Outbreak Investigation Video Series
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https://www.youtube.com/playlist?list=PLvgkamPnkczlPMSlXhNfqaW4KcFWyoW9q

Transcript, Video 4: Immunological Tools for Outbreak Investigations

Immunological techniques could be extremely useful for both identifying pathogens and also determining whether patients were exposed (and responded) to the pathogens in question.

Depending on your question, you would want to collect either samples containing your pathogen of interest, or blood samples that contain antibodies, which are produced by the immune system in response to the recognition of the pathogen. Therefore, your tests are going to be either direct (searching for the pathogen of interest) or indirect (searching for the evidence of the immune system's response to the pathogen). Please realize that just like culture-based and nucleic acid-based approaches, immunological techniques have their inherent limitations. Specifically, direct tests do not tell you whether a particular pathogen is present or absent, rather they test for the presence of a specific antigen. Indirect tests tell you not whether a patient's immune system responded to a particular pathogen, but whether it responded to an antigen that is typically characteristic of a particular pathogen.

Any structure on a surface of a pathogen could be an antigen, and many commercially available kits were designed to detect surfaces structures characteristic of specific pathogens. Most commonly, ELISA (enzyme-linked immunoassays) are used for the detection of antigens or antibodies. They are called enzyme-linked, because in this system, binding of an antigen or an antibody is detected by an enzymatic assay.

Indirect immunological tests, which involve detection of antibodies produced by the immune system, require blood samples. Be aware, however, that serum from patients who have been immunized will test positive for antibodies against particular pathogens. For example, vaccine against Hepatitis A is quite popular, and therefore, immune systems of those who were vaccinated would have produced the antibodies that could be detected with an indirect immunological assay. In a direct ELISA, an antigen (such as a fragment of viral capsule or bacterial cell wall, for example) is absorbed onto a plastic plate. The serum sample potentially containing antibodies is applied to the same plates, and binding of the antibody to the antigen-coated plates is detected using secondary antibodies to which an enzyme is conjugated. The addition of a chromogenic, or color-producing, substrate allows us to detect the presence of the bound antibody-enzyme complex.

Sandwich ELISA immunological tests could be used to detect antigens associated with viral, bacterial and eukaryotic pathogens, as well as many toxins. They could be used quite successfully on fairly complex samples. Clearly, however, the main disadvantage of the immunological approach is that they cannot be used (or will have a low success rate) for the detection of pathogens which tend to accumulate mutations rapidly. For example, Vibrios (including *Vibrio cholera*) become naturally competent when grown on chitin, and this ability has allowed Vibrios to acquire genes that change its immunogenic properties. Sandwich ELISA kits contain specific "capture" antibodies bound to the surfaces of the plastic plates. Samples containing the antigen are added, left to bind to the antibodies and then detected using another set of antibodies, conjugated with an enzyme. The addition of a chromogenic (color-producing) substrate allows us to detect the presence of the bound antibody-enzyme complex. The main

advantage of the sandwich ELISA is that any sample (water, bodily fluid, stool, food, etc,) containing the antigen of interest could be tested.

Over the recent years, immunomagnetic separation gained popularity as a tool that could be used to selectively culture specific pathogens from complex samples. In immunomagnetic separation, antibodies against specific antigens (representative of specific pathogens) are conjugated onto paramagnetic beads. These antibody-coated beads could be mixed with a complex sample, the beads are then pulled out with a magnet and rinsed to remove any contaminants. These complexes containing beads, antibodies and pathogens bound to the antibodies could than be directly plated on selective media to culture pathogens of concern or could be used in PCR reactions as templates.