Adherence
Part II – Identification of Host Cell Receptors

In the following exercise you will use the agglutination reaction to identify the specific molecule acting as the host cell receptor for a pathogen or to identify which adhesin/receptor combination is responsible for adherence.

You will be assigned one of four scenarios. You will read about a pathogen and what is known about its adherence, adhesins, and receptor molecules. You will also learn about techniques used to identify receptors. You will then design an experiment to identify the host cell receptor, perform the experiment, analyze the data, and present your results to the class.

You will use the scientific method when performing these experiments. The scientific method follows the steps listed below.

1. Choose a question.
2. Design a hypothesis (possible answer) to the question.
3. Make testable predictions based on the hypothesis.
4. Design experiments to answer the question and see whether the predictions are met.
5. Perform experiments and collect data.
6. Decide whether the data support or falsify the hypothesis.
7. Present your scenario to the class.

Step 1 – Choose a question
In this case the question is chosen for you by the scenario you are assigned. Read your scenario carefully before proceeding to the steps below. Base all the following steps on your scenario.

Write the question presented in your scenario below in one sentence.

Step 2 – Design a hypothesis
Design a hypothesis related to your question. Remember that a hypothesis is a possible answer to the question. A hypothesis is also often described as an educated guess. A guess, because you do not know in advance whether your hypothesis will be correct or not, and educated because you base it on the knowledge you already have.

You can also design a series of hypotheses (different possible answers) for the question. This technique is often used because one way of adding support to a hypothesis is to falsify other possible hypotheses (answers).

For example, for the previous experiment using agglutination reactions to determine which *E. coli* strains can adhere to intestinal cells, some possible hypotheses would be:
Strain 1 will adhere to intestinal cells.
Strain 1 will not adhere to intestinal cells.
Strain 2 will adhere to intestinal cells.

Write your hypothesis (or hypotheses) below.

Step 3 - Make predictions based on your hypothesis.
Predictions are experimental outcomes that will be true if your hypothesis is correct. Predictions can easily be written as if – then statements.

For example for the first hypothesis above a prediction would be
If E. coli strain 1 can bind intestinal cells, then agglutination will be seen when intestinal cells are mixed with E. coli strain 1.

Write your prediction(s) below.

Step 4 – Design an experiment to answer your question

In this case you will design an experiment using an agglutination reaction to answer your question. Be sure to include controls that will show that your reagents are good and that the procedure is working.

For example, for the experiment presented in part I – good controls would be
- *Intestinal cells alone (negative control to show that intestinal cells alone do not agglutinate)*
- *E. coli strain 1 alone (negative control to show that E. coli alone do not agglutinate)*
- *Intestinal cells plus an E. coli strain known to bind intestinal cells (positive control to show the agglutination reaction works for a strain known to adhere).*

On a separate sheet of paper, design an experiment to answer your question.

Step 5 – Perform you experiment and collect your results!
On a separate sheet of paper, note the results of your experiment, including results to all controls.

Step 6 – Decide whether your results support or falsify your hypothesis.
Results of an experiment never prove a hypothesis. Hypotheses can never be proven. Instead, experimental results support hypotheses.

Hypotheses can, however, be falsified. Results can definitely show that a hypothesis is false. Note that it is not considered bad or a failure to prove a hypothesis false. By proving a hypothesis false, you eliminate one possible answer to your question.

Often the results of an experiment neither support nor falsify a hypothesis. There are many ways in which this happens.

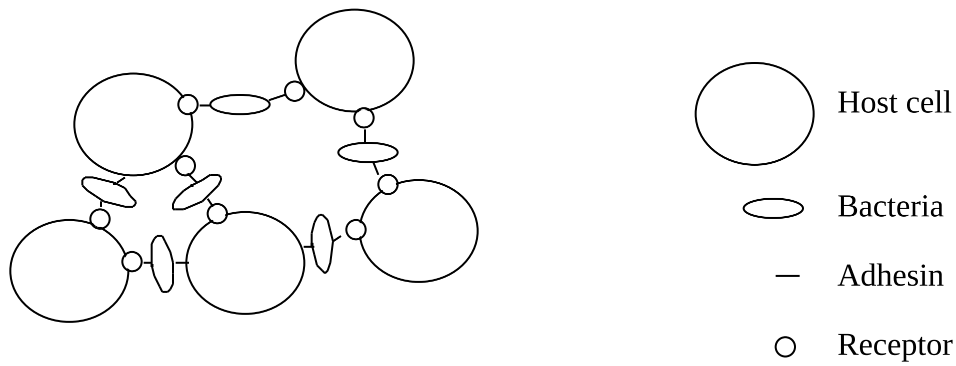
Controls show that one or more of the reagents were not working.
Controls show that the experiment was not designed to answer the intended question.
Results show that the experiment was not designed to answer the intended question.

