Unit 15: Microbiological Techniques

Unit code: D/502/5555

QCF Level 3: BTEC National

Credit value: 10

Guided learning hours: 60

Aim and purpose

The aim of this unit is to enable learners to develop knowledge and skills of key microbiological concepts and techniques that underpin vocational applications, including pharmaceuticals, medical diagnostics, food production, environmental health and forensic science. Learners will look into the characteristic features of cells and will practise aseptic techniques to culture and determine the factors that influence the growth of micro-organisms.

Unit introduction

Micro-organisms are essential to the effective functioning of the world around us and their beneficial uses have long been exploited in traditional biotechnology such as brewing and yogurt production. More recent biotechnological applications include those involved with novel food products, metal extraction from ores and genetic engineering.

Unfortunately, micro-organisms are also responsible for numerous diseases and many millions of people have their health impaired or are killed every year by them. As a result of their continued evolution new epidemics arise, and so biomedical scientists are involved in the constant struggle to produce new antibiotics, antiseptics and improved preventative measures.

As-yet-undiscovered microbial species may contain biological molecules that could form the basis of future biotechnological applications in many industries and services.

Microbiology skills are clearly much in demand and the work of microbiologists is central to developments in: food production, biochemical production, forensic evidence, medical health, environmental impact, crop health and production, livestock health and improvement, and genetic engineering.

This unit introduces learners to the fundamental differences between prokaryotic and eukaryotic cells, classification and identification of micro-organisms and factors affecting microbial growth. Learners will also be given the knowledge and vocational practical skills to work competently and safely in a microbiological laboratory setting. As this is a highly practical unit involving the handling of live organisms, learners need to develop manipulative skills involving good aseptic techniques; they also need to analyse the level of risk. Whilst cultures used are low risk, learners should approach any micro-organism with the respect afforded to serious pathogens.

Learning outcomes

On completion of this unit a learner should:

1 Be able to identify the characteristic features and functions of akaryotes, prokaryotic and eukaryotic cells

2 Be able to use aseptic techniques to culture micro-organisms

3 Be able to determine the factors that influence the growth of micro-organisms

4 Know how to identify micro-organisms.

Unit content

1 Be able to identify the characteristic features and functions of akaryotes, prokaryotic and eukaryotic cells

Techniques: light microscopy (phase contrast, oil immersion); electron microscopy (scanning, transmission)

Eukaryotic: nucleus; Golgi apparatus and secretory vesicles; rough and smooth endoplasmic recticulum; cell membrane; nuclear membrane; nucleolus; ribosomes; mitochondria; chloroplasts; centrioles; cilia; flagella

Prokaryotic: nucleoid; ribosomes; cell wall; capsule; mesosome; cilia; flagella

Functions: energy conversion; synthesis of biological molecules; transport of substances; motility

Identification: key characteristics of bacteria (prokaryotes), viruses (akaryotes), fungi (eukaryotes); electron microscopy (scanning, transmission, advantages, disadvantages); light microscopy (advantages, disadvantages)

2 Be able to use aseptic techniques to culture a range of micro-organisms

Micro-organisms: bacteria (prokaryotes); viruses (akaryotes); fungi (eukaryotes)

Biocontainment: micro-organisms, eg positive (protect sample), negative (protect operator/environment); laminal flow; clean air cabinets

Requirements for growth: nutrients; gaseous environment; temperature; pH

Techniques: disinfection and sterilisation techniques preparing sterile growth media; aseptic technique; inoculation of liquid media; inoculation of solid media, eg pour plates, streak plates, lawn plates, mycelial discs, fungal spore plate inoculation; viral plaque counts (lysis on solid media or lysis in liquid media using a colorimeter); haemocytometer counts; safe disposal methods

3 Be able to determine the factors that influence the growth of micro-organisms

Factors: affecting growth, eg nutrients; aerobic and anaerobic conditions; temperature; pH; osmotic potential; irradiation; antibiotics, antifungals; antivirals; disinfection; sterilisation

Growth: measurement techniques, eg serial dilution; viable counts; total counts; microbiological assays; dry mass determination; growth of mycelial discs; viral plaque counts (liquid or solid media); use of colorimetry to determine turbidity in liquid media

