### **Questions**

#### Q1.

Bacteria were cultured in two different types of medium.

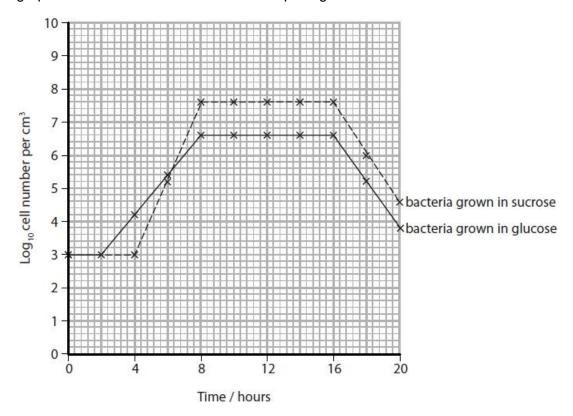
One medium contained glucose as the energy source and the other contained sucrose.

The same molar concentrations of glucose and sucrose were used.

Bacteria were grown in each medium for 20 hours.

Every two hours, the numbers of bacteria were determined using dilution plating and an optical method (turbidity).

The graph shows the results from the dilution plating.



(i) Calculate how many times faster the bacteria in sucrose reproduce than the bacteria in glucose, during the log phase.

Angwar

(2)

(ii) Analyse the data to explain why the growth curve for sucrose is different from the growth curve for glucose.
(2
(iii) On the graph, draw a curve to show the number of bacteria grown in glucose as determined by the optical method.
(1
(Total for question = 5 marks

Antibiotics are widely used to treat infections.

Q2.

Some antibiotics are bactericidal.	
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Describe how you would use aseptic techniques to transfer bacterial cells growing on an

agar plate to a tube containing a sterile broth.

(5)

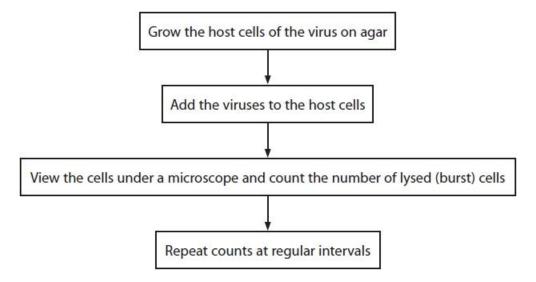
(Total for question = 5 marks)

### Q3.

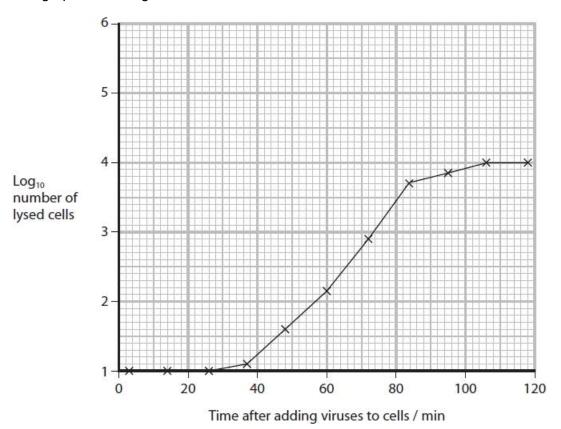
Bacteria are the host cells for  $\lambda$  (lambda) phage viruses.

Viruses can be cultured and a growth curve can be produced.

The flow chart shows how this can be done.



The graph shows a growth curve for viruses.



(i) Explain why there was a delay b	pefore the number of lysed cells started to increase.
	(3)
(ii) Calculate the mean rate of incrementation (iii)	ease in the actual number of lysed cells between 50
	(2)
	Answer cells min -1
(iii) A sketch has been made of this	s growth curve.
Log₁₀ number of lysed cells	

Time after adding viruses to cells

Complete this sketch to predict the shape of the growth curve after 120 minutes, assuming there is an excess of host cells.

(2)

(Total for question = 7 marks)

Q4.

Skin flora include bacteria that live on the skin.

Different parts of the body have different types of bacteria present.

Skin flora are beneficial as they inhibit the growth of pathogenic bacteria on the skin.

