Teacher Prep

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

Preparation

Make 500 mls of each solution below

Solution A – 1 M MgSO₄

Solution B – 0.5 M NaOH

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

Solution C – water

\[
\text{MgSO}_4 + \text{water} \rightarrow \text{no precipitate}
\]

Solution D – 1 M HCl

\[
\text{MgSO}_4 + \text{HCl} + \text{NaOH} \text{ will not form Mg(OH)}_2 \text{ precipitate}
\]

(A brief precipitate may form upon addition of NaOH before mixing with the toothpick)
Distribute solutions A-D into bottles as follows. Very little solution is needed in each bottle. Label each bottle with the scenario number. **One** of each dropper bottle is needed.

**Scenario 1**
Bladder cells (solution A) MgSO4
*E.coli* with type I fimbriae (Solution B) NaOH
Glucose (Solution C) water
Mannose (Solution D) HCl
Galactose (Solution C)
Fructose (Solution C)

**Scenario II**
Bronchial Cells NCI-H292 (Solution A) MgSO4
Wild Type Bordetella (Solution B) NaOH
Fimbriae mutant (Solution B)
Filamentous Hemagglutinin mutant (Solution C) water
Pertussis Toxin mutant (Solution B)
Hemolysin mutant (Solution B)
Adenylate Cyclase mutant (Solution B)

**Scenario III**
Laryngeal Cells HEp-2 (Solution A) MgSO4
Wild Type Bordetella (Solution B) NaOH
Fimbriae mutant (Solution C) water
Filamentous Hemagglutinin mutant (Solution C)
Pertussis Toxin mutant (Solution B)
Hemolysin mutant (Solution B)
Adenylate Cyclase mutant (Solution B)

**Scenario IV**
T-cells (Solution A) MgSO4
HIV (Solution B) NaOH
Anti-CD3 (Solution C) water
Anti-CD4 antibody (Solution D) HCl
Anti-CR5 (Solution C)
Anti CRCX4 (Solution D)
Possible Answers and Experimental Designs

Scenario I

Possible Hypotheses
Mannose is the receptor on bladder cells for *E. coli* with type I pili
Galactose is the receptor on bladder cells for *E. coli* with type I pili
Etc

Prediction for first hypothesis above
If mannose is the receptor, it will inhibit agglutination.

Experimental design

Bladder cells + mannose + *E. coli*
Bladder cells + galactose + *E. coli*
Bladder cells + glucose + *E. coli*
Bladder cells + fructose + *E. coli*

Negative controls
Bladder cells alone
Each sugar alone
*E. coli* alone

Positive control
Bladder cells + *E. coli*

Note – if you add the *E. coli* to the bladder cells before adding the sugar, the sugar will not be able to inhibit the reaction and agglutination will be observed.

Answer –
Mannose is the receptor for this type of fimbriae and should inhibit agglutination in this test
Scenarios II and III

Note
The filamentous hemagglutination mutant is a strain lacking filamentous hemagglutinin etc.
The *B. pertussis* wild type strain is a strain with all possible adhesins present.

Possible Hypotheses
Filamentous hemagglutinin is responsible for the adherence of *B. pertussis* to bronchial/laryngeal cells.
Fimbrae are responsible for the adherence of *B. pertussis* to bronchial/laryngeal cells.
Etc.

Prediction based on the first hypothesis
If filamentous hemagglutinin is responsible for the adherence of *B. pertussis* to bronchial/laryngeal cells then a mutant lacking filamentous hemagglutinin will not cause agglutination.

Experimental
Filamentous hemagglutinin mutant + bronchial/laryngeal cells
Fimbrae mutant + bronchial/laryngeal cells
Pertussis Toxin mutant + bronchial/laryngeal cells
Hemolysin mutant + bronchial/laryngeal cells
Adenylate Cyclase mutant + bronchial/laryngeal cells

Negative controls
Each bacterial strain alone
Bronchial or laryngeal cells alone

Positive control
*B. pertussis* wild type + bronchial/laryngeal cells

Answer
For scenario II – binding to bronchial cells is mediated by filamentous hemagglutinin (no agglutination observed with the FHA mutant).
For scenario III – binding to laryngeal cells is mediated by fimbrae and filamentous hemagglutinin (no agglutination observed with the FHA mutant or the fimbrae mutant).
**Scenario IV**

**Possible hypotheses**
CD3 is the co-receptor for HIV on T cells
CXCR4 is the co-receptor for HIV on T cells

**Prediction for first hypothesis**
If CD3 is the co-receptor for HIV on T cells then antibodies to CD3 should inhibit agglutination.

**Experimental**

T-cells + anti-CD3 + HIV
T-cells + anti-CXCR4 + HIV

Positive controls
T-cells + HIV

Negative controls
T-cells alone
HIV alone

Other controls
T-cells + anti-CD4 + HIV (expect no agglutination confirming that CD4 is part of co-receptor – ie both CD4 and another molecule serve together as the receptor)
T-cells + anti-CCR5 + HIV (expect no agglutination confirming that CCR5 is not involved in binding – this is expected since it is thought that there is no CCR5 on T-cells)

Note – if you add the HIV to the T-cells before adding the antibody, the antibody will not be able to inhibit the reaction and agglutination will be observed.

**Answer**
CXCR4 is the co-receptor for HIV on T-cells (antibodies to CXCR4 inhibited agglutination).