

Bio257 Immunology Problem Set #1

1. An enterprising young bioengineer decides to develop a new indirect ELISA assay to detect HIV contamination of donated blood. Since the lab down the hall works on HIV reverse transcriptase (the enzyme that copies the HIV RNA genome into DNA), the engineer decides to base the new indirect ELISA on this key viral protein. In addition to normal reagents for indirect ELISA assays, the following reagents are available to develop this test:

- purified reverse transcriptase (RT), kindly offered by the neighboring lab
- serum from patients who tested positive in a gp120-based assay
- serum from patients who tested negative in a gp120-based assay

A. Outline a protocol to set up and test the new RT-based assay. Include both the reagents listed above and the normal reagents for indirect ELISA assays (diagram optional).

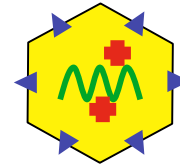
B. Describe the expected results for the test run outlined in (A). Would this assay work? Why or why not?

The engineer decides simultaneously to try to develop an RT-based sandwich ELISA test for HIV infection.

C. What additional reagents will be needed for this assay? Outline a protocol for this RT-based sandwich ELISA assay.

D. Describe the expected results for the test run outlined in (C). Would this assay work? Why or why not? What are the advantages and disadvantages of this assay over the indirect ELISA assay?

HIV virion

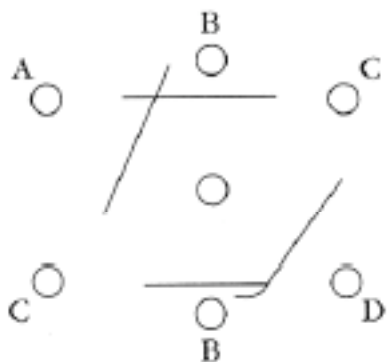


+ reverse transcriptase

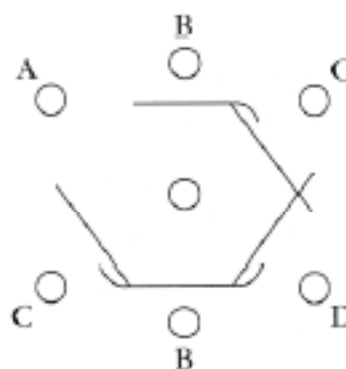
~ HIV RNA genome

▶ gp120

2. “The labels from four bottles (A, B, C and D) of hapten-carrier conjugates were accidentally removed. However, it was known that each bottle contained either hapten 1-carrier 1 (H1-C1), hapten 1-carrier 2 (H1-C2), hapten 2-carrier 1 (H1-C1), or hapten 2-carrier 2 (H1-C2). Double-immunodiffusion (Ouchterlony) assays with either anti-H1-C2 or anti-H2-C2 were performed. From the precipitin patterns shown below, determine which conjugate is in each bottle.” (Goldsby *et al.*, 2000)



Anti-H1-C2 in central well



Anti-H2-C2 in central well

This question may sound familiar; they updated the new edition of the textbook to make it an immunoblot rather than an Ouchterlony experiment, since immunoblotting is much more common these days.

3. A class of aspiring immunologists decides to develop an **assay to monitor the presence of Lyme disease** in local populations. Since Lyme disease is caused by the bacterium *Borrelia burgdorferi*, the researchers decide to start by using **rabbits to produce antisera** that recognize a **57 kDa bacterial protein** called **Bb1**, which forms **homodimers** in the bacterium.

With a divide-and-conquer mentality, the class splits into three groups to make immunogens with which to inject the rabbits. The hard-working biochemists of **Group A purify Bb1 from bacteria**, while the savvy and well-funded **Groups B and C** each order a **peptide**. **Group B's peptide** corresponds to the **N-terminal 15 amino acids of Bb1**, whereas **Group C's peptide** corresponds to the **C-terminal 15 amino acids of Bb1**. Each group injects its immunogen into a separate rabbit (also called A, B and C) to elicit an immune response. After several booster shots, blood is withdrawn from each rabbit and is allowed to coagulate. The groups then join forces to **test their antisera in two assays**: a **Western blot** (Fig. 1) and a **double-diffusion assay** (Fig. 2). Surprisingly, their **antisera give different results** in the two assays!