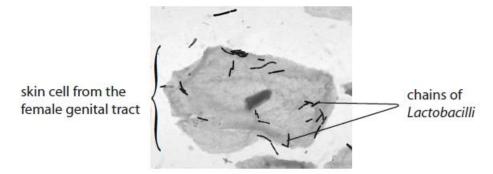
Skin flora compete for nutrients and space or secrete toxic chemicals.

The following are types of bacteria found in the female genital tract:

- Escherichia coli
- Klebsiella species
- Lactobacillus species
- Staphylococcus species.

(i) Describe how these types of bacteria present in the female genital tract could be identified.	
	4

(ii) The photograph shows Lactobacilli on a skin cell from the female genital tract.



Source: © Janice Carr Content Providers (s): CDC/Dr. Mike Miller

Lactobacilli metabolise glycogen into lactic acid. Explain how Lactobacilli inhibit the growth of pathogenic bacteria in the female genital tract.	
 	(3)
	•

(Total for question = 7 marks)

#### Q5.

An investigation was carried out to compare the aerobic respiration of different sugars by yeast.

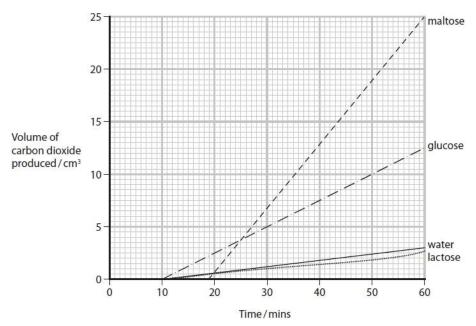
A suspension of yeast was used to produce three cultures, each one with a different sugar.

A control was set up that had a suspension of yeast cultured with water only.

The volume of carbon dioxide produced was measured.

The mass of yeast and the concentration of each sugar were controlled.

The graph shows the results of this investigation.



(i) Give one difference between the structure of glucose and the structure of maltose.	(1)
(ii) Explain the results for the yeast cultured with glucose and the yeast cultured with maltose.	
	(2) 

(Total for question = 3 marks)

Q6.

An investigation was carried out to compare the aerobic respiration of different sugars by yeast.

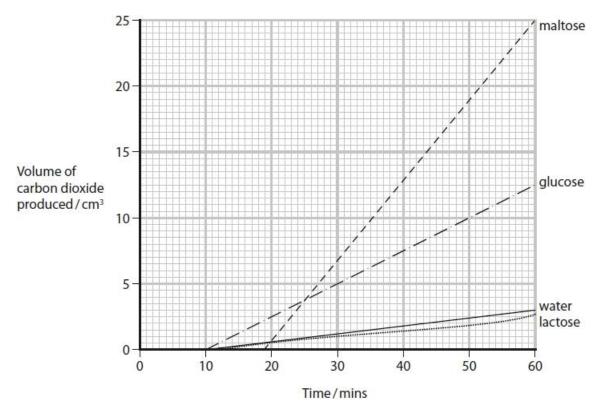
A suspension of yeast was used to produce three cultures, each one with a different sugar.

A control was set up that had a suspension of yeast cultured with water only.

The volume of carbon dioxide produced was measured.

The mass of yeast and the concentration of each sugar were controlled.

The graph shows the results of this investigation.



Explain the results for the yeast cultured with lactose.

	(Total for question = 2 ma	rks)
 	 	(2)

Q7.

Antibiotics can affect bacteria.

The table describes the mode of action of four antibiotics.

