※ 注意：請於試卷內之「非選擇題作答區」依序作答，並應註明作答之部份及題號。

Part A. (30 pts) Read the following stories, summarize their central theme precisely, and provide at least 6 take-home messages for each.

[Topic of story and the source]
New Diet in 2025? Basic Nutrition Is the Best Place to Start
Learn how understanding basic nutrition can help you achieve a healthier and more enjoyable New Year's resolution.
(https://www.discovermagazine.com/health/new-diet-in-2025-basic-nutrition-is-the-best-place-to-start, accessed 1/2025).

[Hint] You may include but not limited to
1. Basics of nutrition
2. Balance between weight loss and wellness

The survey team polled 2,174 American adults about the diet they would start in 2025. The results showed that 40 percent would eat fewer calories, 26 percent said they would eat a low-carb diet, such as keto or Atkins, and 17 percent would try intermittent fasting, among other diets.

The surveyors noticed that only 8 percent of participants were starting the Mediterranean diet, and 7 percent were going to focus on a plant-based or vegan diet. Both of these diets are considered some of the best in terms of weight loss and wellness, primarily because they consist of fresh foods versus overly processed foods.

But overall, trying a new diet can be difficult, and there is so much information out there that the choice can feel overwhelming and can trigger negative emotional responses towards food.

"Counting calories can be time-consuming and create a negative relationship with food for some people. And low-carbohydrate diets come with a range of side effects," said Roxanne Becker, MBChB, DipIBLM, with the Physicians Committee, a health advocacy group with 17,000 physician members, in a press release.

"Research has shown that plant-based diets are effective for weight loss without purposefully restricting or counting calories. This is because plants tend to be naturally lower in calorie density and higher in fiber, which promotes a feeling of fullness," said Becker in the release.

Finding the right diet isn't easy. It takes a lot of time and effort. And if the desired results aren't happening fast enough, it's easy to feel negative about the process. However, something that is crucial to remember is that changes to your health take time.

According to the Massachusetts General Hospital, if you're looking to make changes to your diet, start with basic nutrition. Instead of jumping into crash or fad diets, which can be extremely restrictive, focus on a diet that's well-balanced, sustainable, realistic, and something you can enjoy in the long term.

For some, a well-balanced diet can include Mediterranean and vegan diets, especially because they are full of whole foods, delicious recipes, and are sustainable.

"...While a plant-based diet is effective for weight loss, it also reduces the risk of obesity-related diseases like heart disease, hypertension, high cholesterol, and certain cancers, can save money, and is best for the environment," Becker said in the release.

Finding the right diet is complicated. Certain diets may help you lose weight but could be negatively impacting your mental health. That's why it's important to remember that a healthy diet is more about a lifestyle change than a quick fix. It's about finding

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balance and moving away from overly processed foods, and moving towards foods that are in the Mediterranean and plant-based diets. On top of a balanced diet, making sure you're being active can assist in your health resolutions.

(This article is not offering medical advice and should be used for informational purposes only)

[Topic of story and the source]
Health Benefits or Negative Impacts Still Uncertain for Most Supplements
Understand why there is still little scientific research behind dietary supplements and vitamins.
(https://www.discovermagazine.com/health/health-benefits-or-negative-impacts-still-uncertain-for-most-supplements, accessed 1/2025).

[Hint] You may include but not limited to
1. Are there health benefits from supplements?
2. Can supplements cause harm?

Dietary supplements are any substances that you take in addition to your regular meals and snacks. They can be chemicals, extracts from plants or animal parts, minerals, acids, herbs, or vitamins.

Supplements are typically marketed to provide consumers all kinds of different benefits, whether it's daily immune system boosts, an increase in milk production for pregnant mothers, or specific conditions.

Part of the issue for the dietary supplement industry is that companies have freedom of what to sell, as "the U.S. Food and Drug Administration (FDA) does not determine whether dietary supplements are effective before they are marketed," according to the National Institutes of Health's (NIH) Office of Dietary Supplements.

