



The Biotechnology Education Company ®

EDVO-Kit

141

Blood-Based Cancer Diagnostics

**Store entire experiment at
room temperature.**

EXPERIMENT OBJECTIVE:

The objective of this experiment is to learn and understand the concept of blood-based screening for cancer.

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Material Safety Data Sheets can be found on our website:
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This experiment is designed for 10 student groups.

No actual blood or blood products are used in this experiment.

Experiment Components

Store entire experiment at room temperature.

- Control samples (High & Normal PSA, Positive & Negative HPV)
- Simulated blood samples from six male patients (M1, M2, M3, M4, M5, & M6)
- Simulated blood samples from six female patients (F1, F2, F3, F4, F5, & F6)
- Antibody for PSA
- Antibody for HPV
- Red dye concentrate (for coloring)
- Transfer pipets
- Microtiter plates
- Microcentrifuge tubes

**** NOTE: All Control blood samples and Simulated Patients Blood Samples will be prepared by instructor just prior to use.**

Requirements

- Optional: Automatic micropipet (5 - 50 μ l) and tips

Background Information

Causes of human cancers are beginning to be understood at the molecular level and cures for certain cancers are beginning to be routinely available. As with any disease the key to cancer cures and survival is early detection and the selection of the proper treatment. Physicians order various tests to detect the type of cancer or to assess the progression of cancer in patients. There are several cancer biomarkers in blood that are currently being used as cancer diagnostic blood tests that can assess the health of various organs and systems in the human body. Cancer biomarker based tests detect early cancer activity based on the presence of levels of specific proteins that serve as early indicators of cancer progression or reoccurrence. These biomarkers are also utilized to monitor and assess the state of cancers and response to treatments and complement various imaging tests such as x-rays and CAT scans. As with many diagnostic tests cancer biomarkers can have limitations where normal levels of a marker may not prove that a person is free from a particular cancer or elevated levels of a biomarker may not correlate to progression or reoccurrence of a cancer.

An example of a broad cancer biomarker is the Carcinoembryonic antigen (CEA), a glycoprotein involved in cell adhesion. This protein is present in blood during the development stages of a fetus. Expression of CEA levels in blood stops before birth and the protein is present in trace amounts (essentially absent) in the blood of healthy adults. High levels of the CEA protein in blood serves as a broad biomarker that includes breast, thyroid, lung, ovarian, pancreatic, stomach, liver and colon/rectal cancers

In men, prostate specific antigen (PSA) is an enzyme produced by prostate cells. PSA levels can be used to diagnose prostate cancer, however there are other conditions that also can cause an increased PSA levels that include enlargement of the prostate gland in older men and prostatitis, a condition caused by infection of the prostate. If a patient PSA level is higher than normal, follow up tests are recommended to monitor unusual continued increases in PSA level that further confirms the presence of prostate cancer. PSA as a cancer biomarker is useful only if coupled to other tests to assure accuracy that usually includes medical follow-up examinations, various scans, and, if required, a biopsy to microscopically examine the presence of cancer cells.

In women, ovarian cancer is detected by a tumor biomarker known as CA 125 that is present in blood. So far, CA 125 does not appear to be sensitive or specific enough to be used to screen for ovarian cancer. CA 125 is primarily used to monitor response to treatment and to check for recurrence of ovarian cancer. Cervical cancer is also caused by specific strains of human papilloma virus (HPV) of which there are various strains. Only specific strains are linked to cervical cancer. HPV is a sexually transmitted disease (STD) that cannot be detected by traditional PAP smears. An HPV vaccine recently became available that holds promise to protect against most but not all cervical cancer-causing HPV strains.

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Background Information

Blood A and B antigens are common in the human population, as well as in nature, including bacteria to which we are exposed. When exposed to bacteria with the same blood group antigen, the immune system of the individual will recognize that antigen as “self” and no immune response will be mounted against it. By contrast, when exposed to bacteria with different blood group antigens, the human immune system will see that antigen as foreign and produce antibodies against it. These serum antibodies can then agglutinate RBCs from individuals with a different blood type. For example, anti-A antibodies from one individual's serum will agglutinate another person's RBCs that have the A antigen on their surface. Anti-B antibodies will agglutinate RBCs that have the B antigen on their surface as demonstrated in Table A, below.