Antibiotic	Mode of action
benzylpenic <mark>i</mark> llin	disrupts peptidoglycan structure
streptomycin	binds to 70 S ribosomes
ciprofloxacin	inhibits enzymes involved in prokaryotic DNA replication
rifamycin	inhibits prokaryotic RNA polymerase

A student compared the effectiveness of these four antibiotics on the growth of bacteria. Devise a method that the student could use to obtain valid results. (5)

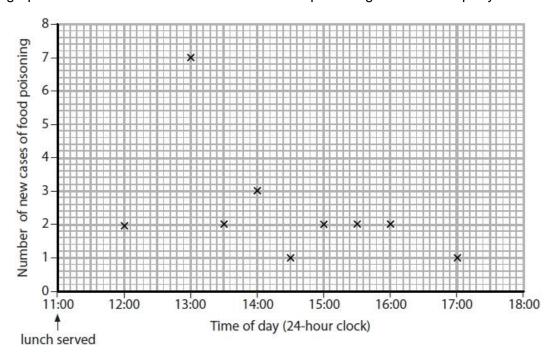
(Total for question = 5 marks)

#### Q8.

Food poisoning can be caused by food that is contaminated with pathogenic microorganisms.

A number of different bacteria can cause food poisoning.

The graph shows the number of new cases of food poisoning after a lunch party.



(i) Explain why it is likely that this food poisoning resulted from food contaminated with *Staphylococcus* rather than with *Salmonella*.

(3)

(ii) Describe the techniques microbiologists could use to confirm that this food poisoning was caused by <i>Staphylococcus</i> .	
	(4)
(Total for question = 7 mark	s)

Q9.

Salmonella are Gram negative bacteria found in the large intestine of humans
--

A scientist studied the growth of Salmonella.

i) Salmonella was isolated from a mixed culture of bacteria, using streak plating onto selective media.
Explain why this is a suitable method for isolating the Salmonella.
(4)
ii) The esigntist made a broth gulture of Salmonalla at a concentration of 5 × 10 <sup>3</sup> calls nor
(ii) The scientist made a broth culture of <i>Salmonella</i> at a concentration of $5 \times 10^3$ cells per cm <sup>3</sup> .
Ten hours later the concentration of Salmonella was $4 \times 10^6$ per cm <sup>3</sup> .
Calculate the exponential growth rate constant (k) for this culture of Salmonella using the formula
(3)
$k = \frac{\log_{10} N_{t} - \log_{10} N_{0}}{0.301 \times t}$
0.301×t

Answer .....

(iii) In this calculation, the scientist did not allow for the time that the <i>Salmonella</i> spent in the lag phase.
Explain the effect that this will have on the calculated value for the growth rate constant. (3)
(Tatal for supportion 140 secules)
(Total for question = 10 marks)

Q10.

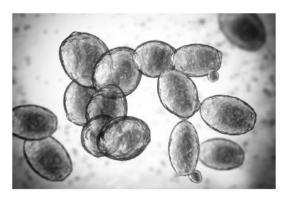
Aseptic conditions are used when growing bacteria in laboratories.	
lustify two methods, other than UV radiation, for ensuring aseptic conditions.	
	(2)
	(-)
(Total for question = 2 mark	ks)

### Q11.

bacteria.

Yeast, Saccharomyces cerevisiae, is a single-celled organism used in brewing and baking.

The photograph shows yeast cells seen using a light microscope.



(Source: © Kateryna Kon/Shutterstock)

A student counted the number of yeast cells, at 90-minute intervals, growing in a liquid culture at 30°C.

Describe two precautions that would prevent the yeast culture becoming contaminated with

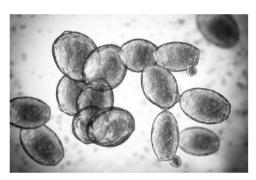
(2)

(Total for question = 2 marks)

#### Q12.

Yeast, Saccharomyces cerevisiae, is a single-celled organism used in brewing and baking.

The photograph shows yeast cells seen using a light microscope.



(Source: © Kateryna Kon/Shutterstock)

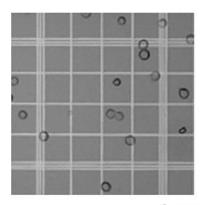
A student counted the number of yeast cells, at 90-minute intervals, growing in a liquid culture at 30°C.

The student counted the yeast cells using a haemocytometer.

This is a slide with chambers containing a known volume of liquid. The yeast cells are counted when viewed using a light microscope.

Any yeast cells totally within the counting square should be counted, along with those which overlap the top or left-hand side of the square.

(i) The volume of liquid in the counting square shown is 0.004 mm<sup>3</sup>.