Actual peer-reviewed studies on the purported effects of supplements are slow to follow the marketing. This is due to a lack of funding and time, and there is difficulty in isolating any one activity as the cause of a positive health outcome — especially when consumers mix supplements to address their health issues.

As a result, the list of vitamins and supplements that actually have scientific evidence of any real benefit is surprising small, given the number of these products available. NIH's Office of Dietary Supplements lists just a few positive effects from a handful of supplements.

Calcium and vitamin D can improve your bone health, for example while folic acid can decrease the risk of birth defects. Omega-3 fatty acids from fish oils can help people with heart disease and a combination of vitamins C, E, zinc, copper, lutein, and zeaxanthin may slow down the vision loss of people with age-related macular degeneration.

NIH's National Center for Complementary and Integrative Health (NCCIH) notes that many vitamins and minerals are essential for our bodies, including vitamins A, C, D, E, K, and various types of vitamin B. Essential minerals include calcium, phosphorus, potassium, sodium, chloride, magnesium, iron, zinc, iodine, sulfur, cobalt, copper, fluoride, manganese, and selenium. But it's important to note that we get many of these vitamins and minerals through eating healthy food.
Whether taking these as supplements actually helps our bodies still needs more research. Nonetheless, in some cases, taking supplements doesn't hurt. NCCIH notes that taking your daily dose of multivitamin is unlikely to pose any risks for healthy people, though most studies have shown little or no effects from multivitamins on the risk of health problems.

Some research has revealed that even some of the most widely marketed supplements have little or no value. A Canadian study

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published in Current Nutrition Reports in 2013, for example, reported that echinacea had little or no evidence to shorten the symptoms of colds or the flu, despite marketing.

Even primrose oil appeared to have no value in treating eczema, though it may be useful for rheumatoid arthritis and breast pain, and more research is needed. Gingko doesn't have much evidence of improving memory for Alzheimer's patients, older adults or those suffering from dementia, and there is little evidence that ginseng does anything.

"[Ginseng] is among the most popular of herbs, and it is used for a variety of reasons, the most common of which are increased sense of well-being, stamina, and improved mental and physical performance," the authors wrote in the study. "Overall, there is very little solid evidence that ginseng lives up to the many lofty claims of its efficacy."

There are a number of potential ways that supplements can cause harm, directly and indirectly. Taking more minerals, vitamins or other supplements than your liver can handle can cause problems — and it doesn't matter whether the ingredients are chemical or natural.

"Many dietary supplements (and some prescription drugs) come from natural sources, but 'natural' does not always mean 'safe,'" according to the NCCIH. "For example, the kava plant is a member of the pepper family but taking kava supplements can cause liver disease."

There are also some specific examples of negative effects from supplements. The Office of Dietary Supplements notes that vitamin K can reduce the ability of some blood thinners to prevent clotting, while St. John's wort can reduce the effectiveness of medicines like birth control pills, antidepressants, and heart and anti-HIV medicines. Supplements that are antioxidants can reduce the effectiveness of some types of chemotherapy for cancer treatments.

Even daily multivitamins may have negative effects to certain consumers. According to the NCCIH, smokers or former smokers may want to avoid multivitamins with high levels of vitamin A, which some studies have linked to increased risk of lung cancer.

In fact, too much vitamin A can create headaches, liver damage, reduce bone strength, and even cause birth defects. Meanwhile, too much iron can induce nausea and may also cause liver and other organ damage.

More research is needed. Indirectly, reliance on supplements may have a negative effect on the motivation of consumers to engage in other healthy activities like exercising, healthy eating in general, or limiting intake of alcohol, tobacco, or other harmful substances.

One consumer study found evidence that "drug marketing undermines intentions to engage in health-protective behaviors."

The authors found that essentially, some may believe that supplements can compensate for not going to the gym or drinking too much on the weekends. They also found that, paradoxically, some people who take a lot of supplements may also associate this supplement-taking behavior with their own poor health.