When the results of an experiment neither support nor falsify a hypothesis it is necessary to repeat the experiment (if there is a problem with the reagents), redesign the experiment (if it is not answering the intended question), or sometimes even to rethink the question and hypothesis. As was the case with falsifying hypotheses, this is not bad science or a failure. Rather it is good science to recognize the problems with experiments and questions and to repeat and redesign them as necessary.

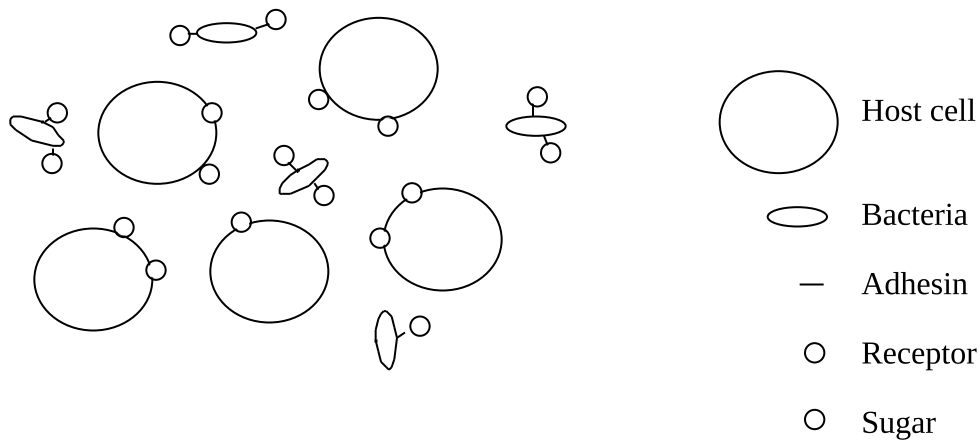
Below write a brief paragraph summarizing how your results support or falsify your hypothesis. If necessary, include how you would repeat or redesign your experiment to answer the intended question.

Scenario I – Identification of receptor for *E. coli* on human bladder cells.
Strains of *E.coli* that cause urinary tract infections have fimbrae called Type I fimbrae that are known to be the adhesin by which these bacteria attach to bladder cells. The specific part of the fimbrae that mediates attachment is called FimH. It is known that FimH binds to a sugar containing receptor. Your goal is to identify the sugar that is serving as the receptor.

A technique commonly used to identify structures serving as receptors is agglutination inhibition. In this type of experiment, different sugars are tested for their ability to inhibit agglutination. Sugars that are parts of the receptor will inhibit agglutination when added to the agglutination reaction by binding to the pathogen so the pathogen is unable to bind the receptor.



Normal Agglutination Reaction



Inhibition of Agglutination by a Sugar

As shown above, sugars that serve as the receptor on the host cell will inhibit agglutination by binding to all the adhesin on the bacteria. Once all the adhesin is bound, it can no longer bind to the receptor on the host cells, and will no longer be able to agglutinate the host cells.

You will design an experiment to test which of the four following sugars – fructose, galactose, glucose, or mannose- is serving as the receptor for attachment of *E. coli* to bladder cells.

You have the following materials to work with.

Solutions of:
Bladder cells
E. coli with Type I fimbrae
Fructose
Galactose
Glucose
Mannose

Toothpicks
Trays with indentations

Scenario II – Identification of adhesins responsible for the binding of *Bordetella pertussis* to bronchial cells.

Bordetella pertussis causes whooping cough. Studies using techniques similar to agglutination tests have shown that many of the molecules on the surface of this bacterium bind to various receptors on human cells.

Adhesin	Receptor(s)
Fimbrae	VLA-5
Filamentous Hemagglutanin	integrin, carbohydrates
Pertussis Toxin	heparin
Hemolysin	unknown
Adenylate Cyclase	unknown

Although it is known that many of molecules found on the surface of *B. pertussis* can bind to host cells, it is not known which of these several molecules is most important for attachment. To study the relative importance of the different surface molecules of *B. pertussis*, scientists have generated mutant strains of *B. pertussis*, with each strain lacking one potential adhesin. By comparing the binding of these different mutants, scientists can determine which adhesin(s) is(are) most important.

Your goal is to determine which of the potential adhesins *B. pertussis* uses to adhere to bronchial cells. You have the following materials to work with.

Bronchial Cells (NCI-H292)
Wild Type *Bordetella* (non-mutant)
Fimbrae mutant
Filamentous Hemagglutanin mutant
Pertussis Toxin mutant
Hemolysin mutant
Adenylate Cyclase mutant

Toothpicks
Trays with indentations

When you have completed your study, compare your results to those with people working with scenario III - Identification of adhesin responsible for the binding of *Bordetella pertussis* to laryngeal cells.

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Your goal is to determine which of the potential adhesins *B. pertussis* uses to adhere to laryngeal cells. You have the following materials to work with.