(Source: © Joseph Elsbernd/Flickr)

Calculate the number of yeast cells per mm<sup>3</sup>. Give your answer in standard form.

(2)

_		,
Answer	 ner	mm
AIISWCI		

(Total for o	question = 4 marks)
	(2)
(ii) Identify two causes of inaccuracy in this method of counting cells.	(2)

### Q13.

Yeast, Saccharomyces cerevisiae, is a single-celled organism used in brewing and baking.

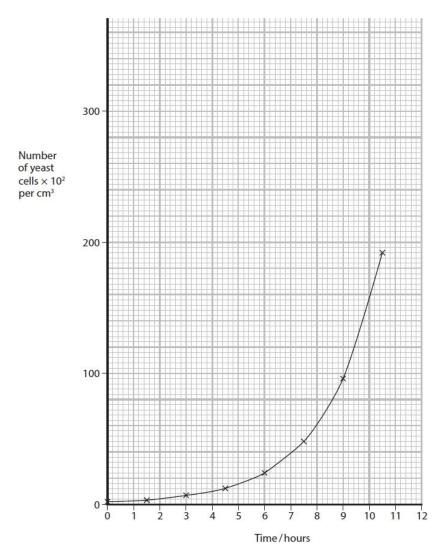
The photograph shows yeast cells seen using a light microscope.



(Source: © Kateryna Kon/Shutterstock)

A student counted the number of yeast cells, at 90-minute intervals, growing in a liquid culture at 30°C.

The graph shows the results.



(1)	Calculate the percentage increase in the number of yeast cells from 2 hours to 6 hours.	
		2)
	Answer	%
(ii)	Predict the number of cells per cm <sup>3</sup> of the culture at 12 hours.	
	Assume that the culture continues to grow at the same rate.	
		1)
		")
	Answer × 10 <sup>2</sup> per cr	$n^3$
		•
	(Total for question = 3 mark	s)
	·	-

#### Q14.

Yeast, Saccharomyces cerevisiae, is a single-celled organism used in brewing and baking.

The photograph shows yeast cells seen using a light microscope.



(Source: © Kateryna Kon/Shutterstock)

A student counted the number of yeast cells, at 90-minute intervals, growing in a liquid culture at 30°C.

The student counted the yeast cells using a haemocytometer.

The table shows the number of cells counted using a haemocytometer.

Time	Number of cells						
/hours	1	2	3	4	5	Mean	Standard deviation (SD)
0.0	1	2	2	1	2	1.6	0.5
1.5	2	3	3	3	2	2.6	0.5
3.0	4	9	9	3	7	6.4	
4.5	13	10	12	9	16	12.0	2.7
6.0	43	31	18	14	15	24.2	12.5

(i) The student calculated the standard deviations using the following formula.

$$SD = \sqrt{\frac{\sum (x - \overline{x})^2}{n - 1}}$$

Calculate the standard deviation for the sample at 3 hours.

(Total for question = 6 marks)

# Edexcel (B) Biology A-level - Microbial Techniques

II)	The student concluded that the yeast population doubles every 90 minutes.	
	Comment on the validity of this conclusion.	
		(3)
		•
• • •		•
• • •		•

# Mark Scheme

Q1.

Question Number	Answer Additional Guidance		Mark
(i)	<ul> <li>values read from graph per unit time and converted back from logs</li> <li>subtraction of each pair and division done</li> </ul>	(7.6=) 39 810 717 and (6.6=) 3 981 072 and (3.0=) 1000 or (4.2=) 15849 10 / 10.04	
		ACCEPT 1.9 / 1.92 / 2 / 82.5 / 82.54 / 83 for 1 mark	
Question Number	Answer	Additional Guidance	Mark
(ii)	An explanation that makes reference to the following     (lag phase longer as) {sucrose has to be brokdown / enzymes to breakdown sucrose had to be synthesised} (1)     (reproduction faster) because sucrose has measured {sugar / monosaccharides / energy (1)	ACCEPT sucrose made of glucose and fructose / sucrose is a disaccharide and glucose is a monosaccharide	
Question Number	Answer	Additional Guidance	Mark
(iii)	a line that continues to go up after 8 hours and then levels off (around) 16 hours without dropping	Namerica grows in success  Starteria grows in success  Starteria grows in success  Starteria grows in success	

