latex accompanied by a lack of agglutination of the Control Latex indicates the presence of either coagulase, protein A or antigens commonly found on S. aureus in the culture under test. Most positive reactions will be almost instantaneous. Follow positive results can occur if the test is read after more than 30 seconds.

Negative Result

Lack of agglutination in both reagents means that the culture under test is unlikely to be S. aureus.

Non-Interpretable Result

Visible agglutination of the Control Latex, whether stronger or weaker than the Test Latex, indicates a non-specific reaction.

QUALITY CONTROL

The following procedures should be carried out with each shipment of test kits and periodically throughout the life of the kit. Local regulations may require that quality control procedures are carried out with every run of testing. A run may be defined as a period of up to 24 hours.

Any departure from the expected results indicates that there may be a problem with the reagents, which must be resolved before further use with clinical samples.

Visual Inspection

The latex suspensions should always be inspected for aggregation as they are dispensed onto the Reaction Card. If there is evidence of clumping before addition of the test sample the suspension should not be used. After prolonged storage some aggregation or drying may occur around the top of the bottle. If this is observed the bottle should be shaken vigorously for a few seconds until reaggregation is complete.

Control Procedure

The performance of the Test and Control Latex reagents should be confirmed using fresh, overnight cultures of reference strains of bacteria, following the method described in Test Procedure. Suitable reference strains are shown below.

S. aureus (ATCC 25923)
S. epidermidis (ATCC 12228)

INTERPRETATION OF RESULTS

A positive reaction indicates the presence of one or more of the following: 21-170, protein A or cell surface antigens in the culture under test and a negative result indicates their absence.

LIMITATIONS OF THE PROCEDURE

1. Specimens grown on media containing antibiotics or a high-salt-supplemented medium such as mannitol-salt agar may give an agglutination containing stringy aggregates.

2. Some species of staphylococci in addition to S. aureus notably S. hyicus, S. intermedius, S. lugdunensis and S. sciuri may give positive results in coagulase tests and may also react in rapid latex procedures. If necessary, these species may be identified by biochemical test procedures. S. hyicus, S. intermedius and S. sciuri are encountered rarely in the clinical laboratory.

3. Some coagulase-negative staphylococcal species, such as S. epidermidis and some species of staphylococci, may also give positive reactions.

4. Some streptococci and possibly other organisms possess immunoglobulin or other plasma protein binding factors which can react in the latex test and there are several bacteria such as S. epidermidis, which are able to non-specifically agglutinate latex particles. To eliminate potential interference from these organisms, a Gram stain and catalase test should be performed to identify any organisms with staphylococcal morphology.

5. All questionable results should be checked for purity and identified by an alternative method.

EXPECTED RESULTS

Strong agglutination with S. aureus cultures, no agglutination with staphylococci which possess neither clumping factor, protein A or surface antigens characteristic of S. aureus.

The School of Biotechnology & Biomolecular Sciences, UNSW 2017
SPECIFIC PERFORMANCE CHARACTERISTICS

The performance of Staphylococcus Plus has been evaluated in four North American and two European microbiological reference laboratories on a total of 648 routine (presumed staphylococcal) clinical isolates and 607 stored cultures. The cultures were tested in parallel with the tube coagulase procedure. Gram stain and at least one alternative rapid test for the identification of S. aureus. The results are summarised in Tables 1 and 2.

CLINICAL ISOLATES

**Methicillin Resistant S. aureus (MRSA)**

A total of 151 fresh S. aureus cultures shown to be resistant to one or more antibiotics were tested in the American and European reference laboratories. Staphylococcus Plus correctly identified 120 of these isolates. The discrepant isolate was positive with a tube coagulase test and an alternative rapid latex test.

The sensitivity of Staphylococcus Plus on this group of MRSA cultures is estimated to be 99.3% (150/151).

**Methicillin Sensitive S. aureus (MSSA)**

Staphylococcus Plus** correctly identified 235 of 257 confirmed S. aureus cultures from the microbiological reference laboratories. The discrepant isolates included one which also gave a negative result with the alternative rapid latex test.