This perception "reduces self-efficacy and perceived ability to engage in complementary health-protective behaviors," the authors wrote in the study.

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Part B. (20 pts) Read the following paragraph and answer the questions below.

Bacteria can be divided into four groups—psychrophiles, psychrotrophs, mesophiles, and thermophiles —according to their growth and reproduction responses to environmental temperature. The minimal growth temperature refers to the lowest temperature at which bacterial reproduction occurs. The optimum growth temperature represents the temperature at which bacteria reproduce most efficiently, while the maximum growth temperature is the highest temperature at which bacterial growth can occur; beyond this point, reproduction ceases entirely. Tables 1 and 2 provide an overview of bacterial types with their respective growth temperature ranges (Table 1) and common examples of each group (Table 2).

Table 1 Classification of Bacteria

Growth temperatures or ranges (°C)			
Categories	Minimum	Optimum	Maximum
Psychrophiles	< 0	0-15	<24
Psychrotrophs	0-5	15	30
Mesophiles	5-25	20-45	30-50
Thermophiles	25-45	50-60	>80

Table 2: Cardinal Growth Points for Various Bacteria

Cardinal growth points (°C)			
Bacteria name	Minimum	Optimum	Maximum
Streptococcus pyogenes	20	38	41
Streptococcus pneumoniae	24	38	42
Clostridium perfringens	15	46	50
Escherichia coli	11	38	45
Listeria monocytogenes	1	34	45
Micrococcus cryophilus	0	14	30
Staphylococcus aureus	11	38	45
Anoxybacillus flavithermus	30	61	72
Bacillus flavothermus	30	59	72

1. Which of the following bacteria listed does not belong to the mesophiles?
   A. *Streptococcus pneumoniae*
   B. *Staphylococcus aureus*
   C. *Listeria monocytogenes*
   D. *Clostridium perfringens*

2. The most commonly occurring category of bacteria listed in **Table 2** is:
   A. Psychrophile
   B. Psychrotroph
   C. Mesophile
   D. Thermophile

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3. The average human body temperature is 37°C. Based on **Table 2**, which of the following bacteria is most likely to grow optimally in the human body?

A. *Anoxybacillus flavithermus*
B. *Clostridium perfringens*
C. *Listeria monocytogenes*
D. *Escherichia coli*

4. Microbiologists have discovered a new bacterium that reproduces most efficiently at 55°C and stops growing at temperatures exceeding 65°C. This bacterium is most likely classified as a:

A. Psychrophile
B. Psychrotroph
C. Mesotroph
D. Thermophile

5. Which of the following bacteria is a psychrotroph?

A. *Listeria monocytogenes*
B. *Escherichia coli*
C. *Anoxybacillus flavithermus*
D. *Micrococcus cryophilus*

6. The type of bacteria mentioned in Table 2 that does not align precisely with any of the categories outlined in Table 1 is:

A. *Clostridium perfringens*
B. *Listeria monocytogenes*
C. *Micrococcus cryophilus*
D. *Streptococcus pneumoniae*

7. Based on the provided information, at what temperature does *Listeria onocytogenes* cease reproduction?

A. >1°C, but <34°C
B. >10°C, but <34°C
C. >1°C, but <45°C
D. >34°C, but <45°C

8. According to the information in Table 2, which type of bacteria has the narrowest growth temperature range?

A. *Listeria monocytogenes*
B. *Streptococcus pneumoniae*
C. *Streptococcus pyogenes*
D. *Micrococcus cryophilus*

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Part C. (30 points) Reading comprehension. Please read each paragraph and answer the following questions.

