Lab skills

1 Which technique could be used to quantify the concentration of a pigmented compound extracted from plant tissue?

A Centrifugation
B Colorimetry
C Electrophoresis
D Immunoassay

2 A researcher studying a protein with an isoelectric point of pH 8.2 wanted to separate it from a mixture of proteins and determine its molecular mass (size). A solution containing the mixture of proteins was adjusted to pH 8.2 then centrifuged.

The most appropriate next steps would be to

A collect the protein from the pellet and carry out SDS-polyacrylamide electrophoresis (SDS-PAGE)
B collect the protein from the supernatant and carry out SDS-polyacrylamide electrophoresis (SDS-PAGE)
C collect the protein from the pellet and carry out native gel electrophoresis
D collect the protein from the supernatant and carry out native gel electrophoresis.

3 Which row in the table describes properties of proteins that allow them to be separated using the techniques shown?

	Protein separation technique	
	Centrifugation	*Gel electrophoresis*
A	density	charge
B	charge	density
C	shape	charge
D	charge	shape

4 Cell components can be separated by a technique called differential centrifugation.

This technique uses a series of centrifugation steps at specific centrifugation forces (g) for a given time. After each step, the supernatant is removed and the cell components in the pellet identified. The supernatant is re-centrifuged at a higher centrifugation force for a longer time.

The table gives information about a differential centrifugation experiment.

Step	Centrifuge conditions		Cell component(s) in pellet
	Force (g)	Time (minutes)	
1	600	10	nucleus, cytoskeleton
2	15 000	15	mitochondria
3	100 000	60	plasma membrane, endoplasmic reticulum fragments

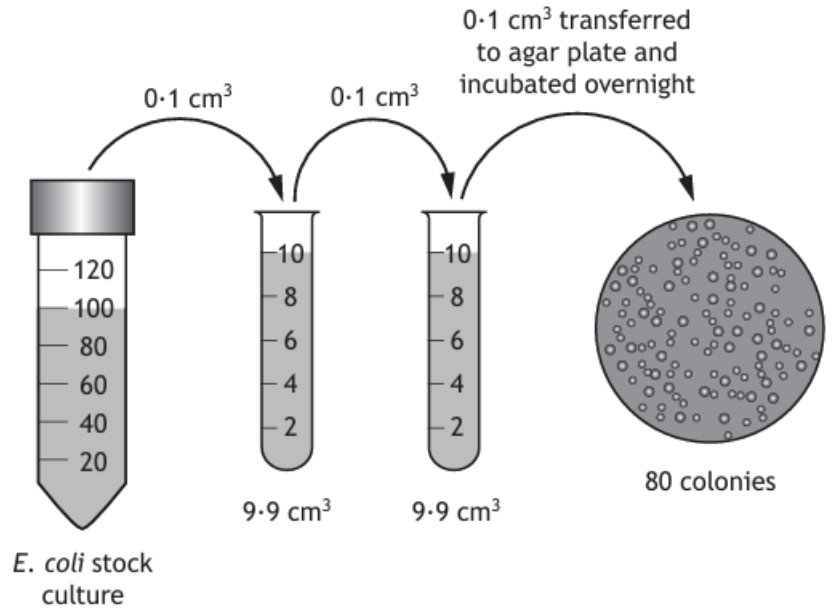
Plasma membrane would be present in the supernatant of which centrifugation step(s)?

A 1 only
B 2 only
C 1 and 2 only
D 3 only

5 SDS-PAGE separates proteins by:

A shape
B size
C charge
D isoelectric point.

6 The figure shows how a biologist used serial dilution followed by plating to estimate the number of cells in a 100 cm^3 stock culture of *E.coli*.



How many *E.coli* cells were present in the original stock culture?

A $8{\cdot}0 \times 10^4$

B $8{\cdot}0 \times 10^6$

C $8{\cdot}0 \times 10^7$

D $8{\cdot}0 \times 10^8$

7 Primary cell lines have

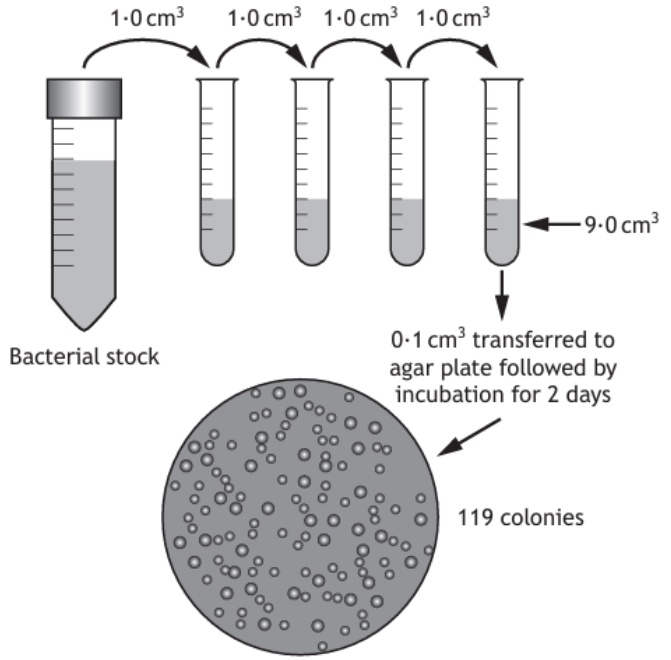
A a limited number of cell divisions and are sourced from tumours

B a limited number of cell divisions and are sourced directly from normal animal tissue

C an indefinite number of cell divisions and are sourced from tumours

D an indefinite number of cell divisions and are sourced directly from normal animal tissue.

8 The figure shows how a scientist used serial dilution followed by plating to check the number of bacteria in a stock culture.



How many bacteria were there in 1 cm^3 of the original bacterial stock?

A $1{\cdot}19 \times 10^5$

B $1{\cdot}19 \times 10^6$

C $1{\cdot}19 \times 10^7$

D $1{\cdot}19 \times 10^8$

9 Trypan blue is used as a vital stain to identify viable cells when viewed in a haemocytometer.

A vital stain

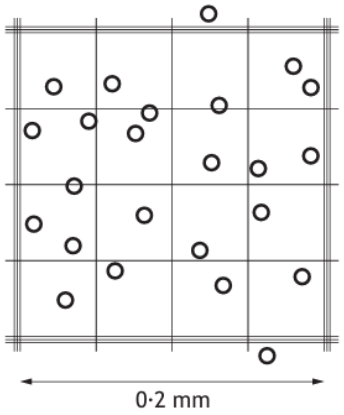
A stains all cells

B only stains dead cells

C only stains living cells

D only stains the culture medium.

10 The diagram shows a haemocytometer grid that was used to estimate the number of cells in a 10 cm^3 microbial culture. The depth of the counting chamber is 0·2 mm.



The number of cells in the 10 cm^3 culture was

A $2{\cdot}75 \times 10^7$

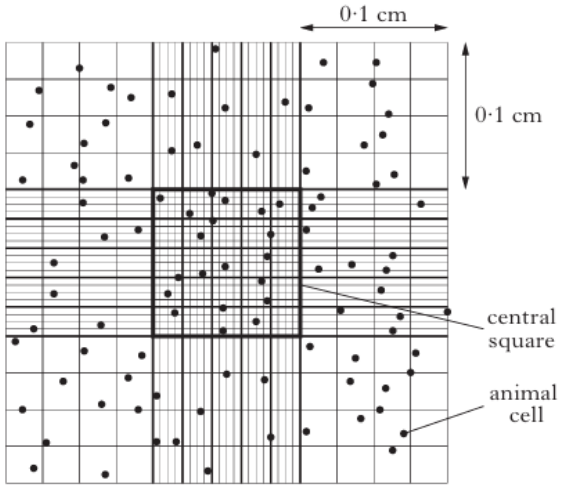
B $2{\cdot}5 \times 10^7$

C $2{\cdot}25 \times 10^7$

D $1{\cdot}6 \times 10^3$

11 The diagram below shows a haemocytometer counting chamber containing animal cells.

The depth of the chamber is 0·01 cm.