Contexts: biotechnological, biomedical

4 Know how to identify micro-organisms

Classification: key characteristics of the main subgroups of bacteria, viruses and fungi

Identification: microscopic examination; colony characteristics; Gram staining

Techniques: light and binocular microscopy, electronmicrographs; Gram staining; membrane filtration; streak plating

Assessment and grading criteria

In order to pass this unit, the evidence that the learner presents for assessment needs to demonstrate that they can meet all the learning outcomes for the unit. The assessment criteria for a pass grade describe the level of achievement required to pass this unit.

Assessment and grading criteria		
To achieve a pass grade the evidence must show that the learner is able to:	**To achieve a merit grade the evidence must show that, in addition to the pass criteria, the learner is able to:**	**To achieve a distinction grade the evidence must show that, in addition to the pass and merit criteria, the learner is able to:**
P1 use light microscopy techniques to identify the characteristic features and functions of prokaryotic and eukaryotic cells	**M1** describe the function of prokaryotic and eukaryotic cell components	**D1** relate the characteristic features of prokaryotic and eukaryotic cell components to their function
P2 use data from electron microscopy to identify the characteristic features and functions of akaryotes, prokaryotic and eukaryotic cells		
P3 carry out practical activities to cultivate micro-organisms using aseptic techniques [RL2,3]	**M2** explain the principles underlying the cultivation and aseptic techniques used	**D2** evaluate the growth conditions in terms of the cultivation techniques used compared with large scale industrial growth
P4 carry out practical investigations of factors that influence the safe growth of micro-organisms [CT1,3]	**M3** compare the calculated growth rates of micro-organisms grown under varying conditions	**D3** draw valid conclusions from the growth rate calculations suggesting how this knowledge may be applied in a biotechnological or biomedical context
P5 describe the main groups of micro-organisms by their principal taxonomic characteristics [SM2].	**M4** outline how the techniques used to identify micro-organisms relate to their structure.	**D4** outline the potential usefulness of a variety of identification techniques in a specific application.

PLTS: This summary references where applicable, in the square brackets, the elements of the personal, learning and thinking skills applicable in the pass criteria. It identifies opportunities for learners to demonstrate effective application of the referenced elements of the skills.

Key	IE – independent enquirers	RL – reflective learners	SM – self-managers
	CT – creative thinkers	TW – team workers	EP – effective participators

Essential guidance for tutors

Delivery

A strong emphasis on health and safety is essential from the start of this unit. Learners should practise all the basic processes involving aseptic techniques and inoculation of media. They should also observe the centre's technicians preparing media and safely disposing of materials and cultures since insurance limitations may prevent learners doing this themselves.

A visit to an industrial laboratory is strongly recommended. Where this is not possible, suitable CD ROMs/DVDs/internet sites may be used to convey the differences between industrial/service-based laboratories and centre-based laboratories. Such differences include clear demarcation of 'clean' and 'contaminated' areas, together with clean air operation to ensure effective bio-containment. Additionally, a visit to an actual manufacturing facility (eg brewery, antibiotic producer) to illustrate the difficulties associated with scaling up production from the laboratory would also be useful in demonstrating the vocational applications.

For learning outcome 1, learners should be able to prepare temporary slides, set up a microscope and use it to locate micro-organisms with reasonable resolution at low and high power. They should be able to use oil immersion and calibrate eyepiece graticules. Labelled biological drawings of their light microscope investigations could be used as evidence of bacterial morphology and simple anatomy of some moulds. Electron micrographs should be used to assess learners' ability to identify correctly cell ultrastructure. This can be achieved through filling in blank labels, creating a key either manually or electronically.

Learners should be aware of how samples are prepared for electron microscope examination in order that they can interpret images obtained from scanning electron microscopes (SEMs) and transmission electron microscopes (TEMs). The internet provides a large number of excellent websites with suitable images.

Learning outcome 2 requires an induction into the importance of using aseptic techniques and carrying out risk assessments prior to handling live specimens. Simulating aseptic techniques using fluorescent dye as a substitute for a live culture is a useful starting point.