The specificity of Staphylococcus Plus on this group of MSSA cultures is estimated to be 99.4% (256/257).

**Other Staphylococci**

A total of 157 fresh non-S. aureus staphylococcal isolates were also tested. Staphylococcus Plus gave a negative result with 150 of these isolates which included S. epidermidis, S. epidermidis, S. haemolyticus, S. hominis and S. saprophyticus. The remaining seven cultures which gave a positive result with Staphylococcus Plus included three which were also positive with an alternative rapid latex test.

The specificity of Staphylococcus Plus on this group of non-S. aureus staphylococcal cultures is estimated to be 95.2% (150/157).

**Overall Performance of Staphylococcus Plus in Comparison with Tube Coagulase on S. aureus Isolates**

- **Relative Sensitivity**: 99.4%
- **Relative Specificity**: 95.2%
- **Overall Agreement**: 98.4%

NOTE: Staphylococcus Plus gave a non-interpretable result with 0.15% (1/648) of the fresh cultures, which have been excluded from the summary above.

STORED CULTURES

**Methicillin Resistant S. aureus (MRSA)**

A total of 252 stored S. aureus cultures shown to be resistant to one or more antibiotics were tested. Staphylococcus Plus correctly identified 251 of these isolates. The discrepant culture was positive with a tube coagulase test and negative with an alternative rapid latex test.

The sensitivity of Staphylococcus Plus on this group of MSSA cultures is estimated to be 99.6% (251/252).

**Methicillin Sensitive S. aureus (MSSA)**

Staphylococcus Plus correctly identified 242 of 257 confirmed S. aureus cultures from the microbiological reference laboratories. The discrepant cultures included four which also gave a negative result with the alternative rapid latex test.

The sensitivity of Staphylococcus Plus on this group of MSSA cultures is estimated to be 97.8% (242/257).

**Other staphylococci**

A total of 109 stored non-S. aureus staphylococcal cultures were also tested. Staphylococcus Plus gave a negative result with 102 of these isolates which included S. saprophyticus, S. epidermidis and S. homolyticus. The remaining seven cultures which gave a positive result with Staphylococcus Plus included two which were also positive with an alternative rapid latex test.

The specificity of Staphylococcus Plus on this group of non-S. aureus staphylococcal cultures is estimated to be 95.6% (102/106).

**Overall Performance of Staphylococcus Plus on Presumed Staphylococcal Clinical Isolates**

- **Relative Sensitivity**: 99.7%
- **Relative Specificity**: 95.0%
- **Overall Agreement**: 97.9%

NOTE: Staphylococcus Plus gave a non-interpretable result with 0.3% (2/671) of the stored cultures, which has been excluded from the summary above.

**Table 1**

<table>
<thead>
<tr>
<th>Staphylococcus Plus result</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methicillin Resistant S. aureus (MRSA)</td>
<td>140</td>
<td>1</td>
<td>141</td>
</tr>
<tr>
<td>Methicillin Sensitive S. aureus (MSSA)</td>
<td>212</td>
<td>2</td>
<td>214</td>
</tr>
<tr>
<td>Non-S. aureus cultures*</td>
<td>7</td>
<td>102</td>
<td>109</td>
</tr>
</tbody>
</table>

*Staphylococcus Plus gave a non-interpretable result with 1 sample. This has been excluded from the table.

**Table 2**

<table>
<thead>
<tr>
<th>Staphylococcus Plus result</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methicillin Resistant S. aureus (MRSA)</td>
<td>231</td>
<td>1</td>
<td>232</td>
</tr>
<tr>
<td>Methicillin Sensitive S. aureus (MSSA)</td>
<td>234</td>
<td>6</td>
<td>240</td>
</tr>
<tr>
<td>Non-S. aureus cultures*</td>
<td>7</td>
<td>102</td>
<td>109</td>
</tr>
</tbody>
</table>

*Staphylococcus Plus gave a non-interpretable result with 2 samples. These have been excluded from the table.

