A Forensic Analysis of Food Poisoning using a Dip-stick Immunoassay

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I. Overview :

Science Concepts: antigen - antibody reactions
food microbiology
types of bacterial toxins
linking bacterial toxins to clinical symptoms
solving a simulated forensic investigation

VI. Advance Preparations

Mix to dissolve and adjust pH to 7.4 and then add 5 ml of Tween 20

Store this solution at room temperature. Dilute 1:10 with distilled water before use and adjust pH if necessary.

Tween 20 can be purchased at Sigma-Aldrich P9416-50ML 16.40

4. BSA and Anti-BSA. Many manufactures: Sigma Aldrich BSA2-153-10g $57.70; Anti-BSA B1520 @$103.50. Other suppliers like Carolina Biological Supply have antigen-antibody kits for less.

VII. Materials and Equipment

a. Designed for a class of 30 students

b. Complete list in section VI

C. Costs in section VI

D. Any antigen-antibody combination can be used

E. Students may need training on micropipettes but calibrated plastic eye droppers can be used.

VIII. Student Prior Knowledge

a. Prior knowledge of antigen-antibody reactions is

XII. Assessment

a. Photograph of end results, or video of steps in this procedure

b. Test on basic concepts of immunoassay

References

1. Amesen, Lotte, Annette Fagerlund and Per Elinar Granum. 2008. From Soil to Gut: Bacillus cereus and its Food Poisoning Toxins. FEMS Microbiology Reviews. 32(4): 579-606.

2. Hilal Colak , Ali Aydin, Bulent Nazli and Ozer Ergun. 2006 š š] } v } (‰ Œ • v } (} Á [• u] o • Z ‰ [• Z by immunochromatography . Food Control. 17(1): 905-908.

3. Haggblom Max M. et al. 2002. Quantitive Analysis of Cereulide, The Emetic Toxin of Bacillus cereus Produced under various Conditions. Applied and Engineering Microbiology. 68(5): 2437-2439

4. Foodborne Illness-Causing Organisms in the U.S. What You Need to Know. October 2008. Found at http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm103263.htm

References for Food Investigative Report

1. Food Investigative Report

t http://www.co.polk.or.us/sites/default/files/community_development/Food%20Investigation%20Report%20Form.pdf

2. Standard Operating Procedure for the Investigation of Food Poisoning Outbreaks
http://www.public.health.wa.gov.au/cproot/1585/2/SOP_Regional.pdf

3. Annex 6. Investigation report forms
http://www.who.int/foodsafety/publications/foodborne_disease/Annex_6.pdf

4. Polk County, Oregon Food Investigation Report

Student Section

I. Rationale

This activity was designed to teach within an Immunology Unit with the goal of integrating

3. Confer with colleagues to determine source of contaminated food.

* Worksheets attached.

Part B. Disk Assay (See Teacher Notes in Appendix)

Preparation of Disks

1. Fix a 2.5 mm wide strip of nitrocellulose to an acetate strip (Transparency or any plastic surface) using double faced tape so that a piece of the nitrocellulose hangs off the end of the double faced tape and acetate. See Figure 1.

Double-faced tape

Nitrocellulose

4. Soak reaction end in 5% Non-fat dairy milk (Powered milk) overnight. Cross reactivity between secondary antibodies and other proteins leads to high background signals, therefore,.). Non fat dry milk Is used as a blocking agent to block non -specific antibody binding and reduce background signal

« «

Dump off b locking solution.

5. Apply sample antibody in 5% powdered milk to the reaction end, Incubate for 20 minutes @ 37.. This can incubate overnight.

6. Rinse in 5% non-fat dairy milk solution three times.

8. Place strips in the HRP conjugate diluted in a 5 % no n fat dairy milk sample and incubate for 20 minutes . (HRP is an enzyme that is conjugated to secondary antibodies and aids in the detection of the bound proteins).

10. Wash with PBS-Tween. (Tween-20 is a nonionic detergent that is used in washing buffers and antibody solutions to help reduce background).