The concentration of animal cells, based on the cell count from the central square, is

A $2{\cdot}0 \times 10^4$ cells per cm^3

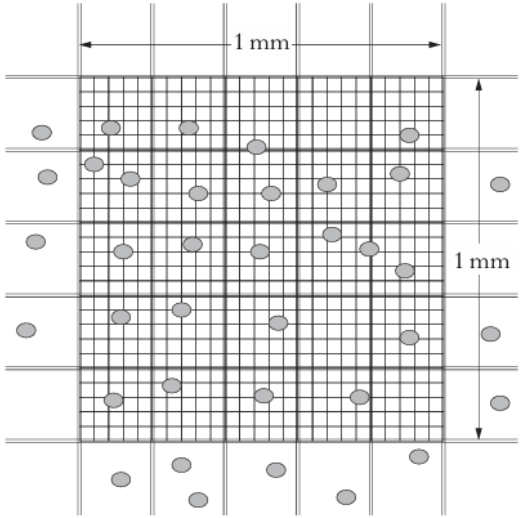
B $2{\cdot}0 \times 10^5$ cells per cm^3

C $2{\cdot}0 \times 10^6$ cells per cm^3

D $2{\cdot}0 \times 10^7$ cells per cm^3.

12 The diagram below represents red blood cells in a haemocytometer.

The grid is **0·1 mm** in depth.



The number of red blood cells per **cm^3** of blood sample is

A $2{\cdot}4 \times 10^3$

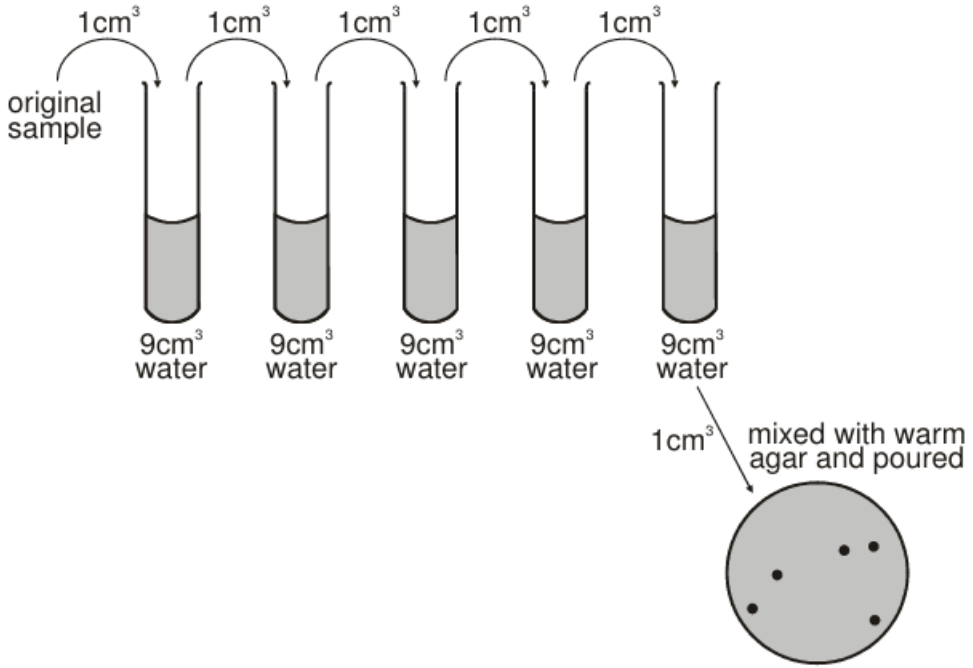
B $2{\cdot}4 \times 10^4$

C $2{\cdot}4 \times 10^5$

D $2{\cdot}4 \times 10^6$.

13 In microbiology, the total cell count can be estimated by measuring turbidity, using a haemocytometer or by flow cytometry. These methods cannot distinguish between living and dead cells. To estimate the number of viable cells, dilution plating is used.

The dilution plating stages used to estimate the number of viable *Escherichia coli* in a culture are shown below.



Which of the following is the estimated number of *E. Coli* per cm^3 in the original sample?

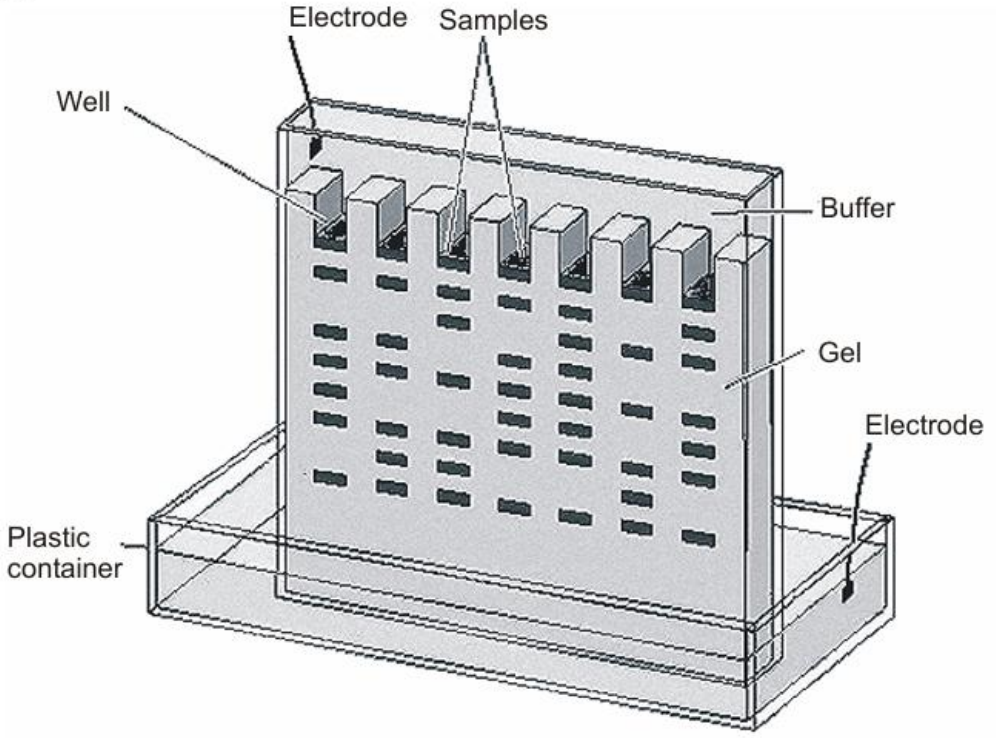
A 5×10^5

B 1×10^6

C 5×10^6

D 1×10^7

14 The diagram represents a gel electrophoresis apparatus that uses current flowing through a buffer solution to separate proteins. The protein samples are treated to give all a negative charge.



Which of the following is correct?

A The smaller proteins migrate faster to the negative electrode.

B The larger proteins migrate faster to the negative electrode.

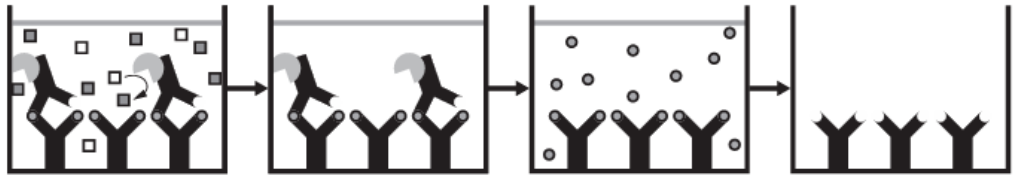
C The smaller proteins migrate faster to the positive electrode.

D The larger proteins migrate faster to the positive electrode.

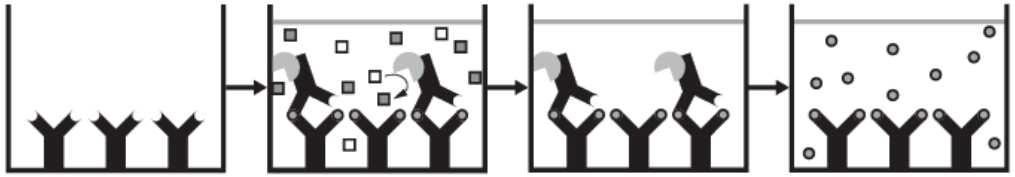
15 The diagrams represent stages in an immunoassay used to detect the presence of a poisonous toxin in food samples. The test shown is positive.

Which of the following shows the sequence of stages in the immunoassay?