**Includes S. saprophyticus, S. epidermidis and S. haemolyticus.**

**BIBLIOGRAPHY**


*Hanks, J.K. A., Manufacturers Information and Safety Data Sheet for Oxoid® L.*
**TECHNICAL SPECIFICATION**

**OXOID**

**OXOID Australia Pty Limited ©**

<table>
<thead>
<tr>
<th>PP2272</th>
<th>CHROMOGENIC CANDIDA AGAR PLATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FORMULA</td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 gm per litre</td>
</tr>
<tr>
<td>Peptone</td>
<td>10.2</td>
</tr>
<tr>
<td>Chromogenic mix</td>
<td>22.0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.5</td>
</tr>
<tr>
<td>pH</td>
<td>6.1± 0.2</td>
</tr>
</tbody>
</table>

**DESCRIPTION**

Chromogenic Candida agar is a differential isolation medium for the presumptive identification of clinically important *Candida* species. Chromogenic Candida agar will not only allow the growth and detection of yeasts – like traditional media such as Sabouraud, but in addition, the colour of the colony will instantly allow differentiation of various species of *Candida*. Studies have shown (1) that the viability of yeasts on Chromogenic Candida Agar is equal to that on Sabouraud. Chloramphenicol is added to inhibit bacterial growth, making it ideal for culturing swabs from areas harbouring a high level of bacterial flora, where overgrowth can make isolating *Candida spp* difficult.

**QUALITY CONTROL**

**ORGANISMS:**

- *C. albicans* MVQC 0007 (ATCC 10231), *C. krusei* MVQC 0083 (ATCC 14243), *C. tropicalis* MVQC 0084 (ATCC 750), *C. glabrata* MVQC 0086 (ATCC 2001), *C. parapsilosis* MVQC 0085 (ATCC 22019).

**SAMPLE NUMBER:**

Sample size is determined in accordance with NATA Technical Notes Number 4 (2) and ASM Guidelines (3).

**STERILITY:**

Those plates not used for bacteriological testing and other quality assurance procedures must be incubated at 30°C for 7 days after which they are examined for sterility.

**INOCULUM:**

As described in Oxoid WI 37, inoculate the specified test organisms onto the media using Working Culture B (<10⁵ cfu).

**INCUBATION:**

24 – 48 hrs / 35°C / aerobically
EXPECTED RESULTS:

- **C. albicans**: 4 – 5+ green colonies
- **C. tropicalis**: 4 – 5+ blue colonies with a purple halo
- **C. krusei**: 4 – 5+ rose/pink rough colonies
- **C. glabrata**: 4 – 5+ mauve colonies
- **C. parapsilosis**: 4 – 5+ pale pink colonies

ALSO CHECK AND RECORD

1. Batch number correct
2. Colour
3. Clarity
4. Final pH – 6.1 ± 0.2
5. Gel strength
6. Sterility
7. Correctly labelled – CHROM CANDIDA

STORAGE

A shelf life of 8 weeks applies when this product is stored at 2°C – 8°C in its original packaging. This medium must be stored in the dark.

REFERENCES

3. Guidelines for Assuring Quality of Medical Microbiological Culture Media. 1996. Media Quality Control Special Interest Group, Australian Society for Microbiology.
Chromogenic Salmonella Agar.

TECHNICAL SPECIFICATION
Oxoid Australia Pty Limited ©

PP2269 CHROMOGENIC SALMONELLA AGAR PLATE

FORMULA

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Special Peptone</td>
<td>10.0 gm per Litre</td>
</tr>
<tr>
<td>Chromogenic Mix</td>
<td>28.0 gm per Litre</td>
</tr>
<tr>
<td>Agar No 1</td>
<td>12.0 mg per Litre</td>
</tr>
<tr>
<td>Cefsulodin</td>
<td>6.0 mg per Litre</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>2.5 mg per Litre</td>
</tr>
</tbody>
</table>

pH = 7.2 ± 0.2

DESCRIPTION (1)

Salmonella Chromogenic Agar is designed to identify Salmonella species based on their utilisation of one chromogenic substrate. Their inability to utilise another chromogenic substrate, that most other members of the family Enterobacteriaceae can, enables rapid and reliable identification of Salmonella species.