Appendix A- Teacher Notes

1. Nitrocellulose membranes are the most popular membranes for immunoblotting (Western blotting) of proteins and nucleic acids. The basis of protein binding to nitrocellulose is primarily due to hydrophobic interactions. For standard nitrocellulose, pre-wetting the membrane in water is sufficient. If using PVDF (polyvinylidene) membranes, the membrane must first be soaked in alcohol (preferably methanol) for a few minutes, followed by washing in water for approximately 5 minutes in a 37°C oven.

2. Part 2 of this lab involves an antigen-antibody reaction. The antigen, which could be a protein or a carbohydrate, elicits the production of antibodies by activated B cells (antibody-producing B cells are called plasma cells). Such antibodies can react specifically with the antigen used and therefore be used to detect the antigen. In this lab the antigen is BSA or Bovine serum albumin. After applying a drop of BSA to the nitrocellulose on the Dipstick, let it dry at room temperature or a 37°C oven. This antigen is the mystery antigen in the simulated food samples. Food samples can be made from dilutes samples of BSA and distilled water.

3. Since antibodies are proteins, the non-fat dry milk is used as a blocking agent to prevent non-specific antibody association with the nitrocellulose membrane, which would obscure the detection of the BSA antigen. [This is a spot where the Dipsticks can be left in the blocking solution overnight.]

4. After removing the Dipsticks with the BSA antigen from the blocking solution, apply sample antibody diluted in 5% powdered milk to the reaction/nitrocellulose end. Incubate for 20 minutes @ 37°C or overnight at 4°C.

5. After removing the Dipsticks rinse in 5% non-fat dairy milk solution three times.

6. Place strips in the HRP-antibody conjugate diluted in a 5% non fat dairy milk sample and Incubate for 20 minutes. The horseradish peroxidase (HRP) antibody conjugate is the detection (BSA-specific) antibody fused with a peroxidase enzyme. This conjugate enables specific detection of BSA on the nitrocellulose while providing an enzymatic readout of that detection (see below).

7. Washing with PBS-Tween. Tween-20 is a nonionic detergent that is used as a washing buffer in antibody solutions to help reduce background.

8. The activity of the HRP enables specific detection of antibody recognition of the BSA on the dipstick. Briefly, HRP catalyzes the oxidation of the TMB (3,3',5,5'-tetramethylbenzidine) substrate, which makes a color change in the spots where the antibody bound its target. Because this is an enzymatic reaction, it is important to not allow the reaction to go too long, as that can cause the entire nitrocellulose portion of the dipstick to darken and obscure the signal. Rinsing the dipstick in water several times is usually sufficient to stop the reaction once the color development is complete.

What You Need to Know About Foodborne Illness-Causing Organisms in the U.S.

Available in PDF (313KB). [1]

Also available en Español (Spanish) [2].

While the American food supply is among the safest in the world, the Federal government estimates that there are about 48 million cases of foodborne illness annually –the equivalent of sickening 1 in 6 Americans 16.7aericthese illnesses result in 128,00 hospitali

The chart below includes foodbo rne disease-causing o chart shows, the threats are numerous and varied, with sy serious,life-threatening illness. While the very young, greatest risk of serious consequences from most food threats to all persons.

Organism	Common Name of Illness	Onset Tim After Ingestin
Bacillus cereus		

E. coli O157:H7	Hemorrhagic colitis or E. coli O157:H7 infection	1-8 days	Severe (often bloody) diarrhea, abdominal pain and vomiting. Usually, little or no fever is present. More common in children 4 years or younger. Can lead to kidney failure.	5-10 days	Undercooked beef (especially hamburger), unpasteurized milk and juice, raw fruits and

liver disease or weakened
immune systems.

For more information, contact: The U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition Food Information Line at 1-888-SAFEFOOD (t oll free), 10 AM to 4 PM ET , Monday through Friday.

Links on this pa g

FOOD INVESTIGATION REPORT

NAME _____ DATE _____

Address _____ City_____ State _____ Zip Code _____

Phone Numbers _____ Age _____ Sex ____

E-mail _____

OTHER QUESTIONS AS NEEDED

What time did you eat?

How many drinks with ice did you have?

Any drinks with water?

Let me ask you about the meals you have eaten over the last few days.