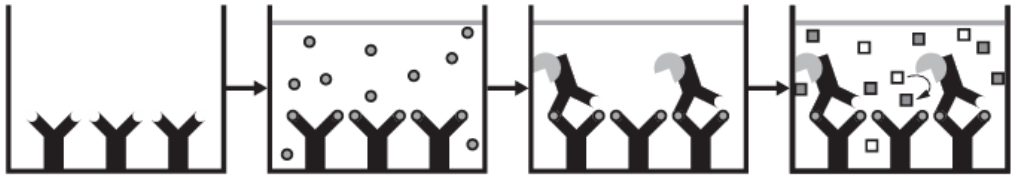
A



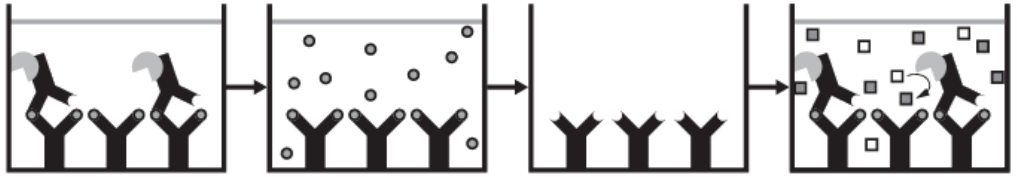
B



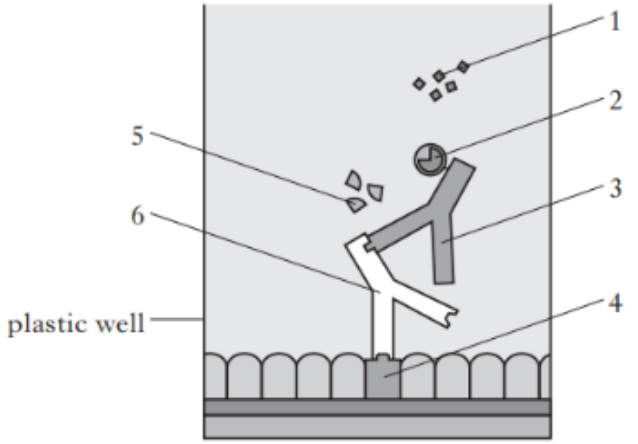
C



D



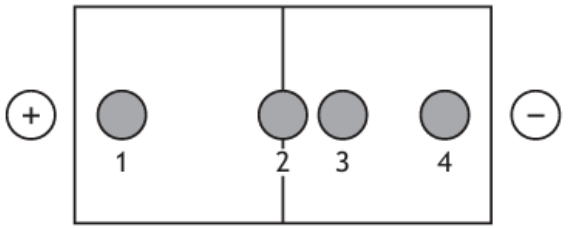
16 The diagram below represents a well in an immunoassay kit testing a blood sample from a person who may have been exposed to a virus. The substrate has been broken down to form a coloured product, so the result is positive.



Which line in the table correctly identifies the roles of numbered components?

	Antigen	*Antibody*	*Enzyme*	*Substrate*
A	4	3	2	1
B	5	2	3	6
C	4	6	5	2
D	6	3	2	5

17 A buffered solution of four amino acids was applied to the midline of a strip of electrophoresis gel. The result of running the gel is shown.



Which amino acid was at its isoelectric point?

A 1

B 2

C 3

D 4

18 An explanation for the separation of different size fragments of DNA during SDS-PAGE electrophoresis is that

A fragments are negatively charged and smaller fragments move faster towards the negative terminal

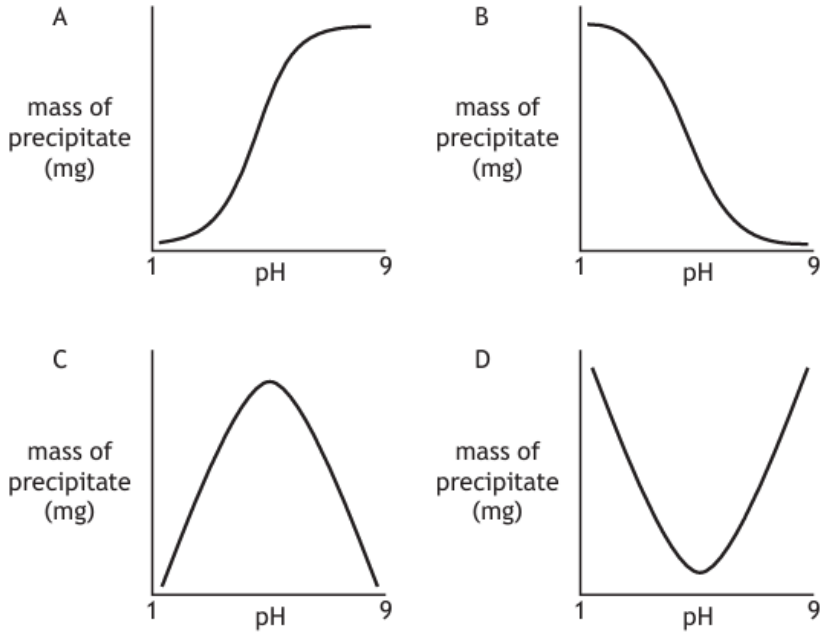
B fragments are positively charged and smaller fragments move faster towards the positive terminal

C fragments are positively charged and smaller fragments move faster towards the negative terminal

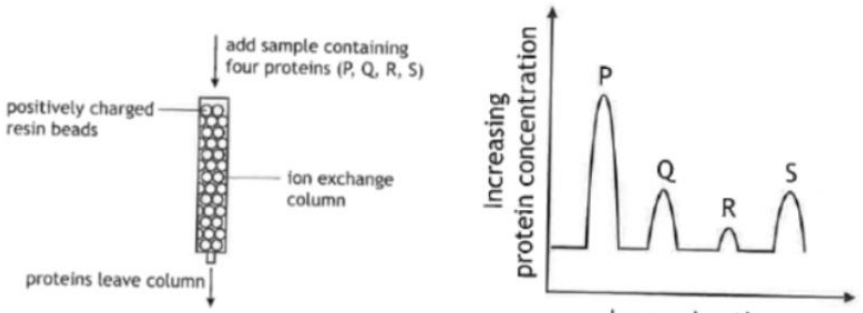
D fragments are negatively charged and smaller fragments move faster towards the positive terminal.

19 Bovine insulin is a soluble protein with an isoelectric point of pH 5·4.

Which of the following graphs represents the level of precipitate formed as the pH of a bovine insulin solution is changed?



20 Immobilised metal ion affinity chromatography (IMAC) works by negatively charged molecules being retained in a column containing immobilised positive beads. The more negatively charged the protein, the higher the affinity for the positive ions & the longer it takes to pass through the column.



Which conclusion can be drawn from this experiment.

- A Protein P is more negatively charged than protein R
- B Protein S is more negatively charged than protein Q
- C Protein R is less negatively charged than protein Q
- D Protein S is less negatively charged than protein R

Immunoassay

1. The label in this ELISA is a reporter enzyme that results in a colour change in a substrate.

   Name another type of label that can be used in immunoassays. **1**

   _____

2. Describe how a test for a person not infected by *Borrelia* would differ from the positive test illustrated. **2**

   _____

   _____

   _____

   _____

   _____

3. Protein kinase A (PKA) is an enzyme that is involved in this type of signalling. To test the hypothesis that PKA is found in a variety of cell types, cell extracts were prepared from different cell types and the proteins in the extracts separated by electrophoresis in a gel. The proteins were blotted onto a solid support and an antibody recognising PKA (anti-PKA antibody) was used to detect the presence of PKA.

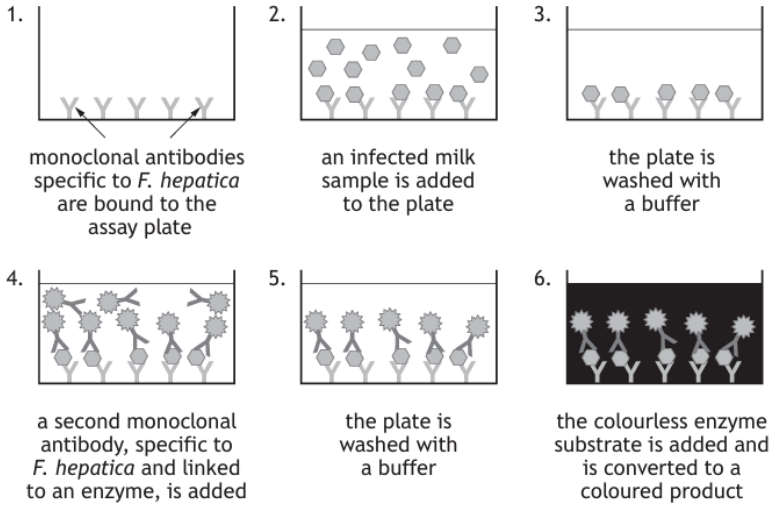
   Explain how the anti-PKA antibody would be used to detect the presence of PKA. **1**

   _____

   _____

   _____

   _____



4. If the procedure was not carried out correctly a positive result could occur in the absence of *F. hepatica* antigens; a *false positive* result.

   Suggest a possible cause for this false positive result.