Learners should be taught a wide range of techniques and skills for learning outcome 2 from which they could select appropriate procedures for learning outcome 3. These techniques should include:

- preparing culture media and pouring plates aseptically
- sterilisation and disinfection techniques
- aseptic inoculating methods on solid media
- aseptic inoculating methods in liquid media including serial dilution
- viral plaque counts (a phage kit is available from suppliers whereby simple experiments using T4B Bacteriophage with E.coli strain B may be used to demonstrate viral lysis on either solid or liquid media)
- haemocytometer counts.

Wherever possible, industrial applications should be considered; for example, a water authority suspecting sewage pollution could use serial dilution to make a realistic assessment of E.coli numbers.

For learning outcome 3, learners should be encouraged to consider a relevant vocational application as this will help them focus on their choice of micro-organism(s), growth factor and measurement technique. Learners may decide to focus on one particular micro-organism or select an example from all three main groups they have been investigating. Learners should also be introduced to a variety of growth factors they could investigate and techniques they could use to measure the growth. A planning aspect could be incorporated into this learning outcome with learners given help to plan their activities by tutors and the laboratory technicians usually involved in preparing microbiology practicals.

Learning outcome 4 can be partially demonstrated through practical techniques like Gram staining and growing and observing colony characteristics, and partially through research into microbial taxonomy. The research could be more structured by asking small groups of learners to investigate different taxonomic groups and related applications or setting learners specific questions to answer.

Outline learning plan

The outline learning plan has been included in this unit as guidance and can be used in conjunction with the programme of suggested assignments.

The outline learning plan demonstrates one way in planning the delivery and assessment of this unit.

Topic and suggested assignments/activities and/assessment
Introduction to unit content and programme outline. **Learning outcome 1** Theory input: prokaryotes and eukaryotes. Practical demonstration: General use of the light microscope and oil immersion. Preparation of temporary slides. Learner activity: Risk assess and carry out individual cell side preparations (eg bacteria, moulds, yeasts) and view under the microscope. Use electron micrographs to observe features of prokaryotes and eukaryotes. **Assignment 1 – (LO1) The Akaryotes, Prokaryotes and the Eukaryotes (P1, P2, M1, D1)** Learners to produce drawings and labels/annotations of visible features of prokaryotes and eukaryotes from light microscopes and akaryotes, prokaryotes and eukaryotes from electron micrographs.
Learning outcome 2 Theory input: Aseptic techniques. Practical demonstration: Simulation of aseptic techniques. Learner activity: Risk assess and practise simulation of aseptic techniques. Learner home study task: Read through booklet on safe handling of micro-organisms.
Theory input: Inoculation techniques – pour, streak, and lawn plates. Practical demonstration: Inoculation of agar plates by pour, streak and lawn methods. Learner activity: Risk assess and practise inoculation of agar plates by pour, streak and lawn methods. **Assignment 2 – (LO2) Cultivating Micro-organisms (P3, M2, D2) – Task 1** Carry out inoculation and incubation of agar plates by pour, streak and lawn methods and produce a report on own results. Group discussion: Evaluate the inoculated plates' results using the OHP projector/Motic camera/Viewcam.
Theory input: Inoculation techniques – liquid media using bacteria or fungi. Practical demonstration: Inoculation of liquid media using bacteria or fungi. Learner activity: Risk assess and practise inoculation of liquid media using bacteria or fungi. **Assignment 2 – (LO2) Cultivating Micro-organisms (P3, M2, D2) – Task 2** Carry out inoculation and incubation of liquid media using bacteria or fungi.
Theory input: Using haemocytometers to count yeast populations. Practical demonstration: Counting yeast populations with haemocytometers. **Assignment 2 – (LO2) Cultivating Micro-organisms (P3, M2, D2) Task 3** Carry out haemocytometer yeast count and report on results.

