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**Adherence**  
**Part 1**  
**Agglutination Reactions to Observe Attachment**  
**Teacher instructions**

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## **Introduction**

Attachment of bacteria to cells in our body is an important first step to causing infection or disease. In this exercise you will learn one of the ways scientists use to measure attachment or adherence of bacteria to human cells. You will then use what you have learned to identify specific attachment sites (receptors) to which pathogens bind.

## **Host Defenses**

Start by reviewing what you know about the defenses of the following parts of your body. What ways does your body use to try to keep microbes from infecting these areas? If you want you can use the following web sites to help you.

### Intestines

<http://www.Colorado.EDU/Research/hughes/actidhpsstomach.html>

*peristalsis – contraction of the smooth muscles of the intestines that moves material through normal flora – competition for space and nutrients*

### Throat and Bronchi

<http://www.Colorado.EDU/Research/hughes/actidhpsmucociliary.html>

*mucus – covers surfaces and prevents potential pathogens from attaching mucociliary escalator – cilia move pathogens trapped in mucus up and out throat has normal flora – compete with pathogens for space and nutrients*

Note that both the intestines and the throat and bronchi have many defenses to prevent potential invaders from attaching. Our normal flora plays a big role in this. There are so many bacteria in our intestines that the entire surface is completely covered with bacteria such that there is no space for potential invaders to get in. In addition, our intestines are constant contracting, a process called peristalsis, such that bacteria unable to attach to our cells are immediately swept out of the intestines. Our upper respiratory tract is covered with a mucus layer, to help prevent invading organisms from attaching to our cells and cilia of the mucociliary escalator push trapped bacteria up and out of our body.

## Attachment

In order to remain in the host, potential invaders must first find a way to attach or adhere to the host's cells. Thus, the initial step in all infections is attachment or adherence. If a potential invader can find an open site and attach, it can remain in place and not be swept out of the host.

Attachment is a very specific process. Potential invaders do not just bind randomly to any surface using a general sort of glue. This would be ineffective since microbes would then be stuck to surfaces other than the host they were trying to infect. Rather microbes have specific molecules on their surface, called **adhesins**, that bind to specific **receptors** on the host cell. The way that adhesins on invaders bind to host cell receptors is analogous to a key fitting into a lock. A specific adhesin (or key) will only recognize and bind to a receptor (or lock) with the correct shape. As shown below the adhesin on the invader has a specific triangular shape that fits into the receptor on the host cell.



A key characteristic of molecules that serve as either adhesins or receptors is that they have a very unique, specific 3-dimensional structure that allows the key in lock fit.

Adhesins on microorganisms are often proteins, although they can also be polysaccharides. Viral adhesins are most often proteins that are part of the viral capsid or protein shell. Bacterial adhesins are often fimbriae, which are long, thin filaments that are made of protein. (Note that fimbriae are sometimes called pili). In bacteria, other surface proteins also serve as adhesins and rarely capsular polysaccharides serve as bacterial adhesins as well. Adhesins are molecules that evolved specifically to allow a pathogen to bind to host cells.

Host cell receptors can also be either proteins or carbohydrates. However, host cell receptors did not evolve in order to allow pathogens to bind to them. Rather host cell receptors serve some function for the host cell, and the pathogen takes advantage of this receptor molecule in order to attach. For example, HIV attaches to a molecule called CD4 on the surface of T-cells. The CD4 molecule plays an important role in helping the T-cell function as an immune system cell by serving as a signal to other immune system cells with which the T-cell interacts.

Some examples of pathogens, the adhesins they use to attach, and the specific receptors they bind to are shown in the table below

Pathogen	Adhesin	Host Cell Receptor
Influenza virus	Hemagglutinin	Neuraminic Acid
<i>Plasmodium vivax</i> (malaria)	Merozoite protein	Duffy blood group antigen
Rhinovirus (common cold)	Capsid protein	ICAM-1
<i>Haemophilus influenza</i> (meningitis)	HifE fimbriae	Sialylyganglioside-GM1
<i>Klebsiella pneumoniae</i>	MrkD fimbriae	Type V Collagen
HIV	Gp120 protein	CD4

The specificity of the attachment process can be a possible explanation for both host range and tissue tropism.

**Host range** refers to the different species of hosts a given pathogen can infect. One of the factors limiting host range is which hosts have the receptor to which the pathogen binds (factors other than attachment may also limit host range). Some pathogens have a very **narrow host range** and can infect only one host species. Examples of disease with a narrow host range that infect only humans are smallpox and polio. Other pathogens can infect many different host species and are thus said to have a **broad host range**. Examples of diseases that infect more than one species are *Yersinia pestis* (bubonic and pneumonic plague) that infects both humans and many different rodents and rabies virus which infects humans, dogs, and several rodents.