Genomes across the tree of life are organized and constantly reshaped by active processes within the cell. Structural maintenance of chromosomes (SMC) protein complexes are important, owing to their major role in chromosome organization. The three major members of the SMC family in eukaryotes are condensin, cohesin, and SMC5/6. All three are capable of manipulating DNA via an ATP-dependent process called DNA loop extrusion, where loops are created and enlarged stepwise by ATP-binding events to the SMC complex. Condensin mainly extrudes DNA loops during mitosis to compact sister chromatids and facilitate chromosome segregation. Cohesin organizes interphase chromosomes by looping DNA between convergently oriented CCCTC-binding factor (CTCF) proteins, giving rise to topologically associated domains in population-average chromosome-conformation-capture as well as imaging experiments, which contributes to transcriptional regulation as well as genome integrity. SMC5/6 has poorly understood functions in chromosome segregation and genome maintenance.

The composition and architecture of the three SMC complexes are highly similar. Two SMC subunits dimerize at their hinge domain and are connected via coiled-coil arms to their heads. The heads harbor ATP-binding domains related to those of ATP-binding cassette (ABC) transporters, which dimerize upon ATP binding to catalyze its hydrolysis. A flexible kleisin subunit bridges the SMC ATPase heads and provides binding sites for two HAWK (HEAT protein associated with a kleisin) proteins in cohesin and condensin or KITE (kleisin-interacting tandem winged-helix element) proteins in SMC5/6.

Despite the overall highly conserved architecture of eukaryotic SMCs and their common ability to extrude DNA loops, they appear to significantly differ in their loop extrusion directionality. Here, we use the following terminology to describe the directionality: asymmetric loop extruders incorporate DNA only from one side at a time into the loop, whereas symmetric extruders reel in DNA from both sides simultaneously into the loop. Unidirectional extruders undergo subsequent phases of asymmetric extrusion (possibly interrupted by pauses) that always occur in the same direction, whereas bidirectional extruders exhibit phases of asymmetric extrusion but, here, the side from which DNA is incorporated into the loop switches direction over time. Yeast condensin is an asymmetric and unidirectional extruder, that is, it reels DNA into the loop strictly from one side, whereas human cohesin was reported to reel DNA from both sides into the loop and was thus considered to be a symmetric DNA loop extruder. Dimeric yeast SMC5/6 similarly was reported to be a symmetric extruder. Given the structural similarity of these complexes, the reason for these differences is unclear. This variability undermines whether the loop extrusion mechanism is shared among all SMC complexes. [Modified from Cell, 2025, doi: 10.1016/j.cell.2024.12.020]

9. Which of the following SMC protein complexes is primarily involved in compacting sister chromatids during mitosis?
   A. Cohesin
   B. Condensin
   C. SMC5/6
   D. HAWK

10. What is the primary function of cohesin during interphase?
   A. Facilitating chromosome segregation
   B. Compacting sister chromatids
   C. Organizing chromosomes by looping DNA between CTCF proteins
   D. Performing ATP hydrolysis

11. What distinguishes asymmetric loop extruders from symmetric ones in the context of DNA loop extrusion directionality?
   A. Asymmetric extruders reel in DNA from both sides simultaneously
   B. Symmetric extruders incorporate DNA from one side only
   C. Asymmetric extruders incorporate DNA from one side at a time
   D. Symmetric extruders can only participate in ATP hydrolysis

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Researchers from Caltech and Princeton University have discovered that bacteria growing in polymer-rich solutions, such as mucus, form extended cable-like structures that twist and interlock, resembling a "living Jell-O." This discovery is significant for studying and treating conditions like cystic fibrosis, where thickened lung mucus can foster severe bacterial infections. It also holds implications for understanding biofilms—clusters of bacteria in a self-produced polymer matrix, which are often found on surfaces like river rocks and in industrial settings, where they can lead to operational issues and health concerns.

"We found that when bacteria grow in fluids with spaghetti-like polymers, such as lung mucus, they create cable-like formations that interact like living gels," says Sujit Datta, a chemical engineering professor at Caltech. Datta, who recently transitioned from Princeton University, explored with his graduate student, Sebastian Gonzalez La Corte, how changes in mucus concentration influence bacterial growth in cystic fibrosis patients, who have elevated polymer levels. By observing E. coli growth in regular and cystic fibrosis-mimicking samples, they noted that in polymer-heavy solutions, bacteria stick together end-to-end after division, forming lengthy "cables" that eventually entangle as networks.