Topic and suggested assignments/activities and/assessment
Theory input: Viral plaques. Practical demonstration: Viral plaque assay using plaque agar overlays on solid or liquid media and colorimetry (Phage kits for these are available from suppliers). Learner activity: Risk assess and practise viral plaque assay using plaque agar overlays or liquid media and colorimetry. Carry out viral plaque assay using plaque agar overlays or liquid media and colorimetry and report on results.
Learning outcome 3 Theory input: Factors that influence growth and ways to measure growth. Biotechnological and biomedical applications. Visit to biotechnological/biomedical laboratory or manufacturing facility. Learner activity and home study task: Research an individual factor and how to measure its growth, then present this information to rest of group. Learner activity: Select different growth factors to investigate with each of the following – bacteria, fungi, and viruses. Research, plan and design the three investigations. **Assignment 3 – (LO3) Factors Influencing Growth of Micro-organisms (P4, M3, D3)** Carry out and report on findings of investigations.
Learning outcome 4 Theory input: Characteristics and identification of bacteria, fungi and viruses. Practical demonstration: Gram staining. Learner activity: Practise Gram staining of a mixed bacterial culture and view under oil immersion. Learner activity: using freshly grown plates of bacteria and fungi, use binocular microscopes to observe and record colony characteristics of bacteria and structural features of moulds. Learner activity: Observe and record information from electron micrographs of microbes, particularly viruses. Learner home study task: Carry out additional research on the characteristics of micro-organisms. **Assignment 4 – (LO4) Classifying Micro-organisms (P5, M4, D4)** Produce a pamphlet to demonstrate key characteristics of bacteria, fungi and viruses.
Workshop sessions to complete/revise evidence for unit and reflect on learner achievement.

Assessment

The highly practical nature of this unit requires that a significant part of this assessment involves records of learners' practical activities. There should be evidence of tutor observation of the key microbiological techniques used. This could take the form of a witness testimony; checklist and commentary sheet; comments on a general feedback sheet; or indeed, annotations on learners' own records of their activities.

All the pass grade criteria must be met in order for learners to achieve this unit.

To achieve P1 and P2, learners must identify the key characteristic features and functions of bacteria, viruses and microscopic fungi, eg yeasts and moulds, using light microscope techniques and evidence from electron micrographs. The evidence could be in the form of informative posters. For P3 learners must also carry out safely, and report on, the cultivation of micro-organisms. Assessment can be by direct tutor observation, written report or presentation for the practical tasks; similarly, the report may be a written document or verbal presentation. To achieve P4, learners have to investigate safely and report on growth factors affecting micro-organisms. They may decide to focus on one particular micro-organism or investigate more than one. By setting the investigation in a biomedical or biotechnological context learners will give themselves

the opportunity to apply their findings to D5. For example, learners could investigate the effect of antibiotics compared with natural remedies, such as garlic or tea tree oil, on the growth of bacteria. An investigation into alcohol production during fermentation by various species of yeasts could also provide the basis for a worthwhile investigation. To demonstrate their ability to describe the main groups of micro-organisms for P5, learners could produce a pamphlet that would highlight the essential features.

For a merit grade, all the pass grade criteria and all the merit grade criteria must be met. For M1 learners must describe the specialist cell functions of the prokaryotes and eukaryotes and functions of akaryotes. This may be done through drawings/diagrams with annotations; in written reports or via a combination of both. When carrying out practical activities, involving cultivation and growth conditions, learners should demonstrate a degree of independence and competence. For M2 they must explain the principles underlying the microbiological techniques used both in terms of relevant aseptic techniques and method of cultivation. This could include why and how aseptic techniques prevent cross contamination and how streak plating can provide a way of separating bacteria in a mixed culture. Learners must include their calculations in the report and comment on the optimum conditions for growth to achieve M3. Techniques used to identify micro-organisms must be related to their structure for M4. For example, they could relate Gram staining to cell wall structure.

For a distinction grade, all the pass, merit and distinction grade criteria must be met. Distinction-grade learners have to produce additional evidence and demonstrate a greater degree of autonomy in their work. For D1 learners must indicate how internal structures link together for cell operation. They could consider how the nucleus, rough endoplasmic recticulum, Golgi apparatus and vesicles are involved in the production and secretion of an antibody. A written report or a verbal presentation could be applied. The benefits of electron microscopy as an aid to micro-organism identification could be evidenced in a similar way.