   _____

   _____

5. A pH buffer was used in all reagents and wash solutions.

   Explain why it is important to control pH in immunoassays.

   _____

   _____

   _____

   _____

Cell Culture

1. Give one way the equipment can be sterilised as part of aseptic technique. 1

2. Explain the benefit of aseptic technique when culturing yeast cells. 1

3. State one way in which tumour cell lines differ from primary cell lines in culture. 1

4. State why the cell culture medium in which the neurons were cultured should contain serum. 1

5. Plating out allows the number of **viable** cells in a liquid culture to be estimated.

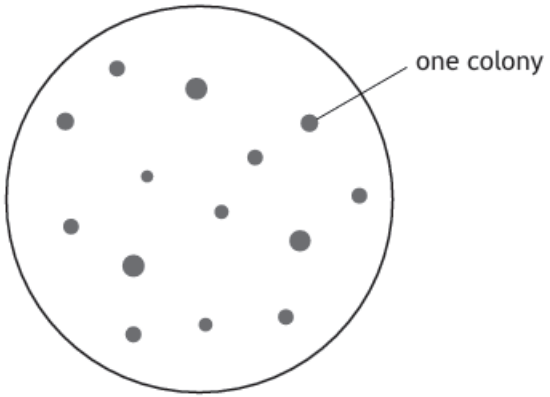
   Describe another method that can be used to estimate the number of **viable** cells in a liquid culture. 1

6. What method, other than vital staining, can be used to determine the number of viable bacterial cells in a liquid culture? 1

7. Bright field microscopy was used to view the cells grafted into the site of spinal injury.

   State another type of biological material that can be viewed using bright field microscopy. 1

8. The diagram represents colonies on a solid culture medium following plating out of a diluted yeast culture. The original sample was diluted by a factor of 10^5 and a 0.2 cm^3 sample of this diluted culture was placed on the solid medium plate.



   (i) Calculate the number of yeast cells in 1 cm^3 of the original undiluted yeast culture. 1

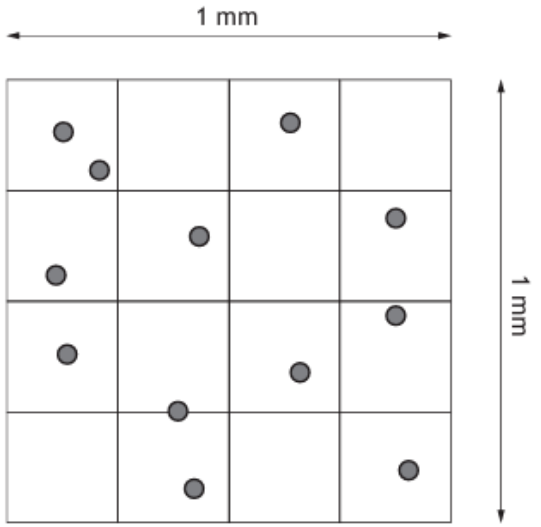
   Space for calculation

   _____ cells per cm^3

Cell Culture

9. A haemocytometer can be used to estimate the number of bacterial cells in a liquid culture.

The figure represents bacterial cells from a culture, placed in a haemocytometer that has a depth of 0.1 mm.



Calculate the number of cells per cm^3 of the liquid culture. **1**

Space for calculation

_____ cells per cm^3

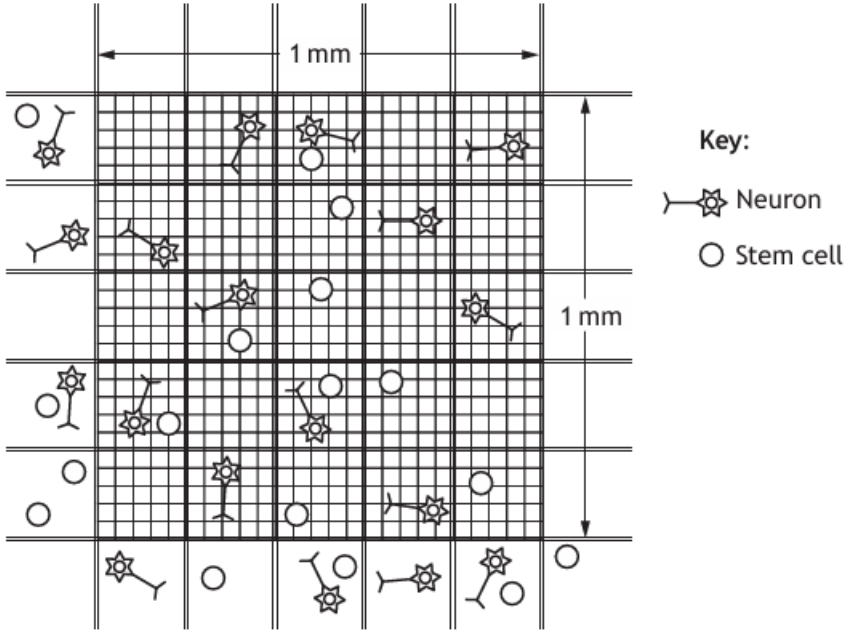
10. Suggest **one** disadvantage of cell counts performed using the haemocytometer. **1**

_____

11 Scientists used a haemocytometer to perform a cell count to calculate the number of stem cells that developed into neurons.

The diagram below represents a sample from a culture placed in a haemocytometer and viewed under a microscope.

The grid is **0·1 mm** in depth.



(i) Calculate the number of **neurons** in 1 cm^3 of the culture. **1**

Space for calculation

_____ neurons

12 In an SDS-PAGE gel, proteins are separated by size alone.

Describe how SDS-PAGE separates proteins on the basis of size. **2**

13 Specific antibodies are used to detect proteins from *Borrelia* bacteria. This cannot be done directly on the SDS-PAGE gel.

State what must happen after electrophoresis to make detection by antibodies possible during Western blotting. **1**

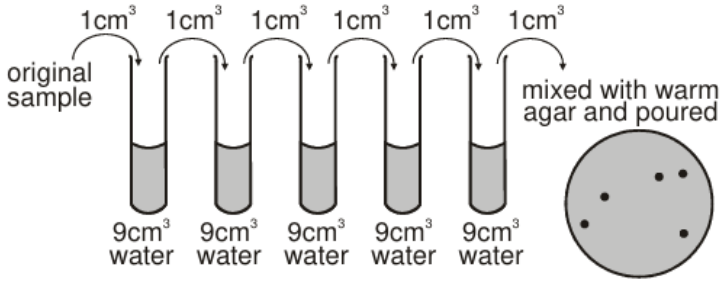
14 SDS-PAGE following purification stage 3 showed the presence of three different proteins.

These were transferred onto a solid medium, and an antibody linked to a reporter enzyme was used to identify the enzyme being purified.

Name the technique described. **1**

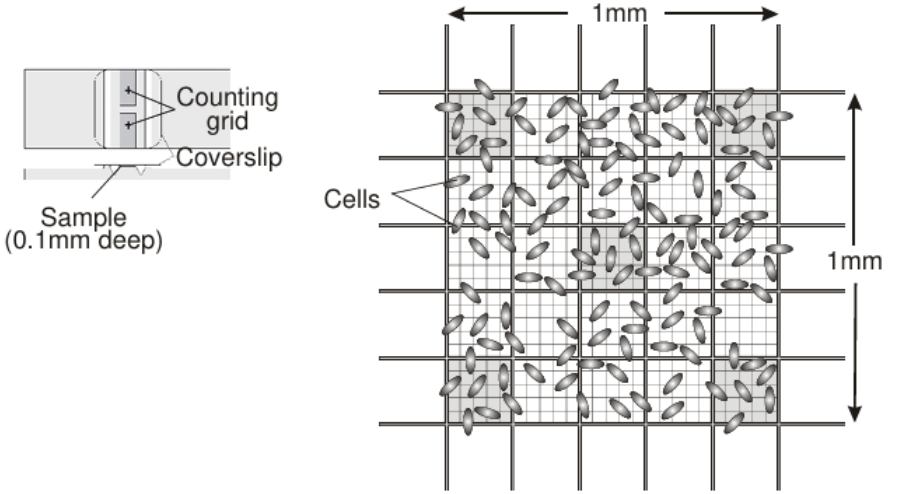
15 Describe how protein electrophoresis is used to separate proteins. **1**

16 An alternative technique that can be used to estimate the number of living *E coli* in a culture is shown below.



Name this type of dilution.