These cables keep elongating as long as nutrients are available, sometimes reaching thousands of bacterial cells in length, regardless of the bacterial species or the exact nature of the polymer solution used—similar outcomes appeared even with synthetic polymers. While initially focused on cystic fibrosis, this finding is broadly important, as mucus is vital in multiple body sites like the gut and cervicovaginal area. It also informs biofilm research, relevant both within the human body and in external environments where they are notoriously tough to manage and treat.

Datta highlights that it is the external pressure from surrounding polymers that keeps cells together—a phenomenon described in physics as depletion interaction. Gonzalez La Corte applied this concept to develop a theoretical model predicting bacterial cable formation in polymer environments. This discovery prompts new questions about the biological roles of cable formation: it might be a defense against immune cells by increasing bacterial size, or it could potentially ease their removal from the body. The ongoing research is aimed at unraveling these possibilities. [Modified from ScienceDaily & Sci. Adv., 2025, doi: 10.1126/sciadv.adq7797]

12. What role do polymers play in the formation of bacterial cables?
    A. They separate bacterial cells after division
    B. They facilitate the bacteria's ability to swim
    C. They create an external pressure that keeps bacteria connected
    D. They provide nutrients that allow bacteria to grow indefinitely

13. What potential biological purpose might the cable formation serve for bacteria?
    A. Enhance bacterial motility
    B. Increase bacterial visibility to immune cells
    C. Make bacteria larger, possibly helping to evade immune cells
    D. Cause bacteria to disperse rapidly in mucus

14. Why is the discovery of cable formation in bacteria significant for cystic fibrosis research?
    A. It demonstrates a new way to cure the disease
    B. It shows how bacterial infections might thrive in thickened mucus
    C. It suggests that bacteria need a dry environment to form cables
    D. It reveals mucus has little effect on bacterial behavior

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Obesity is a global health concern and is associated with a higher risk of developing chronic conditions such as diabetes, cardiovascular disease and cancer, imposing a substantial strain on healthcare systems. Although energy-restricted diets are effective tools for improving body weight and cardiometabolic health, their clinical application is limited by their poor long-term adherence. Time-restricted eating (TRE) has emerged as an alternative and promising dietary intervention to address obesity. TRE limits the daily energy intake to a predetermined eating window (≤10 h) and fasting for the rest of the day (≥14 h). Current literature suggests that TRE is well tolerated, has high adherence rates with minimal side effects and results in modest reductions in body weight and slight improvements in cardiometabolic health in individuals with overweight or obesity.

However, some important aspects related to the effects of TRE remain uncertain, such as its impact on specific ectopic fat deposition6, particularly on visceral adipose tissue (VAT), an important risk factor for cardiometabolic morbidity and mortality. Another important question regarding TRE is whether the timing of the eating window affects its efficacy. While previous TRE studies have typically used arbitrary clock times to determine the timing of food intake, pilot clinical studies have suggested that earlier eating windows during TRE may yield better cardiometabolic benefits, despite similar body weight loss. However, drawing definitive conclusions from existing studies comparing early with late TRE is challenging owing to shortcomings such as their relatively short duration (≤8 weeks), small sample sizes or lack of randomization. Furthermore, there is a notable gap in the literature regarding comparing early or late TRE with a self-selected TRE, which may be of great interest. Allowing participants to select the TRE window that aligns with their personal preferences and schedule may further improve adherence and acceptability and, thus, enhance efficacy. Consequently, more research is needed to provide more conclusive evidence on the optimal timing of the eating window during TRE and its effects on VAT and cardiometabolic health.