**Tissue tropism** refers to the different tissues within a given host that are infected by the pathogen. For example, cold viruses infect only the upper respiratory tract. Attachment is one of several factors that determine tissue tropism. Some pathogens can bind only one type of tissue. Others can attach to several different tissue types within given host.

Similarly, variations in both hosts and pathogens lead to differences in virulence (how sick a given pathogen can make us). For example, within humans, differences in the level of expression of a molecule serving as a receptor for a pathogen will lead to varying levels of susceptibility to that pathogen. Some individuals may completely lack a given receptor and consequently will be resistant to infection with that pathogen. Others may express high levels of that receptor and thus be highly susceptible to infection by that pathogen. Differences in the levels of adhesin expressed by different strains of the same species of pathogen will similarly lead to different levels of virulence.

Answer the questions on host range and variations in virulence on the following pages.

### Questions on Host Range

Study the diagram on the following page and then answer the following questions.

1. Pathogen A will bind to cells from which species?

*Human*

2. Pathogen B will bind to cells from which species?

*All*

3. Which pathogen has a broad host range?

*Pathogen B*

4. Which pathogen has a narrow host range?

*Pathogen A*

5. Can pathogen A infect mice? Explain your answer.

*No, because it is unable to attach to mouse cells because mouse cells do not have a receptor to which pathogen A can bind.*

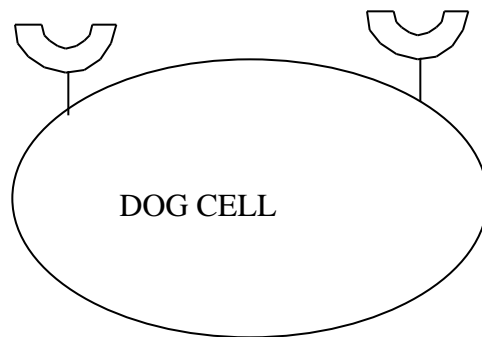
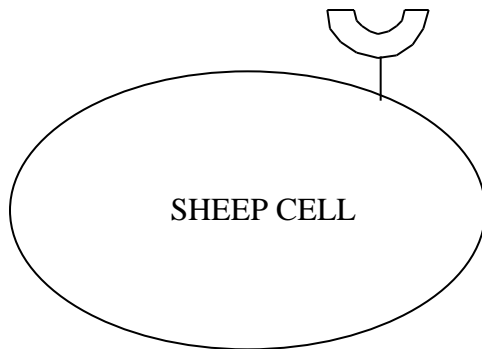
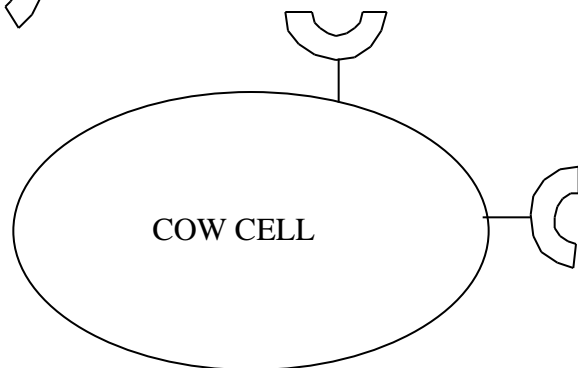
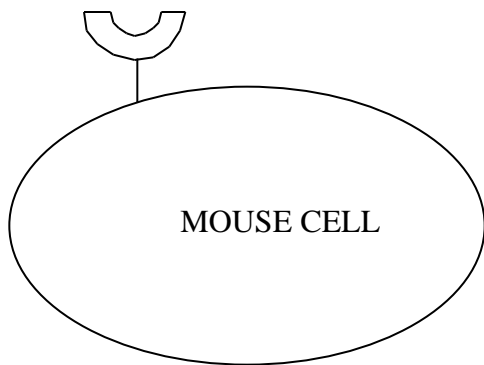
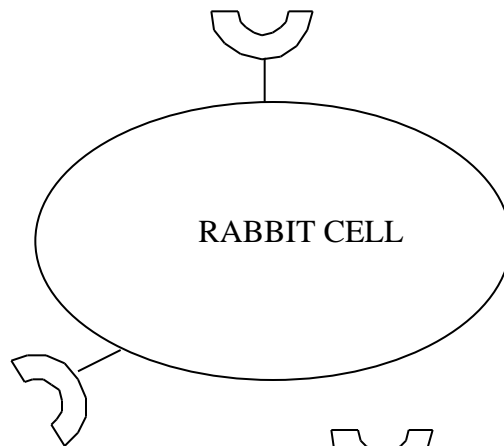
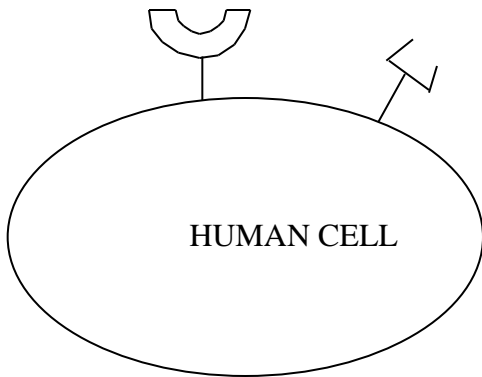
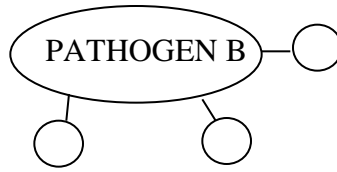
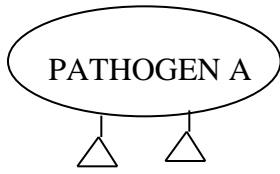
6. For pathogen B attaching to human cells, describe the shape of both the adhesin and the receptor that mediate attachment. Be sure to identify which is the adhesin and which is the receptor.

