Overview of Activity
Koch's Postulates, Causality, and Contemporary Epidemiology

1. Summary of Activity.

Using visual and written sources, students will explore both traditional and new techniques for tracking pathogens and investigate the causality of diseases. Using the process of scientific inquiry, students will propose their own strategies for determining the causality of diseases.

2. Intended Grade Level.

Assignment 1 is intended for high school biology classes. Assignments 2 and 3 are recommended for AP or advanced biology classes.

3. Recommended Prior Knowledge.

Student understanding of the following terms would be helpful.

Virus	bacterium
immune system	epidemiology
inoculation	host
vector	pathogen
autoimmune disorder	prion

Previous experience using the Chi square test is advised, but not necessary for AP or advanced biology students.

4. Background information.

Koch's postulates are a set of criteria for experimentally determining whether a particular pathogen is the cause of a particular disease. These resources may be used to give students a working understanding of Koch's postulates.

- The introductory page to this activity **"TRACKING THE SOURCE OF DISEASE: Koch's Postulates, Causality, and Contemporary Epidemiology."**
- HHMI Holiday Lecture on DVD: "2000 and Beyond, Confronting the Microbe Menace", Lecture 1: Microbe Hunters, Tracking Infectious Agents, Chapter 27 lecture on Koch's Postulates.
- Any currently used classroom textbook that contains information regarding Koch's Postulates.

A previous activity acquainting AP and advanced biology students with the purpose and function of the Chi square statistic would be worthwhile, but not necessary.

5. **Materials list.**

Activity 1: DVD copy of HHMI Holiday Lecture "Confronting the Microbe Menace", and student copies of "Tracking the Source of Disease", and handout "Criteria for Determining Cause-effect Relationships in the Study of Disease."

Activity 2 (for AP and advanced biology students): copies of "Coxsackie Virus and Insulin Dependent Diabetes Mellitus (IDDM)."

Activity 3 (for AP and advanced biology students): student copies of data list for IDDM / Coxsackie B antibodies/diabetes correlations.

6. **Activity Outline.**

I. Assignment 1 for all biology students:
   - A. Introduce Koch's Postulates using handouts "Tracking the Sources of Disease", and HHMI Holiday Lecture DVD: Lecture 1 "Microbe Hunters, Tracking Infectious Agents", Chapter 27.
   - B. Develop hypotheses for testing pathogenic causality for which Koch's Postulates would not work.
   - C. View Lecture 1, Chapters 19-30, to observe a real example for determining causal pathogenicity using non- Koch's Postulates techniques.
   - D. Reevaluate proposed hypotheses based on the new information obtained from watching the DVD.

II. Assignments 2 and 3 for AP and advanced biology students:
   - A. Read the introduction of Contemporary Epidemiology in Assignment 2.
   - B. Read and discuss the article, "The Enemy Within."
   - C. Students write a hypothesis regarding the role of Coxsackievirus B in Type I diabetes and design experimental studies to support their hypotheses in order to complete Assignment 2.
   - D. Teacher may wish to review the Chi square test as an analytical tool prior to beginning Assignment 3.
   - E. Using data from "Patient data list for IDDM / Coxsackie B antibodies /diabetes correlations", students use Chi square to determine likelihood of disease causality in order to complete Assignment 3.

III. Optional Summary Activity for all students
   - A. View lecture 4 on DVD.
   - B. Discuss how the use of Koch's Postulates and post-Koch's postulates techniques are useful in tracking, and potentially treating/preventing epidemics.

7. **Teacher Answer Key**

I. Use the questions included with the assignments in "Tracking the Source of Disease," Koch's Postulates.

II. Suggested answers to questions 1-6, Assignment 1

A. **Question 1 referring to all 3 case studies:** Considering these scenarios, were there any techniques common to all three? Explain your answer.

1. Attempts were made to culture the organism in all three cases.
2. An attempt was made to correlate the presence of the disease with exposure to a common environmental factor in each case.

B. **Case One: Questions 2-4**

1. Question 2: In each case, what facts did you need to establish in order to test whether the pathogen(s) was the actual **cause** of the disease?

a. Establish similarities in symptoms.
b. Establish commonality of a possible exposure source (i.e. air in older part of building).
c. Inability to culture *F. slimeii* from sick individuals.

2. Question 3: What role, if any, did the timing of events play in establishing whether a pathogen was the **cause** of the disease.

There is a direct correlation between time spent in the older part of building and the onset of the illness.

3. Question 4: In each case, what pattern of association between the presence of the pathogen in victims and presence of the disease is strong enough to say that the suspected pathogen actually **caused** the disease?

a. Look for *F.slimeii* in blood samples or search for its DNA using molecular subtraction techniques.
b. Screen for antibodies to *F. slimeii* in all victims. The absence of antibodies in ill individuals would preclude that it was the cause of the disease, but the presence of antibodies alone would not establish causality, only exposure.
c. Search for a nonhuman host in which to culture *f. slimeii* and attempt to observe an onset of the disease symptoms.

The presence of antibodies in all infected individuals, coupled with the presence of its DNA, along with possible success in duplicating the disease in an alternate host would show a high degree of probability that *F. slimeii* was the cause of the disease.

C. **Case Two: Questions 2-4**

1. Question 2

a. There is an increase in the number of cases, typical of the spread of a contagious organism.

b. There is a high correlation between the frequency of exchange of body fluids (frequency of injected drug use and/or number of sex partners) and the likelihood of having the disease.

2. Question 3.

The disease occurs after involvement in the identified behaviors. Disease occurs after exposure to known infected body fluids (ex: after sharing needles or having sex with an infected person).