Last 24 hours, starting with most recent meal:

All items you ate: Dinner (time:_____)

Lunch (time:_____)

Breakfast (time:_____)

Anything you drank. (time for each)

Did you drink water at any location different than normal? Where?

24 to 36 previous hours, starting with most recent meal:

All items you ate: Dinner (time:_____)

Lunch (time:_____)

Breakfast (time:_____)

FO ANCH

Standard Procedure for th tion of Food P utbreaks

Ru l Edition

1.0 Introduction

Why investigate outbreaks?

Investigation of food poisoning outbreaks is an important function of any environmental health service in Western Australia. Interviewing as many of the affected persons as possible is essential to develop hypotheses about a possible source/s of infection amongst associated cases and may lead to the identification of the source of gastrointestinal illness in the community.

Part IX of the Health Act 1911 deals with Infectious Diseases (& outbreaks) and their management within the community.
The desired outcomes are:

1. To identify the source of the outbreak
2. To stop further infection
3. Reduce the risk of future outbreaks
4. Encourage those affected to seek medical attention

Public Health Units, Food Safety Section and local government EHOs work in a cooperative arrangement to investigate most outbreaks. The level of involvement of the Food Safety Section will vary, depending on the nature and size of the outbreak (Outbreak Flow Chart (Chart 1))

The Food Safety Section offers expanded administrative / field support and coordination for outbreak investigations in the following cases:

- x

Chart 1

Chart 2

2.0 Data objectives

1. Outbreak determination

Investigating Officers should endeavour to confirm the existence of an outbreak from an initial complaint. The Department of Health considers two different people (not related) who attended the same function or ate at the same premises and who experienced similar symptoms as a possible outbreak.

From the first contact with a complainant it should be established what the perceived source is. By following the questions outlined on the Enteric Disease Investigation Report (EDIR) (see attachment 1), an officer should determine if the case being reported is a self limiting incident, in which case a follow up by the local EHO is appropriate, or if the person is reporting an outbreak with multiple cases and therefore wider public health implications.

Reported cases that fit the outbreak criteria must be investigated as per the stated protocol. Refer to Chart 2 for the Outbreak Decision tree.

2. Ensure the relevant Public Health Unit is notified of event.

If the incident is reported at a local government level, an officer from that municipality must advise the Public Health Unit, once the determination of an outbreak has been made.

The Food Safety Section in Perth should also be advised, for assistance with faecal submission forms, or other administrative duties.

PUBLIC HEALTH UNITS

Coastal & Wheatbealt (08) 9622 0120
Gascoyne (08) 9941 0560
Goldfields (08) 9021 2622
Great Southerm (08) 9841 8244
Kimberley (08) 9191 1144
Midwest (08) 9964 4299
Pilbara (08) 9140 2377
Southwest (08) 9792 2500

The Food Safety reception number is (08) 9388 4903
or fax Food Safety on (08) 9382 8119

3. Referral to Local Government

Public Health Unit / Disease Control staff may conduct secondary patient interviews on dietary histories, to confirm epidemiological data.

If this information has been reported to a local government EHO (after consulting with their PEHO), the EHO should advise the Public Health Unit and the Food Safety Section of the situation. By reporting the incident sooner, a coordinated response may be organised more swiftly.

5. Identify probable source of the infection

By using the EDIR form, an investigating officer should attempt to discern the probable source of the infection. All fields must be

7. Obtain relevant food samples

If any food remains from a function or event where an alleged food poisoning outbreak has taken place, t

The briefing should consider the following points where appropriate:

- What event took place (day, date, time, location)
- Who and how many attended
- What happened / who was sick
- When the local government / Food Safety Section was approached
- Investigation of premises undertaken
- Results of laboratory testing of food and faecal samples
- Control measures recommended and implemented (e.g. seizure and destruction of food on premises, etc.)
- Any recommendations the Dept of Health should consider (e.g. issuing a recall order, declaring the food to be dangerous.)

3.0 Civil Litigation

Some affected persons may feel that civil litigation is an important step in recovering monies for medical treatment, days lost to work and any mental distress their illness has caused. Information may be sought through approved channels, by lawyers acting on the affected person's behalf or herself, through the Freedom of Information Act.