The main aim of the present study was to investigate the effects of three distinct TRE schedules—an 8 h eating window in the early part of the day (early TRE), an 8 h window later in the day (late TRE) and a participant-selected eating window (self-selected TRE)—combined with usual care (UC, which included 2-monthly sessions of a group nutritional education program based on the Mediterranean diet) versus UC alone, over 12 weeks, focusing on VAT changes (measured by magnetic resonance imaging (MRI)) and cardiometabolic health among men and women with overweight or obesity.

The primary outcome was VAT changes measured by magnetic resonance imaging. A total of 197 participants were randomized to UC ($n = 49$), early TRE ($n = 49$), late TRE ($n = 52$) or self-selected TRE ($n = 47$). No significant differences were found in VAT changes between early TRE (mean difference (MD): −4%; 95% confidence interval (CI), −12 to 4; $P = 0.87$), late TRE (MD: −6%; 95% CI, −13 to 2; $P = 0.31$) and self-selected TRE (MD: −3%; 95% CI, −11 to 5; $P \geq 0.99$) compared with UC, nor among the TRE groups (all $P \geq 0.99$). No serious adverse events occurred; five participants reported mild adverse events. Adherence was high (85–88%) across TRE groups. These findings suggest that adding TRE, irrespective of eating window timing, offers no additional benefit over a Mediterranean diet alone in reducing VAT. TRE appears to be a safe, well-tolerated and feasible dietary approach for adults with overweight or obesity. [Modified from Nat. Med., 2025. doi: 10.1038/s41591-024-03375-y]

15. What is the primary outcome measured in the study investigating TRE?
    A. Body mass index changes
    B. VAT changes
    C. Cardiometabolic health improvements
    D. Adherence rates to the diet

16. How did the adherence rates to the time-restricted eating (TRE) schedules compare among participants?
    A. Low adherence rates were noted across all groups
    B. Adherence was moderately high (60-70%)
    C. High adherence rates (85-88%) were observed across TRE groups
    D. Participants completely failed to adhere to the schedules

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17. What conclusion can be drawn about the efficacy of different TRE schedules compared to usual care in reducing VAT?
    A. Early TRE was significantly more effective than late TRE
    B. All TRE schedules provided significant reductions in VAT compared to usual care
    C. There were no significant differences in VAT changes among TRE schedules compared to usual care
    D. Self-selected TRE resulted in the greatest improvement in VAT

Snakebite envenoming represents a public health threat in many developing regions, notably low-resource settings in sub-Saharan Africa, South Asia, Papua New Guinea and Latin America. With over two million annual cases, snakebites result in 100,000 fatalities and 300,000 permanent disabilities. In 2017, the World Health Organization listed snakebite envenoming as a highest-priority neglected tropical disease. Nonetheless, limited resources have been dedicated to improving the current antivenom treatments. These therapies rely on plasma-derived polyclonal antibodies from hyperimmunized animals, complemented by medical and surgical care. Although instrumental in saving lives, antivenom accessibility is hindered by high production costs and inadequate cold-chain infrastructure in remote areas. Serious adverse effects, including anaphylaxis and pyrogenic reactions, represent additional challenges during antivenom administration. Furthermore, these treatments are often ineffective in counteracting neurotoxicity and tissue necrosis owing to suboptimal concentrations of neutralizing antibodies against three-finger toxins (3FTxs). This inefficacy stems from the limited immunogenicity of 3FTxs in antivenom-producing animals, resulting in a failure to elicit a strong antibody response. Additional issues arise because of the delayed administration of antivenom treatment. Antibody and non-antibody-based therapeutics have been tested in preclinical studies, but the development of these types of molecules requires either immunization of animals or the development of large libraries that require extensive selection, screening and optimization efforts.