17 The apparatus that is often used to estimate the number of *Escherichia coli* in a culture is shown below.



Name this type of apparatus. **1**

Lab skills

1 Which technique could be used to quantify the concentration of a pigmented compound extracted from plant tissue?

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B Colorimetry
C Electrophoresis
D Immunoassay

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The most appropriate next steps would be to

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B	charge	density
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D	charge	shape

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This technique uses a series of centrifugation steps at specific centrifugation forces (g) for a given time. After each step, the supernatant is removed and the cell components in the pellet identified. The supernatant is re-centrifuged at a higher centrifugation force for a longer time.

The table gives information about a differential centrifugation experiment.

Step	Centrifuge conditions		Cell component(s) in pellet
	Force (g)	Time (minutes)	
1	600	10	nucleus, cytoskeleton
2	15 000	15	mitochondria
3	100 000	60	plasma membrane, endoplasmic reticulum fragments

Plasma membrane would be present in the supernatant of which centrifugation step(s)?

A 1 only
B 2 only
C 1 and 2 only
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5 SDS-PAGE separates proteins by:

A shape
B size
C charge
D isoelectric point.

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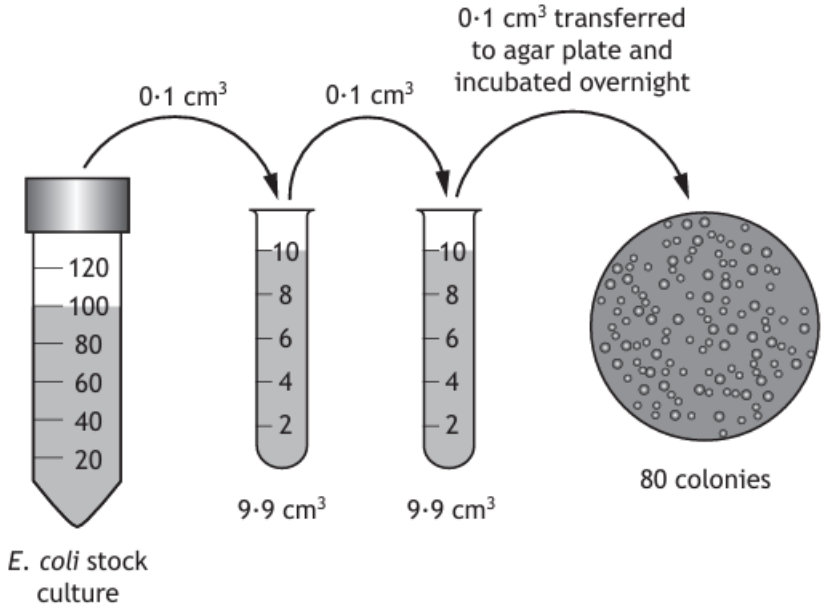

How many *E.coli* cells were present in the original stock culture?

A $8{\cdot}0 \times 10^4$

B $8{\cdot}0 \times 10^6$

C $8{\cdot}0 \times 10^7$

D $8{\cdot}0 \times 10^8$

Df 10^{-7}

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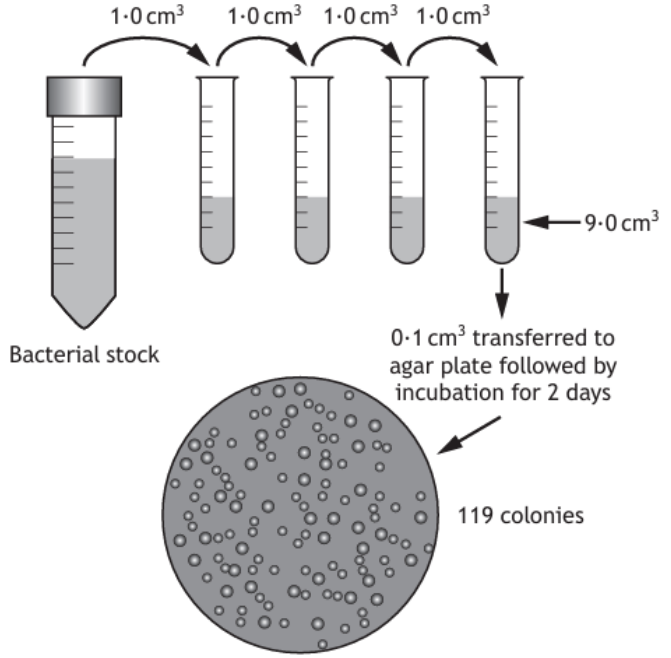

How many bacteria were there in 1 cm^3 of the original bacterial stock?

A $1{\cdot}19 \times 10^5$

B $1{\cdot}19 \times 10^6$

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D $1{\cdot}19 \times 10^8$

Df 10^{-5}

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A vital stain

A stains all cells

B only stains dead cells

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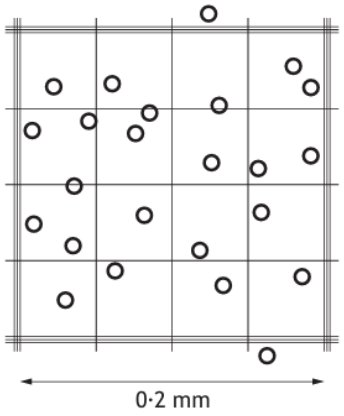


The number of cells in the 10 cm^3 culture was

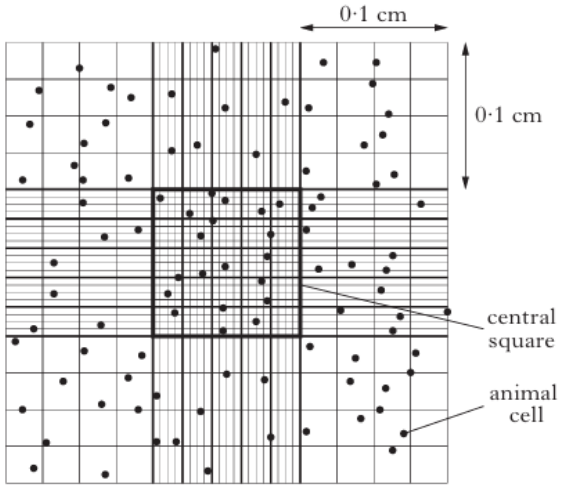
A $2·75 \times 10^7$
B $2·5 \times 10^7$
C $2·25 \times 10^7$
D $1·6 \times 10^3$

$0.02 \times 0.02 \times 0.02 = 0.000008\ cm^3$

$0.000008\ cm^3 \rightarrow 22$ cells
$10cm^3 \rightarrow 27\,5000\,000$

11 The diagram below shows a haemocytometer counting chamber containing animal cells.

The depth of the chamber is 0·01 cm.



The concentration of animal cells, based on the cell count from the central square, is

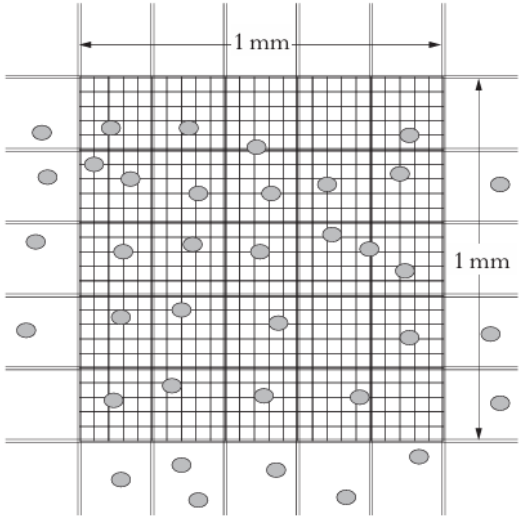
A $2·0 \times 10^4$ cells per cm^3
B $2·0 \times 10^5$ cells per cm^3
C $2·0 \times 10^6$ cells per cm^3
D $2·0 \times 10^7$ cells per cm^3.

$0.1 \times 0.1 \times 0.01 = 0.0001\ cm^3$

$0.0001\ cm^3 \rightarrow 20$ cells
$1cm^3 \rightarrow 200\,000$

12 The diagram below represents red blood cells in a haemocytometer.

The grid is **0·1 mm** in depth.