*The adhesin, found on pathogen B is shaped like a circle.*

*The receptor found on the host cell is shaped like an arc that will fit around the outside of the circle.*

7. On a separate piece of paper draw pathogens and host cells to demonstrate tissue tropism -- why some pathogens might cause infect both respiratory and gastrointestinal tract cells whereas other can infect only one or the other. Assume that the difference is at the level of attachment, not some later step in the pathogenic process.

# Pathogens and Cells for Host Range Questions



### Questions on Differences in Virulence

Study the diagram on the following page and then answer the following questions.

1. Pathogen A can bind to cells from which individuals?

*Pathogen A can bind cells from individuals 1 and 2*

2. Can individual 3 be infected by pathogen A? Explain your answer.

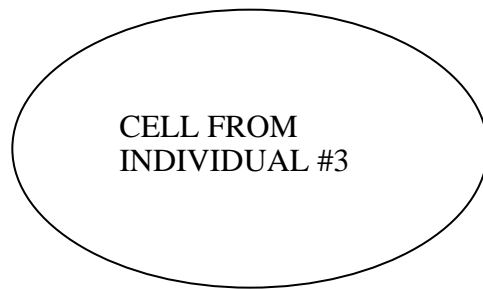
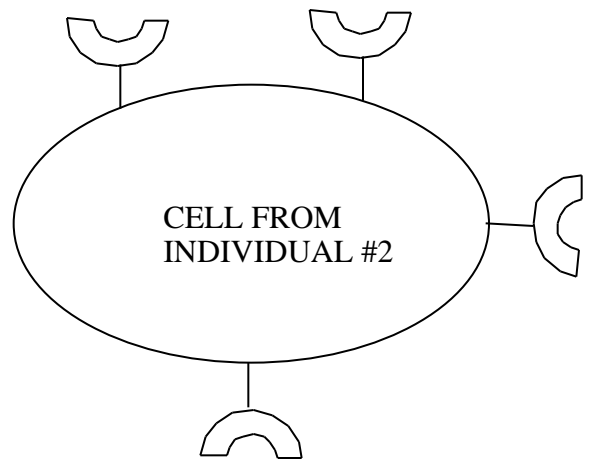
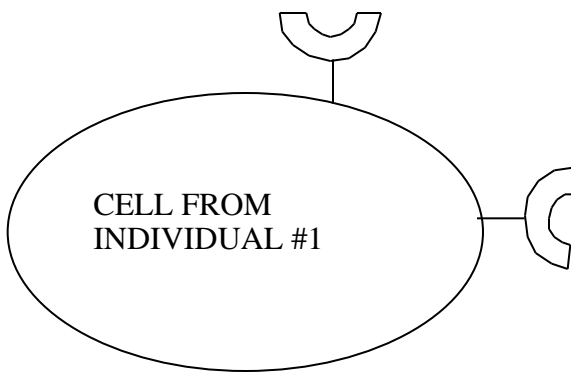
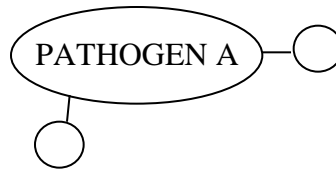
*No, individual 3 does not have the receptor for pathogen A. Thus pathogen A can not attach and will be eliminated before it can cause infection.*

3. Which individual do you predict would experience more symptoms of infection with pathogen A – individual 1 or individual 2? Explain your answer.