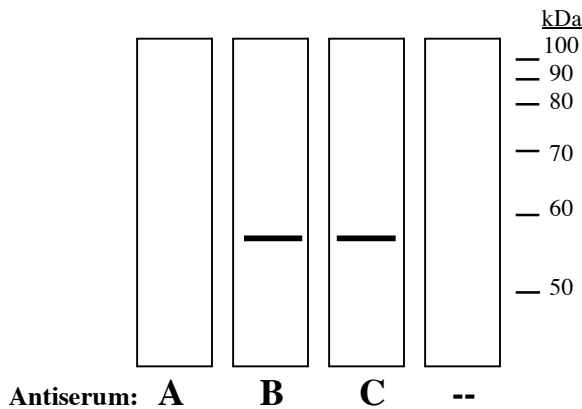


Fig. 1: Comparison of anti-Bb antisera by Western blot analysis

A *B. burgdorferi* culture was grown overnight and lysed with a detergent buffer. Bacterial proteins were treated with a reducing agent before being separated by SDS-PAGE. Proteins were transferred to a membrane and the blot probed with antiserum from rabbit A (A), antiserum from rabbit B (B), antiserum from rabbit C (C), or no antiserum (-) and a enzyme-linked sheep anti-rabbit secondary antibody, followed by chemiluminescence detection. Migration of molecular weight markers (kDa) is shown.

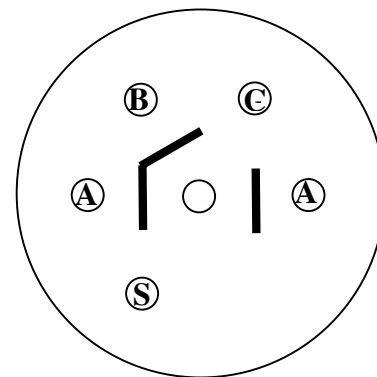


Fig. 2: Comparison of anti-Bb antisera by double diffusion assay

A *B. burgdorferi* culture was grown overnight and lysed gently to release bacterial proteins. Samples were loaded as follows: bacterial proteins (center well), antiserum from rabbit A (well A), antiserum from rabbit B (well B), antiserum from rabbit C (well C), saline (well S). The double-diffusion plate was incubated overnight prior to viewing.

- Explain **why the results** from the two assays are **different**. What do these results tell you about the **epitopes recognized by each antiserum**?
- Give **one advantage** and **one disadvantage** for **each assay** as a **method to detect infection** with *B. burgdorferi*.
- In all likelihood, none of these antisera will be too useful for the detection of Lyme disease in patients. If you were developing an assay to test for **exposure** to *B. burgdorferi*, **how would you design the assay**? Describe what **reagents** would you need, the **steps** of the assay, and what the **results** would look like for **someone who was exposed vs. someone who was not exposed**. Give **one advantage** and **one disadvantage** for your assay.

Additional questions from the textbook

Starred questions are those that are the most similar in format to exam questions. Other questions will be useful to make sure you have mastered the material we have covered. Some important material may not be covered by these questions, so make sure to go over your notes thoroughly and ask me questions about topics you find confusing!

Chapters 1 & 2: Some of these questions cover material we discussed, but since we will discuss most of the material in more detail later, I don't particularly recommend any of these questions (but feel free to look at them if you wish).

Chapter 4: 6*, 8, 10, 12[^]-14, 16*, 17 (+ clinical focus question)

[^]Can you detect incorrect answers in the back of the book for this question?

Chapter 6: 3*, 7, 10*, 13*, 14*

Here's a (non-starred) question from an earlier edition of the textbook:

You are given two solutions, one containing protein X and the other containing antibody to protein X. When you add 1 ml of anti-X to 1 ml of protein X, a precipitate forms. But when you dilute the antibody solution 100-fold and then mix 1 ml of the diluted anti-X with 1 ml of protein X, no precipitate forms.

- a) Explain why no precipitate formed with the diluted antibody.
- b) Which species (protein X or anti-X) would likely be present in the supernatant of the antibody-antigen mixture in each case?

Some of the other questions in Ch. 3, 4 and 6 may be helpful for reviewing ideas we have discussed, but they are not similar in format to exam questions and they also include plenty of material we didn't discuss, which is why I have not specifically indicated more questions.

Reference

Goldsby, R.A., Kindt, T.J. and Osborne, B.A. (2000) Kuby Immunology, 4th ed. (New York: Freeman) p. 147, 171.