4.0 Disclosure of information

It is vitally important that NO sensitive information regarding the matter is disclosed directly to lawyers acting on an affected person's behalf, Ministers, Media personnel or Non-investigative personnel during and after the case of any media statement or FOI request. An officer may only confirm a released media statement.

Any Ministerial enquiries should be directed to the Media Relations Section, Department of Health or the Chief Executive Officer of a local government.

All media enquiries should also be directed to the Department of Health's Media Liaison Officer, or the Local Council's Media Liaison Officer / Legal personnel (if applicable).

Procedure for Food Safety Branch Officers

Purpose

This SOP defines the initial steps taken by officers of

Administrative action – Food Safety Branch

1. Discuss situation with Food Safety officers and determine if this is considered to be an outbreak.
2. E-mail to Food Safety Branch PHIS – Food safety in outlook list) & Medical Epidemiologist listing the above details.

Procedure for Local Government EHOs

Purpose

This SOP defines the initial steps taken by local government Environmental Health Officers in regional and rural areas when an alleged food poisoning incident is reported.

x Initial inquiry (for possible outbreak)

(Receive phone call from complainant [s]

1. Ask name, DOB & contact details?
2. What symptoms have been experienced?
3. At what time was the onset of symptoms noticed?
4. Who is affected / how many in the party / strike rate?
5. Who ate the food?
6. What food was consumed?
7. Where food was consumed? (Name of premises? What address?).
8. Has anyone visited their GP, if so what GP, have faecals been submitted?
9. Is there any food left over or brought home?
10. Does the person have contact details for others who attended? (if applicable)

x Refer to

Annex 6

Investigation report forms

Outline of an outbreak investigation report

Cover page

€ **Title of report**

Indicate whether this is a preliminary or a final report. Keep the title short and memorable, but include information on the type of problem under investigation, the location and date.

€ **Methods**

Epidemiology:

- description of study population
- type of study conducted
- case definition
- procedures for case-ascertainment and selection of controls (if any)
- methods of data collection, including questionnaire design, administration and contents
- methods of data analysis.

Medical laboratory testing:

- methods of specimen collection and processing
-

- conclusions with justification for those conclusion and rejection of alternative explanations
- relationship of these results to other studies and the literature
- implications of the findings
- an assessment of control measures
- needs for future research.

€ **Recommendations**

Initial recommendations and those for future prevention and control should be listed numerically.

€ **References**

Select appropriate references, including reviews in major scientific journals. Follow a standard style of referencing (e.g. Vancouver style), numbering the references in the order in which they appear in the text.

€ **Appendices**

Questionnaires and/or other survey forms
Appropriate field reports
Any other relevant documents, including press releases.

Example of an outbreak form used in England and Wales for investigation of general outbreaks of infectious intestinal diseases

OUTBREAK NO. 97\.....

Name: _____ Address: _____

9. Etiology (Name the bacteria, virus, parasite, or toxin. If available, include the serotype and other characteristics such as phage type, virulence factors, and metabolic profile. Confirmation criteria available at http://www.cdc.gov/ncidod/dbmd/outbreak/ or MMWR 2001;50(RR-2):49)				
Etiology		Serotype	Other characteristics (e.g. phage type)	Detected in (see codes just below)
1)	…Confirmed			
2)	…Confirmed			
3)	…Confirmed			

15. If cohort investigation conducted:

Attack rate* = _____ / _____ x 100 = _____%

Exposed and ill Total number exposed for whom you have illness information

Part 4: Ground beef
1. What percentage of ill persons (for whom information is available) ate ground beef raw or undercooked? _____% 2. Was ground beef case-ready? (Ground beef that comes from a manufacturer packaged for sale and not altered or repackaged at retail by the …Yes …No …Unknown or undetermined 3. Was the beef ground or reground by the retailer? …Yes …No …Unknown or undetermined If yes, was anything added to the beef during grinding (e.g. shop trim or any product to alter the fat content)? _____

Part 5: Mode of transmission

(enterohaemorrhagic