Traditionally, media used to differentiate salmonellae from other members of the family Enterobacteriaceae depend upon the ability of Salmonella species to produce hydrogen sulphide coupled with their inability to ferment lactose. Salmonella Chromogenic Agar combines two different chromogens for the detection of Salmonella species, 5-Bromo-6-Chloro-3-Indolyl caprylate (Magenta-caprylate) and 5-Bromo-4-Chloro-3-Indolyl β-D galactopyranoside (X-gal). X-gal is a substrate for the enzyme β-D-galactosidase. Hydrolysis of the chromogen, Mag-caprylate, by lactose negative Salmonella species results in magenta colonies.

The medium contains bile salts to inhibit the growth of Gram-positive organisms and the addition of the Salmonella Selective Supplement is recommended to increase the selectivity of the medium. This uses novobiocin to inhibit Proteus spp. growth and cefsulodin to inhibit growth of pseudomonads.

QUALITY CONTROL

ORGANISMS:

- S. Typhimurium MVQC 0009 (ATCC 14028), S. Salford MVQC 0044 (IMVS 1710), E. coli MVQC 0004 (ATCC 25922), P. mirabilis MVQC 0008 (ATCC 12453), P. aeruginosa MVQC 0055 (ATCC 27853)

SAMPLE NUMBER: Sample size is determined in accordance with NATA Technical Note Number 4 (2) and ASM Guidelines (3).

STERILITY: Those plates not used for bacteriological testing and other quality assurance procedures must be incubated at 30°C for 3 days after which they are examined for sterility.

INOCULUM: As described in Oxoid WI 37, inoculate the specified test organisms onto the media using Working Culture B (≤10⁵ cfu) or Working Culture A (≥10⁵ cfu).

INCUBATION: 24 – 48 hours / 35°C / aerobically
TECHNICAL SPECIFICATION

EXPECTED RESULTS:

- **S. Typhimurium**: 4-5+ colonies with pink centres and a colourless edge using Working Culture B
- **S. Salford**: 4-5+ pink colonies using Working Culture B
- **E. coli**: 2-3+ pale blue colonies using Working Culture B
- **P. mirabilis**: 4-5+ colourless colonies using Working Culture B
- **P. aeruginosa**: <1+ inhibited using Working Culture A

ALSO CHECKED AND RECORDED

1. Batch number correct
2. Colour
3. Clarity
4. Final pH = 7.2 ± 0.2
5. Gel strength
6. Sterility
7. Correctly Labelled – CHROM SALMONELLA

STORAGE

A shelf life of 6 weeks applies when this product is stored at 2° – 8°C in its original packaging.

REFERENCES

3. Guidelines for Assuring Quality of Medical Microbiological Culture Media. 1996. Media Quality Control Special Interest Group, Australian Society for Microbiology.
Chromogenic UTI agar plates are prepared using a complex substrate mix containing the following elements:

- Phenylalanine and Tryptophan substrates for the indication of the deaminase activity found in *Proteus spp.*, *Providencia spp.* and *Morganella spp.*. The end products of tryptophan deamination result in the formation of brown coloured colonies.
- X-Gluc – a chromogenic substrate for the detection of β – Glucosidase activity found in enterococci. The hydrolysis of this substrate results in the release of an insoluble blue chromogen.
- Red – Gal – a second chromogenic substrate which is hydrolysed by the enzyme β – Galactosidase. This enzyme is produced by *E. coli* and a number of other members of the family *Enterobacteriaceae*. Hydrolysis of this substrate results in the release of an insoluble pink/ red chromogen. The presence of pink/ red colonies suspected of being *E. coli* may be confirmed by the performance of a spot indole test using DMACA Reagent.