## Q2.

Question Number	Answer	Additional Guidance	Mark
	A description that makes reference to five of the following:		
	disinfect surfaces / use benchcoat (1)		
	work near Bunsen flame / work in a sterile cabinet (1)		
	flame top of tube (1)		
	heat wire loop (1)		
	open lid of Petri dish to a small extent (1)		
	allow wire loop to cool (1)		(5)

## Q3.

Question Number	Answer	Additional Guidance	Mark
(i)	An explanation that makes reference to three of the following:  • virus to attach to host cells / {genetic material / DNA} to enter host cells (1)  • synthesis of DNA (1)  • {protein synthesis / transcription and translation} (1)  • assembly of new viruses (1)	ACCEPT virus {enters / infects} DO NOT ACCEPT RNA  IGNORE nucleic acid DO NOT ACCEPT RNA unless already penalised in mp 1 / in the context of {proviruses / latency}  ACCEPT capsid / capsomeres / tail / base plate / tail (fibres) / collar / J protein / enzymes / protease / permease / other named proteins will need to be checked DO NOT ACCEPT reverse transcriptase / integrase	(3) EXP
		NB ACCEPT (time for) {replication / multiplication} of the virus in correct context if no other marks awarded.	

Question Number	Answer	Additional Guidance	Mark
(ii)	<ul> <li>correct values read from graph and divided by the time (1)</li> <li>mean rate calculated (1)</li> </ul>	3.8 and 1.7 and a division by 40 / 0.0525 / 3.147 IGNORE ref to logs  156  ecf wrong time value but correct answer to whole number e.g. (30 mins) 209  Correct answer only = 2 marks	(2) EXP

Question Number	Answer	Additional Guidance	Mark
(iii)	<ul> <li>line going up and (generally) levelling off (1)</li> <li>increase greater than the first increase (1)</li> </ul>	IGNORE a third increase  Log10 number of lysed cells  Time after adding viruses to cells	(2) EXP

### Q4.

Question Number	Answer	Additional Guidance	Mark
(i)	A description that makes reference to four of the following:		
	sample taken from the female genital tract (	1)	
	bacteria grown on {selective / indicator} media (	1)	
	bacteria grown on media containing different type	os	
	of antibiotics (	1)	
	use of {antibodies / DNA profiling}	(1)	
	using {Gram staining / cell shape / colony shape /	200	
	colony colour}	(1)	(4)

Question Number	Answer	Additional Guidance	Mark
(ii)	An explanation that makes reference to three of the following:	j.	
	because they grow on the skin cells preventing pathogenic bacteria from doing so     (1)		
	<ul> <li>because they use the glycogen so less {glucose / energy / glycogen} available for the pathogenic bacteria (1)</li> </ul>		
	because they produce lactic acid which inhibits the growth of pathogenic bacteria     (1)		
	<ul> <li>because the low pH denatures the enzymes of the pathogenic bacteria (1)</li> </ul>	ACCEPT below optimum pH	(3)

### Q5.

Question Number	Answer	Additional Guidance	Mark
(i)	glucose is a monosaccharide and maltose is {a disaccharide /made of two glucoses / made of two monosaccharides} (1)	ACCEPT maltose has a glycosidic bond and glucosedoes not glucose has 6 carbons and maltose has 12 glucose has the formula C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> and maltose C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> ACCEPT a labelled diagram	(1)

Question Number	Answer	Additional Guidance	Mark
Number (ii)	An explanation that makes reference to two of the following:  • neither sugars respired immediately because the yeast need to {acclimatise / adjust to the conditions}  (1)	ACCEPT glycosidic bonds have to be broken	
	<ul> <li>delay is longer for maltose as {enzymes need to be synthesised / maltose needs to be broken down into glucose} (1)</li> </ul>	ACCEPT because maltose contains more energy / maltose is a disaccharide and glucose is a monosaccharide	(2)
	<ul> <li>respiration of maltose is faster (than glucose) as maltose has {twice the number of sugar units / more glucoses / more hydrogens} (1)</li> </ul>		