Learners should be able to reflect critically on their skill acquisition and application when aseptically culturing micro-organisms in a safe manner for D2. This review could also involve a written or verbal presentation. A consideration of industrial and commercial applications for growth conditions is required. Learners could present their findings by comparing and contrasting small scale laboratory manufacture and large scale production via a presentation to the directors/managers of their company. They are also required to justify why specific measurement techniques were chosen for particular micro-organisms. For example, the usefulness of measuring the diameter or area of a mould which grows through the development of a mycelium; or the value of serial dilution and colony counts with bacteria. When drawing valid conclusions from their calculations of growth rate for D3, learners must also comment on how such data may be applied in an industrial context. For example, the recommendation of particular antibiotics as narrow or broad spectrum; the use of various sugar substrates when making alcoholic beverages. Finally, learners must provide an outline of the considered usefulness of the techniques used to identify micro-organisms for a specific application in order to achieve D4. They could consider to what taxonomic level an organism would require identifying, or how quickly results are produced and the significance of this timing in an industrial or medical situation.

Programme of suggested assignments

The table below shows a programme of suggested assignments that cover the pass, merit and distinction criteria in the assessment and grading grid. This is for guidance and it is recommended that centres either write their own assignments or adapt any Edexcel assignments to meet local needs and resources.

Criteria covered	Assignment title	Scenario	Assessment method
P1, P2, M1, D1	The Prokaryotes and the Eukaryotes	Microbiologists preparing images for an open day promoting careers in microbiology.	Biological drawings and electron micrographs.
P3, M2, D2	Cultivating Micro-organisms	Scientists working in a biotechnological or biomedical laboratory.	Carrying out investigation and reporting on the cultivation of micro-organisms
P4, M3, D3	Factors Influencing Growth of Micro-organisms	A pilot scale laboratory operation within a biotechnological or biomedical company or service.	Carrying out and reporting on the factors influencing growth of micro-organisms
P5, M4, D4	Classifying Micro-organisms	An information leaflet produced by a biotechnological or biomedical company/ service for newly appointed science technicians.	A pamphlet identifying the main groups of micro-organisms.

Links to National Occupational Standards, other BTEC units, other BTEC qualifications and other relevant units and qualifications

This unit forms part of the *BTEC Applied Science sector* suite. This unit has particular links with the units shown below in the BTEC Applied Science suite of qualifications:

Level 1	Level 2	Level 3
Defeating Disease (FLT)	Biology and Our Environment	Genetics and Genetic Engineering
	Biotechnology Procedures and Applications	Diseases and Infections

Essential resources

Learners should have access to a range of microbiological resources, similar to those used for 'A' level biology courses. Learners also require access to a science laboratory suitable for growing, isolating and identifying micro-organisms safely. The use of laminar flow cabinets is desirable but not essential. Learners also need pre-irradiated plastic Petri dishes; pipettes and syringes; autoclave; incubator, inoculating loops and spreaders; suitable cultures and media and culture bottles. A colorimeter would be useful to assess viral lysis activity. Standard laboratory microscopes that allow for the use of oil immersion will also be needed. For research purposes and the professional production of evidence, access to suitable books, periodicals, CD ROMs, internet and suitable software packages is desirable.

Employer engagement and vocational contexts

Visits to and from industrial and service industries involved in biotechnological and biomedical processes would be useful in delivering this unit. Sectors of particular interest could be food and drink manufacturers, such as yogurt makers and brewers; and pharmaceutical manufacturers of antibiotics or enzymes.