A number of species of *Enterobacteriaceae* produce both β–Glucosidase and enzyme β–Galactosidase activity. The release of both of these chromogens results in the formation of a purple insoluble precipitate. In addition, this medium is electrolyte deficient which inhibits the swarming of *Proteus spp.*

Chromogenic UTI agar has been demonstrated to provide a valuable cost-effective mechanism to improve the detection of mixed cultures in urines, thereby improving the reliability of reporting of significant isolates when compared with CLED agar (2).
### Typical Reactions:

<table>
<thead>
<tr>
<th>Organism</th>
<th>β − Glucosidase</th>
<th>β − Galactosidase</th>
<th>Tryptophan Deaminase</th>
<th>Colony Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococci</td>
<td>+</td>
<td></td>
<td></td>
<td>Blue</td>
</tr>
<tr>
<td>E. coli</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Pink</td>
</tr>
<tr>
<td>Coliforms</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Purple</td>
</tr>
<tr>
<td>Proteus, Morganella, Providencia</td>
<td></td>
<td></td>
<td>+</td>
<td>Brown</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td></td>
<td>+</td>
<td></td>
<td>Pink</td>
</tr>
</tbody>
</table>

### QUALITY CONTROL

**ORGANISMS:**

- *K. pneumoniae* MVQC 0081 (ATCC 13883), *E. coli* MVQC 0004 (ATCC 25922), *P. mirabilis* MVQC 0008 (ATCC 12453), *S. aureus* MVQC 0003 (ATCC 25923), *S. saprophyticus* MVQC 0033 (ATCC 15905), *E. faecalis* MVQC 0005 (25922)

**SAMPLE NUMBER:**

Sample size is determined in accordance with NATA Technical Note Number 4 (3) and ASM Guidelines (4).

**STERILITY:**

Those plates not used for bacteriological testing and other quality assurance procedures must be incubated at 30°C for 3 days after which they are examined for sterility.

**INOCULUM:**

As described in Oxoid WI 37, inoculate the specified test organisms onto the media using Working Culture B (≤10⁶ cfu).

**INCUBATION:**

24 - 48 hours / 35°C aerobically

**EXPECTED RESULTS:**

- *K. pneumoniae* 4 - 5+ dark blue/purple colonies
- *E. coli* 4 - 5+ pink colonies
- *P. mirabilis* 4 - 5+ straw/brown colonies, no swarming
- *S. saprophyticus* 4 - 5+ small pink/white colonies
- *S. aureus* 4 - 5+ small, white colonies
- *E. faecalis* 4 - 5+ small blue colonies

**ALSO CHECKED AND RECORDED**

1. Batch number correct
2. Colour
3. Clarity
4. Gel strength
5. Final pH 6.8 ± 0.2
6. Sterility
7. Correctly Labelled — CHROMOGENIC UTI

**STORAGE**

A shelf life of 6 weeks applies when this product is stored at 2° - 8°C in its original packaging.

**REFERENCES**

4. Guidelines for Assuring Quality of Medical Microbiological Culture Media. 1996. Media Quality Control Special Interest Group, Australian Society for Microbiology.
XLD (Xylose Lysine Desoxycholate) Agar.

TECHNICAL SPECIFICATION
Oxoid Australia Pty Limited ©

PP2004  XLD AGAR PLATE

FORMULA
Yeast Extract  3.0 gm per litre
L-Lysine HCl   5.0
Xylose         3.75
Lactose        7.5
Sucrose        7.5
Sodium desoxycholate  1.0
Sodium chloride  5.0
Sodium thiosulphate 6.8
Ferric ammonium citrate 0.8
Phenol red     0.08
Agar No. 1     12.5
pH 7.4 ± 0.2

DESCRIPTION (1)
Xylose Lysine Desoxycholate (XLD) Medium was originally formulated for the isolation and identification of shigellae from stool specimens. It has since been found to be a satisfactory medium for the isolation and presumptive identification of both salmonellae and shigellae. It relies on xylose fermentation, lysine decarboxylation and production of hydrogen sulphide for the primary differentiation of *Shigella* spp. and *Salmonella* spp. from non-pathogenic bacteria.