We reasoned that de novo design approaches could have advantages over the traditional methods of antivenom development. First, de novo protein design does not rely on animal immunization and yields proteins that can be manufactured using recombinant DNA technology, thereby creating a source for the continuous production of products with limited batch-to-batch variation. Second, computational design enables the creation of binding proteins with high affinity and specificity without needing extensive experimental screening programmes that often rely on pure toxins, which can be challenging to isolate from whole venoms or generate via recombinant expression. Third, the small size of the designed proteins could offer enhanced tissue penetration compared to large antibodies, enabling rapid toxin neutralization and thereby being more effective in neutralizing local tissue damage. Fourth, designed proteins can have high thermal stability and can be produced using low-cost microbial fermentation strategies, which could help enable the development and deployment of new antivenom therapeutics at reduced cost.

Hence, we used the deep learning-based RFdiffusion method to design antivenoms for short-chain and long-chain α-neurotoxins and cytotoxins from the 3FTx snake venom toxin family. The designed proteins effectively neutralized all three 3FTx subfamilies in vitro and protected mice from a lethal neurotoxin challenge. Such potent, stable and readily manufacturable toxin-neutralizing proteins could provide the basis for safer, cost-effective and widely accessible next-generation antivenom therapeutics. Beyond snakebite, our results highlight how computational design could help democratize therapeutic discovery, particularly in resource-limited settings, by substantially reducing costs and resource requirements for the development of therapies for neglected tropical diseases. [Modified from Nature, 2025, doi: 10.1038/s41586-024-08393-x]

18. What significant public health issue is associated with snakebite envenoming, especially in low-resource settings?
    A. High rates of infection
    B. Limited access to healthcare
    C. Over two million annual cases resulting in 100,000 fatalities
    D. Widespread use of antivenoms

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19. What are the main challenges associated with current antivenom treatments mentioned in the text?
    A. High effectiveness against all venom types
    B. High production costs and inadequate cold-chain infrastructure
    C. Immediate availability and low cost
    D. Safe administration without any adverse effects

20. How does the de novo protein design approach offer advantages over traditional antivenom development?
    A. It requires animal immunization to generate antibodies
    B. It uses large antibodies for better efficacy
    C. It doesn't depend on animal immunization and allows for continuous production using recombinant DNA technology
    D. It focuses solely on generating experimental screenings of pure toxins

Part D. (20 points) Experimental comprehension. Please read the procedures and answer the following questions.

Protocol A: Western Blot (WB) – Antibody Screening

Overview: This protocol outlines the steps for performing a Western Blot to screen multiple antibodies against specific protein targets. [Modified from Nat. Protoc., 2024, doi: 10.1038/s41596-024-01095-8]

Procedures:

I. Prepare protein samples (WT and KO) and molecular weight markers. Ensure all samples are diluted to the same final concentration using a compatible loading sample buffer (1×).
II. Load the samples into a 12-well polyacrylamide gel in the following order: molecular weight marker, WT samples, and KO samples. Up to 4 antibodies can be tested on a single gel.
III. Run the gel using the appropriate running buffer until the dye front reaches approximately 3 mm from the bottom of the gel.
IV. Transfer the proteins from the gel to a nitrocellulose membrane using a wet transfer system. Follow the manufacturer's recommended transfer conditions (e.g., 45 V for 1 hour).
V. Block the membrane with WB blocking solution for 1 hour at room temperature to prevent non-specific binding of antibodies.
VI. Dilute the primary antibodies in the blocking solution according to the manufacturer's recommendations. Incubate the membrane with the diluted primary antibodies overnight at 4 °C.
VII. Wash the membrane 3 times with TBST (Tris-buffered saline with Tween-20) for 5 minutes each to remove unbound antibodies.
VIII. Incubate the membrane with a suitable HRP-conjugated secondary antibody for 1 hour at room temperature. Dilute the secondary antibody in blocking solution.
IX. Wash the membrane again 3 times with TBST for 5 minutes each to remove excess secondary antibody.
X. Apply the chemiluminescent substrate to the membrane and visualize the protein bands using a suitable imaging system.