Blood Type	Antigen on Red Blood Cells	Antibody in Serum
A	A	anti-B
B	B	anti-A
AB	both A and B	neither anti-A nor anti-B
O	neither A nor B	both anti-A and anti-B

Table A: RBC Agglutination

Type O blood is often referred to as the universal donor, and type AB blood is generally referred to as the universal recipient. Neither is correct. There is no universal donor nor a universal recipient in the case of whole blood transfusions. Type O blood is commonly said to be the universal donor because type O RBCs do not have either A or the B antigen on their surface. Thus Type O red blood cells were incorrectly assumed to be safe for transfusing individuals with Type A, B or AB blood. This assumption is incorrect and can have serious medical consequences where the anti-A antibodies from the donor would react with the recipient's red blood cells. Therefore, Type O blood is a universal donor only if red blood cells (and not serum) are being transfused.

If Type O blood was transfused into a person who has Type A blood, the following ABO antigens and antibodies would be present in various recipient's blood following transfusion.

- red blood cells with the A antigen (from recipient)
- red blood cells with neither A nor B antigen (from donor)
- anti-B antibodies (from recipient)
- anti-A and anti-B antibodies (from donor)

Therefore, in reality, only blood of the same type should be transfused into a patient. In fact, since there are subtypes of some of the blood groups and since there are other blood groups besides ABO which may cause transfusion problems, even this conservative approach is an oversimplification and may result in complications for the patient.



Background Information

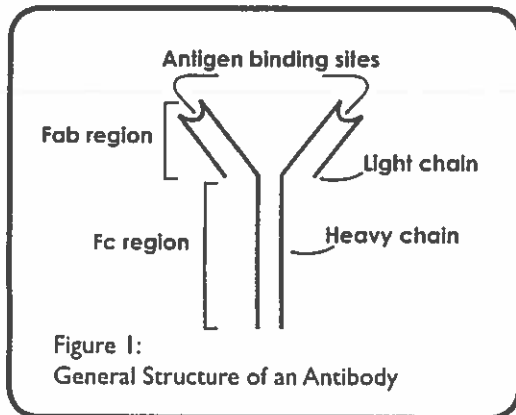


Figure 1:
General Structure of an Antibody

Most biomarkers are identified by polyclonal or monoclonal antibodies. All antibodies belong to a group of serum proteins known as globulins. Each antibody is made up of a heavy and light polypeptide chain (see Figure 1). In general, antibodies are produced in response to the presence of a "non-self" antigenic proteins. Antibodies obtained from animals, such as rabbits, in response to an antigen are known as polyclonal antibodies.

Polyclonal antibodies are heterogeneous in structure and vary in their ability to bind to antigens. Antibodies that have high affinity for antigens may give unwanted cross-reactions that can result in high backgrounds. Such antibodies can also give false negative results while antibodies with weak binding constants may not be as sensitive. It should be noted that polyclonal antibody preparations for a given antigen can have variable binding affinities due to differences in immunological responses. Immunizations with the same antigen in different animals can produce antibodies with variable binding affinities. The use of monoclonal antibodies that are directed against a single epitope (specific parts of a protein cancer biomarker) eliminates potential variability of polyclonal antibodies.

In this experiment a set of simulation blood samples will be provided that represent various blood samples obtained from patients that are to be tested for a particular cancer. The experiment includes a PSA test for men and an HPV test for women. Polystyrene microtiter plates will be used for the reactions to detect positive versus negative samples. Positive tests will form a precipitate between the antibody and the biomarker.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to learn and understand the concept of blood-based screening for cancer.

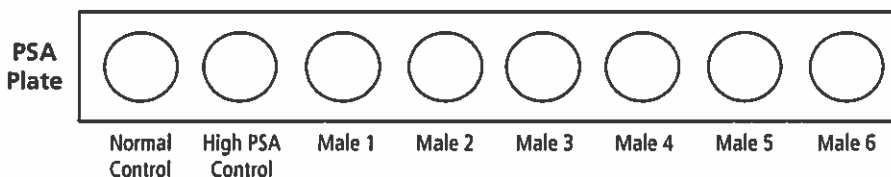
LABORATORY SAFETY

No human materials are used in this experiment. Gloves and safety goggles should be worn as good laboratory practice.