Laryngeal Cells (HEp-2)
Wild Type *Bordetella* (non-mutant)
Fimbrae mutant
Filamentous Hemagglutanin mutant
Pertussis Toxin mutant
Hemolysin mutant
Adenylate Cyclase mutant

Toothpicks
Trays with indentations

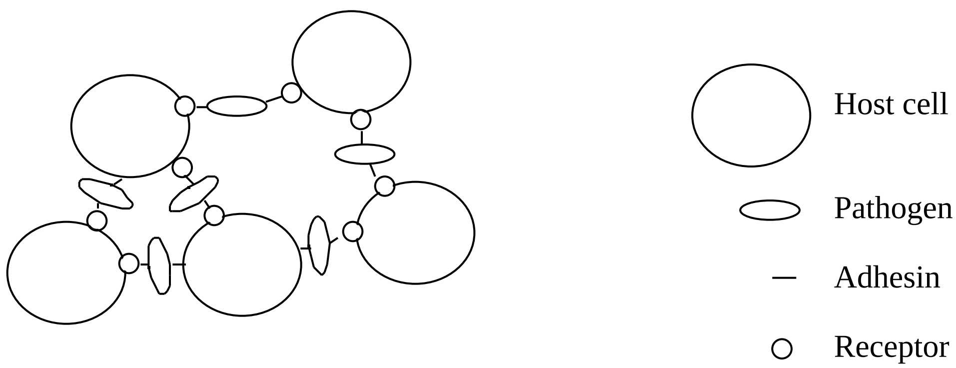
When you have completed your study, compare your results to those with people working with scenario II - Identification of adhesin responsible for the binding of *Bordetella pertussis* to bronchial cells.

Scenario IV – Identification of a coreceptor for HIV virus on human T cells using antibodies to inhibit agglutination.

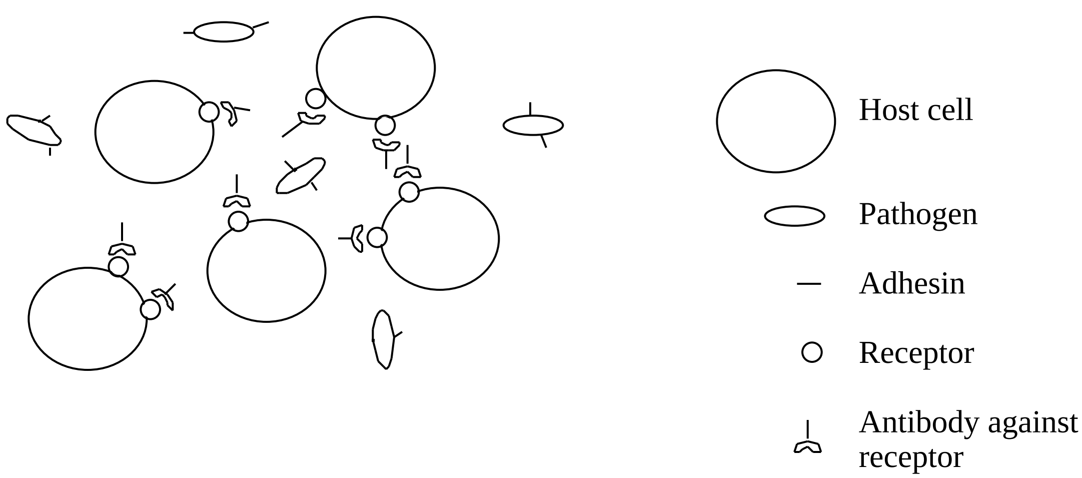
It is known that early in infection with HIV, the virus infects primarily macrophages. As infection progresses, the HIV gradually infects more T cells and less macrophages. As more and more T cells are infected and destroyed, the patient's immune system collapses.

It is also known that the receptor for HIV is CD4, a molecule found primarily on a subclass of T cells. This molecule is also found on macrophages. It is also known that additional molecules serve as a receptor for HIV. By studying individuals at high risk for HIV who did not seem to become infected with the virus, scientists identified a coreceptor molecule – a second molecule to which HIV binds. This molecule, CCR5 (chemokine receptor 5), is found on macrophages but not T cells. Your goal is to determine what molecule is serving as the HIV coreceptor on T cells.

One method scientists use to identify receptors is to block adherence with antibodies.



Normal Agglutination Reaction



Inhibition of Agglutination by an Antibody Against the Receptor

As shown on the previous page, antibodies that are specific for the molecule serving as the receptor on the host cell will inhibit agglutination by binding to all the receptor sites on the host cells. Once all the receptor is bound, it can no longer bind to the adhesin on the pathogen. Thus, the pathogen will no longer be able to agglutinate the host cells.

You will design an experiment to test which of the three following molecules – CD3, CCR5, or CXCR4 - is serving as the receptor for attachment of HIV to T cells. CD3 is an immune system marker found on T cells. CCR5 and CXCR4 are both chemokines, small molecules that signal the immune system.

You have the following materials to work with.

Solutions of:
HIV (fake of course)
Anti-CD4
Anti-CD3
Anti-CCR5
Anti-CXCR4
T cells

Toothpicks
Trays with indentations