*Salmonella* species are differentiated from non-pathogenic xylose-fermenters by the incorporation of lysine in the medium. The presence of *Salmonella*, *Arizona* and *Edwardsiella* species is differentiated from that of shigellae by a hydrogen sulphide indicator.

The high acid level produced by fermentation of lactose and sucrose, prevents lysine-positive coliforms from reverting the pH to an alkaline value, and non-pathogenic hydrogen sulphide producers do not decarboxylate lysine. The acid level also prevents blackening by these micro-organisms until after the 18 - 24 hour examination for pathogens.

Sodium desoxycholate is incorporated as an inhibitor in the medium. The concentration used allows for the inhibition of coliform without decreasing the ability to support *Shigella* spp. and *Salmonella* spp.

COLONIAL APPEARANCES

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>APPEARANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>Red colonies with black centres</td>
</tr>
<tr>
<td><em>Arizona</em></td>
<td></td>
</tr>
<tr>
<td><em>Edwardsiella</em></td>
<td></td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>Red colonies</td>
</tr>
<tr>
<td><em>Providencia</em></td>
<td></td>
</tr>
<tr>
<td><em>H2S-negative Salmonella</em> (eg <em>S.Paratyphi A</em>)</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia</em></td>
<td>Yellow, opaque colonies</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td></td>
</tr>
<tr>
<td><em>Citrobacter</em></td>
<td></td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td></td>
</tr>
<tr>
<td><em>Serratia</em></td>
<td></td>
</tr>
</tbody>
</table>

(NB: False-positive, red colonies may occur with some *Proteus* and *Pseudomonas* species.)
Bacteria & Disease 159

Eosin Methylene Blue Agar

TECHNICAL SPECIFICATION
Thermo Fisher Scientific®

PP2169 EMB AGAR PLATE

FORMULA

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.0 gm per litre</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.0</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>2.0</td>
</tr>
<tr>
<td>Eosin Y</td>
<td>0.4</td>
</tr>
<tr>
<td>Methylene Blue</td>
<td>0.065</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
</tbody>
</table>

pH 6.8 ± 0.2

DESCRIPTION

This medium is prepared to the formula specified by APHA and is used for the enumeration and differentiation of the coliform group of organisms. It may also be used for the rapid identification of Candida albicans, when incubated for 24 - 48 hours at 35°C in a 10% carbon dioxide atmosphere (1). EMB is also specified in Australian Standards methods for examination of waters (2).

QUALITY CONTROL

ORGANISMS:  E. coli MVOC 0014 (ATCC™ 11775), E. aerogenes MVOC 0013 (ATCC™ 13048), P. aeruginosa MVOC 0023 (ATCC™ 10145).

SAMPLE NUMBER:  Sterility sample size is determined in accordance with AS/NZ Guidelines (3).

STERILITY:  Sampled plates are incubated at 30°C for 3 days after which they are examined for sterility.

INOCULUM:  As described in TFS MBD WI 37, inoculate the specified test organism/s onto the media using working culture B (≤10⁵ cfu).

INCUBATION:  24 - 48 hours / 35°C / aerobically.

EXPECTED RESULTS:

- E. coli:  4 – 5+ green colonies with metallic sheen using Working Culture B
- P. aeruginosa:  4 – 5+ colourless colonies using Working Culture B
- E. aerogenes:  4 – 5+ mucoid colonies, no sheen using Working Culture B

ALSO CHECKED AND RECORDED

1. Batch number correct
2. Colour
3. Clarity
4. Gel strength
5. Final pH 6.8 ± 0.2
6. Sterility
7. Correctly labelled - EMB

The School of Biotechnology & Biomolecular Sciences, UNSW 2017