RECOGNISING ACHIEVEMENT

2805/04 Microbiology and Biotechnology

January 2005

Mark Scheme

ADVICE TO EXAMINERS ON THE ANNOTATION OF SCRIPTS

1. Please ensure that you use the **final** version of the Mark Scheme. You are advised to destroy all draft versions.

2. Please mark all post-standardisation scripts in red ink. A tick (✓) should be used for each answer judged worthy of a mark. Ticks should be placed as close as possible to the point in the answer where the mark has been awarded. The number of ticks should be the same as the number of marks awarded. If two (or more) responses are required for one mark, use only one tick. Half marks (½) should never be used.

3. The following annotations may be used when marking. No comments should be written on scripts unless they relate directly to the mark scheme. Remember that scripts may be returned to Centres.

   x = incorrect response (errors may also be underlined)
   ^ = omission mark
   bod = benefit of the doubt (where professional judgement has been used)
   ecf = error carried forward (in consequential marking)
   con = contradiction (in cases where candidates contradict themselves in the same response)
   sf = error in the number of significant figures

4. The marks awarded for each part question should be indicated in the margin provided on the right hand side of the page. The mark total for each question should be ringed at the end of the question, on the right hand side. These totals should be added up to give the final total on the front of the paper.

5. In cases where candidates are required to give a specific number of answers, (e.g. 'give three reasons'), mark the first answer(s) given up to the total number required. Strike through the remainder. In specific cases where this rule cannot be applied, the exact procedure to be used is given in the mark scheme.

6. Correct answers to calculations should gain full credit even if no working is shown, unless otherwise indicated in the mark scheme. (An instruction on the paper to 'Show your working' is to help candidates, who may then gain partial credit even if their final answer is not correct.)

7. Strike through all blank spaces and/or pages in order to give a clear indication that the whole of the script has been considered.

8. An element of professional judgement is required in the marking of any written paper, and candidates may not use the exact words that appear in the mark scheme. If the science is correct and answers the question, then the mark(s) should normally be credited. If you are in doubt about the validity of any answer, contact your Team Leader/Principal Examiner for guidance.

Abbreviations, annotations and conventions used in the Mark Scheme	/	=	alternative and acceptable answers for the same marking point
	;	=	separates marking points
	NOT	=	answers which are not worthy of credit
	R	=	reject
	( )	=	words which are not essential to gain credit
	___	=	(underlining) key words which **must** be used to gain credit
	ecf	=	error carried forward
	AW	=	alternative wording
	A	=	accept
	ora	=	or reverse argument

Question			Expected Answers	Marks
1	(a)	(i)	**A -** partially, permeable membrane **;** **A** selectively, semi, differentially **B -** biological recognition layer **;** **C -** transducer **;**	**3**
		(ii)	(only allows) glucose through membrane **;** by diffusion **;** immobilised enzyme **;** glucose oxidase **;** immobilised glucose oxidase = 2 marks glucose / substrate, binds to enzyme **;** **A** enzyme-substrate complex forms specific / complementary shape / AW **;** named product formed (gluconic acid / hydrogen peroxide) **;** reduces concentration of oxygen **;** ref to platinum oxygen electrode **;** transducer produces, electrical signal / current **;** ref to level related to glucose concentration **;**	**max 5**
		(iii)	*accept any three relevant comments* quantitative measurement of blood glucose **/** AW **;** rapid **;** accurate / sensitive **;** portable / can test anywhere / can test at home **;** using small volumes of blood **;** allows correct dose of insulin to be calculated **;** AVP **;;;** e.g. automated with mini-pump re-useable ref to cost no need to go to clinic / hospital	**max 3**
	(b)	(i)	hybridoma **;**	**1**
		(ii)	B, lymphocyte / cell **;** produce (specific) antibody **;** division limited **/** AW **;** myeloma, cell divides rapidly **;** and continuously **/** AW **;**	**max 3**

(c) **(i)** protein / glycoprotein on, the surface of virus / envelope **;**
antigen **;**
complementary shape / specific tertiary structure **;**
to, attach / bind, to (HIV) antibody **;**
AVP **;** e.g. ELISA technique
named protein
normally binds to receptors on host cells **max 3**

(ii) pregnancy test / test for HCG / identification of drugs / identification of cancers / drug delivery / tissue typing / blood typing / fertility test / passive vaccine / AVP **;**
R magic bullets unqualified **max 1**

[Total: 19]