The number of red blood cells per **cm^3** of blood sample is

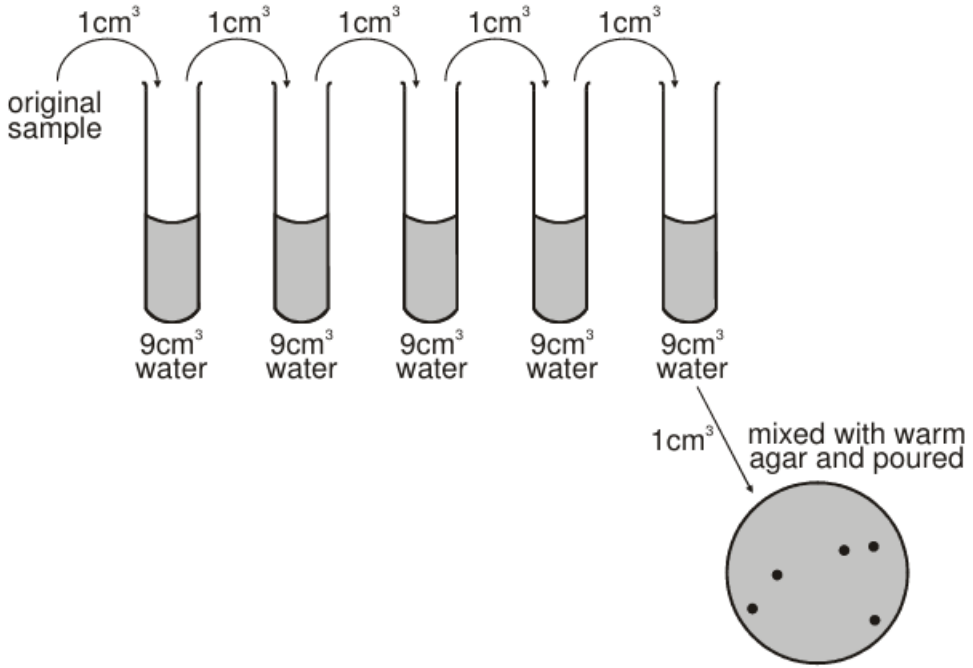
A $2·4 \times 10^3$
B $2·4 \times 10^4$
C $2·4 \times 10^5$
D $2·4 \times 10^6$.

$0.1 \times 0.1 \times 0.01 = 0.0001\ cm^3$

$0.0001\ cm^3 \rightarrow 24$ cells
$1cm^3 \rightarrow 240\,000$

13 In microbiology, the total cell count can be estimated by measuring turbidity, using a haemocytometer or by flow cytometry. These methods cannot distinguish between living and dead cells. To estimate the number of viable cells, dilution plating is used.

The dilution plating stages used to estimate the number of viable *Escherichia coli* in a culture are shown below.



Which of the following is the estimated number of *E. Coli* per cm^3 in the original sample?

A 5×10^5

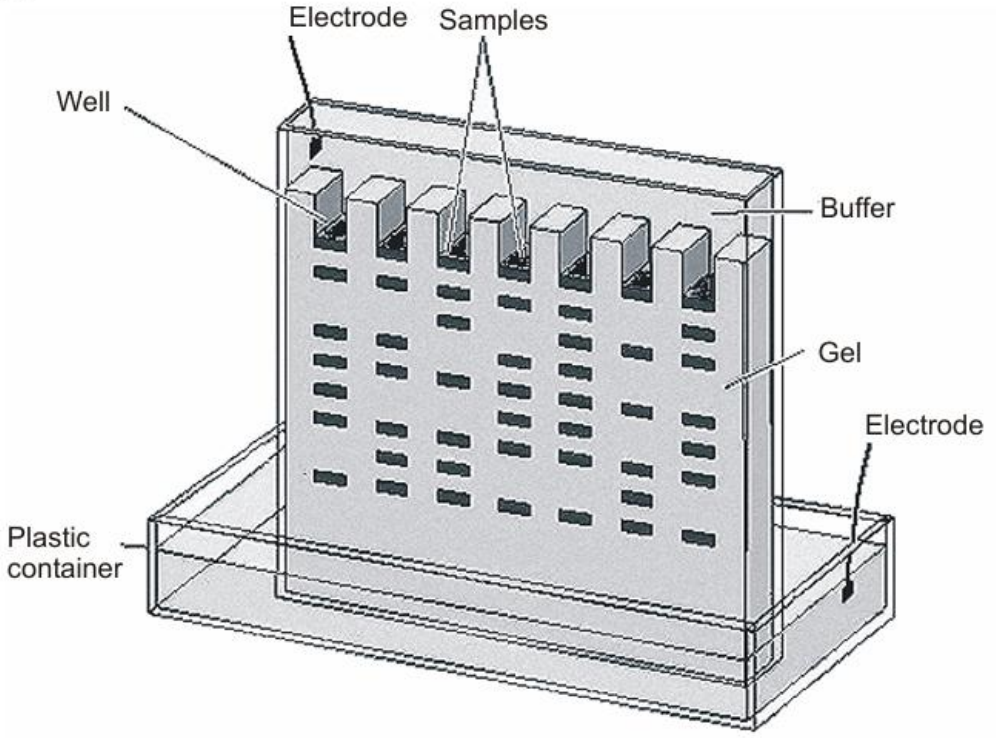
B 1×10^6

C 5×10^6

D 1×10^7

Df 10^{-5}

14 The diagram represents a gel electrophoresis apparatus that uses current flowing through a buffer solution to separate proteins. The protein samples are treated to give all a negative charge.



Which of the following is correct?

A The smaller proteins migrate faster to the negative electrode.

B The larger proteins migrate faster to the negative electrode.

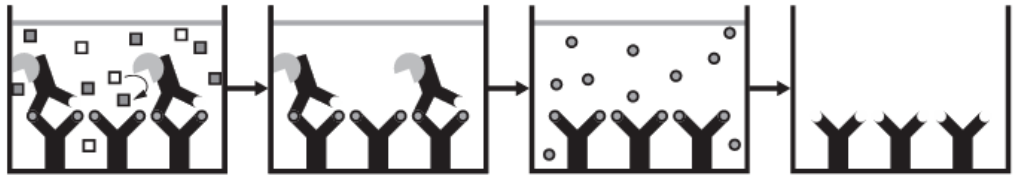
C The smaller proteins migrate faster to the positive electrode.

D The larger proteins migrate faster to the positive electrode.

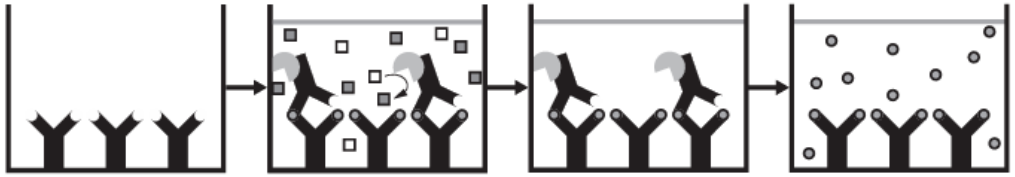
15 The diagrams represent stages in an immunoassay used to detect the presence of a poisonous toxin in food samples. The test shown is positive.

Which of the following shows the sequence of stages in the immunoassay?

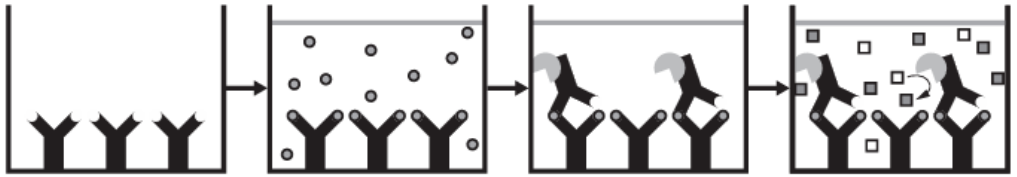
A



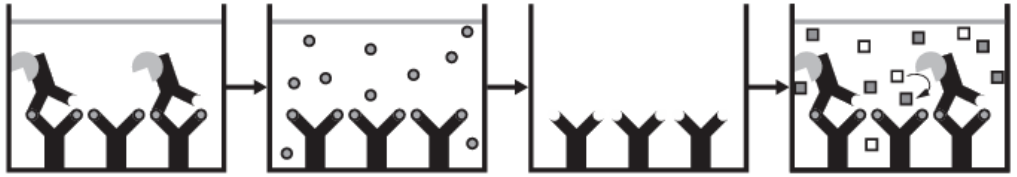
B



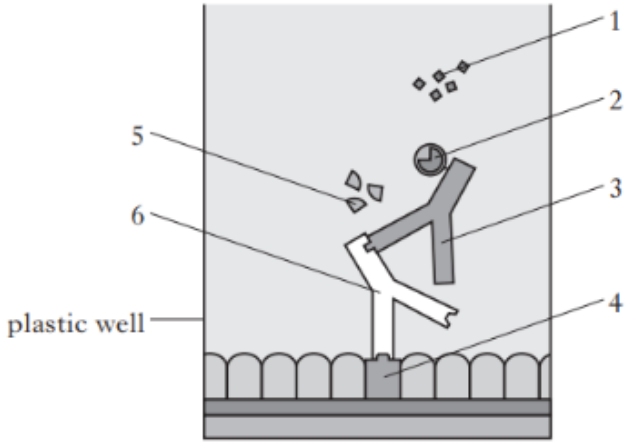
C



D



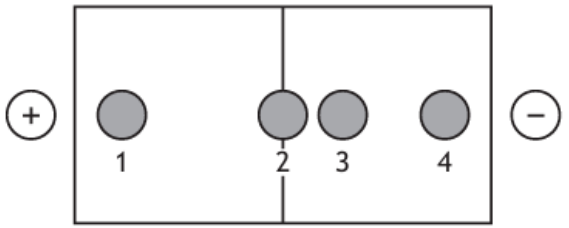
16 The diagram below represents a well in an immunoassay kit testing a blood sample from a person who may have been exposed to a virus. The substrate has been broken down to form a coloured product, so the result is positive.