3. Question 4.

Use Molecular Subtraction techniques to search for the DNA and test whether new virus is found in all victims.

D. **Case Three: Questions 2-4**

1. Question 2

a. There is an increase in the number of cases reported. This could be explained by perhaps more people being around cats, the appearance of a new pathogen, or a new combination of pathogens.
b. Wounds are extremely difficult to treat. This would be consistent with multiple organisms as a cause as each could potentially be resistant to different antibiotics and be exchanging resistance between bacterial strains present.

2. Question 3

People who have been wounded by cats are the only ones to express the disease. Disease occurs after cat wound.

3. Question 4

a. Culture oral and claw bacterial fauna from cats suspected of being the carriers. Isolate strains and grow pure cultures of each bacterial strain.
b. Use human tissue cultures, as well as non-human hosts to test for disease symptoms with each strain and with all combinations of strains in a systematic fashion. Resulting damage to cultured cells or onset of symptoms in alternate hosts would indicate likelihood of an individual strain or a specific combination of strains being the source of the disease.
c. Use molecular techniques to characterize strains of bacteria present as well as the microbial community as a whole.

E. **Question 5 referring to all 3 case studies:**

Develop a list of at least three criteria that you could use in a variety of situations to show whether a pathogen is the cause of a disease.

Answers will vary.

F. **Question 6 referring to all 3 case studies**:

AFTER WRITING DOWN YOUR OWN CRITERIA, compare your criteria with those described in the handout from *Epidemiology in Medicine* by Hennekens and Buring, 1987. Which of your criteria overlap? Which did the handout list that you did not? Which did you list that the handout did not?

Answers will vary.

III. Assignment 2

Researchers are now recognizing the strong association of certain autoimmune conditions with prior exposure to specific pathogens. Design an experiment to investigate the possibility of exposure to Coxsackievirus B as a causal factor in the development of Type I (insulin dependent) diabetes mellitus – an autoimmune disorder. Read the article provided entitled "The Enemy Within" paying close attention to the section on Coxsackievirus. With your partner, write a hypothesis regarding the role of Coxsackievirus B in Type I diabetes. Then write an outline of how you would design a study to determine whether Coxsackievirus B is a causal factor in the development of Type I diabetes. Using the types of analytical studies outlined above, identify which type of study you developed (case-control or cohort).
Present your study design to the rest of the class.

A. Null Hypothesis for Case-Control Study

If a case group of Diabetes Type I patients is compared to a control group without Diabetes Type I for seroreactivity to *Coxsackie virus B*, then there will be no statistically significant difference in rates of antibody production between the two groups.
Data that show a higher seroreactivity in Diabetes Type I patients would refute this hypothesis and would be evidence in support of *Coxsackie virus B* being a causal agent for the onset of Diabetes Type I.

B. Null Hypothesis for Cohort Study

If 2 cohorts, one seroreactive (+) and the other seroreactive (-) for *Coxsackie virus B*, neither of which have Diabetes Type I are compared in a long term study, then there will be no statistically significant difference in the rates of the onset of Diabetes Type I between the 2 cohorts.
Data that shows the seroreactive (+) cohort having a significantly higher incidence of Diabetes Type I would refute this hypothesis and be evidence in support of *Coxsackie virus B* being a causal agent for the onset of Diabetes Type I.

IV. Assignment 3

A. **Question 1**

Decide whether or not you will include all individuals observed or exclude some. If you decide to exclude some individuals, what is your rationale? Draw a single line through the rows containing individuals you choose to exclude.

Since antibodies were first tested at age 5, person who developed diabetes at age 5 or younger might be eliminated from the analysis. It cannot be determined whether or not they were exposed to *Coxsackie virus B* before or after the onset of Diabetes Type 1.

B. **Question 2**

A common tool for analyzing data from case-control and cohort studies is the two-by-two table comparing exposure and presence of the disease. Using the blank two-by-two table as a guide, construct a similar table for the IDDM/Coxsackievirus B study and fill in the appropriate count values.

Assuming that all data, including age 5 or younger is used, here are the observed results:

	Disease (+)	**Disease (-)**	**Row total**
Exposure (+)	**35**	**16**	**51**
Exposure (-)	**5**	**23**	**28**
			Grand Total
Column total	**40**	**39**	**79**

C.**Question 3**

Calculate the odds ratio for your data.

OR = (ad) / (bc) = (35 X 23) / (16 X 5) = 10.06

D. **Question 4**

Calculate a Chi-square value for your data, and determine whether the pattern observed between the case and control data is statistically significant.

Expected Results

	Disease (+)	**Disease (-)**
Exposure (+)	51 X40 /79 = **26**	51 X 39/79 = **25**
Exposure (-)	28 X 40/79 = **14**	28 X 39/79 = **14**

Chi-square Statistic

$X^2 = \sum (O - E)^2/E = (35–26)^2/26 + (16–25)^2/25 + (5–14)^2/14 + (23-14)^2 = 17.927$

Chi-square (from table) = 3.841

Chi-square (calculated) = 17.927

Significant **Yes**/No

E. **Question 5**

What conclusions about the suspected disease pathogen can you make based on your data and statistical analysis? How well do these data meet the criteria for establishing disease causality previously discussed?

There is a slightly more than 1 in100,000 chances that this distribution occurred by chance. The Chi-square test shows that the development of Diabetes Type I is connected to previous exposure to *Coxsackie virus B*. There is not enough evidence to say that exposure alone causes Diabetes Type I, since 16 people were exposed but did not have the disease. Some may develop the disease in the future, however. In addition, 5 people had the disease yet tested negative for *Coxsackie virus B* antibodies. These 5 lend a degree of uncertainty to the virus being the sole cause of the disease.