Question		Expected Answers	Marks
2	(a)	*description to max 2* heat milk ; ref to temperature above 60 °C with a length of time ; e.g. accept values given in yoghurt production - 85 to 95 °C for 15 to 30min, *or* older batch method 63 °C for 15 to 30 mins, *or* current method of 72 °C for 15 sec named method / ref to method ; e.g. batch method / stirring sample, *or* HTST (high temperature short time) system / cooled rapidly, through pipes / thin stream between metal plates in heat exchanger *explanation* kills pathogens ; **A** bacteria / named example e.g. *M. bovis* / *E. coli* / *Brucella melitensis* **R** microorganisms / inactivates pathogens	**max 3**
	(b)	**1** haemocytometer / microscope slide, is gridded ; **A** diagram of grid **2** use a clean, slide / cover slip ; **3** breathe onto cover slip / cover slip moistened ; **4** push cover slip horizontally onto the slide (and press down) ; **5** 'Newton's rings' / (6) rainbow patterns seen (when correctly in place) ; **6** chamber is 0.1 mm deep ; **7** idea of dilution / dilution described e.g. add 1 cm^3 to 9 cm^3 ; **8** mix / agitate, culture / milk sample (before application) ; **9** to, disperse cells / avoid clumping / give even distribution ; **10** fill the chamber / description of method ; e.g. Pasteur pipette / syringe with needle **11** add only enough to fill the, platform / chamber ; **A** avoid running into grooves **12** allow to settle / leave five minutes ; **13** ref to use of microscope ; e.g. low to high power, focusing **14** magnification x 400 ; **15** count number of cells in triple-lined squares ; **16** selected sample squares at random / method of selecting squares to count ; e.g. count 4 corner and centre square **17** count using North-West / South–East rule ; **A** description e.g. count as "in" those cells that lie on (or just touch) the top and left sides of middle line (of triple lines) **18** calculate the, number / density, of cells per (unit) volume ; **19** ref to detail of computation ; e.g. mean number, per square in 0.004mm^3 , multiply number in 5 squares by 5 to give number in 0.1 mm^3, multiply up by dilution factor **20** AVP ; e.g. further detail of computation (if 19 is awarded) ref to stain, further detail of method **R** explanations	**max 9**
		QWC – legible text with accurate spelling, punctuation and grammar	**1**
	(c) (i)	(indicates number of) live / viable, bacteria ; **A** haemocytometer shows dead and alive	**1**
	(ii)	colony / colonies ; **R** bacteria ref to different appearance of colony ; e.g. shape, texture, colour	**2**
	(iii)	takes too long / milk will go off while waiting for results ;	**1**
			[Total: 17]

Question			Expected Answers	Marks
3	(a)	(i)	penicillin ; **A** other named antibiotic	1
		(ii)	(complex organic molecules) produced after / not produced during, the (log / rapid / main) growth phase ; not essential for normal, cell growth / reproduction ;	max 1
		(iii)	batch / fed batch ;	1
			nutrients only added at start ; short / rapid, growth phase ; required product made, during stationary phase / late in life cycle ; ora **R** death phase shortage / depletion of, nutrients / named nutrients ; cell division / reproduction, no longer occurring ; ref to addition of, glucose / lactose, at intervals (to avoid death of culture) ;	max 2
	(b)	1	air pressure will push the medium into the culture vessel ;	
		2	medium / nutrients, added to the culture at a constant rate **/** AW ;	
		3	<u>algae / cells / *Chlorella*</u>, removed / harvested, from the sample port ;	
		4	at the same rate as / to match, the nutrients added ;	
		5	so volume in fermenter remains constant ;	
		6	removal of, waste / toxic products ;	
		7	that could affect, growth / reproduction ;	
		8	(cells kept in) exponential / log / rapid / main, growth phase ;	
		9	algae are photosynthetic ;	
		10	light <u>energy</u> required ;	
		11	ref to use of fluorescent light to avoid overheating ;	
		12	ref to monitoring temperature ;	
		13	ref to optimum conditions ; **A** 'conditions for maximum growth'	
		14	air bubbles to mix culture with nutrients / AW ;	
		15	air bubbles to allow algae to get sufficient light ;	
		16	air bubbles provide oxygen for (aerobic) respiration ;	
		17	and CO_2 for photosynthesis ;	
		18	air flowing into the culture vessel flows out through an outflow tube ;	
		19	preventing build-up of pressure ;	
		20	AVP ; e.g. sampling to check for mass of *Chlorella*	max 6

(c) difficulty maintaining a constant temperature **;**
difficulty maintaining a constant pH **;**
} one mark for ref to difficulty of controlling environmental factors

heating / cooling, qualified **;**
foaming **;**
blocking of, inlet / outlet, tubes **;**
difficulties with, mixing / stirring **;**
contamination / keeping it sterile **;**
conditions need to be continuously monitored **;**
nutrient requirements may change **;**
AVP **;**
AVP **;** e.g. algal growth on glass
difficulties in providing sufficient light
errors lead to loss of several days production of *Chlorella* **max 4**

[Total: 15]