Which line in the table correctly identifies the roles of numbered components?

	Antigen	*Antibody*	*Enzyme*	*Substrate*
A	4	3	2	1
B	5	2	3	6
C	4	6	5	2
D	6	3	2	5

17 A buffered solution of four amino acids was applied to the midline of a strip of electrophoresis gel. The result of running the gel is shown.



Which amino acid was at its isoelectric point?

A 1
B 2
C 3
D 4

18 An explanation for the separation of different size fragments of DNA during SDS-PAGE electrophoresis is that

A fragments are negatively charged and smaller fragments move faster towards the negative terminal

B fragments are positively charged and smaller fragments move faster towards the positive terminal

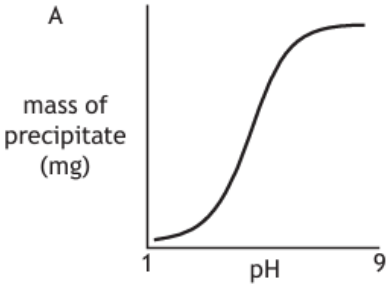
C fragments are positively charged and smaller fragments move faster towards the negative terminal

D fragments are negatively charged and smaller fragments move faster towards the positive terminal.

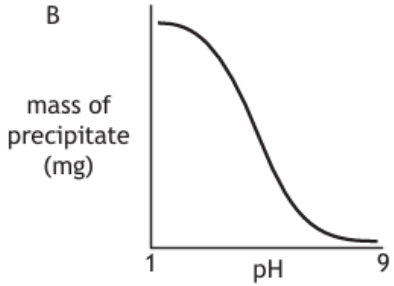
19 Bovine insulin is a soluble protein with an isoelectric point of pH 5·4.

Which of the following graphs represents the level of precipitate formed as the pH of a bovine insulin solution is changed?

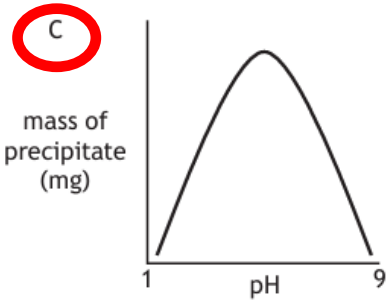
A



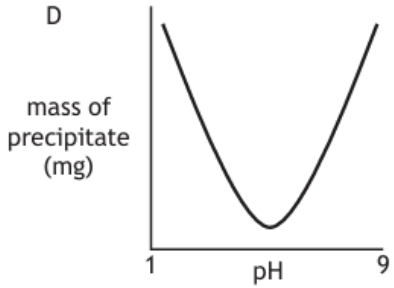
B



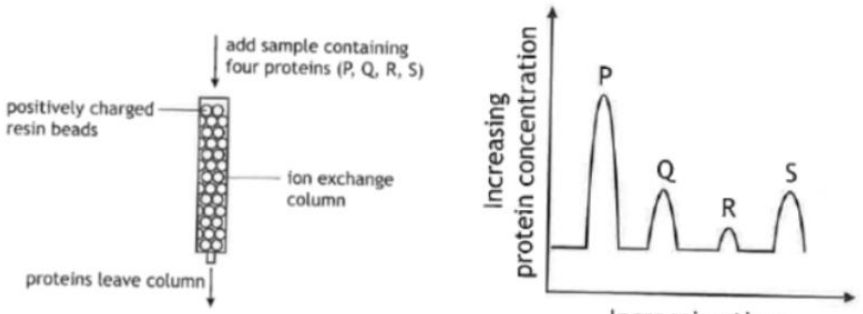
C



D



20 Immobilised metal ion affinity chromatography (IMAC) works by negatively charged molecules being retained in a column containing immobilised positive beads. The more negatively charged the protein, the higher the affinity for the positive ions & the longer it takes to pass through the column.



Which conclusion can be drawn from this experiment.

- A Protein P is more negatively charged than protein R
- B Protein S is more negatively charged than protein Q
- C Protein R is less negatively charged than protein Q
- D Protein S is less negatively charged than protein R

Immunoassay

1. The label in this ELISA is a reporter enzyme that results in a colour change in a substrate.

Name another type of label that can be used in immunoassays. **1**

Fluorescence/ Chemiluminescence/radioisotope

2. Describe how a test for a person not infected by *Borrelia* would differ from the positive test illustrated. **2**

No antibodies to bacteria in serum so no binding to assay plate.

Second MCA specific to human antibodies will not bind

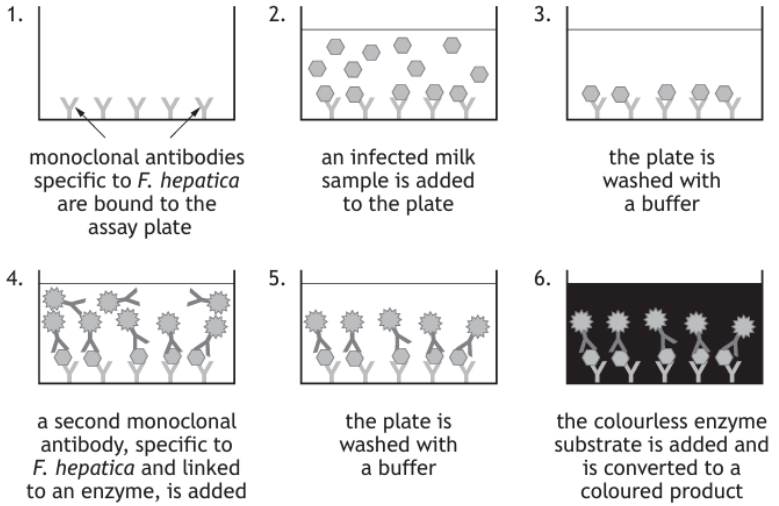
No colour change when the substrate is added.

3. Protein kinase A (PKA) is an enzyme that is involved in this type of signalling. To test the hypothesis that PKA is found in a variety of cell types, cell extracts were prepared from different cell types and the proteins in the extracts separated by electrophoresis in a gel. The proteins were blotted onto a solid support and an antibody recognising PKA (anti-PKA antibody) was used to detect the presence of PKA.

Explain how the anti-PKA antibody would be used to detect the presence of PKA. **1**

Anti PKA MCA with chemical labelled (fluorescence/ reporter enzyme). (1)

Fluorescence/colour/label detected if anti PKA MCA has bound to PKA



4. If the procedure was not carried out correctly a positive result could occur in the absence of *F. hepatica* antigens; a *false positive* result.

Suggest a possible cause for this false positive result.

Plate not washed adequately at stage 5

5. A pH buffer was used in all reagents and wash solutions.

Explain why it is important to control pH in immunoassays.

pH would affect interactions between R groups (1)

Protein with altered structure will have reduced affinity for antigens (1)

Cell Culture

1. Give one way the equipment can be sterilised as part of aseptic technique. 1

heat/chemicals/alcohol/UV

2. Explain the benefit of aseptic technique when culturing yeast cells. 1

eliminates unwanted microbes

3. State one way in which tumour cell lines differ from primary cell lines in culture. 1

Only tumour cell lines are immortal

4. State why the cell culture medium in which the neurons were cultured should contain serum. 1

provides growth factor that promotes cell proliferation

5. Plating out allows the number of **viable** cells in a liquid culture to be estimated.

Describe another method that can be used to estimate the number of **viable** cells in a liquid culture. 1

Use a vital stain to detect living cells then use a haemocytometer to count.