## Q6.

Question Number	Answer	Additional Guidance	Mark
	An explanation that makes reference to the following:  • lactose is not {respired / used} as the yeast do not produce {theappropriate enzymes / lactase} (1)	ACCEPT yeast cannot break lactose down	(2)
	<ul> <li>sugars in the cytoplasm are {used / respired} (1)</li> </ul>	NB at least one reference to respiration for bothmarks to be awarded	

### Q7.

Question Number	Answer	Additional Guidance	Mark
	An answer that makes reference to five of the following points:		
	<ul> <li>same species of bacteria</li> <li>(1)</li> </ul>		
	<ul> <li>same {concentration / volume} of antibiotic</li> <li>(1)</li> </ul>		
	<ul> <li>use {agar / broth / culture medium} (1)</li> </ul>	DO NOT ACCEPT less than 24 hours	
	<ul> <li>incubate between 20 °C and 25 °C for stated time (1)</li> </ul>	<b>e.g.</b> use of Bunsen burner / flame loop / swab bench	
	<ul><li>measure {zone of inhibition / turbidity}</li><li>(1)</li></ul>	swab bench	
	<ul> <li>use of described aseptic technique (1)</li> </ul>		(5)

## Q8.

Question Number	Answer	Additional Guidance	Mark
(i)	An explanation that makes reference to three of the following:	000	
	<ul> <li>because the food poisoning was happening {after one hour /within a few hours} of the meal</li> <li>(1)</li> </ul>	Accept soon after meal / quickly / by {12 / 1} o clock	
	<ul> <li>indicating that exotoxins caused the food poisoning</li> <li>(1)</li> </ul>		
	<ul> <li>because endotoxins are only present after {about 12 hours / several hours / when the bacterial cells are estroyed}</li> <li>(1)</li> </ul>		
	Staphylococcus releases exotoxins / endotoxins come from Salmonella (1)	Do not accept Staphylococcus is an exotoxin / Salmonella is an endotoxin	
			(3)

Question Number	Answer	Additional Guidance	Mark
(ii)	A description that makes reference to any four of the following:		
	<ul> <li>isolate the bacteria from the {food / patient / faeces / vomit}</li> <li>(1)</li> </ul>	Accept a description of how this is done e.g. streak plating from a faecal sample	
	<ul> <li>look at the colonies to see if they have a {characteristic / named characteristic} (of Staphylococcus)</li> <li>(1)</li> </ul>	,	
	use Gram stain to show presence of Gram positive bacteria     (1)	Accept Staphylococci will appear purple with Gram stain	
	<ul> <li>grow on selective media that identify {Staphylococcus / eliminates other bacteria}</li> <li>(1)</li> </ul>	Accept differential media / selective agar / named example e.g. mannitol salt agar / with antibiotics	
	use antibodies against Staphylococcus (1)		(4)

## Q9.

Question Number	Answer	Additional Guidance	Mark
(i)	An explanation that makes reference to four of the following:  • medium allows only {Salmonella / certain bacteria} to grow  (1)  • {culture / colonies / bacteria} is spread out on the {agar / medium} (1)  • because this separates out individual bacteria (1)  • so that colonies are {discrete / separate / individual} (1)  • so only one type of {bacteria /	ACCEPT in context of {named media / antibiotic-containing media}	
	colony} can be picked up (1)		(4)

Question Number	Answer	Additional Guidance	Mark
(ii)	<ul> <li>log of the values taken</li> <li>(1)</li> </ul>	$\log 5 \times 10^3 = 3.6989700043$ and $\log 4 \times 10^6 = 6.6020599913$	
	<ul> <li>substitution of values into equation (and evaluating correctly)</li> <li>(1)</li> </ul>	(6.6020599913 - 3.6989700043) ÷ (0.301 × 10) = 0.9644817233 <b>ACCEPT</b> correct value for rounded up values substituted 0.964 / 0.96	
	<ul> <li>k given to an appropriate number of decimal places         <ul> <li>(1)</li> </ul> </li> </ul>	CE applies from mp 1 only	(3)