Question		Expected Answers	Marks
4	(a)	less insecticides used **thus** cost implications **;** less insecticides used **thus** fewer deaths of, beneficial / useful / other, insects or other organisms **;** less insecticides used **thus** lower environmental impact **;** protein within plant cells **thus** no danger to, humans / animals (of free *Bt* protein) **;** protein within plant cells **thus** specific to plant-eating insect **;** degrades rapidly **thus** no pollution of soil / low environmental impact **;** no bioaccumulation / does not build up in food chains / does not enter human food chain **;** AVP **;** e.g. less spoilage only pests are harmed, so safe if consumed by other organisms	**max 4**
	(b) **1**	identify / isolate, the gene **;**	
	2	restriction enzyme **;**	
	3	cuts out gene **;**	
	4	ref to 'sticky ends' **;**	
	5	ref to cloning **;**	
		either	
	6	plasmid vector **;**	
	7	same restriction enzyme used to, open / cleave, plasmid **;**	
	8	gene inserts by complementary base pairing **;**	
	9	ligase **;**	
	10	joins two pieces of DNA together / produces recombinant DNA **;**	
	11	bacteria used to transfer (recombinant) plasmids into plant cells **;**	
		or	
	6	ballistics **;**	
	7	(minute) gold / tungsten, pellets **;**	
	8	coated with, DNA / gene **;**	
	9	special gun used to fire **;**	
	10	pellets stopped by plate **;**	
	11	enough propulsive force for DNA to enter, plant tissue / nucleus **;**	
		allow other techniques to same number of mark points e.g. electroporation	
	12	use plant tissue culture **;**	
	13	ref to techniques **;** e.g. explant, protoplast culture, callus culture	
	14	use of, plant growth regulators / named plant growth regulator **;**	
	15	AVP **;**	
	16	AVP **;** e.g. ref to Ti plasmid / *Agrobacterium tumefaciens* / antibiotic resistance marker / calcium ions for transformation, new gene attached to the plants chromosome / transgenic plants produced / electrophoresis to isolate gene	**max 8**
		QWC – clear well organised, using specialist terms ;	**1**
		award the QWC mark if four of the following are used in correct context restriction, endonuclease, sticky ends, cloning, vector, plasmid, complementary, recombinant, ligase, transformation, transgenic, explant, protoplast, callus, electrophoresis	

(c) ref to higher yield / faster growth ;
ref nitrogen fixation ;
resistance to herbicides ;
resistance to, disease / bacteria / fungi / viruses / pathogens ; **A** disease-free
resistance to extreme(s) of temperature ; **A** frost resistance
resistance to drought ;
tolerant to flooding ;
salt tolerance ;
improved shelf-life (after harvesting) ;
improved nutritional value ;
AVP ;
AVP ; e.g. synthesis of vaccines
improved texture after freezing
growth on poor nutritional soils

NOT insecticide resistance
NOT pest resistance **max 4**

[Total: 17]

Question			Expected Answers	Marks
5	(a)		(stain with) crystal violet ; (wash with) iodine solution ; (clear with) alcohol or acetone ; stains purple ; A violet / blue	4
	(b)		*mark (i) and (ii) together to max 2*	
	(b)	(i)	Gram-positive have thicker layer of, peptidoglycan / murein ; ora	1
		(ii)	ref to, crystal violet-iodine complex / stain, removed, through thinner wall / from Gram-negative *or* not removed, through thicker wall / from Gram positive ;	1
	(c)	(i)	RNA(i) combines with mRNA ; e.g. of base pairing (but not T) A-U / G-C ; stops translation ; ref to stops mRNA combining with ribosomes ; stops protein synthesis ;	max 3
		(ii)	chemicals / enzymes in, mouth / toothpaste / bacteria ; denature / degrade, RNA ; RNA not normally taken up by bacterial cells ; short life of RNA ; RNA not replicated in bacteria when bacteria reproduce ; toothpaste in mouth only for short time ; AVP ; AVP ; e.g. washed away by saliva	max 2
				[Total: 11]

Question			Expected Answers	Marks
6	(a)	(i)	amylase ;	1
		(ii)	glycosidic ; R glucosidic	1
		(iii)	alpha / α ;	1
	(b)	(i)	encapsulation / trapped in alginate beads ; adsorption *or* stuck onto, collagen / clays / resins ; cross linkage *or* covalent / chemical bonding to, cellulose (fibres) ; gel entrapment / trapped in silica gel ; partially permeable membrane microspheres ;	max 2
		(ii)	does not mix with / does not contaminate / stays separate from, the product ; ref to, no / less / easier, downstream processing ; recoverable / not lost during processing ; reusable / cost effective ; matrix stabilises / protects the enzyme ; so activity not affected by changes in, temperature / pH *or* run at a high temperature / wider range of pH ; longer, use / shelf-life ; so suitable for continuous culture / cost effective / greater yield ; AVP ; *points can interchange if valid*	max 4
	(c)		not necessary to start with a pure enzyme ; keeps the enzyme away from oxygen ; more enzymes involved ; cell produces enzymes ; AVP ; e.g. enzyme(s) may be, expensive / difficult to isolate simultaneous processes can occur	max 2
				[Total: 11]