6. What method, other than vital staining, can be used to determine the number of viable bacterial cells in a liquid culture? 1

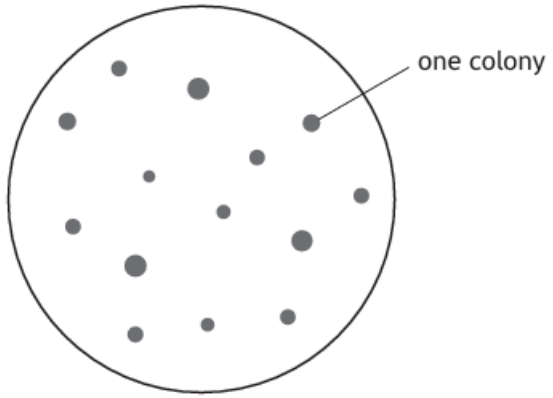
Colony count (after serial dilution)

7. Bright field microscopy was used to view the cells grafted into the site of spinal injury.

State another type of biological material that can be viewed using bright field microscopy. 1

Whole/parts of organisms

8. The diagram represents colonies on a solid culture medium following plating out of a diluted yeast culture. The original sample was diluted by a factor of 10^5 and a 0.2 cm^3 sample of this diluted culture was placed on the solid medium plate.



(i) Calculate the number of yeast cells in 1 cm^3 of the original undiluted yeast culture. 1

Space for calculation

$0.2cm^3$ → 14×10^{-5} df

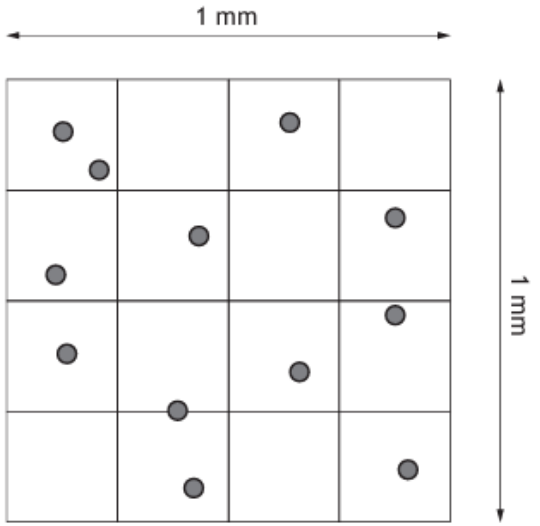
$1cm^3$ → **7×10^6**

_____ cells per cm^3

Cell Culture

9. A haemocytometer can be used to estimate the number of bacterial cells in a liquid culture.

The figure represents bacterial cells from a culture, placed in a haemocytometer that has a depth of 0.1 mm.



Calculate the number of cells per cm^3 of the liquid culture. 1

Space for calculation

$0.1 \times 0.1 \times 0.01 = 0.0001\ cm^3$ → 12 cells

$1 cm^3$ → **120 000**

_____ cells per cm^3

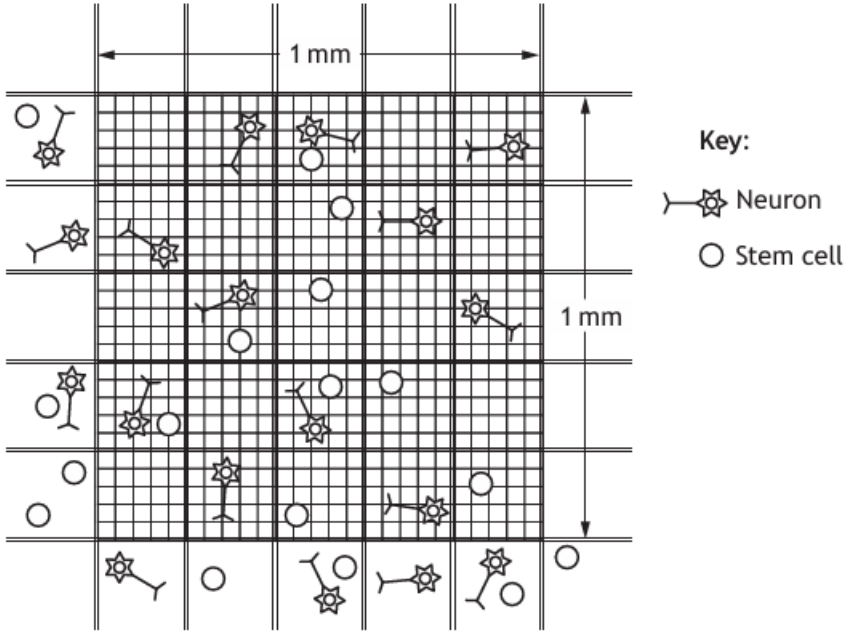
10. Suggest **one** disadvantage of cell counts performed using the haemocytometer. 1

Time consuming/can't distinguish living/dead cells.

11 Scientists used a haemocytometer to perform a cell count to calculate the number of stem cells that developed into neurons.

The diagram below represents a sample from a culture placed in a haemocytometer and viewed under a microscope.

The grid is **0·1 mm** in depth.



(i) Calculate the number of **neurons** in 1 cm^3 of the culture. 1

Space for calculation

$0.1 \times 0.1 \times 0.01 = 0.0001\ cm^3$ → 11 cells

$1 cm^3$ → **110 000**

_____ neurons

12 In an SDS-PAGE gel, proteins are separated by size alone.

Describe how SDS-PAGE separates proteins on the basis of size. 2

SDS-PAGE denatures proteins. (1)

SDS–PAGE gives all proteins a negative charge. (1)

Smaller proteins migrate further/ faster in the electric field.

13 Specific antibodies are used to detect proteins from *Borrelia* bacteria. This cannot be done directly on the SDS-PAGE gel.

State what must happen after electrophoresis to make detection by antibodies possible during Western blotting. 1

Blotting onto a nylon membrane.

14 SDS-PAGE following purification stage 3 showed the presence of three different proteins.

These were transferred onto a solid medium, and an antibody linked to a reporter enzyme was used to identify the enzyme being purified.

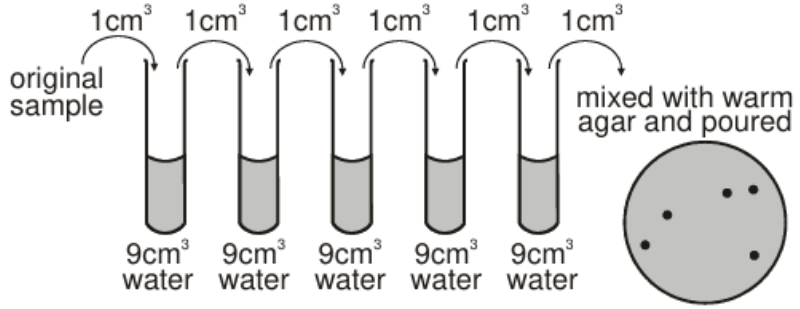
Name the technique described. 1

Western blotting

15 Describe how protein electrophoresis is used to separate proteins. 1

Current flows through buffer in a gel matrix which separates proteins in gel based on size/mass/shape/charge

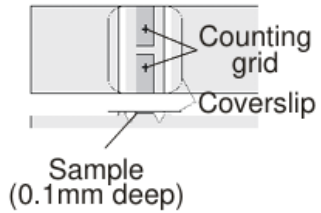
16 An alternative technique that can be used to estimate the number of living *E coli* in a culture is shown below.

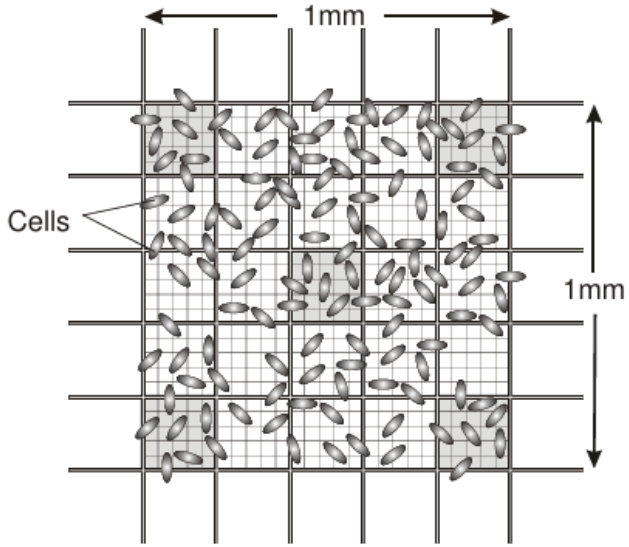


Name this type of dilution.

Serial 1

17 The apparatus that is often used to estimate the number of *Escherichia coli* in a culture is shown below.





Name this type of apparatus.

haemocytometer 1