Indicative reading for learners

Textbooks

Alexander S, Strete D – *Microbiology: A Photographic Atlas for the Laboratory* (Pearson, 2000) ISBN 9780805327328

Deacon J W – *Fungal Biology, Fourth Edition* (Wiley Blackwell, 2005) ISBN 9781405130660

Lammert J – *Techniques for Microbiology: A Student Handbook* (Pearson, 2006) ISBN 97801322401 16

Madigan M, Martinko J, Dunlap P, Clark D and Brock T – *Biology of Microorganisms 12th Edition* (Pearson, 2008) ISBN 9780321536150

Taylor J – *Bath Advanced Science – Micro-organisms and Biotechnology, Second Edition* (Hodder Education, 2001) ISBN 9780174482550

Waites M, Morgan N, Rockey J and Higton G – *Industrial Microbiology – An Introduction* (Wiley Blackwell, 2001) ISBN 9780632053070

Journals

Biological Sciences Review

Microbiology Today

Websites

www.bbsrc.ac.uk/	Biotechnology and Biological Sciences Research Council
www.britmycolsoc.org.uk	British Mycological Society – useful educational resources
www.microbeworld.org	Microbe World
www.microbiologyonline.org.uk	Society for General Microbiology and Microbiology in Schools Advisory Committee
www.saps.plantsci.cam.ac.uk	SAPS Science and Plants for Schools
www.sgm.ac.uk	Society for General Microbiology
www.virology.net	Contains a catalogue of virus pictures and links to other virology websites

Delivery of personal, learning and thinking skills

The table below identifies the opportunities for personal, learning and thinking skills (PLTS) that have been included within the pass assessment criteria of this unit.

Skill	When learners are ...
Creative thinkers	[CT1,3] carrying out and reporting on practical investigations of factors influencing the growth of micro-organisms.
Reflective learners	[RL2,3] carrying out and reporting on practical activities to cultivate micro-organisms
Self-managers	[SM2] describing the main groups of micro-organisms by their principal taxonomic characteristics

Although PLTS are identified within this unit as an inherent part of the assessment criteria, there are further opportunities to develop a range of PLTS through various approaches to teaching and learning.

Skill	When learners are ...
Independent enquirers	[IE2,6] planning and researching factors that influence growth and ways of measuring that growth and appreciating the consequences of the decisions they reach
Creative thinkers	[CT2] visiting a biotechnological or biomedical laboratory or manufacturing facility and asking questions to extend their thinking
Reflective learners	[RL3] simulating and practising aseptic technique processes by reviewing progress and acting on the outcomes
Team workers	[TW6] evaluating the outcomes of the group's various inoculation techniques by providing constructive support and feedback
Self-managers	[SM5] dealing with competing pressures – personal and unit related

Functional Skills – Level 2

Skill	When learners are ...
ICT – Use ICT systems	
Select, interact with and use ICT systems independently for a complex task to meet a variety of needs	selecting and using information from practical investigations to generate reports
Manage information storage to enable efficient retrieval	managing and storing data from carrying out practical investigations of factors influencing the growth of micro-organisms
ICT – Find and select information	
Select and use a variety of sources of information independently for a complex task	selecting and using information to research the key characteristics of the main groups of micro-organisms
ICT – Develop, present and communicate information	
Enter, develop and format information independently to suit its meaning and purpose including: • text and tables • images • numbers • records	obtaining data and images from and for: • microscopy • reports on practical activities to cultivate micro-organisms • practical investigations involving factors influencing the growth of micro-organisms • pamphlet on the main groups of micro-organisms
Bring together information to suit content and purpose	bringing the information together for: • reports on practical activities to cultivate micro-organisms • practical investigations involving factors influencing the growth of micro-organisms • pamphlet on the main groups of micro-organisms
Present information in ways that are fit for purpose and audience	producing drawings, labelled electron micrographs scientific reports and pamphlets
Mathematics	
Identify the situation or problem and the mathematical methods needed to tackle it	analysing data from practical investigations involving factors influencing the growth of micro-organisms
Draw conclusions and provide mathematical justifications	drawing conclusions based on data analysis from practical investigations involving factors influencing the growth of micro-organisms
English	
Reading – compare, select, read and understand texts and use them to gather information, ideas, arguments and opinions	researching the key characteristics of the main groups of micro-organisms
Writing – write documents, including extended writing pieces, communicating information, ideas and opinions, effectively and persuasively	conducting scientific reports on cultivating micro-organisms and practical investigations involving factors influencing the growth of micro-organisms producing a pamphlet on the main groups of micro-organisms.