Student Experimental Procedures

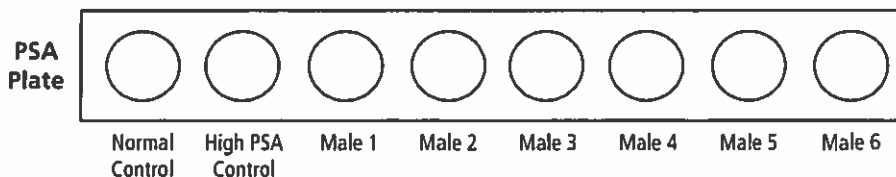
- A. The first experiment is a simulated PSA test for 6 male patients between the ages of 55 and 70.
- Place a microtiter plate strip as shown below. Label the 8 wells across the top or bottom of the plate as follows: Normal, High PSA, M1, M2, M3, M4, M5, & M6 respectively, using a laboratory marking pen. The plate should look like the one pictured below:



IMPORTANT!
Avoid cross-contamination by using a new pipet or pipet tip for each blood sample.

PUT ON YOUR GLOVES NOW

- Using a different transfer pipet or pipet tip (when using an automatic micropipet) for each sample, plate 3 drops or 50 μ l of each control and patient sample into the corresponding wells.
- Use a new transfer pipet to add one drop or 20 μ l of PSA Antibody into each of the wells.
- Let the plate sit undisturbed on the lab bench for 5 minutes.
- Observe the wells for the level or intensity of agglutination. Agglutination has occurred if the mixture appears to be granular rather than smooth. Record your results below.

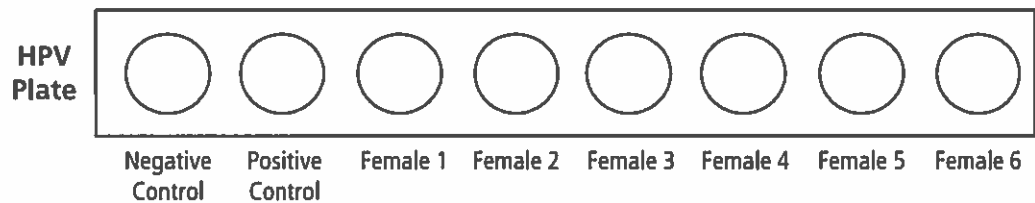


- Based on your observations, which of the six patients would you conclude might have cancer?

Experiment Procedure

Student Experimental Procedures

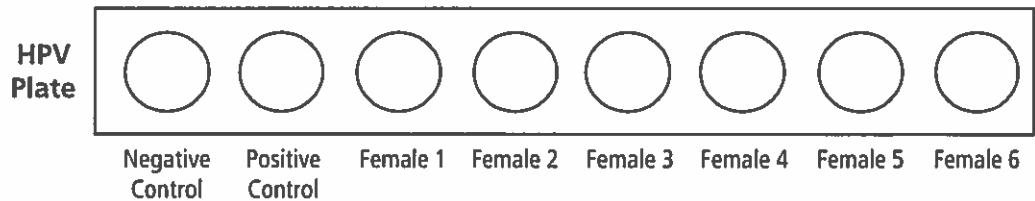
- B. In the second experiment, 6 female patients between the ages of 40 and 55 were tested for HPV, one of the causative agents for cervical cancer.
- Place a microtiter plate strip as shown below. Label the 8 wells across the top or bottom of the plate as follows: Negative, Positive, F1, F2, F3, F4, F5, & F6 respectively, using a laboratory marking pen. The plate should look like the one pictured below:



PUT ON YOUR GLOVES NOW

- Using a different transfer pipet or pipet tip (when using an automatic micropipet) for each sample, plate 3 drops or 50 μl of each control and patient sample into the corresponding wells.
- Use a new transfer pipet to add one drop or 20 μl of HPV Antibody into each of the wells.
- Let the plate sit undisturbed on the lab bench for 5 minutes.
- Observe the wells for the presence or absence of agglutination. Agglutination has occurred if the mixture appears to be granular rather than smooth. Record your results below.

IMPORTANT!
Avoid cross-contamination by using a new pipet or pipet tip for each blood sample.



- Based on your observations, which of the six patients would you conclude is positive for HPV?

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. What are protein biomarkers and how are they used as diagnostic tools for cancers?
2. Why does a doctor order tests in addition to the biomarker based blood cancer tests?
3. Why is a PSA blood test useful but not a definitive diagnostic test for the detection of prostate cancer?
4. How does HPV infect a population and cause cervical cancer in women?


Instructor's Guide

GENERAL INFORMATION

Blood screening is an important clinical assay that health care workers use routinely to properly care for their patients. Students should be made aware of the safety concerns when working with human blood products even though all of the materials in this Edvotek kit are safe chemicals used to simulate blood.

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Please Have the Following Info:

- Experiment number and title
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