21. What is the purpose of blocking the membrane during the Western Blot procedure?
    A. To enhance protein transfer
    B. To prevent non-specific binding of antibodies
    C. To increase the signal intensity
    D. To stabilize the proteins

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22. How long should the primary antibodies be incubated with the membrane in this protocol?
    A. 30 minutes
    B. 1 hour
    C. Overnight at 4 °C
    D. 2 hours at room temperature

23. What is the recommended washing buffer used after primary and secondary antibody incubations in this protocol?
    A. PBS
    B. TBST
    C. RIPA buffer
    D. Deionized water

24. What type of detection method is used in Western Blotting for this protocol?
    A. Colorimetric detection
    B. Fluorescent detection
    C. Chemiluminescent detection
    D. Radioactive detection

Protocol B: Human Cerebellar Organoid Development

Overview: This describes how to make human cerebellum organoids from pluripotent stem cells. Patterning, progenitor growth, and maturation are crucial for organoid development and characterization. [Modified from Nat. Protoc., 2024, doi: 10.1038/s41596-024-01093-w]

Stage 1: Initial Patterning (Days 0-16)

I. Stem Cell Culture: Start with a well-defined human pluripotent stem cell (hPSC) line in a feeder-free system using a specific medium to sustain pluripotency. Ensure cells are healthy, at low passage numbers, and maintain uniform morphology before differentiation begins.

II. Dissociation and Aggregation: hPSCs are broken down into single cells with enzymes and then reassembled into embryoid bodies (EBs) in a non-adhesive culture setting to foster three-dimensional growth. The success of organoid formation hinges on precise cell density and aggregation techniques.

III. Neuroectoderm Induction: EBs are cultivated in a chemically defined medium (gfCDM+i) without growth factors, prompting the pluripotent cells to develop into neuroectoderm. Dual SMAD inhibition with specific inhibitors (such as SB431542 and Noggin) increases efficiency.

IV. Caudalization: Following several days, the neuroectoderm is directed toward hindbrain development by adding specific growth factors like CHIR99021 (a WNT agonist) and FGF8b. These factors, administered in carefully monitored amounts and timelines, promote the emergence of the isthmic organizer, essential for cerebellum development.

Stage 2: Progenitor Cell Expansion (Days 16-30)

V. Transfer to Shaking Culture: The organoids, now neuroepithelial buds, are moved to larger culture dishes and rotated on an orbital shaker to improve nutrient and oxygen distribution, essential for growth.

VI. Expansion Medium: Organoids are grown in a specialized medium (CerDM2) that supports cerebellar progenitor proliferation.

Stage 3: Maturation (Days 30 onwards)

VII. Long-Term Culture: Organoids are continuously cultured in an optimized final medium (CerDM3) that aids in the survival and maturation of diverse cell types.

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VIII. SDF1a Addition: Introducing SDF1a between days 30 and 60 fosters granule cell progenitor migration, enriching the cerebellar structure.

IX. Extended Maturation: Organoids can mature over several months, allowing the development of all major cerebellar cell types, including Purkinje cells.

X. Analysis: After suitable maturation, various techniques like immunohistochemistry, single-cell RNA sequencing, calcium imaging, and electrophysiology are used for organoid characterization.

25. What technique is primarily employed in Stage 1 to direct pluripotent stem cells toward a neuroectodermal identity?
    A. FGF2 stimulation
    B. Dual SMAD inhibition
    C. WNT inhibition
    D. BMP stimulation

26. Which growth factors are utilized in Stage 1 to facilitate caudalization to a hindbrain identity?
    A. TGF-β and BMP
    B. WNT and FGF8b
    C. SHH and RA
    D. FGF2 and Noggin

27. What is the role of SDF1a in the Stage 3 process?
    A. To inhibit Purkinje cell maturation
    B. To encourage the expansion of inhibitory progenitors
    C. To boost migration of granule cell progenitors
    D. To prevent the formation of neuroectoderm

28. What is the primary goal during the maturation stage of human cerebellar organoids?
    A. To induce stem cell differentiation
    B. To allow for the development of all major cerebellar cell types
    C. To minimize cell interactions
    D. To initiate the process of cell dissociation

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