*Individual 2 is likely to experience more symptoms than individual 1 because more pathogens can bind to cells from individual 2. This assumes that both individuals receive the same dose of pathogen A, have the same state of health preceding infection, etc.*

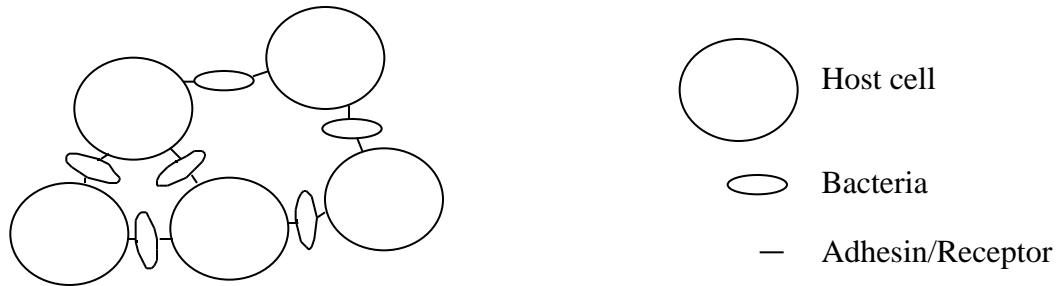
4. On a separate piece of paper draw pathogens and host cells to demonstrate why some pathogens might cause less symptoms than others, even if they are using the same adhesins and receptors. Assume that the difference is at the level of attachment, not some later step in the pathogenic process.

**Pathogens and Cells for Questions on Differences in Virulence**



## Agglutination - Identification of *E.coli* strains that can attach to human cells

In this activity you will test the ability of different strains of *E. coli* to adhere to human intestinal cells. The type of reaction you will be performing is an agglutination reaction. If a bacteria can attach to the host cell, it will cause what is known as agglutination, or clumping of the host cells such that large complexes are formed (see sketch below). This agglutination leads to clumps that are visible to the naked eye. Agglutination reactions are used as a good first test of whether a bacterial strain or species can bind to a particular cell type.



### Procedure

1. Obtain a glass or slide or plate with circular indentations. Label the glass next to the wells to identify the wells 1 – 6.
2. Place a drop of solution containing a suspension of human intestinal cells into wells 1 - 6.
3. Add a drop of bacteria #1 to well 1. Mix with a toothpick.
4. Repeat for bacteria #2 through 5.
5. Note your results in the table below. If the bacteria were able to attach to the intestinal cells, they will cause them to clump.

<i>E.coli</i> Strain	Agglutination Observed?	Ability to Attach (yes or no)
1	<i>yes</i>	<i>yes</i>
2	<i>yes</i>	<i>yes</i>
3	<i>no</i>	<i>no</i>
4	<i>yes</i>	<i>yes</i>
5	<i>no</i>	<i>no</i>
None (negative control)	<i>no</i>	

## Questions

Which strains were able to adhere to intestinal cells?

*Strains 1, 2, and 4 adhered to intestinal cells*

If you had observed agglutination in well 6 (intestinal cells only), what would you be able to conclude about the attachment of the different *E. coli* strains to intestinal cells? Explain your answer.

*Nothing! If intestinal cells alone adhered to each other, it would be impossible to tell whether the agglutination observed in the other wells was due to the bacteria, or simply intestinal cells adhering to each other.*

What additional controls would you add?

*It would be good to test the agglutination of each bacteria strain to itself. However, this control is not absolutely essential since you can look at the bacterial cells in the test tube or dropper bottle and see that there is no agglutination.*

## Teacher Prep

In order to make this experiment simpler, I designed a precipitation reaction to mimic the agglutination reaction.

### **Part I**

#### Components of reaction

Solution A – 1 M  $\text{MgSO}_4$

To represent human intestinal cells

Solution B – 0.5 M NaOH

To represent bacteria that can adhere

$\text{MgSO}_4 + \text{NaOH} \rightarrow \text{Mg(OH)}_2$  (white precipitate)

Solution C – water

To represent bacteria that do not adhere (no precipitate will form)

#### Preparation

Make 500 mls of each solution above

Aliquot reagents into dropper bottles or test tubes as below (below assumes 5 groups)

5 dropper bottles Solution A labeled “intestinal cells”  $\text{MgSO}_4$

5 dropper bottles each of bacteria 1, 2, 4, containing Solution B NaOH  
labeled ‘E. coli strain 1’ ‘E. coli strain 2’ etc

5 dropper bottles each of bacteria 3 and 5 containing Solution C water  
labeled “E. coli strain 3” or “E. coli strain 5”

Note, very little solution is needed in each dropper bottle. 2 mls would be more than enough.