Question Number	Answer	Additional Guidance	Mark
(iii)	An explanation that makes reference to the following:  • the value for t used in the calculated value is greater than the actual value (1)  • therefore the calculated value for k will be smaller (1)  • because the Salmonella will not be replicating (in lag phase)	ACCEPT converse for actual value  ACCEPT converse for actual value  ACCEPT dividing / growing	
	(1)		(3)

### Q10.

Question Number	Answer	Additional Guidance	Mark
	An answer that makes reference to two of the following:		
	{flame / sterilise / autoclave} apparatus to kill bacteria (1)	Accept microbes / spores for all	
	<ul> <li>clean surfaces with {bleach / disinfectant / alcohol} to kill bacteria (1)</li> </ul>	points	
	<ul> <li>open lid of Petri dish slightly to prevent access by bacteria (1)</li> </ul>		
	<ul> <li>work near Bunsen flame to {create updraft / prevent bacteria settling} (1)</li> </ul>		(2)

### Q11.

Question Number	Answer	Additional Guidance	Mark
	A description that includes the following:		
	Two relevant techniques to prevent bacterial growth described (2)	eg use of sterile / autoclaved equipment flaming neck of bottle / transfer loops working next to (lit) Bunsen burner disinfection of bench use of selective medium / antibiotics in medium	Exp
		Ignore partial removal of lid	(2)

### Q12.

Question Number	Answer	Additional Guidance	Mark
(i)		Example of calculation	
	Correct calculation of	7 divided by 0.004 = 1750 per mm <sup>3</sup>	
	number of cells (1)	8 2000 per mm <sup>3</sup>	
	Conversion to standard form (1)	1.75 / 1.8 / 2 x 10 <sup>3</sup>	
	Final and the design of the control	Correct answer with no working gets 2 marks	Grad

Question Number	Answer	Additional Guidance	Mark
(ii)	An answer that includes two of the following:  • {yeast cells may not be evenly distributed / sample may not be representative} (1)	Accept clumping / overlapping of cells	
	yeast cells may be {reproducing / budding / undergoing cell division} (so hard to know whether to count one or two) (1)	Accept this method counts dead cells (as well as live)	
	difficult to decide if they are touching the sides of the counting square (1)	Ignore human error / miscounting unqualified	
		Ignore ref to air bubbles / artefacts / cells moving	(2)

## Q13.

Question Number	Answer	Additional Guidance		Mark
(i)		Example of calculation		
	• calculation of difference (1)	2400 - 400 = 2000	24 - 4 = 20	
	calculation of % increase (1)	2000 x 100 = 500 (%) 400	20 x 100 = 500 4	
		Correct answer with no working gets 2 marks		Grad (2)

Question Number	Answer	Additional Guidance	Mark
(ii)	correct answer (1)	384 (x 10 <sup>2</sup> per cm <sup>3</sup> ) Accept 360 to 400 (x 10 <sup>2</sup> per cm <sup>3</sup> )	
			Grad (1)

### Q14.

Question Number	Answer	Additional Guidance	Mark
(i)	Example of calculation		
	• calculation of $\Sigma (x - \bar{x})^2$ (1)	31.2	
	• divided by {4 / n-1} (1)	7.8	
	• calculation of SD (1)	2.8	
		2.498 / 2.5 gets mp1 if some working shown (31.2 divided by 5) Correct answer with no working gains 3 marks	Exp (3)

Question Number	Answer	Additional Guidance	Mark
(ii)	An answer including the following:  • SD for times 0, 1.5, 3 and 4.5 hours are non-		
	overlapping, so this conclusion is likely to be valid (1)		
	<ul> <li>SD for 6 hours overlaps with SD for 4.5 hours, so this conclusion is not likely to be valid (1)</li> </ul>	Accept SD for 6 hours overlaps with SD for 4.5 hours with comment on difference / significance	MANA.
	description of possible error (1)	Accept eg dilution error, measurement error,	
	value of 43 may be an anomalous result (so this conclusion may not be valid) (1)	counting error	Exp (3)